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International Society for Marginalized Lives
Dr. Mozib Newborn Foundation
The Carlo GNS Center for Saving Lives at Birth
Vishwa Mahesh Parivaar
Autism Care Network Foundation

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SABREE Enrichment Academy: Empowering Ability
The Caribbean Association for Hematology and Oncology
First Breath of Life
GNS Neonatal Radiology Forum
Global Newborn Society, Orthopedic Surgery Section

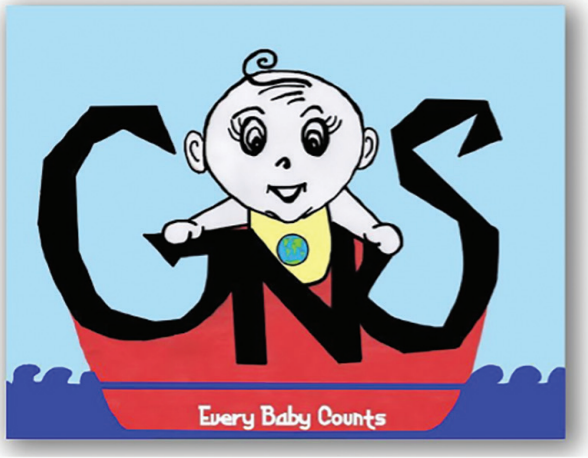


High-throughput Sequencing to Identify Errors in DNA Sequences and Chromosomal Abnormalities

Other Highlighted articles:
Zellweger Spectrum Disorders: Beyond the Peroxisome
Maternal Immunization: Optimizing Passive Immunity in the Newborn

Also available online at
https://www.globalnewbornsociety.org/our-scientific-journal-newborn

Bibliographic Listing:
J-Gate, Scilit, WorldCat, PORTICO, ICMJE



Global Newborn Society

Each time we lose an infant, we lose an entire life and its potential!

Newborn is the official journal of the Global Newborn Society (GNS), a globally active, non-profit organization that is registered as a 501(c)(3) non-profit formation in the United States and is currently being listed as an analogous charity in many other nations. The aim is to enhance research in newborn medicine, understand epidemiology (risk factors) of disease, train healthcare workers, and promote social engagement. The GNS was needed because despite all improvements in medical care, infants remain a high-risk patient population with mortality rates similar to 60-year-olds. We need to remind ourselves that *Every Baby Counts*, and that *Each Time We Lose an Infant, We Lose an Entire Life and its Potential*.

Our logo above, a hand-drawn painting, graphically summarizes our thought-process. There is a lovable little young infant exuding innocent, genuine happiness. The curly hair, shape of the eyes, long eyelashes, and the absence of skin color emphasize that infants need care all over the world, irrespective of ethnicity, race, and gender. On the bib, the yellow background reflects happiness, hope, and spontaneity; the globe symbolizes well-coordinated, worldwide efforts. The age-related vulnerability of an infant, with all the limitations in verbal expression, is seen in being alone in the boat.

The unexpressed loneliness that many infants endure is seen in the rough waters and the surrounding large, featureless sky. However, the shades of blue indicate that the hope of peace and tranquility is not completely lost yet. The acronym letters, GNS, on the starboard are made of cast metal and are pillars of strength. However, the angular rough edges need continued polishing to ascertain adequacy and progress. The red color of the boat symbolizes our affection. The expression "*Every Baby Counts*" seen on the boat's draft below the waterline indicates our commitment to philanthropy, and if needed, to altruism that does not always need to be visible. The shadow behind the picture shows that it has been glued on a solid wall, one built out of our adoption and commitment.

Design of the Journal Cover

The blue color on the journal cover was a careful choice. Blue is the color of flowing water, and symbolizes the abnormalities of blood vascular flow that are seen in many neonatal illnesses. There is a gradual transition in the shades of blue from the top of the cover downwards. The deeper shades of blue on the top emphasize the depth, expertise, and stability, which the renowned authors bring. Light blue is associated with health, healing, tranquility, understanding, and softness, which their studies bring. The small letter "*n*" in the title of the journal, *newborn*, was chosen to emphasize the little size of a newborn baby. The issue editors chose three articles to be specifically highlighted; the two pictures and two titles below reflects an order suggested by them.

Instructions to Authors

The journal welcomes original articles and review articles. We also welcome consensus statements, guidelines, trials methodology, and core outcomes relevant to fetuses/young infants in the first 1000 days. A detailed set of instructions to authors can be seen online at https://www.globalnewbornsociety.org/intructions-for-authors. The manuscripts can be submitted via the online manuscript submission system.

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From Sanger to Single Molecules: How High-throughput Deoxyribonucleic Acid Sequencing Became Possible

For almost three decades, deoxyribonucleic acid (DNA) sequencing relied nearly exclusively on Sanger's chain-termination method, which reads one DNA fragment at a time.[1] This method was accurate and became the basis of the Human Genome Project, but the project took more than a decade and cost about US$3 billion. High-throughput sequencing changed this by reading millions to billions of fragments at once. A human genome can now be sequenced in a day or two for less than US$1,000. This shift did not come from a single invention. It came from advances that converged in chemistry, engineering, enzymology, single-molecule physics and computation, as outlined below (**Fig. 1**):

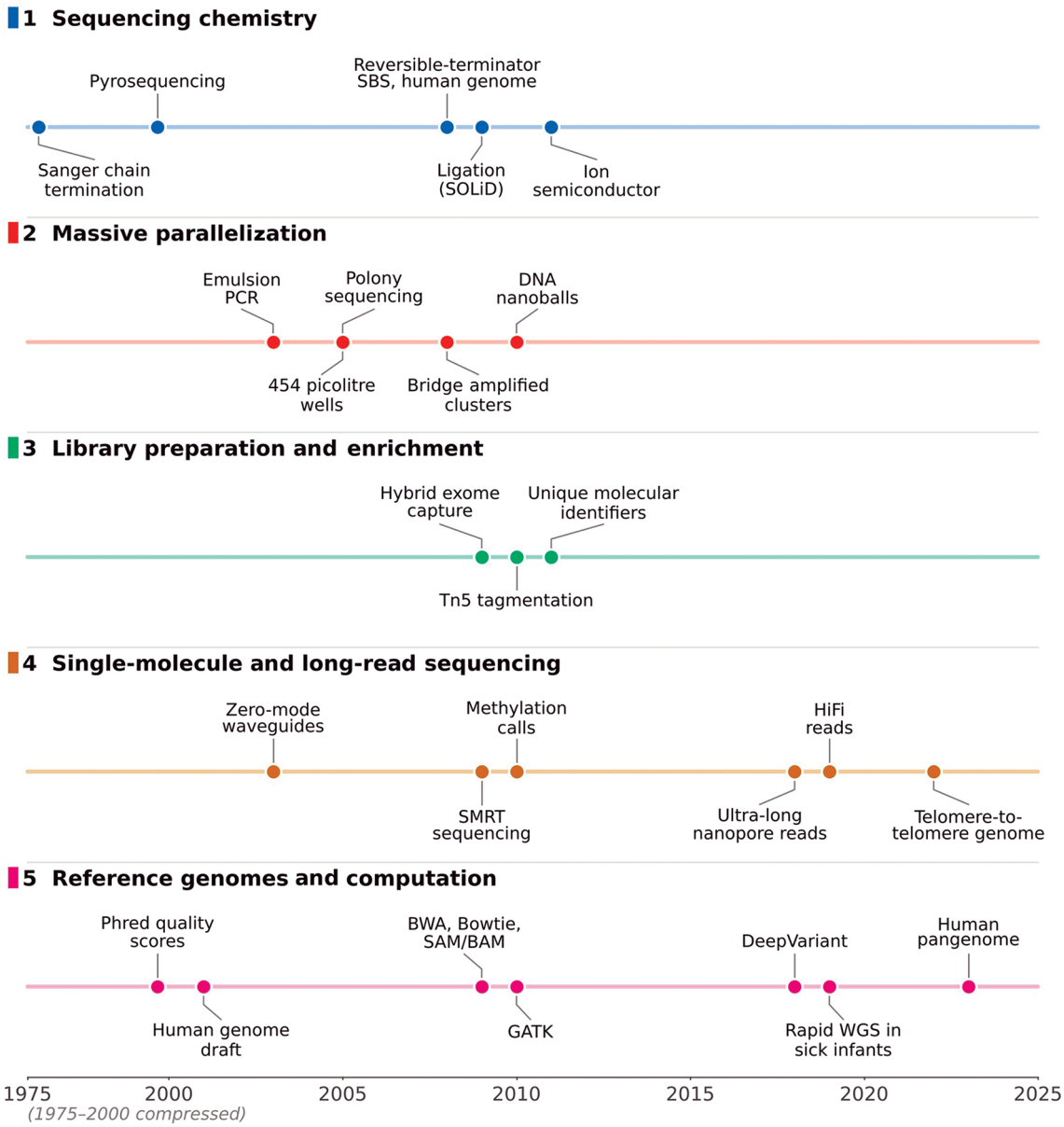

Fig. 1: Timeline of advances that made high-throughput DNA sequencing possible. Each colored lane corresponds to one of the five streams described in the text. Each dot marks a milestone, placed at the year of its key publication. Clusters of dots show where developments in different fields converged, particularly between 2003 and 2011. The time axis is compressed before 2000. *Abbreviations:* BWA, Burrows–Wheeler Aligner; DNA, deoxyribonucleic acid; GATK, Genome Analysis Toolkit; HiFi, high-fidelity (circular consensus) reads; PCR, polymerase chain reaction; SAM/BAM, Sequence Alignment/Map format and its binary version; SBS, sequencing by synthesis; SMRT, single-molecule real-time; SOLiD, Sequencing by Oligonucleotide Ligation and Detection; Tn5, transposon 5; WGS, whole-genome sequencing

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New Sequencing Chemistries: Detecting Each Base As It Is Added

Sanger sequencing needed a separate electrophoresis run for every fragment read. The shift came with cyclic "sequencing by synthesis," in which each nucleotide is detected as a polymerase adds it to a growing strand. The approaches differ in how they detect the added base:

- Pyrosequencing flowed one deoxynucleotide at a time over the template. Each incorporation released pyrophosphate, which ATP sulfurylase converted to ATP and luciferase turned into a flash of light.[2] Apyrase then degraded the excess nucleotides before the next flow. The method struggled with homopolymers, runs of the same base whose length had to be estimated from the brightness of the light.
- Reversible-terminator chemistry solved this problem.[3] Each of the four nucleotides carries a distinct cleavable fluorophore and a 3′-O blocking group (such as azidomethyl), so only one base is added per cycle. The flow cell is imaged, then the fluorophore and block are chemically removed before the next cycle. This gives highly accurate paired-end reads of 100–300 bases. Most errors are substitutions, at rates of about 0.1%.
- Sequencing by ligation (SOLiD) used fluorescent probes that a DNA ligase joins to the strand.[4] A two-base encoding scheme reads each position twice to help separate true variants from errors.
- Ion semiconductor sequencing dispensed with optics altogether.[5] Each incorporation releases a hydrogen ion, and the resulting pH change is detected by an ion-sensitive field-effect transistor beneath each microwell. This brought sequencing onto standard semiconductor chips.

Massive Parallelization: Clonal Amplification on Surfaces and Beads

The signal from a single DNA molecule was too weak for the early optics. Each fragment therefore had to be copied into a clonal population that could be read as one spot, with millions of such spots read in parallel. Four platforms solved this in different ways:

- Emulsion polymerase chain reaction (PCR) copies each fragment onto its own bead inside a water-in-oil droplet that acts as a microscopic reactor.[6] In the 454 system the beads were loaded into a "picotiter" plate of about 1.6 million wells, and 25 million bases were read in a single four-hour run.[7]
- Bridge amplification grafts adapter-complementary oligonucleotides onto a glass flow cell.[3] Each fragment bends over to prime from a neighboring oligonucleotide, forming a cluster of about 1,000 identical copies. Patterned flow cells with ordered nanowells later raised cluster density into the billions per run.
- Polony sequencing showed that a whole bacterial genome could be read accurately by cyclic sequencing of amplified molecules arrayed in a gel.[8]
- Deoxyribonucleic acid nanoballs are made by rolling-circle amplification of circularized templates.[9] These compact, self-contained copies settle into ordered sites on an array, and PCR is not needed.

Library Preparation, Target Enrichment, and Molecular Barcoding

Every fragment must carry adapters at its ends, short known sequences that anchor it to the surface and prime the sequencing reaction. Several advances have made this step faster, cheaper and more informative:

- Conventional library preparation shears DNA acoustically or enzymatically, repairs the ends, adds an A-tail, and ligates the adapters.
- Tagmentation uses a hyperactive transposon 5 (Tn5) transposase to fragment the DNA and insert adapters in one step, cutting time and the amount of input DNA needed.[10]
- Hybrid capture with biotinylated RNA or DNA baits pulls down only the regions of interest.[11] Its use to sequence all protein-coding exons (exome sequencing) showed that clinically relevant variants could be found at a fraction of the cost of a whole genome.[12]
- Sample barcodes (indexes) built into the adapters allow dozens to hundreds of patients to be pooled in one run and separated computationally afterwards.
- Unique molecular identifiers tag each original molecule before amplification.[13] Polymerase chain reaction duplicates and errors can then be recognized, and variants present at very low frequencies can be detected.
- Engineered polymerases, derived from archaeal enzymes and mutated to accept bulky 3′-blocked nucleotides, made reversible-terminator chemistry efficient.

Single-molecule and Long-read Sequencing

Third-generation platforms read single DNA molecules without amplification, which removes amplification bias and produces reads thousands of bases long:

- Single-molecule real-time (SMRT) sequencing places one polymerase at the bottom of a zero-mode waveguide.[14] This is a nanoscale aperture about 70 nm wide that confines observation to a zeptoliter volume. The fluorophore is attached to the terminal phosphate of each nucleotide, so it is released naturally at incorporation, and the strand continues to grow unmodified.[15] The timing of each incorporation also reveals base modifications such as methylation.[16] Circularizing the template allows the same molecule to be read repeatedly. The resulting circular consensus ("HiFi") reads reach about 99.8% accuracy at lengths of more than 13 kilobases.[17]
- Nanopore sequencing ratchets a DNA strand through a protein pore set in a membrane, using a motor protein.[18] Each group of bases passing through the pore produces a characteristic change in the ionic current, which neural-network base callers convert

into sequence. Methylated bases are detected directly. Reads can exceed 1,00,000 bases, and lengths of more than 8,00,000 bases have been reported.[19] The devices can be as small as a USB stick.
- Long reads span repeats, segmental duplications and structural variants that short reads cannot resolve. These capabilities culminated in the first complete, telomere-to-telomere human genome, which added about 200 million bases of previously missing sequence.[20]

Reference Genomes, Base Calling and Computation

High-throughput sequencing depends as much on computation as on chemistry. The main computational advances include:

- Reference genomes: The Human Genome Project provided the reference against which billions of reads are aligned.[21] The draft human pangenome now adds diverse haplotypes, reducing reference bias across populations.[22]
- Base calling: Phred quality scores express the probability that each called base is wrong, which lets downstream tools weigh the evidence statistically.[23]
- Alignment: Burrows–Wheeler transform and FM-index aligners compress the genome into a searchable index, so short reads can be mapped quickly with modest memory.[24,25]
- Data formats: Standard file formats such as SAM/BAM let data move between tools.[26]
- Variant calling: Frameworks such as the Genome Analysis Toolkit (GATK) added base-quality recalibration and haplotype-aware calling of single-nucleotide variants and insertions or deletions.[27] Deep-learning callers now treat read pileups as images and classify genotypes with convolutional neural networks.[28]
- Clinical translation: Combined with automated phenotyping and interpretation, rapid whole-genome sequencing (WGS) can now return a diagnosis for a critically ill infant within days, and in some cases within hours.[29]

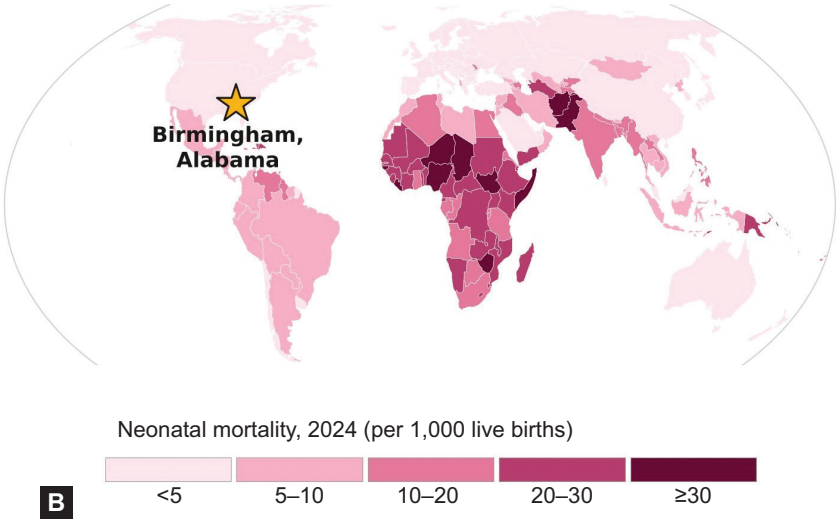
We are pleased to announce the establishment of the *GNS Center for Integrative Thinking*, into which our GNS Center for Computational Scientific Methodology has now merged. Medical training has long rewarded focus on a single organ, disease, or technique. This was a necessity, not a failing: knowledge in each field grew so vast that years of dedicated study were needed to reach real expertise. Yet, to answer the most fundamental questions about health, we need to cross the boundaries between disciplines that we ourselves drew over the past two centuries; nature has never recognized them. The biologist E. O. Wilson called this convergence of knowledge across disciplines "consilience."[30] Understanding rises through a pyramid, from data to information, to knowledge, and finally to wisdom: the judgment to know which questions matter and what the answers mean.[31] The availability of computational tools and large databases has transformed our ability to gather, organize, and analyze data and information, on a scale no individual could once have managed. This frees investigators to spend more of their effort at the summit. The idea is not new: in 1962, Douglas Engelbart proposed that computers could augment human intellect rather than replace it.[32] The Center is intended as a home for that work, which proceeds in three stages. First comes the question. A baby dies in one place and survives in another, and we ask why. Investigators need no longer hold back in thinking about a question because they lack expertise in mathematics, biophysics, biochemistry, anthropology, sociology or evolutionary biology, or other fields; or because they lack access to the right library. For colleagues in low-resource settings, this may matter most of all. Second, the investigator builds a template that captures the structure, standards, and reasoning style of the work. Third, the template becomes a highway. The tools supply speed, and the investigator gallops, repeatedly asking "what more?" and keeping an aerial view until the work reaches the limits of the evidence. Whole populations, rather than samples, can now be studied, and an idea that arrives at midnight need not wait until morning. This matters, because as a global organization, we have to work across time zones. Throughout, the tools remain instruments, not authors, and their outputs can err: every question begins with the investigator, and every result is verified by the investigator, who remains responsible for every conclusion.[33] *Now, at last, the question comes first, not the questioner's ability to answer it.*

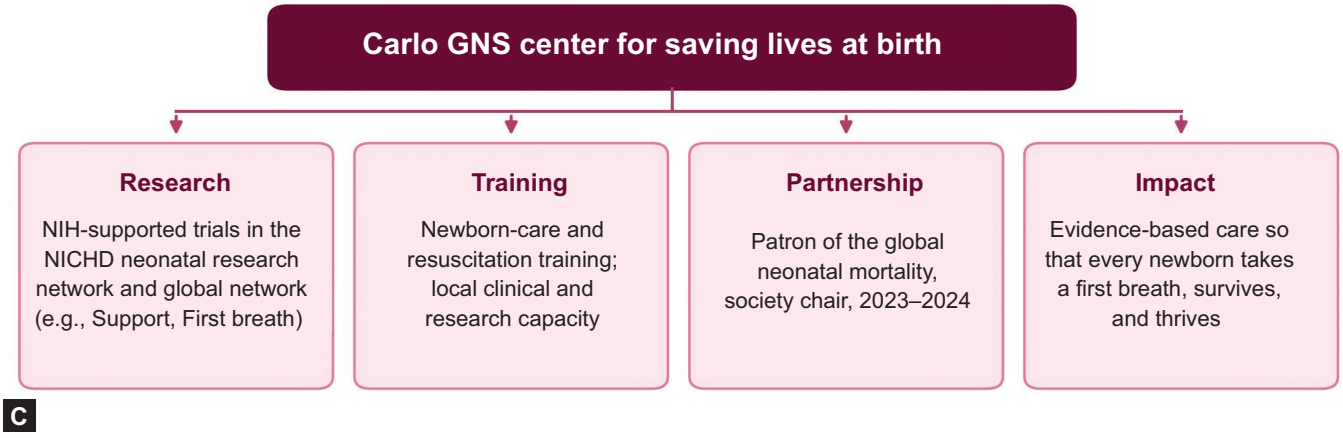
In this issue, we would like to highlight the achievements and contributions of the Carlo GNS Center for Saving Lives at Birth. This virtual center of the Global Newborn Society was established through the generous dedication of time and financial resources by Prof. Waldemar A. Carlo and the GNS. Prof. Carlo is the Edwin M. Dixon Chair in Neonatology and Professor of Pediatrics at the University of Alabama at Birmingham, and has devoted his career to reducing preventable deaths around the time of birth. As a patron of the GNS and one of its chairs during 2023–2024, he has continued to give the Society motivation, direction and counsel.[34] With support from the US National Institutes of Health, through the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Neonatal Research Network and the Global Network for Women's and Children's Health Research, he has led landmark trials. These include the Surfactant, Positive Pressure, and Oxygenation Randomized Trial (SUPPORT) trial of oxygen-saturation targets in extremely preterm infants and the First Breath trial of newborn-care training in low- and middle-income countries.[35,36] Through this work he has also trained clinicians and researchers who carry it forward in their own countries. Building on this legacy, the Center brings together clinicians, researchers, and educators across the world to develop, test, and share practical, evidence-based ways to help every newborn take a first breath, survive, and thrive (**Fig. 2**).

This journal aims to cover fetal/neonatal problems that begin during pregnancy, at the time of birth, or during the first 1,000 days after birth. As in our previous issues, we present eight articles here (**Fig. 3**). A brief summary of the eight articles is presented below:

CRP/albumin ratio in neonatal sepsis due to Gram-negative bacteria: Agroya et al. examined whether the ratio of C-reactive protein to albumin can indicate the class of the infecting organism in 94 neonates with culture-proven sepsis.[37] The ratio was about three times higher in Gram-negative than in Gram-positive infections (36.2 vs 11.8) and was the only independent predictor of Gram-negative sepsis. Values above 35 suggested *Klebsiella*, *Pseudomonas* or *Escherichia coli*. Neonatal sepsis remains a major global burden: a meta-analysis estimated about 2,824 cases per 100,000 live births and a mortality rate of 17.6%.[38] The CRP/albumin ratio had previously been studied as a predictor of neonatal sepsis and its severity, but not of the causative organism.[39] If validated in larger cohorts, this inexpensive







Figs 2A to C: The Carlo GNS Center for Saving Lives at Birth. (A) Prof. Waldemar A. Carlo speaking at the inauguration of the first Global Newborn Society meeting at the Nobel Prize Museum, Stockholm, Sweden (held at the Museum and Uppsala University on November 2–4, 2025); (B) The Center is based in Birmingham, Alabama. The underlying map shows neonatal mortality in 2024, which is highest in sub-Saharan Africa and South Asia, where the Center's work in research and training is most needed; (C) The Center's work spans research, training, partnership, and impact, aimed at helping every newborn take a first breath, survive, and thrive. *Source for (B):* World Bank, World Development Indicators.

marker could help guide the choice of empirical antibiotics while culture results are awaited.

Mother's own milk in an extramural NICU: Joseph et al. report a quality improvement study from an extramural NICU in Chennai, India, where infants are admitted far from mothers who remain at the birth hospital.[40] Over four plan–do–study–act cycles, the team introduced structured counseling, telelactation, transport of expressed milk, and counselor-led monitoring. With these, the proportion of 186 separated infants receiving mother's own milk within 48 hours rose from 25 to 94%. Breastfeeding has well-established benefits for child survival, health and development, and these benefits underpin global recommendations to prioritize mother's own milk.[41] In mothers of very low birth weight infants, starting milk expression within the first hour after birth increased milk volume and brought earlier onset of copious milk production (lactogenesis II).[42] This low-cost bundle offers a model that other units caring for separated mother–infant pairs can readily adopt.

Zellweger spectrum disorders: In their review, Maheshwari and Huisman revisit Zellweger spectrum disorders: autosomal recessive peroxisome biogenesis disorders caused by pathogenic variants in *PEX* genes.[43] These defects disrupt peroxisome assembly and matrix protein import, leading to accumulation of very-long-chain fatty acids, phytanic and pipecolic acids, and bile acid intermediates, with deficient plasmalogen synthesis. Looking beyond the peroxisome, the authors bring neuronal migration, oligodendrocyte and microglial dysfunction, mitochondrial–peroxisomal crosstalk, epigenetic and non-coding RNA responses, and the evolutionary conservation of peroxins into one integrated view of disease mechanisms. A clinical overview of the spectrum has shown that presentations range from lethal neonatal disease to milder forms that survive into adulthood, and that management remains supportive and multidisciplinary.[44]

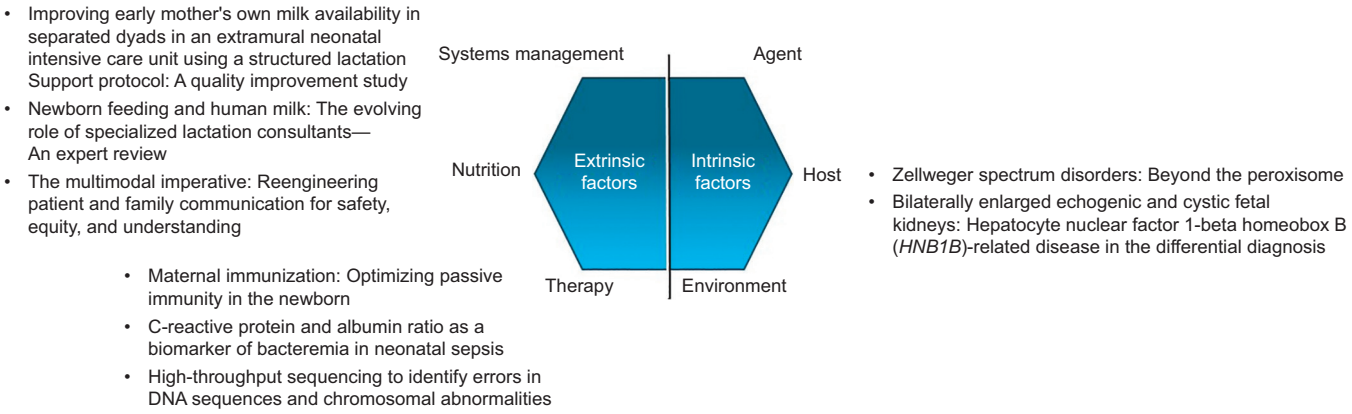


Fig. 3: Areas of focus in the newborn, Volume 5, Issue 3. We have expanded the traditional agent-host-environment trinodal disease model to a hexagonal system. The three additional foci represent extrinsic factors that can affect health - those originating in therapy, nutrition, and systems management are shown. This issue covers 2 nodes, with articles focused on host factors and systems management.

A genetic classification of more than 600 patients mapped the mutational spectrum across the *PEX* genes and identified *PEX1* as the gene most frequently affected.[45] This review gives neonatologists a mechanistic framework for early recognition, family counseling and the targeted therapies that may follow.

High-throughput sequencing: Das and Maheshwari give a practical overview of high-throughput sequencing, from nucleic acid preparation and library construction to the detection of single-nucleotide variants, insertions and deletions, copy-number variants, repeat expansions and structural chromosomal rearrangements.[46] These authors also trace the biological origins of such errors and discuss emerging uses in single-cell genomics, pathogen surveillance, cancer genomics and AI-assisted variant interpretation. Chain-terminating sequencing, introduced by Sanger in 1977, defined DNA analysis for nearly three decades.[1] Massively parallel platforms have since cut the cost and time of sequencing by orders of magnitude.[47] As genomic testing becomes routine in critically ill infants, this review is a timely primer on what sequencing can detect and on the pitfalls of technical artifacts and overdiagnosis.

Multimodal patient and family communication: Dahiya and Patel argue that communication with patients and families is a clinical and patient-safety function.[48] Brief verbal exchanges and text often fail families facing limited health literacy, language barriers, cognitive overload or sensory limitations. The authors propose a Multimodal Communication Principle, which matches verbal, written, visual, audiovisual and AI-enabled formats to the family, the main barrier, the clinical context and the task. A meta-analysis found that poor physician communication was associated with a 19% higher risk of non-adherence, and that communication training increased the odds of adherence 1.62-fold.[49] Adding pictures to written or spoken information has been shown to improve attention, comprehension, recall and adherence.[50] For families in the NICU, who must absorb complex information under great stress, this framework is directly relevant.

Maternal immunization: Michie presents maternal immunization as prenatal neonatal preventive medicine.[51] Vaccination in pregnancy increases the pathogen-specific IgG available for FcRn-mediated transfer across the placenta and complements the mucosal protection of human milk. The review highlights the particular vulnerability of preterm infants, who miss much of the third-trimester IgG transfer, and outlines roles for neonatologists in counseling, research, and collaboration with obstetric and public health teams. In England, maternal pertussis vaccination was 91% effective in preventing pertussis in infants.[52] In the MATISSE trial, a bivalent RSV prefusion F vaccine given in pregnancy reduced medically attended severe RSV lower respiratory tract illness in infants by 81.8% within 90 days of birth.[53] With vaccines against group B *Streptococcus* and cytomegalovirus in development, this article invites neonatologists to take part in protection that begins before birth.

Specialized lactation consultants: Kaushal and Ahmed present newborn feeding as a continuum of care and describe the evolving role of International Board-certified Lactation Consultants in managing ineffective latch, nipple trauma, the mastitis spectrum, low milk supply and infant feeding dysfunction.[54] The authors argue for integrating lactation expertise across maternity, neonatal, pediatric and primary care, supported by telelactation, clear referral pathways and equitable access. The *Lancet* Breastfeeding Series estimated that scaling up breastfeeding could prevent about 823,000 child deaths each year.[41] A recent meta-analysis found that interventions by lactation consultants improved both exclusive and any breastfeeding compared with usual care.[55] This review makes a timely case for treating specialized lactation support as a core clinical service rather than an optional extra.

***HNF1B*-related fetal kidney disease:** Lodha et al. describe a fetus with progressively enlarged, echogenic kidneys and small cortical cysts at 22 weeks' gestation.[56] Chromosomal microarray was normal, but trio sequencing identified a pathogenic *HNF1B* nonsense variant. The same variant was found in the asymptomatic mother, whose subsequent evaluation revealed subtle renal changes and hypomagnesemia, a striking example of variable expressivity within one family. Anomalies of *HNF1B* (then called *TCF2*) were earlier shown to be the main cause of bilateral hyperechogenic fetal kidneys, and the disease is now known to extend beyond the kidney to diabetes, hypomagnesemia and liver involvement.[57,58] The report reminds clinicians that visible fetal renal cysts are a morphological sign rather than a diagnosis, and that a molecular diagnosis establishes the cause without predicting the course of disease.

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References

1. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 1977;74(12):5463–5467. DOI: 10.1073/pnas.74.12.5463.
2. Ronaghi M, Uhlén M, Nyrén P. A sequencing method based on real-time pyrophosphate. Science 1998;281(5375):363–365. DOI: 10.1126/science.281.5375.363.
3. Bentley DR, Balasubramanian S, Swerdlow HP, et al. Accurate whole human genome sequencing using reversible terminator chemistry. Nature 2008;456(7218):53–59. DOI: 10.1038/nature07517.
4. McKernan KJ, Peckham HE, Costa GL, et al. Sequence and structural variation in a human genome uncovered by short-read, massively parallel ligation sequencing using two-base encoding. Genome Res 2009;19(9):1527–1541. DOI: 10.1101/gr.091868.109.
5. Rothberg JM, Hinz W, Rearick TM, et al. An integrated semiconductor device enabling non-optical genome sequencing. Nature 2011;475(7356):348–352. DOI: 10.1038/nature10242.
6. Dressman D, Yan H, Traverso G, et al. Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. Proc Natl Acad Sci U S A 2003;100(15):8817–8822. DOI: 10.1073/pnas.1133470100.
7. Margulies M, Egholm M, Altman WE, et al. Genome sequencing in microfabricated high-density picolitre reactors. Nature 2005;437(7057):376–380. DOI: 10.1038/nature03959.
8. Shendure J, Porreca GJ, Reppas NB, et al. Accurate multiplex polony sequencing of an evolved bacterial genome. Science 2005;309(5741):1728–1732. DOI: 10.1126/science.1117389.
9. Drmanac R, Sparks AB, Callow MJ, et al. Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. Science 2010;327(5961):78–81. DOI: 10.1126/science.1181498.
10. Adey A, Morrison HG, Asan, et al. Rapid, low-input, low-bias construction of shotgun fragment libraries by high-density in vitro transposition. Genome Biol 2010;11(12):R119. DOI: 10.1186/gb-2010-11-12-r119.
11. Gnirke A, Melnikov A, Maguire J, et al. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. Nat Biotechnol 2009;27(2):182–189. DOI: 10.1038/nbt.1523.
12. Ng SB, Turner EH, Robertson PD, et al. Targeted capture and massively parallel sequencing of 12 human exomes. Nature 2009;461(7261):272–276. DOI: 10.1038/nature08250.
13. Kinde I, Wu J, Papadopoulos N, et al. Detection and quantification of rare mutations with massively parallel sequencing. Proc Natl Acad Sci U S A 2011;108(23):9530–9535. DOI: 10.1073/pnas.1105422108.
14. Levene HJ, Korlach J, Turner SW, et al. Zero-mode waveguides for single-molecule analysis at high concentrations. Science 2003;299(5607):682–686. DOI: 10.1126/science.1079700.
15. Eid J, Fehr A, Gray J, et al. Real-time DNA sequencing from single polymerase molecules. Science 2009;323(5910):133–138. DOI: 10.1126/science.1162986.
16. Flusberg BA, Webster DR, Lee JH, et al. Direct detection of DNA methylation during single-molecule, real-time sequencing. Nat Methods 2010;7(6):461–465. DOI: 10.1038/nmeth.1459.
17. Wenger AM, Peluso P, Rowell WJ, et al. Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome. Nat Biotechnol 2019;37(10):1155–1162. DOI: 10.1038/s41587-019-0217-9.
18. Deamer D, Akeson M, Branton D. Three decades of nanopore sequencing. Nat Biotechnol 2016;34(5):518–524. DOI: 10.1038/nbt.3423.
19. Jain M, Koren S, Miga KH, et al. Nanopore sequencing and assembly of a human genome with ultra-long reads. Nat Biotechnol 2018;36(4):338–345. DOI: 10.1038/nbt.4060.
20. Nurk S, Koren S, Rhie A, et al. The complete sequence of a human genome. Science 2022;376(6588):44–53. DOI: 10.1126/science.abj6987.
21. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature 2001;409(6822):860–921. DOI: 10.1038/35057062.
22. Liao WW, Asri M, Ebler J, et al. A draft human pangenome reference. Nature 2023;617(7960):312–324. DOI: 10.1038/s41586-023-05896-x.
23. Ewing B, Green P. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res 1998;8(3):186–194. DOI: 10.1101/gr.8.3.186.
24. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 2009;25(14):1754–1760. DOI: 10.1093/bioinformatics/btp324.
25. Langmead B, Trapnell C, Pop M, et al. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 2009;10(3):R25. DOI: 10.1186/gb-2009-10-3-r25.
26. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25(16):2078–2079. DOI: 10.1093/bioinformatics/btp352.

27. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010;20(9):1297–1303. DOI: 10.1101/gr.107524.110.
28. Poplin R, Chang PC, Alexander D, et al. A universal SNP and small-indel variant caller using deep neural networks. Nat Biotechnol 2018;36(10):983–987. DOI: 10.1038/nbt.4235.
29. Clark MM, Hildreth A, Batalov S, et al. Diagnosis of genetic diseases in seriously ill children by rapid whole-genome sequencing and automated phenotyping and interpretation. Sci Transl Med 2019;11(489):eaat6177. DOI: 10.1126/scitranslmed.aat6177.
30. Wilson EO. Consilience: The Unity of Knowledge. New York: Alfred A. Knopf; 1998.
31. Ackoff RL. From data to wisdom. J Appl Syst Anal 1989;16:3–9.
32. Engelbart DC. Augmenting Human Intellect: A Conceptual Framework. Summary Report AFOSR-3223. Menlo Park, CA: Stanford Research Institute; 1962.
33. Global Newborn Society. Using AI: policy for authors. Newborn. Available from: https://www.globalnewbornsociety.org/our-scientific-journal-newborn/gns-newborn-journal-submit/using-ai. Accessed October 14, 2026.
34. Global Newborn Society. Prof. Waldemar Carlo at the 1st Global Newborn Society Meeting, Uppsala, Sweden. Nov 4, 2025 [Video]. YouTube 2025. Available from: https://www.youtube.com/watch?v=f5-R0wHeDxM. Accessed October 13, 2026.
35. SUPPORT Study Group of the Eunice Kennedy Shriver NICHD Neonatal Research Network; Carlo WA, Finer NN, Walsh MC, et al. Target ranges of oxygen saturation in extremely preterm infants. N Engl J Med 2010;362(21):1959–1969. DOI: 10.1056/NEJMoa0911781.
36. Carlo WA, Goudar SS, Jehan I, et al. Newborn-care training and perinatal mortality in developing countries. N Engl J Med 2010;362(7):614–623. DOI: 10.1056/NEJMsa0806033.
37. Agroya AS, Patil NB, Kavthekar SO. C-reactive Protein and Albumin Ratio as a Biomarker of Bacteremia in Neonatal Sepsis. Newborn (Clarksville, Md) 2026;5(3). DOI: 10.5005/jp-journals-11002-0168.
38. Fleischmann C, Reichert F, Cassini A, et al. Global incidence and mortality of neonatal sepsis: A systematic review and meta-analysis. Arch Dis Child 2021;106(8):745–752. DOI: 10.1136/archdischild-2020-320217.
39. Li T, Li X, Wei Y, et al. Predictive value of C-reactive protein-to-albumin ratio for neonatal sepsis. J Inflamm Res 2021;14:3207–3215. DOI: 10.2147/JIR.S321074.
40. Joseph JE, Muthukumaran N, Sangeetha T. Improving Early Mother's Own Milk Availability in Separated Dyads in an Extramural Neonatal Intensive Care Unit Using a Structured Lactation Support Protocol: A Quality Improvement Study. Newborn (Clarksville, Md) 2026;5(3). DOI: 10.5005/jp-journals-11002-0166.
41. Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. Lancet 2016;387(10017):475–490. DOI: 10.1016/S0140-6736(15)01024-7.
42. Parker LA, Sullivan S, Krueger C, et al. Effect of early breast milk expression on milk volume and timing of lactogenesis stage II among mothers of very low birth weight infants: A pilot study. J Perinatol 2012;32:205–209. DOI: 10.1038/jp.2011.78.
43. Maheshwari A, Huisman TAGM. Zellweger Spectrum Disorders: Beyond the Peroxisome. Newborn (Clarksville, Md) 2026;5(3). DOI: 10.5005/jp-journals-11002-0164.
44. Klouwer FC, Berendse K, Ferdinandusse S, et al. Zellweger spectrum disorders: Clinical overview and management approach. Orphanet J Rare Dis 2015;10:151. DOI: 10.1186/s13023-015-0368-9.
45. Ebberink MS, Mooijer PA, Gootjes J, et al. Genetic classification and mutational spectrum of more than 600 patients with a Zellweger syndrome spectrum disorder. Hum Mutat 2011;32(1):59–69. DOI: 10.1002/humu.21388.
46. Das JK, Maheshwari A. High-throughput Sequencing to Identify Errors in DNA Sequences and Chromosomal Abnormalities. Newborn (Clarksville, Md) 2026;5(3). DOI: 10.5005/jp-journals-11002-0171.
47. Reuter JA, Spacek DV, Snyder MP. High-throughput sequencing technologies. Mol Cell 2015;58(4):586–597. DOI: 10.1016/j.molcel.2015.05.004.
48. Dahiya R, Patel B. The Multimodal Imperative: Reengineering Patient and Family Communication for Safety, Equity, and Understanding. Newborn (Clarksville, Md) 2026;5(3). DOI: 10.5005/jp-journals-11002-0172.
49. Zolnierek KB, DiMatteo MR. Physician communication and patient adherence to treatment: A meta-analysis. Med Care 2009;47(8):826–834. DOI: 10.1097/MLR.0b013e31819a5acc.
50. Houts PS, Doak CC, Doak LG, et al. The role of pictures in improving health communication: A review of research on attention, comprehension, recall, and adherence. Patient Educ Couns 2006;61(2):173–190. DOI: 10.1016/j.pec.2005.05.004.
51. Michie CA. Maternal Immunization: Optimizing Passive Immunity in the Newborn. Newborn (Clarksville, Md) 2026;5(3). DOI: 10.5005/jp-journals-11002-0173.
52. Amirthalingam G, Andrews N, Campbell H, et al. Effectiveness of maternal pertussis vaccination in England: An observational study. Lancet 2014;384(9953):1521–1528. DOI: 10.1016/S0140-6736(14)60686-3.
53. Kampmann B, Madhi SA, Munjal I, et al. Bivalent prefusion F vaccine in pregnancy to prevent RSV illness in infants. N Engl J Med 2023;388(16):1451–1464. DOI: 10.1056/NEJMoa2216480.
54. Kaushal M, Ahmed S. Newborn Feeding and Human Milk: The Evolving Role of Specialized Lactation Consultants—An Expert Review. Newborn (Clarksville, Md) 2026;5(3). DOI: 10.5005/jp-journals-11002-0170.
55. D'Hollander CJ, McCredie VA, Uleryk EM, et al. Breastfeeding support provided by lactation consultants: A systematic review and meta-analysis. JAMA Pediatr 2025;179(5):508–520. DOI: 10.1001/jamapediatrics.2024.6810.
56. Lodha A, Zein H, Al Awad EH, et al. Bilaterally Enlarged Echogenic and Cystic Fetal Kidneys: Hepatocyte Nuclear Factor 1-beta Homeobox B (HNF1B)–related Disease in the Differential Diagnosis. Newborn (Clarksville, Md) 2026;5(3). DOI: 10.5005/jp-journals-11002-0169.
57. Decramer S, Parant O, Beaufils S, et al. Anomalies of the TCF2 gene are the main cause of fetal bilateral hyperechogenic kidneys. J Am Soc Nephrol 2007;18(3):923–933. DOI: 10.1681/ASN.2006091057.

58. Bockenhauer D, Jaureguiberry G. HNF1B-associated clinical phenotypes: The kidney and beyond. Pediatr Nephrol 2016;31(5):707–714. DOI: 10.1007/s00467-015-3142-2.
59. Phoenixmed-at-arizona.edu. Ling He Phoenix, Arizona, United States: University of Arizona; 2025. Available from: https://phoenixmed.arizona.edu/directory/he-ling.
60. sabreeenrichmentacademy.org. Sonji-Fatima St. Louis, Missouri, United States: Sabree Enrichment Academy, St. Louis, United States; 2025. Available from: https://www.drsonjifatima.com/.
61. Collegium Medicum P, Poland. Office of the Dean, Faculty of Health Sciences, Płock, Poland Płock, Poland: Collegium Medicum, Płock, Poland; 2025. Available from: https://mazowiecka.edu.pl/en/collegium-medicum/.

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ORIGINAL RESEARCH

Improving Early Mother's Own Milk Availability in Separated Dyads in an Extramural Neonatal Intensive Care Unit Using a Structured Lactation Support Protocol: A Quality Improvement Study

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Abstract

Objective: To increase the proportion of separated neonates receiving mother's own milk (MOM) within 48 hours of admission from a baseline of 25% to at least 50% over a 6-month period in an extramural neonatal intensive care unit (NICU).

Design: Prospective quality improvement study using the point-of-care quality improvement (POCQI) methodology.

Setting: Extramural NICU of a tertiary care teaching hospital in South India.

Participants: One hundred and eighty-six separated mother–infant dyads admitted within the first 5 days of life.

Intervention: Sequential implementation of four plan–do–study–act (PDSA) cycles consisting of structured counseling, telelactation support, transportation support for expressed breast milk, and lactation counselor-led monitoring.

Main outcome measures: Primary outcome was the proportion of neonates receiving MOM within 48 hours of admission. Secondary outcome was the proportion of MOM in total enteral feeds by day 5. Process measures included counseling within 6 hours of admission and milk expression frequency greater than eight times daily.

Results: A total of 186 mother–infant dyads were enrolled. The proportion of neonates receiving MOM within 48 hours increased from 25% at baseline to 94% during the sustenance phase. Counseling within 6 hours improved from 10% to 98%, while mothers expressing milk more than eight times daily increased from 0 to 96%. The proportion of MOM in total feeds by day 5 showed sustained improvement across successive PDSA cycles.

Conclusion: A structured lactation-support protocol significantly improved early MOM availability among separated dyads in an extramural NICU. The interventions were low-cost, feasible, and sustainable.

Keywords: Breastfeeding, Expressed breast milk, Mother's own milk, Neonatal intensive care unit, Point-of-care quality improvement, Quality improvement, Telelactation.

Newborn (2026): 10.5005/jp-journals-11002-0166

Introduction

Mother's own milk (MOM) is the recommended source of nutrition for all newborn infants and is particularly important for preterm and sick neonates admitted to neonatal intensive care units (NICUs). Human milk reduces the risk of necrotizing enterocolitis, late-onset sepsis, feeding intolerance, bronchopulmonary dysplasia, and adverse neurodevelopmental impairment while improving survival and long-term health outcomes.[1–3] The World Health Organization recommends prioritization of MOM for all hospitalized neonates whenever feasible.[4]

Despite its recognized benefits, establishing and maintaining lactation remains challenging in NICU settings. Mothers of hospitalized neonates frequently experience delayed lactogenesis, maternal illness, psychological stress, and physical separation from their infants.[5,6] Several studies have demonstrated that early initiation of milk expression and frequent milk removal are among the strongest predictors of successful milk production in mothers of preterm infants.[7–9]

Extramural NICUs face additional challenges because mothers often remain admitted in distant healthcare facilities while their newborns are transferred to referral centers. In such situations, inadequate counseling, poor family awareness, transportation barriers, and lack of structured lactation support further compromise MOM availability.[10] Contemporary evidence suggests that successful promotion of MOM in NICUs requires bundled interventions that address maternal, family, and health-system barriers simultaneously.[5,11]

Quality improvement methodology has emerged as an effective strategy for improving breastfeeding-related outcomes in neonatal units. Previous QI initiatives have demonstrated

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significant improvements in MOM utilization through structured education, lactation support, and systematic monitoring.[12–14] To our knowledge, published quality improvement studies specifically addressing early availability of MOM among separated mother–infant dyads in extramural NICUs are scarce. Therefore, we undertook a quality improvement initiative using the point-of-care quality improvement (POCQI) framework to improve early MOM availability among separated mother–infant dyads admitted to our NICU.

AIM OF THE STUDY

To increase the proportion of separated neonates receiving MOM within 48 hours of admission from a baseline of 25% to at least 50% over a 6-month period in an extramural NICU.

MATERIALS AND METHODS

Study Design

This was a prospective quality improvement study conducted using the POCQI methodology. The study was conducted as part of routine clinical care using the POCQI methodology. Institutional ethics committee review was waived according to local policy after Scientific Committee approval.

Study Setting

The study was conducted in the Extramural NICU of the Institute of Child Health and Hospital for Children, Chennai, a tertiary referral center receiving sick neonates from multiple healthcare facilities across the state.

Study Population

All neonates admitted within the first 5 days of life whose mothers were physically separated because of NICU admission were eligible.

During the study period, 186 separated mother–infant dyads admitted within the first five days of life were enrolled. The study population included both term and preterm neonates referred from multiple healthcare facilities across Tamil Nadu.

Inclusion Criteria

- Neonates admitted within 5 days of life.
- Separated mother–infant dyads.

Exclusion Criteria

- Neonates with conditions precluding enteral feeding.
- Mothers with contraindications to breast milk expression.

QI Team

The multidisciplinary team included neonatologists, pediatric residents, nursing staff, a lactation counselor, and a data collection coordinator.

Baseline Assessment

Process mapping was performed to understand existing practices related to breast milk expression and transport. A multidisciplinary team conducted a root-cause analysis using a fishbone diagram to identify barriers to early MOM availability among separated mother–infant dyads.

The identified causes were subsequently prioritized using Pareto analysis. The most important contributors included mother–infant separation, inadequate counseling, poor family awareness, and absence of dedicated lactation support.

OUTCOME MEASURES

Primary Outcome

Proportion of neonates receiving MOM within 48 hours of admission.

Secondary Outcome

Proportion of MOM in total enteral feeds by day 5.

Process Measures

- Proportion of mothers counseled within 6 hours of admission.
- Proportion of mothers expressing milk more than eight times daily.

Interventions

Baseline Phase (Weeks 1–4)

Routine practices without structured intervention.

PDSA Cycle 1 (Weeks 5–8)

Structured counseling of fathers and mothers, introduction of MOM documentation forms, communication with referring facilities, and early maternal contact.

PDSA Cycle 2 (Weeks 9–12)

Telelactation supports daily telephone counseling, WhatsApp educational videos, reminder messages, and educational pamphlets.

PDSA Cycle 3 (Weeks 13–16)

Transportation support including milk-storage education, labelling protocols, and assistance with transport of expressed milk.

PDSA Cycle 4 (Weeks 17–20)

Lactation counselor-led monitoring, daily rounds, troubleshooting, and audit-feedback mechanisms.

Sustenance Phase (Weeks 21–24)

Successful interventions were incorporated into routine NICU workflows.

Data Analysis

Weekly outcome and process measures were monitored using statistical process control (SPC) charts and run charts. Run charts were analyzed using established run-chart rules including shifts, trends, runs, and astronomical data points to identify special-cause variation.

RESULTS

A total of 186 mother–infant dyads were included during the study period.

The proportion of neonates receiving MOM within 48 hours improved progressively following implementation of successive plan–do–study–act (PDSA) cycles. The median proportion of neonates receiving MOM within 48 hours increased from 25% during the baseline phase to 94% during the sustenance phase. Following implementation of structured counseling and telelactation support, sustained improvement was observed, with rates exceeding 70% and reaching 90–100% during the sustenance phase (Figs 1 to 3).

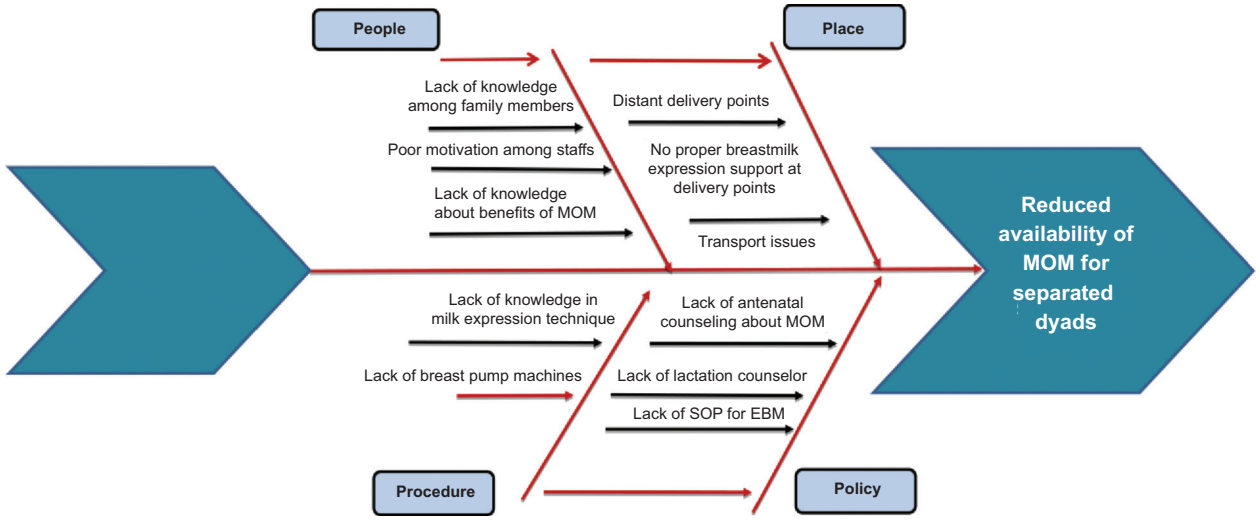

Fig. 1: Fishbone diagram depicting causes of reduced mother's own milk availability

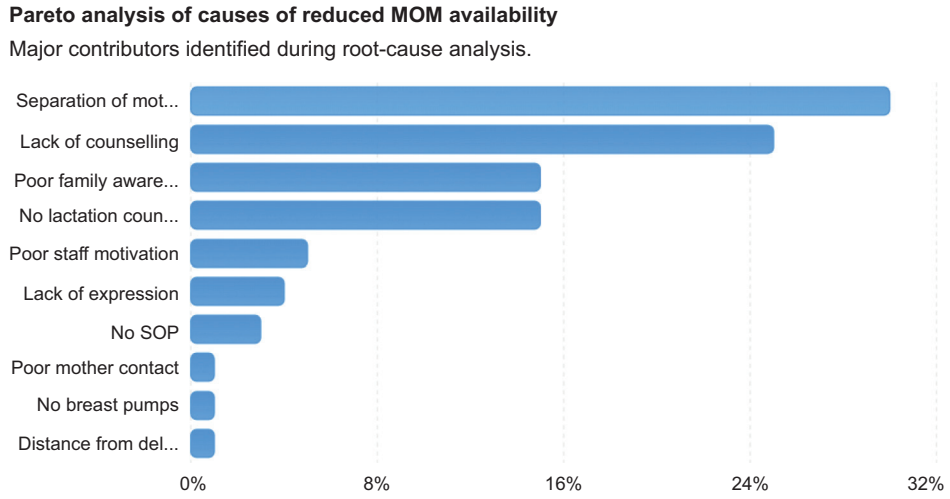

Fig. 2: Pareto chart showing major contributors to reduced mothers' own milk availability

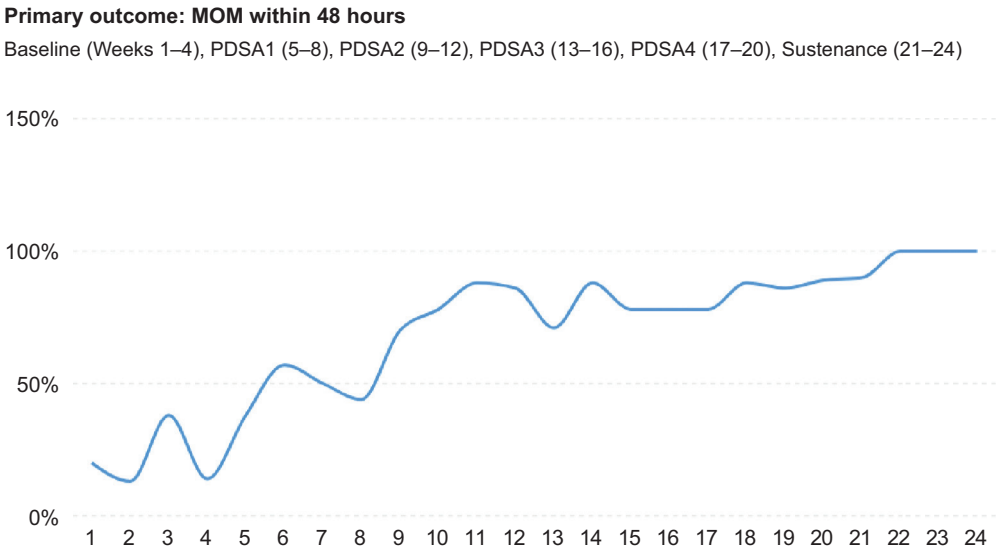

Fig. 3: Weekly trend in babies receiving MOM within 48 hours

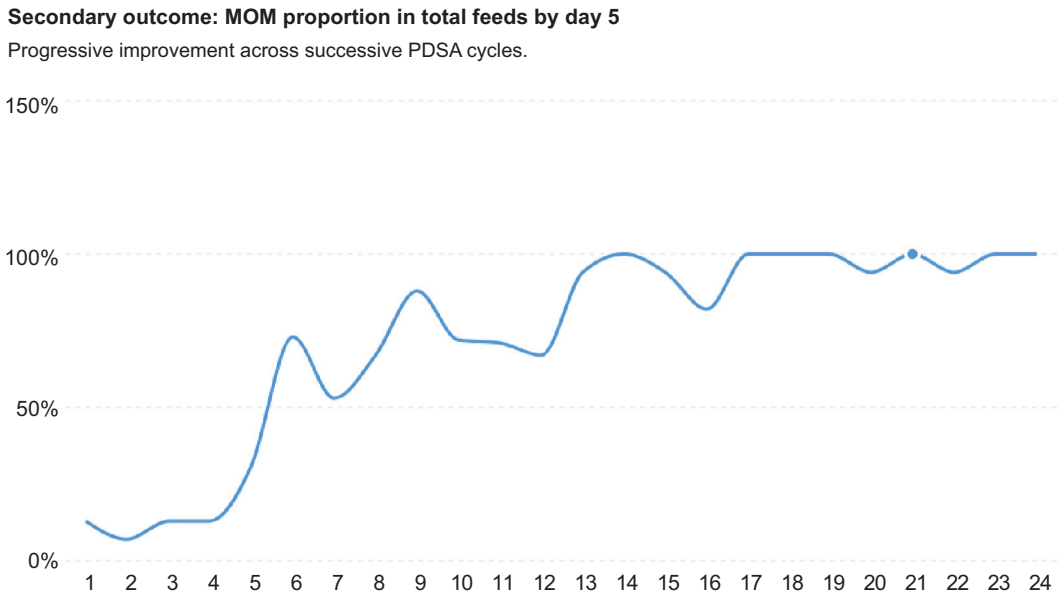

Fig. 4: Weekly trend in proportion of MOM feeds by day 5

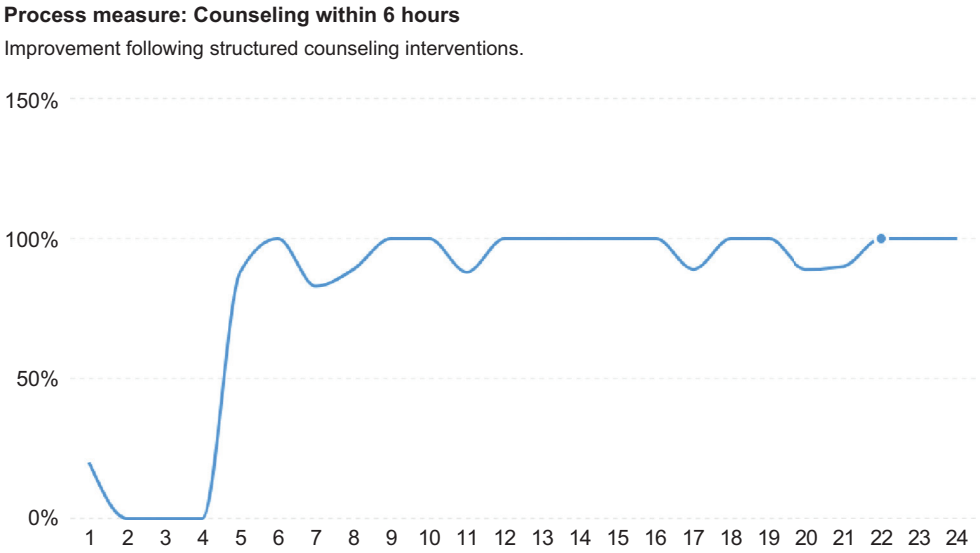

Fig. 5: Mothers counseled within 6 hours

The proportion of MOM in total feeds by day 5 improved steadily across successive intervention phases and approached near-complete MOM feeding during the latter part of the study (Fig. 4).

Counseling within 6 hours improved from baseline values of 0–20% to nearly 100% following implementation of structured counseling and telelactation interventions (Fig. 5).

The proportion of mothers expressing milk more than eight times daily increased from 0% at baseline to nearly 100% during the later intervention phases (Fig. 6).

A summary table of PDSA cycles has been shown in Table 1.

Discussion

In this quality improvement study involving 186 separated mother–infant dyads admitted to an extramural NICU, implementation of a structured lactation-support protocol resulted in substantial and sustained improvement in early MOM availability. The proportion of neonates receiving MOM within 48 hours increased progressively from baseline levels to more than 90% during the sustenance phase. Significant improvements were also observed in counseling rates, frequency of milk expression, and the proportion of MOM in total feeds by day 5. The sustained gains observed during the post-intervention period suggest successful integration of the implemented practices into routine clinical workflows.

Our findings are consistent with previously published evidence demonstrating the effectiveness of structured lactation-support interventions in NICU settings. Early initiation and frequent expression of breast milk have been shown to be strongly associated with improved milk production among mothers of preterm infants.[7–9] Furthermore, availability of MOM during the early postnatal period is associated with improved neonatal

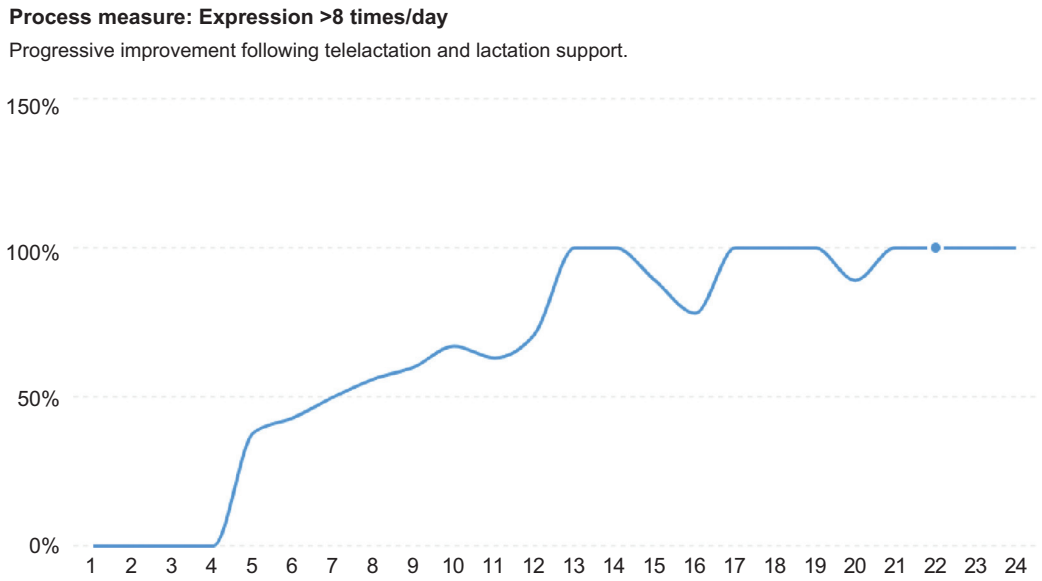

Fig. 6: Mothers expressing milk more than 8 times daily

Table 1: Summary of PDSA cycles

PDSA cycle	*Identified problem*	*Intervention*	*Learning*	*Adaptation*
PDSA 1	Delayed counseling	Structured counseling of fathers and mothers	Counseling improved awareness	Continued in next cycle
PDSA 2	Lack of follow-up	Telelactation support	Improved compliance	Continued
PDSA 3	Difficulty transporting milk	Transportation support	Improved milk delivery	Continued
PDSA 4	Inconsistent monitoring	Lactation counselor rounds	Sustained gains	Incorporated into routine care

outcomes and reduced complications related to prematurity.[2,3,5] Previous quality improvement initiatives from both high-income and low- and middle-income countries have reported significant improvements in MOM utilization through targeted interventions focused on education, milk-expression support, and family engagement.[12–14] The magnitude and sustainability of improvement observed in our study are comparable to these reports and support the effectiveness of bundled lactation-support strategies in resource-constrained neonatal settings.

Our findings are also consistent with the quality improvement initiative reported by Kulkarni et al.,[12] who demonstrated improved utilization of MOM among very-low-birth-weight infants through implementation of structured lactation-support interventions in an NICU setting. Similar to their study, improvements in our project were achieved through a bundled approach involving education, systematic monitoring, and multidisciplinary team engagement. However, our study specifically addressed the unique challenges of an extramural NICU with physically separated mother–infant dyads, where telelactation support and transportation assistance for expressed breast milk played a critical role in improving early MOM availability.

An important observation in our study was that improvement in process measures preceded improvement in outcome measures. Counseling rates improved immediately following implementation of structured counseling and telelactation support, followed by progressive increases in milk-expression frequency and eventual improvement in MOM availability. This pattern is biologically plausible because successful establishment of lactation depends on maternal knowledge, confidence, and frequent milk removal during the early postpartum period.[6–9] The marked improvement observed following telelactation support highlights the potential role of remote lactation interventions in overcoming barriers associated with mother–infant separation and improving continuity of breastfeeding support, findings consistent with recent systematic reviews of digital breastfeeding interventions.[15,16]

The strengths of our study include prospective implementation using a structured POCQI methodology, multidisciplinary team involvement, use of root-cause analysis and Pareto prioritization, and continuous monitoring using SPC and run charts. The interventions were low-cost, feasible, and readily incorporated into routine NICU practice, making them potentially scalable to similar referral centers. However, the study was conducted at a single tertiary care institution and may not be fully generalizable to other settings. The absence of a concurrent control group limits causal inference, and longer-term outcomes such as exclusive breastfeeding at discharge, post-discharge breastfeeding continuation, neonatal morbidities, and growth outcomes were not assessed. Nevertheless, the sustained improvement across multiple outcome and process measures suggests that a structured lactation-support package can effectively improve early MOM availability among separated dyads in extramural NICUs.

Conclusion

A structured lactation-support protocol incorporating counseling, telelactation support, transportation assistance, and lactation counselor-led monitoring significantly improved early availability of MOM among separated mother–infant dyads. The intervention was feasible, sustainable, and readily replicable in similar referral NICU settings.

Acknowledgments

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References

1. Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in the 21st century. Lancet 2016;387:475–490. DOI: 10.1016/S0140-6736(15)01024-7.
2. Patel AL, Meier PP, et al. Strategies to increase the use of mother's own milk. Pediatr Res 2020;88:21–24. DOI: 10.1038/s41390-020-1075-3.
3. Villamor-Martínez E, Pierro M, Cavallaro G, et al. Mother's own milk and bronchopulmonary dysplasia: A systematic review and meta-analysis. Front Pediatr 2019;7:224. DOI: 10.3389/fped.2019.00224.
4. World Health Organization. Protecting, Promoting and Supporting Breastfeeding in Facilities Providing Maternity and Newborn Services. Geneva: WHO; 2017.
5. Meier PP, Johnson TJ, Patel AL, et al. Evidence-based methods that promote human milk feeding of preterm infants. Clin Perinatol 2017;44:1–22.
6. Sankar MN, Weiner Y, Chopra N, et al. Barriers to optimal breast milk provision in the NICU. J Perinatol 2022;42:1076–1082. DOI: 10.1038/s41372-021-01275-4.
7. Parker LA, Sullivan S, Krueger C, et al. Effect of early breast milk expression on milk volume and timing of lactogenesis stage II among mothers of very low birth weight infants: A pilot study. J Perinatol 2012;32:205–209. DOI: 10.1038/jp.2011.78.
8. Parker LA. Optimizing mother's own milk production in mothers delivering critically ill infants. J Perinat Neonatal Nurs 2020;34:13–15. DOI: 10.1097/JPN.0000000000000461.
9. Levene I, Quigley MA, Fewtrell M, et al. Does extremely early expression of colostrum after very preterm birth improve mother's own milk quantity? A cohort study. Arch Dis Child Fetal Neonatal Ed 2024;109:F475–F480. DOI: 10.1136/archdischild-2023-326784.
10. Tomlinson C, Haiek LN. Breastfeeding and human milk in the NICU. Paediatr Child Health 2023;28:510–517. DOI: 10.1093/pch/pxad034.
11. Tran HT, Nguyen TT, Mathisen R. The use of human donor milk. BMJ 2020;371:m4243. DOI: 10.1136/bmj.m4243.
12. Kulkarni DV, Murki S, Pawale D, et al. Quality improvement initiative to improve mother's own milk usage till hospital discharge in VLBW infants. J Perinatol 2020;40:1273–1281. DOI: 10.1038/s41372-020-0707-0.
13. Gupta S, Parikh T. Optimizing own mother's milk supply for NICU babies. Newborn 2020;34:1–2. DOI: 10.1177/0973217920922398.
14. Ministry of Health and Family Welfare. Point of Care Quality Improvement (POCQI) Learner's Guide. New Delhi: Government of India.
15. Demirci J, Kotzias V, Bogen DL, et al. Telelactation via mobile app: Perspectives of rural mothers, their care providers, and lactation consultants. Telemed J E Health 2019;25(9):853–858. DOI: 10.1089/tmj.2018.0113.
16. Gavine A, Marshall J, Buchanan P, et al. Remote provision of breastfeeding support and education: Systematic review and meta-analysis. Maternal Child Nutr 2022;18:e13296. DOI: 10.1111/mcn.13296.

ORIGINAL RESEARCH

C-reactive Protein and Albumin Ratio as a Biomarker of Gram-negative Bacteremia in Neonatal Sepsis

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Abstract

Introduction: Many previous studies often focused on C-reactive protein (CRP) or albumin individually, without considering their combined effect in neonatal sepsis.

Aim: To evaluate the utility of the CRP and albumin ratio (CRP/albumin) for differentiating between Gram-positive or Gram-negative bacteremia in blood culture-positive neonatal sepsis.

Materials and methods: In this cross-sectional study, a total of 94 blood culture-positive (done by the automated BACTEC) septic neonates were included. The serum CRP and albumin levels were measured on a biochemistry analyzer, and the serum CRP/albumin ratio was calculated manually and compared with (Gram-positive/Gram-negative) blood culture reports.

Results: Neonates with Gram-positive and Gram-negative infections had a mean CRP of 32.98 ± 8.52 mg/L and 96.79 ± 13.05 mg/L, respectively. The mean serum albumin was 2.80 ± 0.13 gm/dL and 2.69 ± 0.14 gm/dL among neonates with Gram-positive and Gram-negative infections, respectively. The mean CRP/albumin ratio was 11.80 ± 3.20 and 36.16 ± 5.33 in Gram-positive and Gram-negative neonates, respectively, and was statistically significant ($p < 0.001$). Multivariable logistic regression analysis revealed CRP/albumin as the sole significant predictor of Gram-negative sepsis (OR: 1.291; 95% CI: 1.148–1.451).

Conclusion: The CRP/albumin ratio was significantly higher in Gram-negative neonatal sepsis than Gram-positive sepsis. The higher ratio can be used to predict Gram-negative neonatal sepsis, especially organisms like *Klebsiella*, *Pseudomonas*, and *Escherichia coli* species.

Clinical significance: The CRP/albumin biomarker emerged as a cost-effective, gestational-age-independent tool. A markedly higher CRP/albumin ratio >35 suggested Gram-negative sepsis, and it can be used as an adjunctive tool that complements clinical assessment while awaiting culture report confirmation.

Keywords: Albumin, Blood culture, C-reactive protein, Neonatal sepsis, Organisms.

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Introduction

Neonatal sepsis is a leading cause of mortality in neonatal intensive care units (NICUs) despite many more advances in neonatal care. Its early diagnosis becomes difficult, as clinical signs often overlap with other conditions, and it is similarly difficult to identify the causative pathogen promptly.[1] Blood culture, the gold standard for diagnosis of neonatal sepsis, often takes 48–72 hours, and in a few cases, results may not even identify the causative pathogen.[2] This delay in identification of neonatal sepsis makes early intervention crucial, particularly in high-risk neonates.

C-reactive protein (CRP), which is broadly recognized as a biomarker for infection, is elevated in patients with neonatal sepsis.[3,4] However, its specificity and sensitivity alone are low, and it cannot differentiate between Gram-positive and Gram-negative bacterial infections.[5] Albumin, which is a key protein in the body, is primarily produced by the liver, and during inflammatory processes, it tends to decrease due to both its consumption in the inflammatory response and reduced synthesis by the liver.[6] Many previous studies have often focused on CRP or albumin individually, without considering their combined effect in neonatal sepsis.

The CRP and albumin ratio (CRP/albumin), which combines these two biomarkers, has been suggested as a potential predictor of infection and its severity in neonatal sepsis.[7–9] However, the role of CRP/albumin in the detection of causative microorganisms and as a guide for empirical management in neonatal sepsis remains under-explored. This study aimed to evaluate the utility of CRP/albumin for differentiating between Gram-positive and Gram-negative bacteremia in blood culture-proven neonatal sepsis and to compare its predictive performance against traditional demographic risk factors like birth weight and gestational age.

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Materials and Methods

This cross-sectional prospective study was conducted in the Department of Pediatrics and NICU at a tertiary care center between 1st February 2024 and 30th August 2025 after Institutional Ethics

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Committee approval. Written and informed consent was obtained from at least one of the parents before including the neonate in this study.

A total of 94 neonates admitted to the NICU with blood culture-positive sepsis were included. The demographic data of all neonates were collected. Neonates with fungal sepsis, septic shock, multi-system inflammatory syndrome in neonates (MIS-N), inborn errors of metabolism, and prolonged jaundice were excluded from this study.

The neonates presenting with any of the clinical features of neonatal sepsis as per BMJ 2020 guidelines were suspected of being clinically septic and were confirmed further by blood culture positivity.[10] The blood cultures were done by the automated BACTEC/alert 3D rapid bacterial culture system (Biomerieux, France). At the time of blood culture sample collection, the blood samples were also collected for serum CRP and albumin analysis. The serum CRP and albumin levels were measured by the latex-enhanced immunoturbidimetric method and the bromocresol green endpoint method, respectively, using the AGAPPE/Mispa Nano Plus biochemistry analyzer. The CRP/albumin was calculated manually and then compared with the blood culture positivity and the organisms detected (Gram-positive/Gram-negative).

The sample size was calculated using the formula:

$$n = \frac{\left(z_{1-\alpha/2}\right)^2 p(1-p)}{(d)^2}$$ Where, $Z_{1-\alpha/2}$ is the critical value of the normal distribution at α (confidence level of 95%, α = 0.05, and the critical value is 1.96), prevalence of 41.6%, and the margin of error (*d*) = 10%.[11] The calculated minimum sample size was 94.

Statistical Analysis

The collected data were entered into a Microsoft Excel spreadsheet and analyzed using IBM SPSS Statistics Version 28 (IBM Corp., Armonk, NY, USA) software. The categorical variables were analyzed to determine their frequency and percentages, while continuous variables were assessed for measures of mean and standard deviation. The unpaired *t*-test was used for comparison, where a *p*-value less than 0.05 was considered significant. The multivariable logistic regression analysis was used for the prediction of Gram-negative sepsis.

Results

Out of 94 neonates with culture-positive neonatal sepsis, the mean age of presentation was 13.69 ± 7.82 days. Males 56.38% (*n* = 53) outnumbered females 43.62% (*n* = 41). The pre-term babies accounted for 57.45% (*n* = 54), while 42.55% (*n* = 40) were full-term. The low birth weight (<2.5 kg) neonates comprised 62.77% (*n* = 59), whereas only 37.23% (*n* = 35) were ≥2.5 kg. The mode of delivery was cesarean section at 65.96% (*n* = 62) and vaginal in 34.04% (*n* = 32). The mean CRP was markedly elevated at 55.38 ± 32.29 mg/L, while serum albumin levels averaged 2.76 ± 0.15 gm/dL. The mean CRP/albumin was 20.35 ± 12.37. The blood cultures yielded Gram-positive organisms in 56.38% (53/94) and Gram-negative in 43.62% (41/94), as shown in Table 1.

Neonates with Gram-negative sepsis had a mean CRP of 96.79 ± 13.05 mg/L, whereas those with Gram-positive sepsis had a mean of 32.98 ± 8.52 mg/L. Although a formal *p*-value was not reported for this comparison, the large difference suggests a significantly heightened acute inflammatory response in Gram-negative sepsis. The mean serum albumin concentration among Gram-negative sepsis neonates was 2.69 ± 0.14 gm/dL, as compared with a mean of 2.80 ± 0.13 gm/dL in those with Gram-positive infections.

Table 1: Gram-positive and Gram-negative organisms showing in blood culture of neonates with sepsis

Organisms	*Type*	*Frequency (n)*	*Percentage (%)*
Gram-positive	Coagulase-positive *staphylococcus*	5	9.43
	S. aureus	7	13.21
	S. hominis	3	5.66
	S. epidermidis	11	20.75
	Coagulase-negative *staphylococcus*	14	26.42
	Enterococcus species	10	18.87
	Bacillus species	3	5.66
Gram-negative	*E. coli*	11	26.83
	Klebsiella species	14	34.15
	Pseudomonas aeruginosa	6	14.63
	Salmonella typhi	7	17.07
	Acinetobacter baumannii	2	4.88
Total		94	100

The CRP/albumin was significantly elevated in neonates with Gram-negative sepsis as compared to those with Gram-positive sepsis. The mean CRP/albumin in the Gram-negative septic neonates was 36.16 ± 5.33, while that in Gram-positive septic neonates was 11.80 ± 3.20 ($p < 0.001$), reflecting a marked inflammatory response in Gram-negative sepsis. The CRP/albumin varied significantly across different organisms associated with neonatal sepsis and is shown in Figure 1.

Multivariable logistic regression analysis revealed CRP/albumin as the sole significant predictor of Gram-negative sepsis (OR: 1.291; 95% CI: 1.148–1.451), indicating each unit increase in the ratio increased the odds of Gram-negative infection by roughly 29.1%. Birth weight ≥2.5 kg (OR: 8.431; 95% CI: 0.903–78.723) and term gestation (OR: 2.038; 95% CI: 0.483–8.608) were nonsignificant, with confidence intervals crossing unity as shown in Table 2. Therefore, CRP/albumin outperforms traditional demographic risk factors in differentiating sepsis etiology.

Discussion

The concept of using the CRP/albumin as a biomarker in this study was to assess a more comprehensive view of the inflammatory response. It might not only reflect the degree of inflammation but also assess the severity of the underlying infection.[7] The CRP/albumin would be a potential marker of the inflammatory process, as CRP increases in response to inflammation while albumin levels fall as part of the body's acute-phase reaction. The primary aim of our study was to evaluate the utility of the CRP/albumin as an early, reliable biomarker for differentiating Gram-negative from Gram-positive bacteremia in neonates with culture-positive sepsis and, if it correlated with causative organisms, could potentially serve as

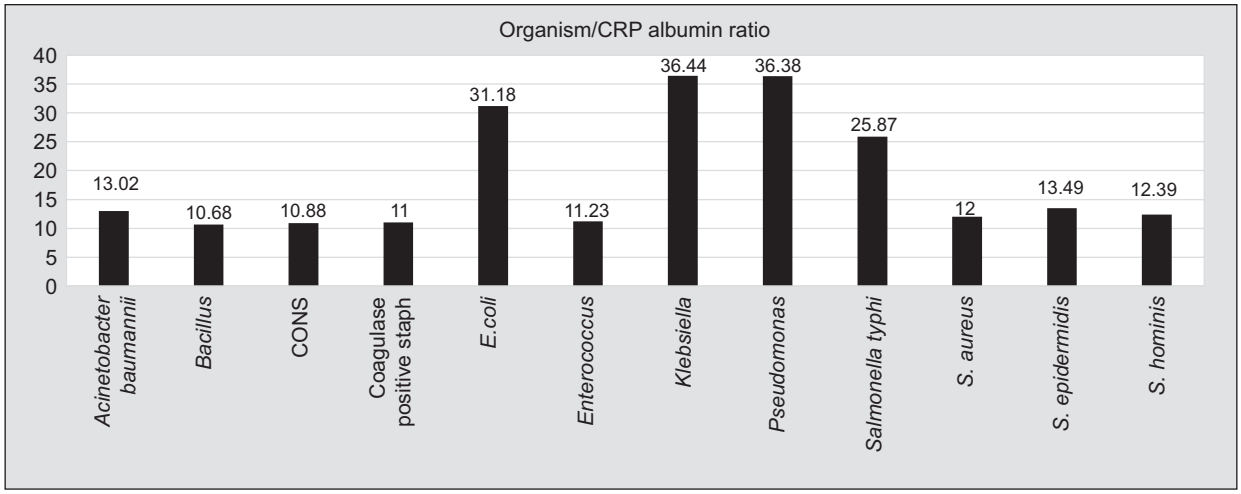

Fig. 1: The CRP/albumin ratio across different organisms associated with neonatal sepsis

Table 2: Multivariable logistic regression analysis for the prediction of Gram-negative sepsis

Effect	*Odds ratio*	*95% CI lower*	*95% CI upper*	*p-value*
CRP/albumin ratio	1.291	1.148	1.451	<0.001*
Birth weight kg ≥2.5 vs <2.5	8.431	0.903	78.723	0.061
Gestational age term vs pre-term	2.038	0.483	8.608	0.333

*Indicates that the results were statistically significant

a useful adjunct to blood cultures, providing clinicians with a faster means of deciding on empiric therapy.

The usefulness of our study remains in addressing a management gap in neonatal sepsis. There is an urgent need for a biomarker that could be cost-effective, rapidly done, and widely available to guide empiric antibiotic therapy until the blood culture report arrives. The traditional culture techniques require 24–72 hours for definitive identification, during which inappropriate or delayed treatment can worsen mortality and foster antimicrobial resistance.

In our study, neonates with Gram-negative sepsis exhibited a mean CRP of 96.79 ± 13.05 mg/L, whereas those with Gram-positive infections had a mean CRP of 32.98 ± 8.52 mg/L. This significantly elevated CRP in the Gram-negative sepsis underscores its responsiveness to endotoxin-mediated inflammation. Gram-negative lipopolysaccharide triggers stronger hepatic synthesis of CRP via interleukin (IL)-6 pathways, yielding higher circulating levels early in sepsis.[12] Omran et al.[13] similarly reported a mean CRP of 78.2 ± 34 mg/L in full-term septic neonates vs 3.3 ± 1.7 mg/L in controls, with higher CRP paralleling greater illness severity and correlating with salivary IL-10 ($r \approx 0.65$, $p < 0.001$). Güneş et al.[14] demonstrated that premature neonates with Gram-negative sepsis had significantly higher mean CRP (≈85 mg/L) as compared to Gram-positive cases (≈40 mg/L), reinforcing CRP's utility in pathogen-type differentiation [area under the curve (AUC) 0.839]. Despite these strengths, CRP kinetics rising 6–12 hours post-insult and peaking ~48 hours would limit its use for early diagnosis and may require serial measurements.

Similarly, in our study, mean serum albumin was modestly lower in Gram-negative (2.69 ± 0.14 gm/dL) as compared to Gram-positive septic neonates (2.80 ± 0.13 gm/dL). This modest but consistent reduction in serum albumin (negative acute-phase protein) in the Gram-negative cohort likely reflects a more intense systemic inflammatory response leading to downregulation of hepatic synthesis and increased capillary permeability with capillary leak due to endotoxin and cytokine storms.[15] Khedr et al.[9] also found moderate diagnostic utility with serum albumin levels. These above findings suggest that albumin measurement was less sensitive compared to CRP, but it provides complementary prognostic information.

The CRP/albumin showed the most significant differentiation by the organisms. The mean ratio was 36.16 ± 5.33 in Gram-negative sepsis while 11.80 ± 3.20 in Gram-positive sepsis ($p < 0.001$). It serves as a promising tool for diagnosing and monitoring neonatal sepsis as its composite index magnifies differences in acute phase response and negative reactant hypoalbuminemia, yielding superior discriminative power compared to CRP or albumin alone.

In our study, Gram-negative bacteria such as *E. coli* (31.18 ± 12.39), *Klebsiella* (36.44 ± 3.02), and *Pseudomonas* (36.38 ± 4.02) showed higher ratios, indicating a more intense inflammatory response. Güneş et al.[14] found a CRP/albumin ratio with a cut-off >35.17 effectively predicted Gram-negative sepsis in premature neonates (specificity 97%, sensitivity 56%; AUC: 0.839), while Khedr et al.[9] observed a ratio of >1.5 in preterm neonates achieved 100% sensitivity and 95% specificity (AUC: 0.99). The higher CRP/albumin ratio >35 can be used as an adjunctive tool that complements clinical assessment while awaiting culture confirmation in neonatal sepsis.

The strength of our study was its cross-sectional design and focus exclusively on culture-positive neonatal sepsis. The CRP/albumin ratio could be a novel biomarker in neonatal sepsis, and our study would probably be the first kind of study in which the differentiation of Gram-negative vs Gram-positive neonatal sepsis was done, to the best of our knowledge, in our region. The limitation of our study was, firstly, it's a single-center study with a moderate sample size, which might limit generalizability to all NICUs with different patient mixes or microbial ecology. Secondly, our chosen CRP/albumin ratio threshold might require external validation in diverse settings, including low- and middle-income countries where baseline albumin and CRP kinetics may differ.

Conclusion

The CRP/albumin ratio was significantly higher in Gram-negative neonatal sepsis than Gram-positive sepsis. The higher ratio can be used to predict Gram-negative neonatal sepsis, especially organisms like *Klebsiella*, *Pseudomonas*, and *E. coli* species.

Clinical Significance

The CRP/albumin biomarker emerged as a cost-effective, gestational-age-independent tool. A markedly higher CRP/albumin ratio >35 suggests Gram-negative sepsis, and it can be used as an adjunctive tool that complements clinical assessment while awaiting culture report confirmation. By enabling more precise early risk stratification, this ratio has the potential to improve antibiotic stewardship, reduce neonatal morbidity and mortality, and optimize resource utilization in NICUs globally, especially in resource-limited settings where advanced molecular diagnostics are unavailable.

Declarations

Ethical Approval

Institutional ethical committee approval No: DYPMCK/IEC/186/2023 dated 29/01/2024 by IEC D.Y. Patil Medical College, Kolhapur.

Clinical Trial Registry of India Approval Number

Not applicable.

Artificial Intelligence (AI) Disclosure Statement

None.

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Nil.

Authors' Contributions

Conception and design, or acquisition, or analysis and interpretation of data: Anjali S Agroya, Nivedita B Patil, Saiprasad O Kavthekar.

Drafting the article or revising it critically for important intellectual content: Saiprasad O Kavthekar.

Final approval of the version to be published: Nivedita B Patil, Saiprasad O Kavthekar.

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: Nivedita B Patil.

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References

1. Fleischmann C, Reichert F, Cassini A, et al. Global incidence and mortality of neonatal sepsis: A systematic review and meta-analysis. Arch Dis Child 2021;106(8):745–752. DOI: 10.1136/archdischild-2020-320217.
2. Ahirrao BM, Dravid N, Ahirrao M, et al. Diagnostic utility of hematological scoring system (HSS) with clinicopathological and bacteriological evaluation in early diagnosis of neonatal sepsis. Ann Pathol Lab Med 2017;4(6):A721–A726. DOI: 10.21276/APALM.1504.
3. Ansar W, Ghosh S. C-reactive protein and the biology of disease. Immunol Res 2013;56(1):131–142. DOI: 10.1007/s12026-013-8384-0.
4. Yogeshpriya S, Selvaraj P. C-Reactive Protein: Is Early Prognostic Marker? Clinical Significance of C-reactive Protein; 2020. pp. 291–313. DOI: 10.1007/978-981-15-6787-2_10.
5. Sharma D, Farahbakhsh N, Shastri S, et al. Biomarkers for diagnosis of neonatal sepsis: A literature review. J Matern Fetal Neonatal Med 2018;31(12):1646–1659. DOI: 10.1080/14767058.2017.1322060.
6. Shi J, Lu ZQ, Lin QM, et al. The role of albumin in the diagnosis of neonatal sepsis over the last 11 years: A retrospective study. J Inflamm Res 2023;16:2855–2863. DOI: 10.2147/JIR.S414611.
7. Li T, Li X, Wei Y, et al. Predictive value of C-reactive protein-to-albumin ratio for neonatal sepsis. J Inflamm Res 2021;14:3207–3215. DOI: 10.2147/JIR.S321074.
8. Yin W, Fang C, Fan X, et al. Albumin and C-reactive protein as diagnostic markers for neonatal sepsis: A retrospective study. J Int Med Res 2024;52(3):3000605241238993. DOI: 10.1177/03000605241238993.
9. Khedr D, Nabil S, Abdelnaby A, et al. The C-reactive protein/albumin ratio as an early diagnostic marker of neonatal sepsis in preterm neonates: A case-control study. Pediatri Sci J 2024;4(2):103–110. DOI: 10.21608/cupsj.2024.297476.1135.
10. Kim F, Polin RA, Hooven TA. Neonatal sepsis. BMJ 2020;371:m3672. DOI: 10.1136/bmj.m3672.
11. Zakariya BP, Bhat V, Harish BN, et al. Neonatal sepsis in a tertiary care hospital in south India: Bacteriological profile and antibiotic sensitivity pattern. Indian J Pediatr 2011;78(4):413–417. DOI: 10.1007/s12098-010-0314-8.
12. Habib SH, Ansar W. Role of C-Reactive Protein (CRP) in Sepsis: Severity and Outcome. Clinical Significance of C-reactive Protein; 2020. pp. 249–290. DOI: 10.1007/978-981-15-6787-2_9.
13. Omran A, Sobh H, Abdalla MO, et al. Salivary and serum interleukin-10, C-reactive protein, mean platelet volume, and CRP/MPV ratio in the diagnosis of late-onset neonatal sepsis in full-term neonates. J Immunol Res 2021;2021(1):4884537. DOI: 10.1155/2021/4884537.
14. Güneş H, Yurttutan S, Çobanuşağı M, et al. CRP/albumin ratio: A promising marker of gram-negative bacteremia in late-onset neonatal sepsis. Turk Arch Pediatr 2021;56(1):32–36. DOI: 10.14744/TurkPediatriArs.2020.99076.
15. Abdelaal MA, Donia A, Kamel Elsayeh AA. Correlation between serum albumin levels and neonatal sepsis in preterm infants. Al-Azhar J Pediatri 2019;22(1):1–20.

REVIEW ARTICLE

Zellweger Spectrum Disorders: Beyond the Peroxisome

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Abstract

Zellweger spectrum disorders (ZSDs) are rare autosomal recessive peroxisomal biogenesis disorders caused by pathogenic variants in *PEX* genes encoding peroxins, proteins essential for peroxisome assembly, maintenance, and matrix protein import. Defective peroxisome biogenesis disrupts multiple metabolic pathways, resulting in accumulation of very-long-chain fatty acids (VLCFAs), phytanic and pristanic acids, pipecolic acid, and bile acid (BA) intermediates, together with impaired plasmalogen and docosahexaenoic acid (DHA) synthesis. These biochemical abnormalities produce a severe multisystem disorder involving the central nervous system, liver, kidneys, eyes, ears, adrenal glands, and skeleton. Clinical manifestations range from the classic neonatal Zellweger syndrome with profound hypotonia, refractory seizures, feeding difficulties, hepatic dysfunction, characteristic craniofacial features, and early mortality, to milder phenotypes that survive into childhood or adulthood. This review summarizes current knowledge regarding the epidemiology, molecular genetics, pathophysiology, developmental biology, and evolutionary conservation of peroxisomes, emphasizing the pivotal roles of peroxins, particularly peroxin 5 (*PEX5*), in peroxisomal protein import. Recent advances in understanding neuronal migration defects, oligodendrocyte maturation, microglial dysfunction, oxidative stress, mitochondrial–peroxisomal interactions, epigenetic regulation, and non-coding RNA (ncRNA) responses are discussed to provide an integrated view of disease mechanisms. Characteristic laboratory abnormalities, neuroimaging findings, ultrastructural changes, and molecular diagnostic strategies are reviewed, together with emerging concepts including uniparental disomy (UPD), genotype–phenotype correlations, and phylogenetic conservation of *PEX* genes. Although treatment remains largely supportive and multidisciplinary, improvements in biochemical testing, next-generation sequencing, newborn diagnosis, and comprehensive clinical care have expanded recognition of milder disease and improved long-term outcomes for selected patients. Continued advances in molecular biology, systems genomics, and targeted therapeutic development are expected to refine diagnosis, enhance prognostic assessment, and provide opportunities for future disease-modifying interventions in this complex group of inherited peroxisomal disorders.

Keywords: Autosomal recessive inheritance, Bile acid metabolism, Ether lipid synthesis, Metabolic detoxification, Pathogenic variants, Peroxins, Peroxisomal biogenesis disorders, Peroxisomal biogenesis factor, Peroxisomal biogenesis factor mutations, Toxic metabolite accumulation.

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Key Points

- Zellweger spectrum disorders (ZSDs) are inherited autosomal recessive peroxisomal biogenesis disorders caused by pathogenic variants in *PEX* genes, resulting in defective peroxisome formation and impaired lipid metabolism.
- Peroxisomal dysfunction leads to accumulation of very-long-chain fatty acids (VLCFAs), phytanic acid, pipecolic acid, and bile acid (BA) intermediates, together with plasmalogen deficiency, producing widespread neurological, hepatic, renal, ocular, auditory, and skeletal abnormalities.
- Diagnosis is based on characteristic biochemical abnormalities, including elevated plasma VLCFAs, and is confirmed by molecular genetic testing of *PEX* genes, supported by neuroimaging and other organ-specific investigations.
- Recent advances have improved understanding of ZSD pathogenesis through insights into peroxin biology, neuronal migration, oligodendrocyte and microglial dysfunction, mitochondrial–peroxisomal interactions, epigenetic regulation, and non-coding RNA (ncRNA) networks.
- Management remains primarily supportive and multidisciplinary, but earlier diagnosis through modern genomic technologies and improved clinical care have expanded recognition of milder phenotypes and provided a foundation for the development of future targeted therapies.

Introduction

Zellweger spectrum disorders are a group of autosomal recessive peroxisomal biogenesis disorders caused by mutations in

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peroxisomal biogenesis factor (*PEX*) genes encoding peroxins.[1,2] These genes are required for the assembly and function of peroxisomes, organelles required for the metabolism of very-long-chain fatty acids (VLCFAs; fatty acids >22 carbon long), bile acid (BA) intermediates, and phytanic acid, and in plasmalogen synthesis.[3] Disruption of peroxisomal biogenesis results in toxic metabolite accumulation and deficiency of key lipids required for normal brain and organ development.[3] These were first identified in conditions such as the classic Zellweger syndrome, neonatal adrenoleukodystrophy (ALD), and infantile Refsum disease.[4] Zellweger syndrome was first described in the mid-1960s by Dr. Hans Zellweger, a Swiss-American pediatrician and geneticist at the University of Iowa.[5] The condition was characterized by profound hypotonia, seizures, craniofacial dysmorphism, hepatic dysfunction, and severe neurodevelopmental impairment.[6] Later, advances in molecular genetics showed Zellweger syndrome, neonatal ALD, and infantile Refsum disease to represent a continuum of severity resulting from defects in the same peroxisomal biogenesis pathway, and are now all grouped as ZSDs.[7]

Zellweger spectrum disorders impair peroxisome biogenesis, leading to characteristic biochemical abnormalities such as elevated levels of VLCFAs and BA intermediates.[8] Clinically, ZSDs are multisystem disorders affecting the brain, liver, eyes, kidneys, and skeleton. Severely affected neonates present with hypotonia, feeding difficulties, seizures, and structural brain and skeletal abnormalities, and have a high mortality rate in early infancy.[1,2,4–7,9–12] Diagnostic advances have improved early recognition, but the treatment is largely supportive.[2] This review summarizes the molecular basis, clinical spectrum, diagnostic approach, and key biochemical and radiologic features of ZSDs, with emphasis on neonatal disease.

Epidemiology

The incidence of ZSDs is generally reported to be approximately 1 in 50,000–1,00,000 live births, with some differences across geographic regions/populations.[1,2,7,13] Zellweger spectrum disorders are seen in all ethnic and racial backgrounds; with its autosomal recessive inheritance, males and females are affected equally.[4] In North America and Europe, some populations show higher frequencies, likely due to local founder mutations (Table 1).[14] Parents of affected infants are typically asymptomatic carriers of a pathogenic variant of the same *PEX* gene; when both are carriers, each pregnancy carries a 25% risk of producing an affected child.[7,13]

The incidence of severe neonatal ZSD disease may have decreased in recent decades.[15] Although encouraging, further observation is needed; it could very well be an artifact of improved molecular diagnosis, carrier screening, and prenatal testing.[17,18] The recognition of milder phenotypes may have broadened the clinical spectrum with increasing identification of patients who may survive into childhood or adulthood.[17,18]

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Pathophysiology

Structure and Function of Peroxisomes

These are small, single-membrane-bound organelles found in nearly all eukaryotic cells and are particularly abundant in the liver, kidneys, brain, and adrenal glands.[19] Peroxisomes play a crucial role in lipid metabolism, cellular detoxification, and the maintenance of oxidative balance.[20] These specialize in metabolic pathways involving fatty acids and other molecules that cannot be efficiently processed elsewhere in the cell.[21]

Peroxisomes measure about 0.1–1 µm in diameter and are seen in close association with the endoplasmic reticulum (ER) and mitochondria (Fig. 1).[22] The number, size, shape, and protein composition vary with the cell type and environmental conditions.[23] There is a single covering phospholipid bilayer membrane containing transporter proteins, and an electron-dense crystalline core or fibrillar matrix containing at least 60 known enzymes such as urate oxidase, D-amino acid oxidase, catalase, and others involved

Table 1: Estimated incidence of ZSDs by continent[4,15]

Continent	*Estimated incidence (live births)*	*Evidence strength*	*Key notes*
North America	1:50,000–80,000	Moderate–high	Strong metabolic/genetic diagnostic infrastructure; most cases due to *PEX1*
Europe	1:50,000–75,000	High	Best population-level data; some regional founder effects*
Asia	1:75,000–1,50,000	Low–moderate	Likely underdiagnosed; wide inter-country variability
Africa	1:50,000–1,00,000 (estimated)	Very low	Many cases likely remain unrecognized
South America	1:50,000–1,00,000	Low	Data limited to tertiary centers
Oceania	1:50,000–80,000	Moderate	Similar to Europe/North America

*Regional founder effects: A small number of individuals establish a new population in a delimited geographic region, carrying only a subset of the genetic variation present in the original population. If one or more founders carry a rare genetic variant, that variant can be seen frequently in their descendants;[16] *PEX*, peroxisomal biogenesis factor; ZSD, Zellweger spectrum disorder

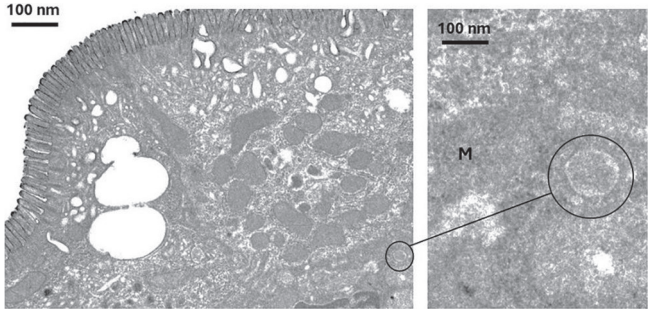


Fig. 1: Peroxisomes are 0.1–1 μm self-replicating structures with a crystalline core containing amorphous gray material. Some may appear similar to the Golgi complex or storage granules, although the formation follows a different process. The structure next to the peroxisome, labeled M, is a mitochondrion

in fatty acid β-oxidation and metabolic pathways.[19,24] There are some fibrils, organized protein/crystalline filamentous structures, which may enhance metabolic efficiency by concentrating enzymes needed for related biochemical reactions.[22,25] The key components of a peroxisome are shown in Figure 2.

Peroxisomes serve many essential roles in lipid metabolism, detoxification, and cellular homeostasis.

(i) Fatty acids: Peroxisomes shorten VLCFAs through β-oxidation, producing medium-chain fatty acids (MCFAs) that can be transferred to mitochondria for complete oxidation.[26] Peroxisomes are also a key site for the α-oxidation of branched-chain fatty acids such as phytanic acid, which is needed because the branched structure prevents direct degradation in conventional β-oxidation pathways (Fig. 3A).[27]

(ii) Pipecolic acid (piperidine-2-carboxylic acid): This is a cyclic, non-proteinogenic amino acid intermediate in lysine degradation.[28] Peroxisomal disorders are characterized by high levels of pipecolic acid, a useful biochemical marker of impaired peroxisomal function (Fig. 3B).[29]

(iii) Bile acids: C27 BA intermediates dihydroxycholestanoic acid (DHCA) and trihydroxycholestanoic acid (THCA) are converted into mature C24 cholic acid and chenodeoxycholic acid (Fig. 3C).[29–31]

(iv) Plasmalogens: These ether phospholipids are synthesized through pathways involving the peroxisome and the ER (Fig. 4; Table 2), and are particularly abundant in the brain, myelin, heart, skeletal muscle, and immune cells.[27,32] There is a distinctive vinyl ether bond at the sn-1 position of the glycerol backbone.[33] Plasmalogens play important roles in maintaining membrane structure and fluidity, facilitating membrane fusion and cell signaling, and protecting cells from oxidative stress.[34]

(v) Polyunsaturated fatty acids (PUFAs): Peroxisomes promote the metabolism and biosynthesis of PUFAs, particularly docosahexaenoic acid (DHA; 22:6n-3), which is essential for brain and retinal development.[35] Although the initial elongation and desaturation steps of PUFA synthesis occur primarily in the ER, the final maturation of DHA requires peroxisomal β-oxidation.[36]
   - Shortens tetracosahexaenoic acid (THA) (24:6n-3) into DHA.[37]
   - Promotes DHA synthesis: α-linolenic acid (18:3n-3) is processed in the ER to form eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3), and finally, THA (24:6n-3).[38] Tetracosahexaenoic acid undergoes β-oxidation to form DHA (22:6n-3) and is incorporated into peroxisomal membrane phospholipids.[38,39]

(vi) DHA: Highly enriched in neuronal membranes, synapses, photoreceptor outer segments of the retina, and myelin.[40] It contributes to membrane fluidity, neurotransmission, signal transduction, neurogenesis, visual function, and modulation of inflammatory responses.[41] During fetal life and infancy, DHA is needed for brain growth and normal neurodevelopment.[40] Peroxisomes play an essential role in the synthesis of DHA.[42]

(vii) Reactive oxygen species (ROS): Peroxisomes both produce and help in detoxification of ROS.[43] Several oxidases generate hydrogen peroxide (H_2O_2) and other ROS, whereas antioxidant enzymes such as catalase detoxify these potentially harmful molecules to maintain cellular redox homeostasis.[43,44]

Peroxisomal Dysfunction

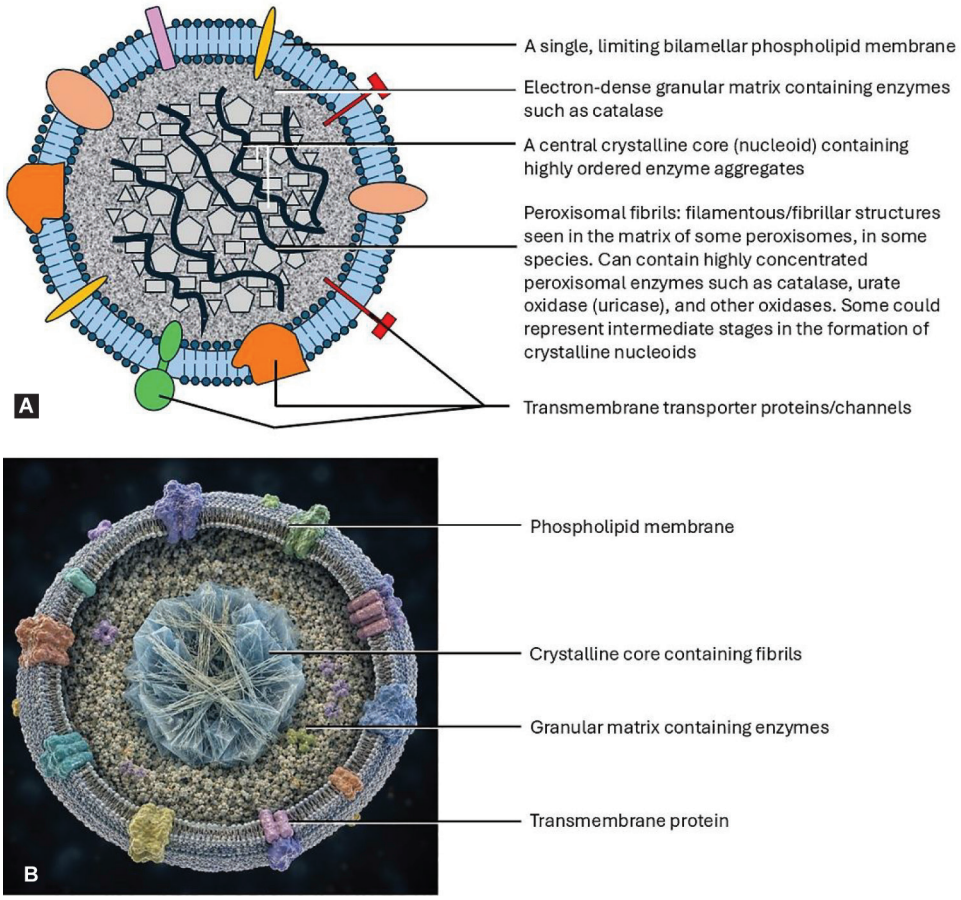
The importance of peroxisomes is highlighted by the severe consequences of peroxisomal dysfunction. Defects in peroxisome formation/function in ZSDs result are characterized by the accumulation of VLCFAs, pipecolic acid, BA intermediates, and reduced plasmalogen synthesis.[2]

Structural Abnormalities

Electron micrographs show striking differences between normal cells and those from patients with ZSDs.[45] In control cells, peroxisomes appear as numerous small, spherical, single membrane-bound organelles with a finely granular, electron-dense matrix distributed throughout the cytoplasm.[19] In ZSDs, there is a major paucity of mature peroxisomes because of defective peroxisome biogenesis. Electron microscopy shows an absence or severe reduction of normal peroxisomes; the remaining ones may show small membrane remnants and have been described as "peroxisomal ghosts."[46] These are structurally abnormal, unstable due to impaired *PEX*-mediated assembly and defective organelle dynamics, and do not interact with other organelles such as mitochondria and ER.[46,47] There are no/minimal internal contents due to defective import and impaired local synthesis of matrix proteins. Enzymes required for β-oxidation, plasmalogen synthesis, and detoxification (acyl-CoA oxidases, catalase, thiolases, *AGPS*) are absent or mislocalized, rendering peroxisomes metabolically inactive.[46–48] Super-resolution stimulated emission depletion (STED) microscopy has verified these abnormalities.[49] These ultrastructural abnormalities correlate with the metabolic defects, including impaired β-oxidation of VLCFAs, defective plasmalogen synthesis, and abnormalities of BA and DHA metabolism.[3,39]

Metabolic Abnormalities

In the central nervous system, ZSDs can disrupt key developmental processes, leading to cortical malformations such as polymicrogyria, pachygyria, and neuronal heterotopias.[50,51] In addition, impaired plasmalogen synthesis and accumulation of toxic metabolites promote oxidative stress, mitochondrial dysfunction, and cellular injury, further exacerbating neurological and systemic disease.[52] Outside the nervous system, patients frequently show fatty changes in the liver. Impaired BA synthesis causes cholestatic liver disease.[1] Disrupted chondrocyte maturation and endochondral ossification contribute to *chondrodysplasia punctata* and other skeletal abnormalities.[53] Renal cortical cysts, retinal dystrophy, optic nerve abnormalities, and sensorineural hearing loss (SNHL) reflect the widespread developmental consequences of peroxisomal failure.[2,4]

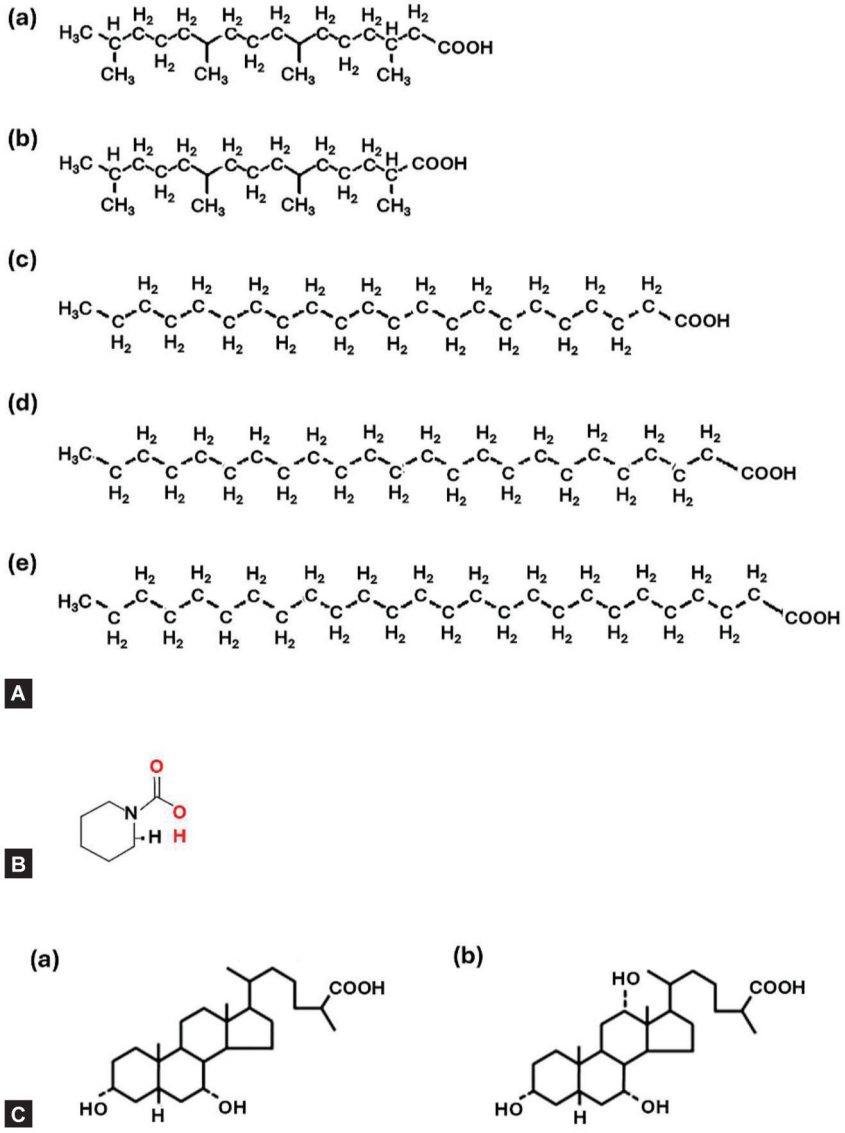


Figs 2A and B: (A) Schematic showing the structural components of a peroxisome. These spherical/ovoid organelles are covered by a single phospholipid bilayer containing transport proteins such as ATP-binding cassette subfamily D (ABCD) transporters, which mediate the import of activated fatty acids, BA intermediates, and other metabolites. The central core of peroxisomes contains a protein-rich matrix, containing enzymes involved in VLCFA β-oxidation, α-oxidation of phytanic acid, plasmalogen biosynthesis, BA synthesis, and ROS metabolism. Matrix enzymes include catalase, acyl-CoA oxidases, D-bifunctional protein, peroxisomal thiolase, PHYH (phytanoyl-CoA hydroxylase), and pipecolate oxidase. Some peroxisomes contain a central electron-dense crystalline core (nucleoid) composed of highly ordered enzyme aggregates. Fine fibrillar structures may also be present, representing organized assemblies of matrix proteins. The peroxisomal membrane encloses these metabolic pathways, allowing efficient substrate channeling and protection of the cytoplasm from hydrogen peroxide generated during oxidative reactions. This specialized ultrastructure enables peroxisomes to play essential roles in lipid metabolism, BA formation, plasmalogen synthesis, and cellular redox homeostasis. (B) An illustration of the above schematic was prepared using artificial intelligence-assisted image generation. The figure legend, including the scientific content and conceptual framework, was prepared by the authors prior to figure development. This legend was then used as the initial prompt for AI-assisted image generation with ChatGPT (OpenAI; GPT-5.5, 2026), which served as an interactive design assistant to iteratively translate the written scientific concept into a visual representation. The resulting artwork was further refined using BioRender with its curated library of biomedical illustrations and icons; Inkscape for precision vector editing, object alignment, and figure assembly; and Adobe Illustrator for graphic design, typography, and publication-quality finishing. Nine iterative refinements were performed through successive text-based prompts to optimize the composition, symbolism, labeling, visual hierarchy, and overall presentation until the illustration achieved the intended design objectives. The final figure was critically reviewed, edited, verified, and approved by the authors, who assume full responsibility for its scientific content and accuracy

Immunofluorescence studies show altered incorporation of peroxisomal membrane proteins.[54] These findings reflect the fundamental defects in peroxisomal protein import in ZSDs and help distinguish these conditions from single-enzyme peroxisomal defects.[1] Nanoscale examination of the peroxisomal surface using atomic force microscopy showed altered membrane topology; there may be increased membrane irregularity, reduced mechanical stiffness, and altered nanomechanical compliance.[46]

Zellweger spectrum disorders show intracellular accumulation of VLCFAs such as docosanoic acid (behenic acid, C22:0), tetracosanoic acid (lignoceric acid, C24:0), and hexacosanoic acid (cerotic acid, C26:0); and branched-chain fatty acids such as phytanic and pristanic acids.[4] Elevated pristanic acid contributes to cellular toxicity, oxidative stress, and neurological injury.[55] Amino acid derivatives such as pipecolic acid are elevated; the findings are not specific for ZSDs but are a useful, supportive biomarker when interpreted alongside VLCFA analysis, plasmalogen measurements, and molecular testing of *PEX* genes.[56]

A major consequence of peroxisomal dysfunction is plasmalogen deficiency.[3] Plasmalogens are critical components of cell membranes, particularly in the brain, myelin, heart, lungs, and skeletal tissues. Reduced plasmalogen synthesis impairs neuronal migration, myelination, synaptic function, membrane stability, and normal organ development.[57]



Figs 3A to C: Metabolic pathways active in peroxisomes. (A) Fatty acids: (a) phytanic acid, a 16-carbon (16_C) branched-chain fatty acid (FA; 3,7,11,15-tetramethylhexadecanoic acid) undergoes α-oxidation in the peroxisomal matrix to produce pristanic acid; (b) this pristanic acid, a C_{19} branched-chain FA (2,6,10,14-tetramethylpentadecanoic acid) metabolite, is then degraded via β-oxidation in the matrix; (c) docosanoic acid, a C_{22} straight-chain saturated long-chain FA (LCFA), is produced in the cytosol and ER and is metabolized in the peroxisome. Peroxisomes can also generate some docosanoic acid locally through chain shortening of very-LCFA (VLCFAs) by β-oxidation; (d) tetracosanoic acid is another straight-chain saturated VLCFA; it contains 24 carbon atoms; (e) hexacosanoic acid is a C_{26} straight-chain saturated VLCFA. (B) Pipecolic acid, a cyclic, non-proteinogenic amino acid (piperidine-2-carboxylic acid) is synthesized from the degradation of lysine in the peroxisomal matrix. (C) Bile acids: An 8-carbon side chain in cholesterol is processed to produce the C_{24} primary BAs, chenodeoxycholic and cholic acid: (a) a C_{27} BA intermediate, 3α,7α-dihydroxy-5β-cholestanoic acid (DHCA), undergoes β-oxidation with shortening of side-chains, to form a 24-carbon primary BA, chenodeoxycholic acid; (b) another C_{27} BA intermediate, 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA), is converted into cholic acid, a primary C_{24} BA required for digestion

ZSDs Show Extensive Abnormalities in Brain Development

Neuronal development is severely disrupted in ZSDs due to impaired peroxisomal biogenesis from *PEX* gene mutations.[9,11,12,58] Abnormal neuronal migration and cortical malformations have been noted due to failure of neurons to reach correct developmental positions.[59] Neuronal connectivity is also compromised, with abnormal axonal growth, dendritic development, and synaptic organization, resulting in disorganized neural networks.[60] Secondary oxidative stress and mitochondrial dysfunction further impair neuronal survival and function.[61]

Oligodendrocyte development is also severely impaired in ZSDs.[60] These cells depend on intact lipid metabolism, particularly plasmalogen synthesis and VLCFA regulation, which are both disrupted in ZSDs.[62] Arrested maturation of oligodendrocyte precursor cells results in a reduced pool of mature myelinating cells and profound hypomyelination.[63] Oxidative stress and mitochondrial–peroxisomal dysfunction further impair

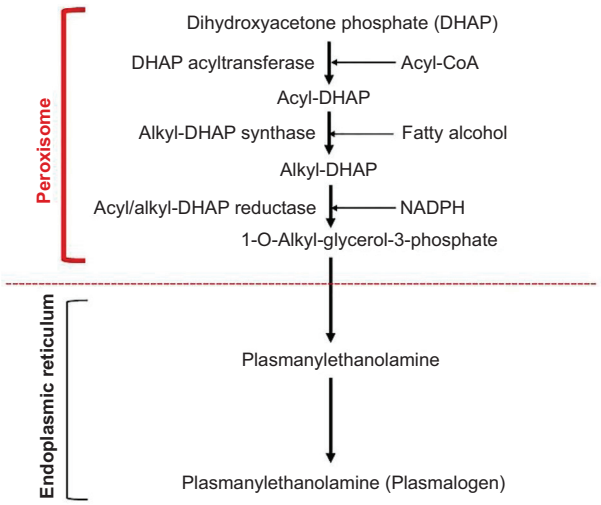


Fig. 4: The first three committed reactions for the biosynthesis of ether phospholipids and plasmalogens occur in the peroxisome. Defects in these reactions lead to severe plasmalogen deficiency in disorders such as ZSDs and Rhizomelic *Chondrodysplasia Punctata*

Table 2: Localization of key ether lipid biosynthetic enzymes

Enzyme	*Gene*	*Localization*
DHAP acyltransferase[64]	*GNPAT*	Inner surface of peroxisomal membrane, active site facing matrix
Alkyl-DHAP synthase[64,65]	*AGPS*	Inner surface of peroxisomal membrane, active site facing matrix
Fatty acyl-CoA reductase[66,67]	*FAR1/FAR2*	Peroxisomal membrane
Acyl/alkyl-DHAP reductase[65]	*DHRS7B*	Cytosolic face of peroxisomal membrane and ER

oligodendrocyte survival by reducing energy availability and increasing cellular injury during critical developmental periods.[68]

Microglial development and function are increasingly recognized as part of broader neuroimmune dysregulation caused by defective peroxisomal biogenesis due to *PEX* gene mutations.[69] Microglia require tightly regulated lipid metabolism and redox balance for their role in synaptic pruning, neuronal migration, and circuit refinement.[70] In ZSDs, these processes are disrupted, leading to a chronically activated, dysregulated microglial state, contributing secondarily to neuronal and white matter injury.[60]

Zellweger spectrum disorders also show impaired synapse development.[60] There are several possible causes, including disrupted lipid metabolism, membrane formation, and redox balance during early brain development.[71] Impaired neuronal migration and dendritic development further reduce synaptic density and organization, resulting in simplified and poorly connected neural networks.[59] Oxidative stress and mitochondrial dysfunction add additional injury by damaging synaptic proteins and limiting synaptic plasticity.[61]

Genetics of ZSDs

Peroxisomal biogenesis factor mutations disrupt peroxisome formation/function and lead to a group of inherited conditions known as the peroxisome biogenesis disorders (PBDs).[72] Zellweger spectrum disorders are the most severe of the PBDs.[2]

The ZSDs are recognized as a heterogeneous group of disorders, with pathogenic variants identified in 13 of the 37 *PEX* genes.[73,74] *PEX1* mutations are seen most frequently, in up to 60–70% of cases in most ZSD cohorts.[75] Variants such as p.Gly843Asp (G843D) frequently show population-specific founder effects, and disease severity varies widely depending on residual protein function.[76] *PEX1* encodes an AAA+ ATPase that, together with *PEX6*, mediates recycling of the peroxin 5 (*PEX5*) receptor from the peroxisomal membrane to the cytosol, a critical step in matrix protein import.[77] *PEX6* mutations underlie 10–15% of the ZSDs and comprise the second most frequently seen set of genetic abnormalities in ZSDs.[11] This gene functions in the same ATPase recycling complex with *PEX1*; severe variants typically produce classic neonatal Zellweger disease due to near-complete loss of import activity.[77] Mutations in *PEX10*, *PEX12*, and *PEX2* account for about 5–10% of the ZSD patients.[58] These affect the Really Interesting New Gene (RING) finger docking/translocation complex that ubiquitinates *PEX5* and enables protein import, usually resulting in severe neonatal phenotypes.[78] *PEX5* mutations disrupt the cytosolic receptor for peroxisomal targeting signal (PTS)1-targeted proteins and also tend to cause severe or intermediate disease.[78] Less common but functionally critical genes include *PEX13* and *PEX14* (docking complex components), *PEX16* and *PEX3* (peroxisomal membrane biogenesis), and *PEX26* (anchors the *PEX1–PEX6* complex). Mutations in these genes generally impair peroxisome formation or import at different stages, although residual function can modify severity.[79]

Zellweger spectrum disorders are mostly inherited in an autosomal recessive pattern. While carrier rates for many common recessive diseases are 1 in 100–200, ZSD may be rarer, with an estimated frequency of pathogenic alleles of 1 in 1198, 1 in 953 likely

pathogenic, and combined frequencies of 1:531.[80] In other cohorts, the frequencies were estimated to be higher, around 1:100.[80] The mutational spectrum is consistent across most populations, except for some founder cohorts.[15,81]

Estimates of carrier frequencies from observed disease incidence have used Hardy–Weinberg equilibrium (HWE) assumptions.[73,82] Hardy–Weinberg equilibrium assumes a large population size, random mating, no mutation, no migration, and no natural selection affecting the studied alleles.[82] Under these conditions, the frequencies of normal and pathogenic *PEX* alleles would remain stable, allowing estimation of carrier frequencies and expected disease incidence for this rare autosomal recessive disorder. However, real populations may deviate from HWE because of factors such as regional founder effects, genetic drift, consanguinity, population isolation, migration patterns, or differences in reproductive fitness.[83,84] These deviations can influence the distribution of specific *PEX* mutations and explain why certain pathogenic variants may be enriched in some populations.

Historically, severe ZSD manifesting in neonates was considered distinct from milder phenotypes such as neonatal ALD and infantile Refsum disease.[7] These entities are now recognized as part of a continuous clinical spectrum, with severity correlating broadly with residual peroxin function and peroxisomal activity. Improved molecular diagnostics have expanded recognition of milder phenotypes, potentially increasing observed incidence in regions with advanced genomic testing.

In Refsum disease, including classic adult Refsum disease and related peroxisomal fatty acid oxidation defects, the primary abnormality lies in defective peroxisomal enzyme function rather than peroxisome biogenesis.[85] Most commonly, mutations affect PHYH (phytanoyl-CoA hydroxylase) or, less frequently, genes involved in peroxisomal protein import such as *PEX7*.[86] Because the machinery required for peroxisome assembly is intact, the ultrastructural appearance of peroxisomes is largely preserved.[20] Subtle secondary changes may be observed in hepatocytes and renal tubular cells, with mild variation in matrix density and the presence of adjacent cytoplasmic lipid droplets, reflecting impaired metabolism of phytanic acid and related BCFAs.[87] However, these findings are nonspecific and do not represent primary structural defects of the organelle.

Development and Biogenesis of Peroxisomes in Cells

Peroxisomes are dynamic organelles present in virtually all eukaryotic cells.[19] Unlike mitochondria, peroxisomes do not contain their own genome; all peroxisomal proteins are encoded by nuclear genes, translated in the cytoplasm, and then translocated into peroxisomes.[88]

Peroxisome biogenesis occurs through two complementary pathways.[89,90] First, new peroxisomes can arise *de novo* from the ER, where pre-peroxisomal vesicles bud off and mature into functional organelles. Second, existing peroxisomes grow and divide through growth and fission, a process regulated by peroxins such as *PEX11*, along with membrane remodeling factors and dynamin-related proteins.

A central feature of peroxisomal ontogeny is protein import.[91] Cytosolic enzymes containing PTS1 or PTS2 are recognized by receptor proteins such as *PEX5* and *PEX7*. These receptors shuttle enzymes and proteins to the peroxisomal membrane docking and translocation machinery (including *PEX13*, *PEX14*, and the RING complex composed of *PEX2*, *PEX10*, and *PEX12*).[88,92] Finally, ATP-dependent recycling of import receptors by *PEX1* and *PEX6* is essential for sustained peroxisomal function.[93]

During embryonic development, peroxisomes are particularly important in tissues with high lipid metabolic demands, including brain, liver, and adrenal cortex.[20] Their functional maturation is closely linked to lipid signaling, ROS regulation, and membrane biogenesis.[94] Disruption of ontogeny, as seen in PBDs such as ZSDs, leads to a multisystem developmental failure.

Phylogeny of Peroxisomes

The evolutionary origin of peroxisomes remains distinct from other organelles because these are believed to have arisen from the endomembrane system rather than endosymbiosis.[95] Unlike mitochondria and chloroplasts, peroxisomes do not have a bacterial ancestor; these have likely evolved from early eukaryotic membranes associated with lipid metabolism and oxidative reactions.[96]

Comparative genomics shows that peroxisomes are conserved across most eukaryotic lineages, including animals, plants, fungi, and many protists, but not in some parasitic species.[74] This suggests that peroxisomes are evolutionarily ancient but secondarily dispensable in certain metabolic contexts. The core set of *PEX* genes needed for peroxisome biogenesis is highly conserved; *PEX5*, *PEX7*, *PEX19*, and *PEX3* are found across diverse species, indicating that the fundamental mechanisms of peroxisomal protein import and membrane assembly arose early in eukaryotic evolution.[74]

There has been some variability in metabolic functions of peroxisomes during evolution, reflecting ecological and metabolic adaptation.[95] In plants, peroxisomes have expanded roles in photorespiration and seed metabolism, whereas these are important in fatty acid utilization in fungi.[97,98] In animals, peroxisomes are especially critical for plasmalogen synthesis, BA metabolism, and VLCFA oxidation, functions that are highly relevant to neurodevelopment and disease.[71]

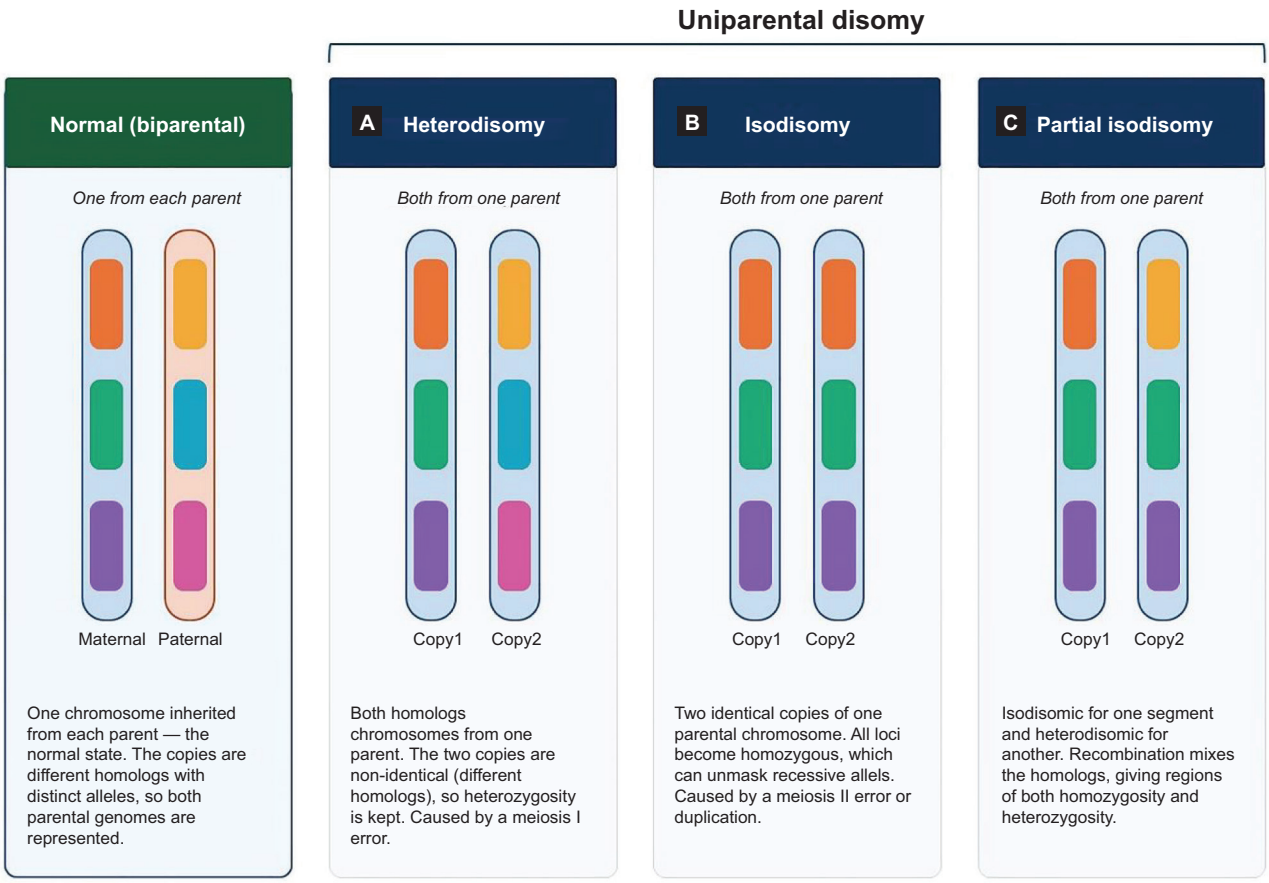
Overall, the ontogeny and phylogeny of peroxisomes are tightly linked.[95] The evolutionary conservation of *PEX* genes underlies the universal mechanisms of peroxisome formation. The developmental regulation of these organelles determines tissue-specific metabolic specialization.

Uniparental Disomy (UPD) can be a Cause of ZSDs

Some patients with ZSDs have shown UPD (Fig. 5). The most clinically relevant mechanism is isodisomy, where identical chromosomal copies unmask a recessive variant.[99] Although any *PEX* gene can be involved, *PEX1* on chromosome 7 seems to be most relevant.[100] Uniparental disomy does not alter disease biology; these patients were indistinguishable from those with conventionally inherited disease. Its main importance is diagnostic and related to genetic counseling, particularly when only one parent appears to be a carrier. Confirmation requires parental testing and chromosomal studies.

Importance of RNAs in ZSDs

Several non-coding RNAs (ncRNAs) can comprise secondary, system-level responses in ZSDs.[101–103] Identification of the exact



Figs 5A to C: Comparison of normal biparental inheritance with the three forms of UPD (both chromosome copies from one parent). Normally, a chromosome pair carries one maternal and one paternal homolog. In UPD, both copies derive from a single parent: (A) Heterodisomy retains two different homologs (meiosis I error); (B) Isodisomy carries two identical copies of one homolog (meiosis II error or post-zygotic duplication), making all loci homozygous and able to unmask recessive alleles; and (C) Partial isodisomy combines isodisomic and heterodisomic segments through recombination. Capsule color denotes parental origin (blue, maternal; salmon, paternal); colored bands mark alleles at chromosomal loci—identical colors indicate homozygous (isodisomic) regions and different colors indicate heterozygous regions

types of RNA could provide valuable mechanistic information (Table 3).

Gene Duplication

The primary genetic mechanism in ZSDs is biallelic loss-of-function mutations in *PEX* genes.[7] Most pathogenic variants are missense, nonsense, splice-site changes, or small insertions/deletions.[104] Gene duplication is not a typical or established cause of ZSD. However, copy-number variations (CNVs) can occasionally involve *PEX* genes.[6,105] These structural variants are uncommon but are included in diagnostic testing because they may disrupt gene integrity or reading frame. Copy-number variation analysis is important because standard sequencing may miss exon-level deletions or duplications.

Associations of ZSDs with Other Genetic Conditions

True co-occurrence of ZSDs with unrelated Mendelian diseases is rare because these are typically a single-gene, autosomal recessive peroxisomal biogenesis disorder caused by *PEX* gene mutations.[106] However, several important overlaps and differential diagnostic relationships exist:

- Zellweger spectrum disorders are viewed in a broader peroxisomal disease spectrum, which includes disorders with related metabolic defects such as *PEX7*-related rhizomelic *chondrodysplasia punctata*, D-bifunctional protein deficiency (HSD17B4), ACOX1 deficiency, and Refsum disease;[1]
- A wide group of phenotypic mimics overlaps with ZSDs in neonates presenting with hypotonia, seizures, dysmorphism, and liver dysfunction. Key examples include mitochondrial disorders such as POLG (DNA polymerase gamma, catalytic subunit) disease, Leigh syndrome, congenital disorders of glycosylation (CDG), Smith–Lemli–Opitz syndrome, and severe amino acid metabolism disorders such as maple syrup urine disease;[7,107]
- At a pathway level, ZSDs share downstream effects with other conditions affecting lipid metabolism, mitochondrial function, oxidative stress, and neurodevelopment, but these can be viewed more as mechanistic overlaps rather than shared genetics.[108]

Epigenetic Changes in ZSDs

In peroxisomal disorders, epigenetic changes are not primary drivers but represent a secondary, metabolically induced dysregulation resulting from lipid imbalance, oxidative stress, and mitochondrial dysfunction.[109] Metabolic stress can indirectly alter DNA methylation, histone modification, and chromatin

Table 3: Potential roles of ncRNAs in ZSDs

ncRNA/RNA technology	*Major findings in ZSDs*	*Biological and clinical significance*
miRNAs	miRNAs represent a secondary, systems-level response to peroxisomal dysfunction rather than a primary consequence of *PEX* gene mutations. Dysregulation results from VLCFA accumulation, plasmalogen deficiency, oxidative stress, mitochondrial dysfunction, and chronic inflammation, leading to altered transcriptional and epigenetic programs.[110] Here, major functional groups of miRs include: • Lipid and BA metabolism: miR-21, miR-33, miR-34a, miR-122; • Oxidative stress and inflammation: miR-146a, miR-155, miR-210, miR-200 family; • Neurodevelopment: miR-9, miR-124, miR-128, miR-132; • Myelination: miR-219, miR-338, miR-23; • Liver injury and fibrosis: miR-122, miR-34a, miR-29 family.	Reflect secondary metabolic adaptation to peroxisomal failure. Altered miRNA networks may contribute to neurodevelopmental abnormalities, dysmyelination, hepatic injury, inflammation, and oxidative stress, while providing potential biomarkers and future therapeutic targets
siRNAs	siRNAs are not a disease feature or current therapy but serve as powerful experimental tools to model peroxisomal dysfunction by transiently silencing *PEX* genes.[111] Knockdown of *PEX1*, *PEX5*, *PEX6* (matrix protein import and receptor recycling), *PEX13* and *PEX14* (docking complex), *PEX2*, *PEX10*, and *PEX12* (RING finger ubiquitination complex), and *PEX3* and *PEX16* (peroxisomal membrane biogenesis) reproduces "ZSD-like" cellular phenotypes. These include defective matrix protein import, formation of peroxisomal "ghosts," VLCFA accumulation, impaired plasmalogen biosynthesis, defective BA metabolism, and increased oxidative stress	Provide an essential research platform for investigating disease mechanisms, validating pathogenic variants, studying peroxisome biogenesis, and evaluating candidate therapies before clinical translation
lncRNAs	lncRNAs are increasingly recognized as components of the secondary transcriptional response to peroxisomal dysfunction.[112] VLCFA accumulation, plasmalogen deficiency, oxidative stress, mitochondrial dysfunction, and inflammation collectively remodel lncRNA expression across multiple tissues • Liver: H19, MALAT1, NEAT1, and LINC00261 are associated with metabolic stress, cholestatic injury, inflammation, and fibrosis. • Developing brain: MEG3, RMST, TUNA, and SOX2OT are linked to neuronal differentiation, migration, and cortical development. • Stress-responsive lncRNAs: MALAT1, NEAT1, HOTAIR, and PVT1 participate in oxidative stress, apoptosis, inflammatory signaling, and mitochondrial-peroxisomal crosstalk	Represent secondary epigenetic remodeling rather than primary disease causation. LncRNAs may regulate lipid metabolism, neurodevelopment, inflammatory signaling, and cellular stress responses, although further study is needed for recognition as diagnostic biomarker/therapeutic targets in ZSDs
circRNAs	CircRNAs are covalently closed non-coding RNAs that regulate gene expression by acting as miRNA sponges, interacting with RNA-binding proteins, and modulating transcription and cellular stress pathways[113] • In the nervous system, ciRS-7 (CDR1as), circHIPK2, circSLC8A1, and circFOXO3 are associated with altered synaptic function, neuronal differentiation, apoptosis, and neurodevelopment. • In the liver, circHIPK3, circFOXO3, and circITCH are linked to metabolic stress, calcium signaling, hepatocellular injury, and neonatal cholestasis.	In ZSDs, circRNAs are considered a secondary regulatory layer influenced by *PEX* gene mutations and peroxisomal dysfunction

circRNAs, circular RNAs; lncRNAs, long non-coding RNAs; miRNAs, microRNAs; ncRNAs, non-coding RNAs; *PEX*, peroxisomal biogenesis factor; RING, Really Interesting New Gene; siRNAs, small-interfering RNAs; VLCFA, very-long-chain fatty acid; ZSD, Zellweger spectrum disorder

organization.[114] These effects are driven by VLCFA accumulation, plasmalogen deficiency, and impaired redox balance, although disease-specific epigenetic signatures remain incompletely defined.

Epigenetic changes are potentially reversible but also heritable modifications that regulate gene expression without altering the DNA sequence.[115] These mechanisms allow cells with identical genomes to develop distinct functions. The major forms of epigenetic regulation include DNA methylation, histone modifications, chromatin remodeling, non-coding RNA (ncRNA) regulation, RNA modifications (epitranscriptomics), genomic imprinting, and three-dimensional chromatin organization.[116,117]

DNA methylation generally suppresses gene expression by adding methyl groups to cytosine residues within CpG sites.[118] Histone modifications, including acetylation and methylation, alter

Table 4: Epigenetic changes in peroxisomes

Epigenetic mechanism	*Major findings in ZSDs*	*Biological and clinical significance*
DNA methylation	• Peroxisomal dysfunction causes oxidative stress and disruption of one-carbon metabolism, which may reduce the availability of methyl donors such as SAM. • DNA methylation can alter gene-specific DNA methylation patterns, particularly in lipid metabolism, oxidative stress responses, inflammation, and cellular homeostasis	• Suggests that secondary DNA methylation changes contribute to disease progression by modifying gene expression without altering the underlying DNA sequence. • DNA methylation may explain some of the phenotypic variability among patients with similar *PEX* mutations
Histone modifications and chromatin remodeling	• Increased ROS, mitochondrial dysfunction, and metabolic stress can alter the balance between histone acetylation and deacetylation, resulting in changes in chromatin accessibility and transcriptional regulation. • May dysregulate genes involved in neuronal migration, oligodendrocyte maturation, myelination, cortical development, and cellular stress responses	• Provides a mechanistic link between metabolic dysfunction and abnormal neurodevelopment. • May contribute to impaired brain development, dysmyelination, and progressive neurological disease in ZSDs
ncRNA regulation	• Peroxisomal dysfunction leads to secondary dysregulation of microRNAs, lncRNAs, and circular RNAs. • ncRNAs may influence lipid metabolism, inflammation, oxidative stress, neuronal differentiation, myelination, apoptosis, and hepatic injury, thereby amplifying downstream cellular dysfunction	• ncRNAs may represent an important secondary regulatory network that integrates metabolic stress with transcriptional control. • Altered ncRNA expression may provide future biomarkers and therapeutic targets, although none has yet been validated clinically
Metabolic–epigenetic coupling	• Metabolite-driven epigenetic remodeling is potentially an important concept in ZSDs. • Accumulation of VLCFAs, depletion of plasmalogens, impaired BA metabolism, oxidative stress, and mitochondrial dysfunction alter nuclear signaling pathways, including PPAR-dependent transcriptional networks. • Metabolic abnormalities reshape epigenetic regulation, particularly in the developing brain and liver	Integrates the metabolic and epigenetic consequences of peroxisomal dysfunction into a unified disease model. This systems-level mechanism helps explain multisystem involvement and highlights potential opportunities for therapies targeting metabolic signaling and epigenetic regulation rather than individual downstream pathways

BA, bile acid; lncRNAs, long non-coding RNAs; ncRNA, non-coding RNA; *PEX*, peroxisomal biogenesis factor; ROS, reactive oxygen species; SAM, S-adenosylmethionine; VLSFAs, very-long-chain fatty acids; ZSD, Zellweger spectrum disorder

chromatin structure to either activate or repress transcription.[116,117] Chromatin remodeling complexes reposition nucleosomes, regulating DNA accessibility for transcription. Non-coding RNAs, including microRNAs, long non-coding RNAs (lncRNAs), and circular RNAs, control gene expression by influencing mRNA stability, translation, and chromatin regulation. Epitranscriptomic modifications alter RNA processing and translation, while genomic imprinting and higher-order chromatin organization regulate parent-specific gene expression and long-range interactions between genes and regulatory elements.[115]

Together, these interconnected mechanisms integrate developmental, metabolic, and environmental signals to fine-tune gene expression. A summary of the current information on the impact of peroxisomes on global epigenetics is shown in Table 4.[119–126]

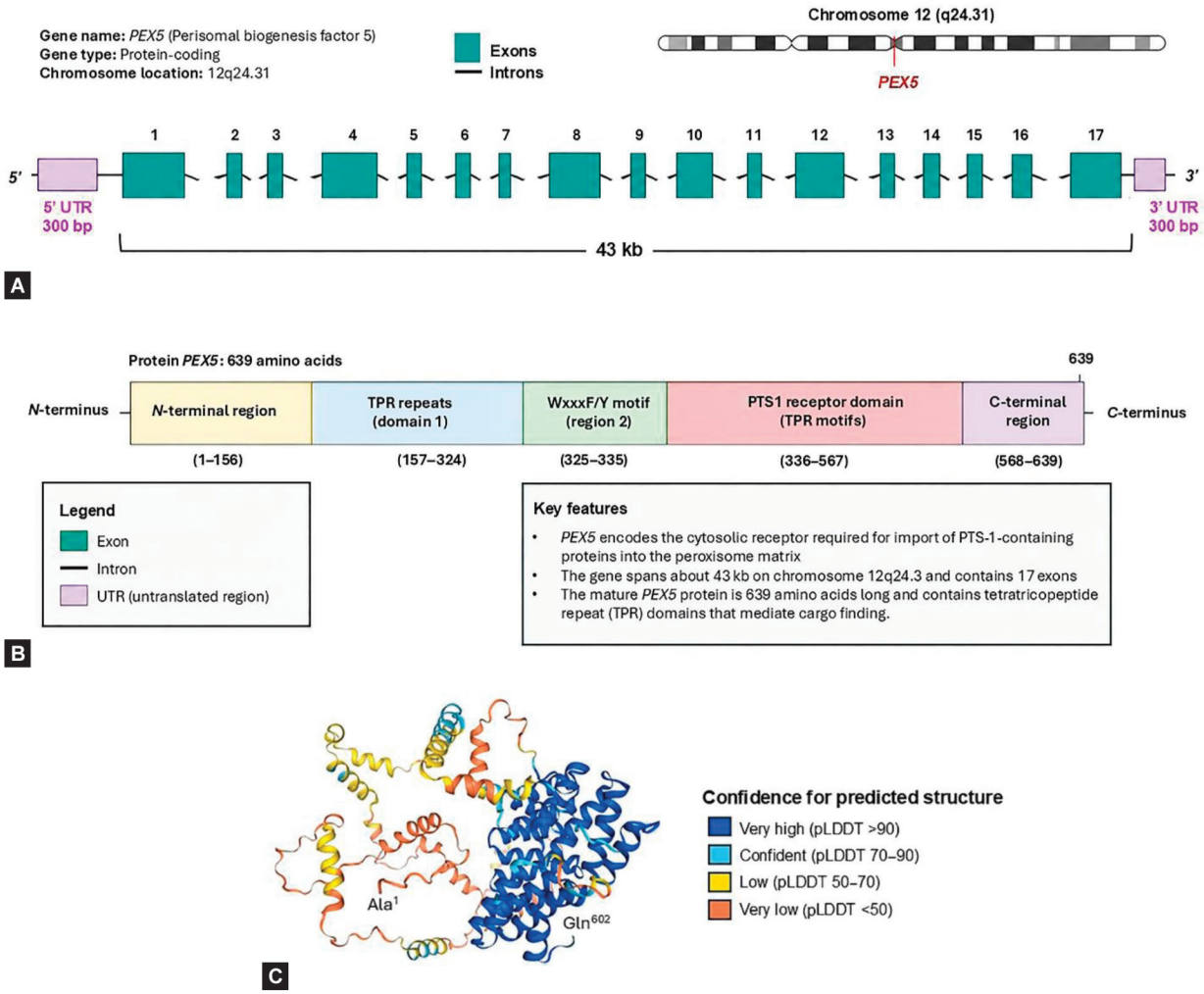
Overall, epigenetic alterations in ZSD are likely to be secondary, metabolically driven effects of peroxisomal dysfunction. These likely amplify neurodevelopmental and multisystem disease processes, but remain incompletely characterized and are not primary epimutations.

Peroxins

Peroxins are proteins encoded by *PEX* genes that are essential for peroxisome biogenesis, maintenance, and function.[127] Over 30 peroxins coordinate the formation of peroxisomal membranes, import of matrix enzymes, receptor recycling, and organelle proliferation.[128] Peroxin 5 is the most abundant of the peroxins inside the peroxisomes (Fig. 6).[129] Table 5 lists the key peroxins involved in peroxisome biogenesis and function.

Peroxin 5 and most related proteins are synthesized on free ribosomes in the cytosol and imported post-translationally via targeting signals and peroxin-mediated transport systems.[79] *PEX5*, the receptor for PTS1 type 1-tagged proteins, and *PEX7*, which recognizes PTS2 signals, are critical for cellular function.[130] These are important targeting pathways used by most of the peroxisomal matrix enzymes, including catalase and those responsible for VLCFA oxidation.[131–133] The two peroxins mentioned above deliver cargo to the peroxisomal membrane, where docking proteins such as *PEX13* and *PEX14* facilitate translocation into the organelle.[79] After cargo delivery, *PEX1* and *PEX6* (AAA-ATPases) recycle *PEX5* back to the cytosol; defects in these proteins are a common cause of ZSDs.[12,134,135]

We first used fast minimum evolution (FME), a distance-based optimization method for analysis (Fig. 7A).[136] These distance matrices were calculated using the amino acid substitution models from Whelan and Goldman (WAG), Jones–Taylor–Thornton (JTT), and Le and Gascuel (LG).[137–140] The tree with the lowest total branch length was chosen with the assumption that evolution generally proceeds with the fewest overall changes.[141,142] The results were then compared with those from the maximum likelihood (ML) model of amino acid substitutions, and the tree with the highest log likelihood in phylogenetic analysis (–1,32,936.63) was selected (Fig. 7B).[143–145] The chosen tree had to have the shortest length in 10 random models; the final dataset in our analysis identified



Figs 6A to C: Structure of the human *PEX5* gene and encoded protein. (A) Schematic representation of the human *PEX5* gene showing the 5′ and 3′ untranslated regions (UTRs), exons, introns, chromosomal location, and overall gene organization. (B) The domain architecture of the *PEX5* protein, including the *N*-terminal region involved in receptor docking and recycling, a WxxxF/Y motif, and the *C*-terminal tetratricopeptide repeat (TPR) domain that recognizes proteins containing the PTS1. *PEX5* functions as the principal cytosolic receptor responsible for the import of most matrix proteins into the peroxisome. The WxxxF/Y motif is a short, highly conserved peptide sequence in the *N*-terminal region of *PEX5* that mediates interactions with the peroxisomal membrane docking complex during matrix protein import. W = tryptophan; xxx = any three amino acids; F/Y = phenylalanine or tyrosine. These motifs enable *PEX5* to bind the peroxisomal docking proteins *PEX13* and *PEX14* after the receptor has captured a PTS1-containing cargo protein in the cytosol. (C) Three-dimensional (3D) structure of the *PEX5* protein, derived from the predicted Local Distance Difference Test (pLDDT). The pLDDT scores are a per-residue confidence metric shown on an incremental color scale. It shows the increasing accuracy of the model in predicting the local 3D structure of a specific amino acid. As seen in the illustration, the 3D structures have been predicted with greater confidence (a) near the C-terminus than the N-terminus; and (b) in alpha-spirals and beta-pleated sheets than in the turns, loops, and coils. The *C*-terminal TPR motifs directly recognize and bind the PTS1 signal on peroxisomal matrix proteins. Because this interaction is essential for importing enzymes into the peroxisome, strong evolutionary pressure has preserved the structure of the domain. Conserved residues within the TPR repeats help create a hydrophobic and charged binding groove that accommodates the PTS1 tripeptide. The base structures for this figure were taken with minor modifications from https://www.proteinatlas.org/ENSG00000139197-*PEX5*/structure

100 amino acid sequences with 707 positions. Evolutionary analyses were conducted using the software program MEGA12 (Molecular Evolutionary Genetics Analysis, version 12), utilizing up to four parallel computing threads.[146–148]

Peroxin 5 is highly conserved across eukaryotes, with homologous proteins clustering according to established taxonomic relationships.[74] Closely related species formed compact clades with short terminal branch lengths, indicating high amino acid sequence conservation, whereas longer internal branches reflected ancient divergence among major eukaryotic lineages.[149] The limited sequence divergence, particularly among vertebrate homologs, suggests that *PEX5* has been subject to strong purifying selection, consistent with the deleterious effects of mutations that disrupt cargo recognition, receptor docking, or receptor recycling. Overall, the phylogenetic topology supports the co-evolution of *PEX5* with species diversification while preserving the structural and functional domains required for PTS1-dependent peroxisomal protein import.[150]

Table 5: Peroxins with important contributions to the peroxisome structure/function

Peroxin	*Contribution to the peroxisomal protein pool*
PEX5	Likely one of the most abundant functional peroxins because it repeatedly cycles between cytosol and peroxisome to deliver most PTS1-containing matrix proteins. High turnover makes it a major component of the import machinery
PEX14	One of the most abundant peroxisomal membrane peroxins; forms the docking site for *PEX5* and *PEX7* cargo receptors.
PEX1	The largest peroxin by amino acid length (1,283 amino acids), but not necessarily the most numerous. It is a key regulator of *PEX5* recycling
PEX6	Forms the *PEX1–PEX6* recycling complex; large in size but less abundant than many matrix proteins

PTS, peroxisomal targeting signal

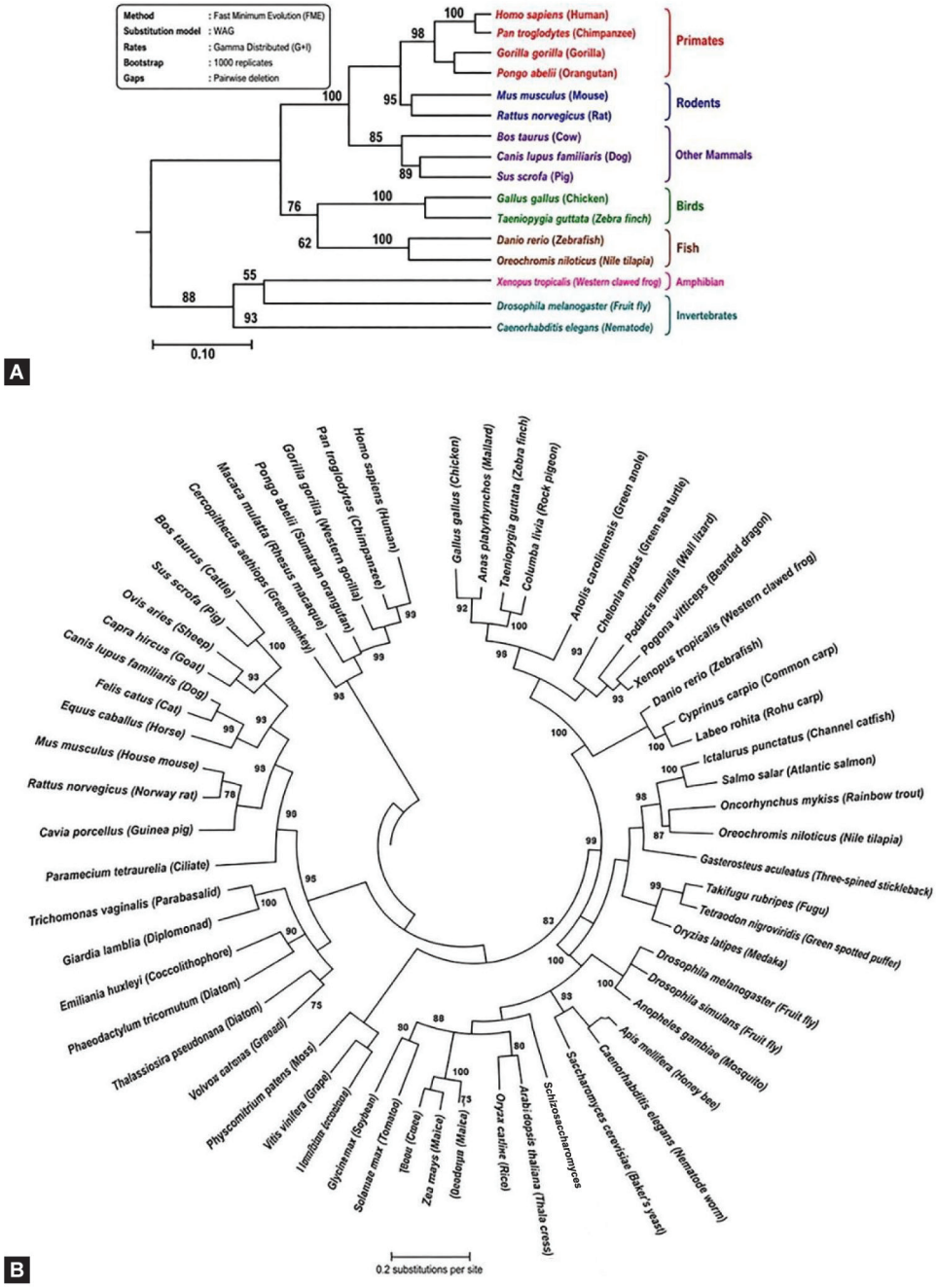
Clinical Features of ZSDs

The following clinical features have been frequently associated with ZSDs:[1,2,6,9,15,17,25,80,151–155]

- Facial dysmorphology: Characteristic craniofacial features are often present at birth and, together with hypotonia, provide early diagnostic clues.[7] These include a high forehead and a large fontanelle. The midface has a flattened appearance, giving a broad and under-projected facial appearance. There can be ocular features such as hypertelorism, sometimes with epicanthal folds and mildly altered palpebral fissure orientation, contributing to widened orbital spacing. There can be a broad, flat nasal bridge and reduced nasal tip projection. There can be micrognathia/retrognathia, which may contribute to feeding and airway difficulties. The ears may be low-set and simplified in contour, though this finding is variable.
- Neurologic dysfunction: Neurologic dysfunction in ZSDs results from abnormal brain development, including impaired neuronal migration and cortical organization.[4] These infants are usually hypotonic from birth—the muscle tone is typically very low, producing the classic "floppy infant" presentation. It reflects primarily central nervous system dysfunction due to abnormal brain development such as polymicrogyria and disrupted neuronal migration. Clinically affected neonates show markedly reduced spontaneous movement, poor axial tone with severe head lag, weak or absent neonatal reflexes, and poor postural control. Tone is often more affected in the axial than limb musculature, consistent with central hypotonia. Bulbar involvement is prominent, leading to weak suck–swallow coordination, feeding difficulty, and high risk of aspiration. Unlike peripheral neuromuscular disorders, muscle bulk is usually preserved early on, but motor responsiveness is significantly reduced. Hypotonia does not improve with stimulation and may worsen with disease progression.
- Seizures: Seizures are a frequent and often early manifestation of ZSDs, particularly in severe neonatal phenotypes.[151–153] They reflect global cortical maldevelopment, neuronal migration defects, and metabolic dysfunction. Early signs may be subtle, including apnea, oxygen desaturation, ocular deviation, lip smacking, brief tonic posturing, or myoclonic jerks.[152] With progression, seizures become multifocal, frequent, and often refractory, evolving into tonic, clonic, or mixed seizure types. Overall, seizure activity is diffuse and non-localizing, reflecting global cortical dysfunction.
- Developmental delay: All developmental domains are affected.[4,7] Gross motor delay is typically most pronounced and earliest, followed by fine motor impairment, absent or minimal social responsiveness, and severely impaired or absent language development. In severe neonatal cases, milestones beyond primitive reflexes are rarely achieved. Motor development is dominated by central hypotonia and impaired motor planning, with poor head control, absent postural responses, minimal antigravity movements, and markedly reduced spontaneous activity. This pattern reflects cortical malformations and corticospinal tract dysfunction rather than primary muscle disease. Cognitive and social development are profoundly impaired due to cortical malformations and white matter abnormalities. Affected infants show reduced alertness, poor environmental engagement, and absent social interaction. Visual and hearing impairment further worsen developmental outcomes.
- Respiratory distress: Respiratory distress (tachypnea, grunting, hypoxemia) can be seen in the early neonatal period, intermediate phenotypes with chronic hypoventilation, and in severe cases, recurrent apnea and respiratory failure.[1,6,151] Neuromuscular hypotonia can further worsen respiratory function, causing weak respiratory effort, poor airway clearance, and increased risk of atelectasis and aspiration. The imaging findings on chest radiographs are nonspecific and may show low lung volumes, atelectasis, or patchy opacities, without a pathognomonic pattern.
- Apneas: Apneas are seen frequently.[1,4] These reflect failure of central respiratory control, brainstem dysfunction, severe hypotonia, and metabolic instability. Most apneas are of central origin, presenting as episodes of absent respiratory effort with oxygen desaturation and bradycardia, though mixed central–obstructive apnea may occur due to airway hypotonia. There may be recurrent cyanotic episodes, poor respiratory effort, feeding intolerance, and weak cry. It may overlap with seizure-related events, requiring EEG for differentiation.
- Hepatic dysfunction: Hepatic dysfunction can present as hepatomegaly, cholestatic jaundice, elevated liver enzymes, coagulopathy, and progressive fibrosis, reflecting impaired peroxisomal BA metabolism.[154,156,157]
- Skeletal abnormalities: Patients with severe Zellweger syndrome often display low bone density, rhizomelia with preferential shortening of the humerus and femur, and developmental hip dysplasia-like changes with hip instability or subluxation.[7,158]
- Ophthalmologic abnormalities: Ophthalmologic abnormalities include cataracts, glaucoma, retinal dystrophy, optic nerve abnormalities, and progressive visual impairment.[4,7]
- SNHL: SNHL is seen frequently and tends to be severe.[4,7]
- Olfactory dysfunction: Olfactory dysfunction can occur in ZSDs. There may be reduced response to odor stimuli and diminished sensory-driven behavior, but the findings are often not detected with overlapping hypotonia and encephalopathy.[159]

Laboratory Diagnosis of ZSDs

The diagnosis of clinically suspected ZSDs is based on biochemical and molecular genetic testing.



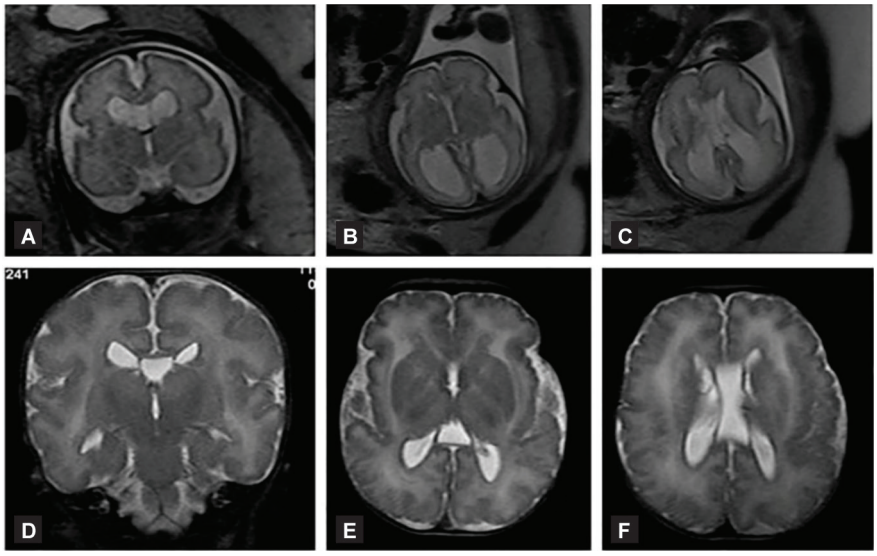
Figs 7A and B: Phylogenetic trees of *PEX5* proteins from representative eukaryotic species were developed using: (A) FME; and (B) Maximum Likelihood (ML) methods. The FME approach utilizes a Neighbor-Joining (NJ) topology to identify a tree with the shortest overall branch length; these methods show more accurate branch lengths and better topological resolution than NJ alone. In the ML pie-chart phylogram, protein sequences were aligned using MUSCLE (Multiple Sequence Comparison by Log-Expectation) and phylogenetic relationships were analyzed using the MEGA12 software program. The ML analysis shows branch lengths proportional to the estimated number of amino acid substitutions per site. The topology demonstrates that *PEX5* proteins cluster according to taxonomic relationships, reflecting the evolutionary conservation of this essential receptor for PTS1-mediated peroxisomal matrix protein import. Numbers at the internal nodes represent bootstrap support values (%), indicating the robustness of the inferred phylogenetic relationships. The human gene is highlighted (red)

Initial Biochemical Evaluation

The first steps include measurement of plasma VLCFAs, which are typically elevated due to impaired peroxisomal β-oxidation.[160] Additional supportive biomarkers include increased phytanic acid, pristanic acid, pipecolic acid, and BA intermediates such as DHCA and THCA, together with reduced erythrocyte plasmalogens.[160,161]

Molecular Genetic Testing

This is the diagnostic gold standard and identifies pathogenic variants in *PEX* genes, most commonly *PEX1* and *PEX6*. Gene panel testing, whole-exome sequencing, or whole-genome sequencing confirms the diagnosis, facilitates genetic counseling, and enables prenatal or preimplantation genetic testing.[13,29,153,162]



Figs 8A to F: Pre- and postnatal neuroimaging findings in ZSD. Representative fetal (A to C) and infant (D to F) T2-weighted MRI shows temporal evolution of cerebral pathology. Prenatally, mild ventriculomegaly, widened Sylvian fissures with suggestion of insular polymicrogyria maturation, and a mildly delayed white matter maturation are evident. Following birth, MRI demonstrates diffuse white-matter T2 hyperintensity reflecting hypomyelination, persistent ventriculomegaly and bilateral perisylvian polymicrogyria. These imaging features reflect impaired neuronal migration, anomalous cortical organization and delayed or abnormal white matter myelination, hallmarks of severe PBDs

Additional Testing

When results are inconclusive, cultured skin fibroblasts can be used to confirm defective peroxisomal matrix protein import and generalized impairment of peroxisomal function.[163] Brain magnetic resonance imaging (MRI), liver and renal ultrasonography, ophthalmologic examination, and audiologic testing help assess the extent of organ involvement but are not diagnostic.[1]

Prenatal Diagnosis

Evaluation is available for families with a previously identified pathogenic *PEX* variant. Targeted molecular testing can be performed using chorionic villus sampling (typically at 10–13 weeks' gestation) or amniocentesis (after 15 weeks' gestation). Preimplantation genetic testing offers an additional reproductive option for at-risk couples undergoing in vitro fertilization.[1,7,164]

Overall, diagnosis of ZSDs requires clinical suspicion, identification of characteristic biochemical abnormalities, and confirmation by molecular genetic testing. Functional studies may be helpful.

Correlation of Laboratory and Imaging Findings in ZSDs

Central and Peripheral Nervous System

Evaluation of the nervous system is important in infants with hypotonia, seizures, dysmorphism, and multisystem disease.[157,164–172]

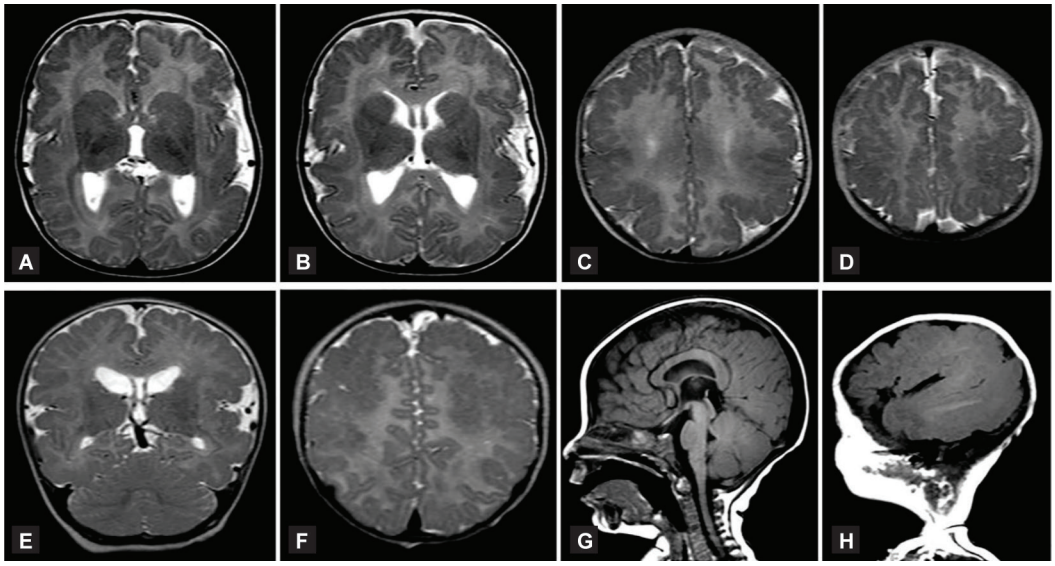
Magnetic Resonance Imaging

Structural sequences can help map anatomical abnormalities and initial myelination delays:

- T1-weighted protocols may show germinolytic subependymal cysts within the caudothalamic grooves, showing them as dark, fluid-filled pockets.[4] Metabolic changes may demonstrate atypical T1 shortening (hyperintensity) in the globus pallidus due to hyperbilirubinemia or underlying hepatic dysfunction.[4,165]
- T2-weighted protocol may show abnormal gray matter architectures. Changes can often be identified before birth (Fig. 8). In young infants, myelination can be noticeably delayed or absent (Fig. 9).[4,165]
- Fluid-attenuated Inversion Recovery (FLAIR) is useful in surviving older infants, children, or adults with milder phenotypes for tracking leukoencephalopathy. Signal enhancement may indicate areas of progressive white matter damage.[165]
- Diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI) can help assess cellular-level white matter tract integrity.[165,173] Diffusion-weighted imaging and apparent diffusion coefficient (ADC) evaluate water molecule movement; subtle restricted diffusion (bright on DWI, dark on ADC maps) shows ongoing myelin breakdown. In fractional anisotropy (FA) maps, DTI metrics show significantly decreased FA in major white matter tracts. These changes confirm the destruction or failed development of the structural channels needed for normal cognitive and motor pathways.
- Magnetic resonance spectroscopy (MRS) can help evaluate biochemical alterations within the brain tissue. It often reveals decreased N-acetylaspartate (NAA) levels, indicating neuronal loss, paired with an elevated Choline (Cho) peak, representing accelerated myelin/membrane turnover. Susceptibility-weighted imaging (SWI)/T2* can help differentiate ZSDs from concurrent vascular insults or trauma.[174]

The following section lists MRI findings frequently noted in ZSDs:[157,164–172]

- Cortical malformations: Neuronal migration abnormalities such as pachygyria (broad, thick gyri) and polymicrogyria (too many small, shallow gyri) around the perisylvian and perirolandic (Rolandic fissure = central sulcus) regions; lissencephaly-like



Figs 9A to H: Representative brain MRI findings in severe ZSD. Axial (A to D), coronal (E and F) T2-weighted images reveal diffuse bilateral symmetric hyperintensity involving the periventricular and deep cerebral white matter, compatible with abnormal myelination. Enlargement of the lateral ventricles is accompanied by diffuse reduction in cerebral white-matter volume. The cerebral cortex demonstrates extensive, symmetrical perisylvian and perirolandic polymicrogyria. Sagittal T1-weighted images (G and H) show frontal bossing and a high-riding forehead as well as a foreshortened but intact corpus callosum. There were no significant posterior fossa abnormalities. These neuroimaging features illustrate the coexistence of prenatal disturbances of neuronal migration, cortical organization, and white matter dysmyelination resulting from peroxisomal dysfunction

cortical simplification in frontal, perisylvian, perirolandic, and occipital lobes; and neuronal heterotopias are seen in the periventricular areas in many patients.

- White matter and myelination: There can be delayed or severely reduced myelination, diffuse white matter abnormalities, and early cerebral atrophy. These changes reflect plasmalogen deficiency and VLCFA toxicity affecting oligodendrocytes.
- Ventricular and structural abnormalities: Some patients may show ventriculomegaly (or enlargement limited to just the occipital horns), hypoplasia/dysgenesis of the corpus callosum, and germinolytic cysts (subependymal pseudocysts, typically located near the lateral ventricles in the caudothalamic groove).
- Basal ganglia: There may be a mild underdevelopment or volume reduction in the caudate nucleus and/or putamen. The deep gray nuclei may show signal abnormalities. These changes reflect global developmental under-formation rather than primary degeneration.
- Brainstem: There may be subtle hypoplasia or abnormal signal (sometimes not prominent on MRI). The resulting functional impairment of respiratory and autonomic centers manifests as central apnea, weak cry, poor suck–swallow coordination, and autonomic instability.
- Spinal cord and peripheral nervous system: ZSDs may manifest as abnormal corticospinal tract maturation, delayed spinal myelination, and peripheral nerve hypomyelination. Assessment of changes in nerves may require further studies such as magnetic resonance neurography.

  Electromyography and nerve conduction studies are usually negative in ZSDs. These are considered when there is diagnostic uncertainty, to differentiate these from conditions such as spinal muscular atrophy, congenital myopathies, neuropathies, or neuromuscular junction disorders.
- Cranial MRI in late survivors: There may be progressive leukodystrophy-like changes, cerebral and cerebellar atrophy, and worsening white matter abnormalities.[159] Magnetic resonance spectroscopy may demonstrate metabolic disturbances, although findings are not disease-specific.

Cranial Ultrasonography

Ultrasonography is useful for an initial assessment of brain abnormalities in ZSDs.[175,176] Ventriculomegaly, enlarged extra-axial spaces, and cystic lesions may be seen. However, ultrasound is less sensitive than MRI for detecting cortical malformations, neuronal migration defects, and white matter abnormalities.

Electroencephalography (EEG)

Electroencephalography often becomes abnormal before clinical seizures are evident.[171,177] There can be diffuse background slowing; poor organization and absent sleep–wake cycling; multifocal epileptiform discharges; generalized spike/polyspike activity; and burst–suppression or low-voltage discontinuous patterns. Electroencephalography abnormalities typically worsen with disease progression, transitioning from slowing to multifocal epileptiform activity and, in severe neonatal disease, to burst–suppression.

Electroencephalography findings closely correlate with MRI abnormalities such as polymicrogyria, pachygyria, delayed myelination, ventriculomegaly, and white matter disruption, forming a diffuse epileptogenic substrate. The seizures in ZSD are frequently refractory to standard antiseizure medications, with treatment primarily supportive.[4] A summary of neurological abnormalities in ZSDs is presented in Table 6.

Liver Involvement

Neonates typically show cholestasis, including conjugated hyperbilirubinemia, elevated transaminases, and often

Table 6: Neurological abnormalities seen in ZSDs

Domain	*Clinical features*	*EEG findings*	*MRI/neuroimaging findings*	*Pathophysiological basis*
Cortical dysfunction (primary encephalopathy)	Severe developmental delay, poor arousal, absent milestones	Diffuse background slowing, poor organization	Polymicrogyria, pachygyria, lissencephaly-like cortex, cortical dysgenesis	Impaired neuronal migration and corticogenesis due to peroxisomal dysfunction
Epileptic phenotype	Neonatal seizures, multifocal clonic jerks, myoclonus, infantile spasms–like events	Multifocal epileptiform discharges, generalized spike/polyspike activity, burst–suppression (severe cases)	Cortical malformations serving as epileptogenic substrate	Disorganized cortical networks with metabolic instability (VLCFA toxicity, membrane dysfunction)
Central hypotonia (motor system failure)	Profound axial hypotonia, weak reflexes, head lag, poor suck–swallow coordination	Non-specific slowing; may show suppression in severe encephalopathy	Delayed myelination, white matter hypoplasia, corticospinal tract immaturity	Impaired corticospinal development, plasmalogen deficiency affecting myelin integrity
Brainstem/autonomic dysfunction	Central apnea, weak cry, feeding failure, autonomic instability	Severe generalized suppression or low-voltage EEG in advanced disease	Brainstem hypoplasia or subtle signal abnormalities (midbrain, pons, medulla)	Developmental failure of respiratory and autonomic nuclei
White matter/myelination failure	Hypotonia, reduced motor responses, global developmental arrest	Diffuse slowing with poor maturation patterns	Marked delayed myelination, hypomyelination, white matter rarefaction	Oligodendrocyte dysfunction due to plasmalogen deficiency and lipid toxicity
Deep gray matter involvement	Severe motor impairment, global encephalopathy	Multifocal epileptiform discharges may be prominent	Basal ganglia underdevelopment, ventricular enlargement	Metabolic vulnerability of high-energy neuronal structures
Global severe neonatal phenotype	Refractory seizures, apnea, feeding failure, poor responsiveness	Burst–suppression or near-continuous epileptiform activity	Diffuse cortical malformations + white matter + brainstem involvement	Global failure of peroxisomal lipid metabolism and neurodevelopment

MRI, magnetic resonance imaging; VLCFA, very-long-chain fatty acid; ZSD, Zellweger spectrum disorder

hepatomegaly.[13,154,178] A key biochemical hallmark is accumulation of atypical BA intermediates (DHCA and THCA), contributing to ongoing hepatocellular injury. With progression, the liver may show steatosis, canalicular cholestasis, and progressive fibrosis, occasionally evolving toward early cirrhosis.[179] Synthetic dysfunction can lead to coagulopathy, hypoalbuminemia, and fat-soluble vitamin deficiencies (A, D, E, K), further worsening systemic instability. Ultrasound imaging may show liver enlargement, fibrosis, and indirect signs of cholestasis.

Renal Abnormalities

Renal ultrasound may show multiple cortical cysts on one or both sides.[4] These can be detected prenatally/in the neonatal period and reflect abnormal tubular differentiation. Large kidneys, increased echogenicity, and poor corticomedullary differentiation may be seen.[4] Functional impairment is usually mild but may include subtle tubular dysfunction and reduced concentrating ability. Renal defects are not an immediate cause of mortality in most infants.

Pulmonary Involvement

PEX gene-related plasmalogen deficiency affects surfactant biology and lung development.[180] In addition, there can be developmental lung abnormalities such as mild pulmonary hypoplasia and/or simplified alveolar architecture. Many infants also show changes in the diaphragm; there is reduced tone and contractility due to central hypotonia, impaired neuromuscular activation, and metabolic energy failure from combined mitochondrial–peroxisomal dysfunction.

Apneas

Apneas are seen frequently in infants with ZSDs. These reflect failure of central respiratory control, brainstem dysfunction, severe hypotonia, and metabolic instability. Most apneas are of central origin, presenting as episodes of absent respiratory effort with oxygen desaturation and bradycardia, though mixed central–obstructive apnea may occur due to airway hypotonia. There may be recurrent cyanotic episodes, poor respiratory effort, feeding intolerance, and weak cry. It may overlap with seizure-related events, requiring EEG for differentiation.

The primary mechanisms of central respiratory dysregulation are related to cortical malformations and abnormal brainstem development. This is compounded by global encephalopathy and reduced arousal responsiveness. Generalized hypotonia further contributes through diaphragmatic and intercostal weakness, reducing ventilatory effectiveness and worsening the severity of apnea severity. Metabolic dysfunction, including VLCFA accumulation and impaired peroxisome–mitochondria interactions, further destabilizes respiratory control.

Olfactory Dysfunction

Olfactory dysfunction in ZSDs is a congenital and under-recognized neurodevelopmental feature in ZSDs. The etiopathogenesis is likely rooted in disrupted neuronal migration, axonal guidance, and synaptogenesis. This leads to olfactory bulb hypoplasia

or dysgenesis, impaired olfactory tract connectivity, abnormal orbitofrontal and limbic cortical development, and global white matter and cortical dysfunction. These central abnormalities are the primary drivers of congenital anosmia or severe hyposmia. Olfactory receptor neurons may also be affected by membrane instability from plasmalogen deficiency, impaired odorant receptor signaling, and dysfunction of olfactory cilia. Generally, peripheral mechanisms are less significant than central defects.

In severe cases, MRI may show olfactory bulb hypoplasia, and olfactory evoked potentials may be absent. Overall, olfactory dysfunction likely contributes to reduced sensory integration and early environmental responses, even though it might not always be clinically obvious.

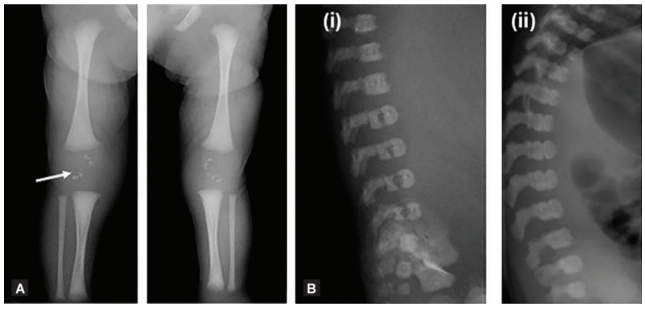
Skeletal Abnormalities

Zellweger spectrum disorders may show generalized skeletal dysplasia with defective chondrocyte differentiation, cartilage matrix formation, and endochondral ossification.[7,153] These skeletal abnormalities are usually part of a broader multisystem phenotype, where they may contribute to positioning difficulties and joint instability. Some of the structural abnormalities include:

- Abnormalities in hip joints: There may be developmental hip dysplasia-like changes (shallow acetabulum, reduced femoral head coverage), delayed or absent femoral head ossification, and hip instability or subluxation. Generalized hypotonia may also play a role with reduced periarticular stabilization. The hip abnormalities can be one facet of a multisystem metabolic disorder, which may contribute to joint laxity. Radiographs may show persistent delayed ossification, epiphyseal irregularities, and *chondrodysplasia punctata*. The acetabulum may appear shallow (abnormal alpha angle) with delayed development of the femoral head.
- Long bone abnormalities: There may be delayed ossification of the femur, tibia, and humerus; small/absent epiphyseal ossification centers; and generalized skeletal immaturity. These reflect impaired growth plate development and chondrocyte differentiation during fetal life. There may be epiphyseal stippling around the knee and elbow joints, and in proximal long bones. Additional findings include metaphyseal flaring and irregularity, poorly defined growth plate margins, and delayed appearance or hypoplasia of epiphyseal centers. Many infants show osteopenia and cortical thinning, reflecting systemic metabolic disruption of bone matrix formation.

Plain radiographs have traditionally been used to evaluate skeletal changes in ZSDs. *Chondrodysplasia punctata* is often seen with stippled or punctate epiphyseal calcifications, involving cartilage and periarticular regions (Fig. 10).[175] These findings are frequently seen in the knees, hips, shoulders, vertebrae, long bones, and the spine. Delayed ossification, shortened long bones, metaphyseal irregularities, hip dysplasia and joint contractures, and occasionally, vertebral anomalies are also seen.[175]

Bone ultrasound can be a useful, non-invasive bedside tool for evaluating/monitoring skeletal abnormalities.[181] It can detect delayed ossification of long bones at epiphyseal and metaphyseal regions. These ossification centers may be absent, could be small-for-gestational age, or not be clearly defined—all suggesting impaired endochondral ossification. Cartilages could also appear thickened or irregular with poorly defined cartilage–bone transition zones. High-resolution ultrasound may also demonstrate epiphyseal cartilage hypoplasia and delayed metaphyseal mineralization.[158]



Figs 10A and B: Radiographic changes in ZSDs. (A) Anteroposterior radiograph of the lower extremities of an affected infant demonstrating characteristic punctate (stippled) calcifications within the epiphyseal cartilage adjacent to the knees (arrow), a hallmark of chondrodysplasia punctata. The long bones also show mild metaphyseal flaring; (B) Lateral spinal radiographs from another patient show chondrodysplasia punctata in the spine: Multiple punctate calcific densities of spinal stippling in the vertebral bodies and posterior spinal elements as well as coronal clefting

Hematological Abnormalities

Hematologic abnormalities in ZSDs are usually a secondary change, reflecting systemic metabolic and hepatic dysfunction rather than primary bone marrow disease.[1] Vitamin K-dependent coagulopathy is seen frequently, caused by cholestasis with impaired fat-soluble vitamin absorption and reduced hepatic synthesis of clotting factors (II, VII, IX, X).[182]

Bone marrow function is typically intact.[7] Some infants may show mild to moderate normocytic anemia, typically due to chronic illness, malnutrition, and impaired erythropoiesis. Occasionally, there might be some nonspecific hemolysis. Platelet counts are usually normal, but subtle platelet dysfunction may occur due to altered membrane lipids. Leukocyte counts are generally preserved.

Ocular Abnormalities

Corneal or anterior segment abnormalities can be seen. There may also be retinal dystrophy and pigmentary changes, attenuated retinal vessels, and optic nerve hypoplasia.[7] These changes reflect impaired photoreceptor and optic pathway development. Functional evaluation may show poor/absent fixation and tracking, low visual responsiveness, and mixed visual loss (retinal, optic nerve, and cortical). Electroretinograms and visual evoked potentials are often markedly abnormal.

Hearing Impairment

Congenital, moderate-to-profound SNHL is seen.[7,183] There may be abnormalities involving the cochlea (outer hair cell and stria vascularis), auditory nerve, and central auditory pathways. Otoacoustic emissions (OAEs) are helpful but can show some variability. Auditory brainstem responses more consistent; these are usually absent or severely abnormal. Many infants have central auditory processing defects due to brain malformations. These can worsen over time.

Developmental Delay

In neonatal variants of ZSDs, developmental delay is global, early-onset, and usually severe.[7] Altered peroxisomal biogenesis leads to secondary abnormalities in brain development, neuronal migration, myelination, and widespread metabolic dysfunction affecting neurological development from fetal life.[71]

Clinical Management

Patients with ZSDs require lifelong, multidisciplinary care.[1] Although no curative therapy is known, appropriate supportive treatment can significantly improve survival and quality of life, particularly for individuals with milder phenotypes. Because ZSDs affect multiple organ systems, including the nervous system, liver, eyes, ears, kidneys, adrenal glands, and musculoskeletal system, care needs to be coordinated with input from metabolic geneticists, neurologists, hepatologists, ophthalmologists, audiologists, endocrinologists, rehabilitation specialists, nutritionists, and genetic counselors.

Neurological care is a major component of management because most patients with ZSDs show hypotonia, developmental delay, and epilepsy.[2] Seizures should be evaluated with EEG and treated using standard antiseizure medications such as levetiracetam, phenobarbital, or other agents selected according to seizure type and hepatic function.[155] Early developmental intervention, including physical, occupational, and speech therapy, should begin soon after diagnosis to maximize motor function, communication, and independence. Individualized rehabilitation programs are needed, tailored to each patient's abilities to improve participation in daily activities.[2,184]

Nutritional management is equally important because feeding difficulties, poor weight gain, gastroesophageal reflux, and swallowing dysfunction are seen frequently in these patients.[2] Infants often require high-calorie nutritional supplementation, careful assessment by feeding specialists, and the risk of aspiration. Some children eventually require gastrostomy tube placement to ensure adequate nutrition and medication administration. Growth should be monitored closely, together with regular assessment of fat-soluble vitamin status, particularly in patients who develop cholestatic liver disease.

Liver disease is one of the defining features of ZSDs and requires regular monitoring through liver function tests, coagulation studies, and abdominal ultrasonography.[1] Progressive fibrosis, cholestasis, and portal hypertension may develop over time. Supplementation with vitamins A, D, E, and K is frequently necessary, particularly in patients with cholestasis. Liver transplantation has been performed in rare, carefully selected individuals, but it does not correct the underlying systemic metabolic defect.

Endocrine surveillance is increasingly recognized as an essential component of long-term care for ZSDs.[185] Many patients develop adrenal insufficiency due to VLCFA accumulation in the adrenal cortex. Routine screening with morning cortisol concentrations and ACTH stimulation testing allows early identification of adrenal dysfunction. Patients with confirmed adrenal insufficiency should receive replacement therapy with hydrocortisone and require stress-dose corticosteroids during illness or surgery. Growth, bone mineralization, thyroid function, and vitamin D status should also be monitored.

Sensory impairments require ongoing evaluation because progressive SNHL and retinal degeneration are common manifestations of ZSDs.[7] Regular audiologic assessments permit timely fitting of hearing aids or consideration of cochlear implantation in selected patients. Ophthalmologic examinations should monitor for retinal dystrophy, cataracts, glaucoma, and refractive errors, allowing early intervention to preserve visual function whenever possible. Rehabilitation services, including low-vision support and communication therapy, can substantially improve quality of life.

Respiratory, renal, and musculoskeletal complications also require regular surveillance in ZSDs.[1] Hypotonia and recurrent aspiration predispose affected children to respiratory infections, making airway clearance techniques, aspiration prevention strategies, and, when necessary, noninvasive ventilatory support important aspects of care. Renal ultrasonography and assessment of kidney function help detect renal cortical cysts, nephrolithiasis, or hyperoxaluria. Physical therapy, stretching programs, orthopedic assessment, and treatment of scoliosis, hip dysplasia, or contractures help preserve mobility and reduce disability. Bone health should be supported through adequate nutrition, vitamin D supplementation, and monitoring for osteopenia.

Routine laboratory monitoring includes measurement of plasma VLCFAs, phytanic and pristanic acids, plasmalogen concentrations, liver enzymes, coagulation parameters, and fat-soluble vitamin levels.[7] These biochemical markers do not always correlate perfectly with disease severity, but the measurements still provide valuable information regarding metabolic control and the development of organ-specific complications.

Genetic counseling is an integral part of management because ZSDs are inherited in an autosomal recessive manner.[1,7] Families should receive counseling regarding the 25% recurrence risk in future pregnancies, and carrier testing should be offered to at-risk relatives when the causative pathogenic variants are known.[186] Prenatal diagnosis and preimplantation genetic testing are available for many families and can facilitate informed reproductive decision-making.[7]

Prognosis

The outlook for infants with ZSDs is currently poor; most patients die during infancy due to progressive neurologic impairment, respiratory failure, feeding difficulties, liver disease, and multisensory deficits.[7,13] Supportive care may improve survival in milder phenotypes, but does not alter the course of classic neonatal disease.[7] Early diagnosis is important for management and genetic counseling.[155]

Future directions

Looking ahead, the integration of modern high-throughput platforms with the conventional clinical/diagnostic methods will inevitably reshape the diagnostic landscape for ZSDs (Table 7).[2] Integration of molecular and biochemical testing has and will further enhance early detection and enable prenatal and preimplantation genetic diagnosis. Next-generation sequencing has enabled rapid identification of *PEX* gene mutations, particularly in *PEX1* and *PEX6*, and offers hope for earlier and more precise diagnosis and recognition of a broader phenotypic spectrum.[162] Genotype–phenotype correlations now show that null variants cause severe neonatal disease, whereas hypomorphic variants are associated with milder phenotypes, supported by functional assays of peroxisomal activity.[187] As for many other rare genetic disorders, gene replacement therapy, mRNA-based therapeutics, nonsense read-through strategies, pharmacological chaperones, metabolic supplements, and compounds that enhance peroxisome biogenesis are being studied.[1,188] Advances in MRI and biomarker profiling may help in earlier diagnosis and disease stratification.[165] Overall, these developments have shifted ZSD toward a genetically defined, mechanism-based disorder, enabling precision diagnostics and hope for effective therapies.

Table 7: High-throughput platforms available for studying ZSDs

Omics platform	*Major findings in ZSDs*	*Clinical and biological significance*
Proteomics	Peroxisomes are central to lipid metabolism, redox homeostasis, and BA synthesis; therefore, proteomic analysis provides a systems-level view of disease pathology. Mass spectrometry demonstrates loss or mislocalization of numerous peroxisomal matrix proteins, including enzymes involved in β-oxidation, plasmalogen biosynthesis, BA metabolism, and reactive oxygen species detoxification. These abnormalities reflect defective *PEX*-dependent protein import rather than isolated enzyme deficiencies	Defines the molecular consequences of impaired peroxisome biogenesis and identifies disrupted metabolic pathways that contribute to multisystem disease
Peroxisomal protein networks	ZSDs are characterized by disruption of entire peroxisomal protein interaction networks, including reduced abundance or abnormal localization of peroxins such as *PEX5* and *PEX14*. Loss of coordinated protein trafficking leads to failure of multiple interconnected metabolic pathways instead of single-enzyme dysfunction	Demonstrates that ZSDs represent organelle-wide disorders affecting multiple biochemical pathways simultaneously
Secondary organelle responses	Proteomic studies reveal extensive remodeling of other organelles. • Mitochondria show compensatory upregulation of β-oxidation enzymes, oxidative phosphorylation proteins, and energy metabolism pathways. • ER exhibits activation of stress signaling and the unfolded protein response.	Highlights strong functional crosstalk between peroxisomes, mitochondria, and the ER, explaining secondary cellular dysfunction
Oxidative stress proteome	Increased expression of antioxidant enzymes, heat shock proteins, inflammatory mediators, and cellular stress-response proteins reflects persistent oxidative injury resulting from defective peroxisomal metabolism	Supports oxidative stress as a major contributor to disease progression and a potential therapeutic target
Tissue-specific proteomic signatures	Distinct tissue-specific protein alterations have been identified • Brain exhibits abnormalities in synaptic proteins, myelin-associated proteins, and neurodevelopmental pathways. • Liver demonstrates altered BA synthetic enzymes and lipid metabolic proteins. • Kidney shows changes in transport proteins and intermediary metabolism.	Explains organ-specific clinical manifestations and identifies tissue-selective biomarkers
Adipose tissue proteomics and lipid remodeling	• Adipose tissue demonstrates abnormal lipid composition with accumulation of VLCFAs and depletion of plasmalogens within adipocyte membranes, resulting in altered membrane fluidity and impaired cellular function. • Severe neonatal ZSD is frequently associated with reduced subcutaneous fat development together with increased oxidative stress and inflammatory signaling.	Indicates that adipose abnormalities arise from systemic peroxisomal dysfunction rather than a primary adipose tissue disorder
Urinary metabolomics	• Can help demonstrate accumulation of atypical BA intermediates, particularly DHCA and THCA, reflecting defective peroxisomal BA side-chain β-oxidation. • Increased dicarboxylic acids result from compensatory ω-oxidation, while branched-chain lipid metabolites accumulate because of impaired α-oxidation. • Additional findings include elevated pipecolic acid, mild organic aciduria, oxidized fatty acids, and experimental increases in F2-isoprostanes, reflecting oxidative stress and secondary mitochondrial dysfunction.	Provides a valuable non-invasive screening tool for failed peroxisomal side-chain cleavage in ZSDs. Although highly suggestive, urinary metabolomics is not diagnostic and should be interpreted alongside plasma VLCFA analysis and *PEX* gene testing
Genomics	Whole-exome and whole-genome sequencing identify pathogenic variants in *PEX* genes, particularly *PEX1*, *PEX6*, and other peroxins responsible for peroxisome biogenesis. Functional variant analysis improves genotype–phenotype correlations and prognostic stratification	Enables definitive molecular diagnosis, carrier detection, prenatal diagnosis, and personalized genetic counseling

(Contd...)

Table 7: *(Contd...)*

Omics platform	*Major findings in ZSDs*	*Clinical and biological significance*
Transcriptomics	Transcriptomic profiling demonstrates widespread alterations in genes regulating lipid metabolism, mitochondrial function, oxidative stress responses, inflammation, and neurodevelopment. Compensatory activation of mitochondrial energy metabolism and antioxidant pathways is seen	Reveals adaptive cellular responses and identifies dysregulated signaling pathways that may represent therapeutic targets
Lipidomics	Lipidomic analyses reveal the characteristic biochemical signature of ZSDs, including accumulation of VLCFAs, depletion of plasmalogens, increased branched-chain fatty acids (phytanic and pristanic acids), and accumulation of BA intermediates resulting from impaired peroxisomal β-oxidation	Provides sensitive biochemical biomarkers for diagnosis, disease monitoring, and therapeutic response assessment
Epigenomics	Emerging evidence suggests secondary alterations in DNA methylation, chromatin organization, and epigenetic regulation driven by chronic metabolic stress and oxidative injury, particularly within the developing nervous system	May explain variability in disease severity and contribute to abnormal neurodevelopment beyond the primary genetic defect
Single-cell omics	Single-cell transcriptomic and proteomic analyses demonstrate cell-type-specific vulnerability, particularly affecting neurons, oligodendrocytes, hepatocytes, renal tubular epithelial cells, and adrenal cortical cells	Can possibly improve understanding of organ-specific disease mechanisms and facilitate identification of cell-targeted therapies
Multi-omics integration	• Integration of genomic, transcriptomic, proteomic, lipidomic, metabolomic, and epigenomic datasets provides comprehensive systems-level models of peroxisomal dysfunction. • Can link defective protein import with biochemical abnormalities such as VLCFA accumulation, plasmalogen deficiency, abnormal BA metabolism, oxidative stress, and secondary mitochondrial dysfunction. • Can help identify candidate biomarkers—altered peroxisomal membrane proteins, stress-response molecules, and metabolic signatures. • Genomics has helped identify *PEX1*/*PEX6* functional variants. Transcriptomics in mapping broad lipid, energy, and neurodevelopmental expression shifts. Epigenomics has shown secondary DNA methylation changes.	• Can possibly improve early diagnosis, biomarker discovery, patient stratification, disease monitoring, prognostic prediction, and identification of novel therapeutic targets for ZSDs. • Can possibly connect upstream genetic mutations to downstream cellular traits, establishing models for biomarker discovery and clinical stratification.

BA, bile acid; DHCA, dihydroxycholestanoic acid; ER, endoplasmic reticulum; *PEX*, peroxisomal biogenesis factor; THCA, trihydroxycholestanoic acid; VLCFA, very-long-chain fatty acid; ZSD, Zellweger spectrum disorder

Statement

This is an extensive article addressing a wide range of subtopics and is a response to a specific request from members of the Global Newborn Society from the Middle East. The current size and format of the manuscript were prepared after due permission from the Editorial Board. The large number of keywords aims to increase accessibility for non-anglophonic members of the readership.

References

1. Klouwer FC, Berendse K, Ferdinandusse S, et al. Zellweger spectrum disorders: Clinical overview and management approach. Orphanet J Rare Dis 2015;10:151. DOI: 10.1186/s13023-015-0368-9.
2. Braverman NE, Raymond GV, Rizzo WB, et al. Peroxisome biogenesis disorders in the Zellweger spectrum: An overview of current diagnosis, clinical manifestations, and treatment guidelines. Mol Genet Metab 2016;117(3):313–321. DOI: 10.1016/j.ymgme.2015.12.009.
3. Argyriou C, D'Agostino MD, Braverman N. Peroxisome biogenesis disorders. Transl Sci Rare Dis 2016;1(2):111–144. DOI: 10.3233/TRD-160003.
4. Elumalai V, Pasrija D. Zellweger Spectrum Disorder. StatPearls 2026.
5. Zellweger H, Maertens P, Superneau D, et al. History of the cerebrohepatorenal syndrome of Zellweger and other peroxisomal disorders. South Med J 1988;81(3):357–364. DOI: 10.1097/00007611-198803000-00017.
6. Kheir AEM. Zellweger syndrome: A cause of neonatal hypotonia and seizures. Sudan J Paediatr 2011;11(2):54–58. PMID: 27493320.
7. Steinberg SJ, Raymond GV, Braverman NE, et al. Zellweger Spectrum Disorder. GeneReviews 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1448/.
8. Fujiki Y, Yagita Y, Matsuzaki T. Peroxisome biogenesis disorders: Molecular basis for impaired peroxisomal membrane assembly: In metabolic functions and biogenesis of peroxisomes in health and disease. Biochim Biophys Acta 2012;1822(9):1337–1342. DOI: 10.1016/j.bbadis.2012.06.004.
9. Borgia P, Baldassari S, Pedemonte N, et al. Genotype-phenotype correlations and disease mechanisms in PEX13-related Zellweger spectrum disorders. Orphanet J Rare Dis 2022;17(1):286. DOI: 10.1186/s13023-022-02415-5.
10. Ebberink MS, Csanyi B, Chong WK, et al. Identification of an unusual variant peroxisome biogenesis disorder caused by mutations in the PEX16 gene. J Med Genet 2010;47(9):608–615. DOI: 10.1136/jmg.2009.074302.
11. Ebberink MS, Mooijer PA, Gootjes J, et al. Genetic classification and mutational spectrum of more than 600 patients with a Zellweger syndrome spectrum disorder. Hum Mutat 2011;32(1):59–69. DOI: 10.1002/humu.21388.

12. Geisbrecht BV, Collins CS, Reuber BE, et al. Disruption of a PEX1-PEX6 interaction is the most common cause of the neurologic disorders Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease. Proc Natl Acad Sci U S A 1998;95(15):8630–8635. DOI: 10.1073/pnas.95.15.8630.
13. Steinberg SJ, Dodt G, Raymond GV, et al. Peroxisome biogenesis disorders. Biochim Biophys Acta 2006;1763(12):1733–1748. DOI: 10.1016/j.bbamcr.2006.09.010.
14. Levesque S, Morin C, Guay SP, et al. A founder mutation in the PEX6 gene is responsible for increased incidence of Zellweger syndrome in a French Canadian population. BMC Med Genet 2012;13:72. DOI: 10.1186/1471-2350-13-72.
15. Bose M, Yergeau C, D'Souza Y, et al. Characterization of severity in Zellweger spectrum disorder by clinical findings: A scoping review, meta-analysis and medical chart review. Cells 2022;11(12):1891. DOI: 10.3390/cells11121891.
16. Flanagan BA, Krueger-Hadfield SA, Murren CJ, et al. Founder effects shape linkage disequilibrium and genomic diversity of a partially clonal invader. Mol Ecol 2021;30(9):1962–1978. DOI: 10.1111/mec.15854.
17. Cheillan D. Zellweger syndrome disorders: From severe neonatal disease to atypical adult presentation. Adv Exp Med Biol 2020;1299:71–80. DOI: 10.1007/978-3-030-60204-8_6.
18. Blomqvist M, Ahlberg K, Lindgren J, et al. Identification of a novel mutation in PEX10 in a patient with attenuated Zellweger spectrum disorder: A case report. J Med Case Rep 2017;11(1):218. DOI: 10.1186/s13256-017-1365-5.
19. Terlecky SR, Walton PA. The biogenesis and cell biology of peroxisomes in human health and disease. Madame Curie bioscience database 2013. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6339/.
20. Lodhi IJ, Semenkovich CF. Peroxisomes: A nexus for lipid metabolism and cellular signaling. Cell Metab 2014;19(3):380–392. DOI: 10.1016/j.cmet.2014.01.002.
21. Talley JT, Mohiuddin SS. Biochemistry, fatty acid oxidation. StatPearls 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK556002/.
22. Smith JJ, Aitchison JD. Peroxisomes take shape. Nat Rev Mol Cell Biol 2013;14(12):803–817. DOI: 10.1038/nrm3700.
23. Smith JJ, Aitchison JD. Regulation of peroxisome dynamics. Curr Opin Cell Biol 2009;21(1):119–126. DOI: 10.1016/j.ceb.2009.01.009.
24. Kumar R, Islinger M, Worthy H, et al. The peroxisome: An update on mysteries 3.0. Histochem Cell Biol 2024;161(2):99–132. DOI: 10.1007/s00418-023-02259-5.
25. Delille HK, Bonekamp NA, Schrader M. Peroxisomes and disease—an overview. Int J Biomed Sci 2006;2(4):308–314. PMID: 23674998.
26. Herzog K, Pras-Raves ML, Ferdinandusse S, et al. Functional characterisation of peroxisomal beta-oxidation disorders in fibroblasts using lipidomics. J Inherit Metab Dis 2018;41(3):479–487. DOI: 10.1007/s10545-017-0076-9.
27. Kleiboeker B, Lodhi IJ. Peroxisomal regulation of energy homeostasis: Effect on obesity and related metabolic disorders. Mol Metab 2022;65:101577. DOI: 10.1016/j.molmet.2022.101577.
28. Hallen A, Jamie JF, Cooper AJ. Lysine metabolism in mammalian brain: An update on the importance of recent discoveries. Amino Acids 2013;45(6):1249–1272. DOI: 10.1007/s00726-013-1590-1.
29. Ferdinandusse S, Ebberink MS, Vaz FM, et al. The important role of biochemical and functional studies in the diagnostics of peroxisomal disorders. J Inherit Metab Dis 2016;39(4):531–543. DOI: 10.1007/s10545-016-9922-4.
30. Ferdinandusse S, Denis S, Faust PL, et al. Bile acids: The role of peroxisomes. J Lipid Res 2009;50(11):2139–2147. DOI: 10.1194/jlr.R900009-JLR200.
31. Ferdinandusse S, Houten SM. Peroxisomes and bile acid biosynthesis. Biochim Biophys Acta 2006;1763(12):1427–1440. DOI: 10.1016/j.bbamcr.2006.09.001.
32. Wu Y, Deng Y, Angelov B, et al. Plasmalogen as a bioactive lipid drug: From preclinical research challenges to opportunities in nanomedicine. FASEB Bioadv 2025;7(8):e70028. DOI: 10.1096/fba.2025-00010.
33. Chen Z, Dong C, Chen L, et al. The changes in plasmalogens: Chemical diversity and nutritional implications-a narrative review. Nutrients 2025;17(22):3497. DOI: 10.3390/nu17223497.
34. Messias MCF, Mecatti GC, Priolli DG, et al. Plasmalogen lipids: Functional mechanism and their involvement in gastrointestinal cancer. Lipids Health Dis 2018;17(1):41. DOI: 10.1186/s12944-018-0685-9.
35. Ferdinandusse S, Denis S, Mooijer PA, et al. Identification of the peroxisomal beta-oxidation enzymes involved in the biosynthesis of docosahexaenoic acid. J Lipid Res 2001;42(1):1987–1995. PMID: 11734571.
36. Gonzalez-Soto M, Mutch DM. Diet regulation of long-chain PUFA synthesis: Role of macronutrients, micronutrients, and polyphenols on delta-5/delta-6 desaturases and elongases 2/5. Adv Nutr 2021;12(3):980–994. DOI: 10.1093/advances/nmaa142.
37. Dyall SC. Long-chain omega-3 fatty acids and the brain: A review of the independent and shared effects of EPA, DPA and DHA. Front Aging Neurosci 2015;7:52. DOI: 10.3389/fnagi.2015.00052.
38. Shao C, Lin H, Yu J, et al. Maternal docosahexaenoic acid and eicosapentaenoic acid supplementation: Effects and mechanisms on lipid metabolism in the offspring. Front Nutr 2026;13:1745358. DOI: 10.3389/fnut.2026.1745358.
39. Chen CT, Shao Z, Fu Z. Dysfunctional peroxisomal lipid metabolisms and their ocular manifestations. Front Cell Dev Biol 2022;10:982564. DOI: 10.3389/fcell.2022.982564.
40. Weiser MJ, Butt CM, Mohajeri MH. Docosahexaenoic acid and cognition throughout the lifespan. Nutrients 2016;8(2):99. DOI: 10.3390/nu8020099.
41. Diaz M, Mesa-Herrera F, Marin R. DHA and its elaborated modulation of antioxidant defenses of the brain: Implications in aging and AD neurodegeneration. Antioxidants (Basel) 2021;10(6):907. DOI: 10.3390/antiox10060907.
42. Li Z, Kaplan ML, Hachey DL. Hepatic microsomal and peroxisomal docosahexaenoate biosynthesis during piglet development. Lipids 2000;35(12):1325–1333. DOI: 10.1007/s11745-000-0649-0.
43. Walker CL, Pomatto LCD, Tripathi DN, et al. Redox regulation of homeostasis and proteostasis in peroxisomes. Physiol Rev 2018;98(1):89–115. DOI: 10.1152/physrev.00033.2016.
44. Lismont C, Revenco I, Fransen M. Peroxisomal hydrogen peroxide metabolism and signaling in health and disease. Int J Mol Sci 2019;20(15):3673. DOI: 10.3390/ijms20153673.
45. Tang X, Islinger M, Schrader M. Visualisation of peroxisomes: A journey through seven decades. Histochem Cell Biol 2026;164(1). DOI: 10.1007/s00418-026-02492-8.
46. Santos MJ, Imanaka T, Shio H, et al. Peroxisomal membrane ghosts in Zellweger syndrome-aberrant organelle assembly. Science 1988;239(4847):1536–1538. DOI: 10.1126/science.3281254.
47. Santos MJ, Henderson SC, Moser AB, et al. Peroxisomal ghosts are intracellular structures distinct from lysosomal compartments in Zellweger syndrome: A confocal laser scanning microscopy study. Biol Cell 2000;92(2):85–94. DOI: 10.1016/s0248-4900(00)89016-4.
48. Wendland M, Subramani S. Presence of cytoplasmic factors functional in peroxisomal protein import implicates organelle-associated defects in several human peroxisomal disorders. J Clin Invest 1993;92(5):2462–2468. DOI: 10.1172/JCI116854.
49. Soliman K, Gottfert F, Rosewich H, et al. Super-resolution imaging reveals the sub-diffraction phenotype of Zellweger syndrome ghosts and wild-type peroxisomes. Sci Rep 2018;8(1):7809. DOI: 10.1038/s41598-018-24119-2.
50. Squier W, Jansen A. Polymicrogyria: Pathology, fetal origins and mechanisms. Acta Neuropathol Commun 2014;2:80. DOI: 10.1186/s40478-014-0080-3.
51. Widjaja E, Griffiths PD, Wilkinson ID. Proton MR spectroscopy of polymicrogyria and heterotopia. AJNR Am J Neuroradiol 2003;24(10):2077–2081. PMID: 14625236.

52. Bozelli JC, Jr., Azher S, Epand RM. Plasmalogens and chronic inflammatory diseases. front physiol 2021;12:730829. DOI: 10.3389/fphys.2021.730829.
53. Bermúdez LO, Noguera V, Del Castillo V, et al. Chondrodysplasia punctata: A rare entity identified incidentally. Cureus 2025;17(8):e90894. DOI: 10.7759/cureus.90894.
54. Wiemer EA, Brul S, Just WW, et al. Presence of peroxisomal membrane proteins in liver and fibroblasts from patients with the Zellweger syndrome and related disorders: Evidence for the existence of peroxisomal ghosts. Eur J Cell Biol 1989;50(2):407–417. PMID: 2697558.
55. Schönfeld P, Reiser G. Brain lipotoxicity of phytanic acid and very long-chain fatty acids. Harmful cellular/mitochondrial activities in refsum disease and X-linked adrenoleukodystrophy. Aging Dis 2016;7(2):136–149. DOI: 10.14336/AD.2015.0823.
56. Peduto A, Baumgartner MR, Verhoeven NM, et al. Hyperpipecolic acidaemia: A diagnostic tool for peroxisomal disorders. Mol Genet Metab 2004;82(3):224–230. DOI: 10.1016/j.ymgme.2004.04.010.
57. Dorninger F, Forss-Petter S, Berger J. From peroxisomal disorders to common neurodegenerative diseases-the role of ether phospholipids in the nervous system. FEBS Lett 2017;591(18):2761–2788. DOI: 10.1002/1873-3468.12788.
58. Krause C, Rosewich H, Thanos M, et al. Identification of novel mutations in PEX2, PEX6, PEX10, PEX12, and PEX13 in Zellweger spectrum patients. Hum Mutat 2006;27(11):1157. DOI: 10.1002/humu.9462.
59. Faust PL, Banka D, Siriratsivawong R, et al. Peroxisome biogenesis disorders: The role of peroxisomes and metabolic dysfunction in developing brain. J Inherit Metab Dis 2005;28(3):369–383. DOI: 10.1007/s10545-005-7059-y.
60. Crane DI. Revisiting the neuropathogenesis of Zellweger syndrome. Neurochem Int 2014;69:1–8. DOI: 10.1016/j.neuint.2014.02.007.
61. Rahim RS, Chen M, Nourse CC, et al. Mitochondrial changes and oxidative stress in a mouse model of Zellweger syndrome neuropathogenesis. Neuroscience 2016;334:201–213. DOI: 10.1016/j.neuroscience.2016.08.001.
62. Barnes-Vélez JA, Yasar FBA, Hu J. Myelin lipid metabolism and its role in myelination and myelin maintenance. Innovation (Camb) 2023;4(1):100360. DOI: 10.1016/j.xinn.2022.100360.
63. Eberhart T, Charles KN, Salumbides-Torres B, et al. Dysregulated lipid metabolism and hypomyelination in postnatal peroxisome-deficient Pex2 knockout Zellweger mice. Front Mol Neurosci 2026;19:1636268. DOI: 10.3389/fnmol.2026.1636268.
64. Dobrowsky RT, Ballas LM. Peroxisomal dihydroxyacetone phosphate acyltransferase. Effect of acetaldehyde on the intact and solubilized activity. J Biol Chem 1987;262(7):3136–3139. PMID: 3818635.
65. Honsho M, Tanaka M, Zoeller RA, et al. Distinct functions of acyl/alkyl dihydroxyacetonephosphate reductase in peroxisomes and endoplasmic reticulum. Front Cell Dev Biol 2020;8:855. DOI: 10.3389/fcell.2020.00855.
66. Honsho M, Asaoku S, Fukumoto K, et al. Topogenesis and homeostasis of fatty acyl-CoA reductase 1. J Biol Chem 2013;288(48):34588–34598. DOI: 10.1074/jbc.M113.498345.
67. Wanders RJ, Waterham HR, Ferdinandusse S. Metabolic interplay between peroxisomes and other subcellular organelles including mitochondria and the endoplasmic reticulum. Front Cell Dev Biol 2015;3:83. DOI: 10.3389/fcell.2015.00083.
68. French HM, Reid M, Mamontov P, et al. Oxidative stress disrupts oligodendrocyte maturation. J Neurosci Res 2009;87(14):3076–3087. DOI: 10.1002/jnr.22139.
69. Raas Q, Tawbeh A, Tahri-Joutey M, et al. Peroxisomal defects in microglial cells induce a disease-associated microglial signature. Front Mol Neurosci 2023;16:1170313. DOI: 10.3389/fnmol.2023.1170313.
70. Guedes JR, Ferreira PA, Costa JM, et al. Microglia-dependent remodeling of neuronal circuits. J Neurochem 2022;163(2):74–93. DOI: 10.1111/jnc.15689.
71. Berger J, Dorninger F, Forss-Petter S, et al. Peroxisomes in brain development and function. Biochim Biophys Acta 2016;1863(5):934–955. DOI: 10.1016/j.bbamcr.2015.12.005.
72. Waterham HR, Ebberink MS. Genetics and molecular basis of human peroxisome biogenesis disorders. Biochim Biophys Acta 2012;1822(9):1430–1441. DOI: 10.1016/j.bbadis.2012.04.006.
73. Waterham HR, Koster J, Ebberink MS, et al. Autosomal dominant Zellweger spectrum disorder caused by de novo variants in PEX14 gene. Genet Med 2023;25(11):100944. DOI: 10.1016/j.gim.2023.100944.
74. Jansen RLM, Santana-Molina C, van den Noort M, et al. Comparative genomics of peroxisome biogenesis proteins: Making sense of the PEX proteins. Front Cell Dev Biol 2021;9:654163. DOI: 10.3389/fcell.2021.654163.
75. Collins CS, Gould SJ. Identification of a common PEX1 mutation in Zellweger syndrome. Hum Mutat 1999;14(1):45–53. DOI: 10.1002/(SICI)1098-1004(1999)14:1<45::AID-HUMU6>3.0.CO;2-J.
76. Zhang R, Chen L, Jiralerspong S, et al. Recovery of PEX1-Gly843Asp peroxisome dysfunction by small-molecule compounds. Proc Natl Acad Sci U S A 2010;107(12):5569–5574. DOI: 10.1073/pnas.0914960107.
77. Pedrosa AG, Francisco T, Ferreira MJ, et al. A mechanistic perspective on PEX1 and PEX6, two AAA+ proteins of the peroxisomal protein import machinery. Int J Mol Sci 2019;20(21):5246. DOI: 10.3390/ijms20215246.
78. Alencastre IS, Rodrigues TA, Grou CP, et al. Mapping the cargo protein membrane translocation step into the PEX5 cycling pathway. J Biol Chem 2009;284(40):27243–27251. DOI: 10.1074/jbc.M109.032565.
79. Ma C, Subramani S. Peroxisome matrix and membrane protein biogenesis. IUBMB Life 2009;61(7):713–722. DOI: 10.1002/iub.196.
80. Vasiljevic E, Ye Z, Pavelec DM, et al. Carrier frequency estimation of Zellweger spectrum disorder using ExAC database and bioinformatics tools. Genet Med 2019;21(9):1969–1976. DOI: 10.1038/s41436-019-0468-3.
81. Shimozawa N, Nagase T, Takemoto Y, et al. Genetic heterogeneity of peroxisome biogenesis disorders among Japanese patients: Evidence for a founder haplotype for the most common PEX10 gene mutation. Am J Med Genet A 2003;120A(1):40–43. DOI: 10.1002/ajmg.a.20030.
82. Abramovs N, Brass A, Tassabehji M. Hardy-Weinberg equilibrium in the large scale genomic sequencing era. Front Genet 2020;11:210. DOI: 10.3389/fgene.2020.0210.
83. Chen YS, Su YC, Pan W. Effect of spatial constraints on Hardy-Weinberg equilibrium. Sci Rep 2016;6:19297. DOI: 10.1038/srep19297.
84. Pemberton TJ, Rosenberg NA. Population-genetic influences on genomic estimates of the inbreeding coefficient: A global perspective. Hum Hered 2014;77(1–4):37–48. DOI: 10.1159/000362878.
85. Kumar R, Hodis B, De Jesus O. Refsum Disease. StatPearls 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560618/.
86. Jansen GA, Waterham HR, Wanders RJ. Molecular basis of Refsum disease: Sequence variations in phytanoyl-CoA hydroxylase (PHYH) and the PTS2 receptor (PEX7). Hum Mutat 2004;23(3):209–218. DOI: 10.1002/humu.10315.
87. Hughes JL, Poulos A, Robertson E, et al. Pathology of hepatic peroxisomes and mitochondria in patients with peroxisomal disorders. Virchows Arch A Pathol Anat Histopathol 1990;416(3):255–264. DOI: 10.1007/BF01678985.
88. Skowyra ML, Feng P, Rapoport TA. Towards solving the mystery of peroxisomal matrix protein import. Trends Cell Biol 2024;34(5):388–405. DOI: 10.1016/j.tcb.2023.08.005.
89. Kim PK, Mullen RL, Schumann U, et al. The origin and maintenance of mammalian peroxisomes involves a de novo PEX16-dependent pathway from the ER. J Cell Biol 2006;173(4):521–532. DOI: 10.1083/jcb.200601036.
90. Tabak HF, Braakman I, van der Zand A. Peroxisome formation and maintenance are dependent on the endoplasmic reticulum. Annu Rev Biochem 2013;82:723–744. DOI: 10.1146/annurev-biochem-081111-125123.
91. Smith MD, Schnell DJ. Peroxisomal protein import. The paradigm shifts. Cell 2001;105(3):293–296. DOI: 10.1016/s0092-8674(01)00337-3.
92. Skowyra ML, Rapoport TA. Import mechanism of peroxisomal proteins with an N-terminal signal sequence. Nat Cell Biol 2025;27(6):948–958. DOI: 10.1038/s41556-025-01662-5.

93. Platta HW, Jeske J, Schmidt N, et al. ATP-Dependent steps in peroxisomal protein import. Annu Rev Biochem 2024;93(1):233–259. DOI: 10.1146/annurev-biochem-030222-111227.
94. Farre JC, Mahalingam SS, Proietto M, et al. Peroxisome biogenesis, membrane contact sites, and quality control. EMBO Rep 2019;20(1):e46864. DOI: 10.15252/embr.201846864.
95. Gabaldon T, Snel B, van Zimmeren F, et al. Origin and evolution of the peroxisomal proteome. Biol Direct 2006;1:8. DOI: 10.1186/1745-6150-1-8.
96. Schluter A, Fourcade S, Ripp R, et al. The evolutionary origin of peroxisomes: An ER-peroxisome connection. Mol Biol Evol 2006;23(4):838–845. DOI: 10.1093/molbev/msj103.
97. Molina-Moya E, Rodriguez-Gonzalez A, Pelaez-Vico MA, et al. Peroxisomal-dependent signalling and dynamics modulate plant stress responses: Reactive oxygen and nitrogen species as key molecules. J Exp Bot 2025;76(13):3706–3721. DOI: 10.1093/jxb/eraf072.
98. Poirier Y, Antonenkov VD, Glumoff T, et al. Peroxisomal beta-oxidation-a metabolic pathway with multiple functions. Biochim Biophys Acta 2006;1763(12):1413–1426. DOI: 10.1016/j.bbamcr.2006.08.034.
99. Turner CL, Bunyan DJ, Thomas NS, et al. Zellweger syndrome resulting from maternal isodisomy of chromosome 1. Am J Med Genet A 2007;143A(18):2172–2177. DOI: 10.1002/ajmg.a.31912.
100. Wallerstein V, Grant L, Wallerstein R. Complete uniparental disomy of chromosome 1 in a child with isolated developmental delay. Clin Case Rep 2022;10(7):e5956. DOI: 10.1002/ccr3.5956.
101. Donda K, Torres BA, Maheshwari A. Non-coding RNAs in neonatal necrotizing enterocolitis. Newborn (Clarksville) 2022;1(1):120–130. DOI: 10.5005/jp-journals-11002-0012.
102. Nair J, Maheshwari A. Non-coding RNAs in necrotizing enterocolitis- a new frontier? Curr Pediatr Rev 2022;18(1):25–32. DOI: 10.2174/1573396317666211102093646.
103. Pitsava G, Hawley M, Auriga L, et al. Genome sequencing reveals the impact of pseudoexons in rare genetic disease. medRxiv 2025. DOI: 10.1101/2024.12.21.24318325.
104. Vitale E, Ricci D, Corrao F, et al. Nonsense mutations in rare and ultra-rare human disorders: An overview. IUBMB Life 2025;77(6):e70031. DOI: 10.1002/iub.70031.
105. Zhang F, Gu W, Hurles ME, et al. Copy number variation in human health, disease, and evolution. Annu Rev Genomics Hum Genet 2009;10:451–481. DOI: 10.1146/annurev.genom.9.081307.164217.
106. Capra AP, La Rosa MA, Briguori S, et al. Coexistence of genetic diseases is a new clinical challenge: Three unrelated cases of dual diagnosis. Genes (Basel) 2023;14(2):484. DOI: 10.3390/genes14020484.
107. Briones P, Vilaseca MA, Garcia-Silva MT, et al. Congenital disorders of glycosylation (CDG) may be underdiagnosed when mimicking mitochondrial disease. Eur J Paediatr Neurol 2001;5(3):127–131. DOI: 10.1053/ejpn.2001.0483.
108. Nuebel E, Morgan JT, Fogarty S, et al. The biochemical basis of mitochondrial dysfunction in Zellweger Spectrum Disorder. EMBO Rep 2021;22(10):e51991. DOI: 10.15252/embr.202051991.
109. Hajri T, Zaiou M, Fungwe TV, et al. Epigenetic regulation of peroxisome proliferator-activated receptor gamma mediates high-fat diet-induced non-alcoholic fatty liver disease. Cells 2021;10(6):1355. DOI: 10.3390/cells10061355.
110. Catanesi M, d'Angelo M, Tupone MG, et al. MicroRNAs dysregulation and mitochondrial dysfunction in neurodegenerative diseases. Int J Mol Sci 2020;21(17):5986. DOI: 10.3390/ijms21175986.
111. Petriv OI, Pilgrim DB, Rachubinski RA, et al. RNA interference of peroxisome-related genes in C. elegans: A new model for human peroxisomal disorders. Physiol Genomics 2002;10(2):79–91. DOI: 10.1152/physiolgenomics.00044.2002.
112. Jilo DD, Abebe BK, Wang J, et al. Long non-coding RNA (LncRNA) and epigenetic factors: Their role in regulating the adipocytes in bovine. Front Genet 2024;15:1405588. DOI: 10.3389/fgene.2024.1405588.
113. Xiao J, Joseph S, Xia M, et al. Circular RNAs acting as miRNAs' sponges and their roles in stem cells. J Clin Med 2022;11(10):2909. DOI: 10.3390/jcm11102909.
114. Wu YL, Lin ZJ, Li CC, et al. Epigenetic regulation in metabolic diseases: Mechanisms and advances in clinical study. Signal Transduct Target Ther 2023;8(1):98. DOI: 10.1038/s41392-023-01333-7.
115. Berger SL, Kouzarides T, Shiekhattar R, et al. An operational definition of epigenetics. Genes Dev 2009;23(7):781–783. DOI: 10.1101/gad.1787609.
116. Singh S, Maheshwari A. Transgenerational epigenetics. Newborn (Clarksville) 2025;4(3):142–152. DOI: 10.5005/jp-journals-11002-0137.
117. Donda K, Maheshwari A. Human milk lipids induce important metabolic and epigenetic changes in neonates. Clin Perinatol 2022;49(2):331–353. DOI: 10.1016/j.clp.2022.02.006.
118. Maheshwari A. Innate immune memory in macrophages. Newborn (Clarksville) 2023;2(1):60–79. DOI: 10.5005/jp-journals-11002-0058.
119. Farr RL, Lismont C, Terlecky SR, et al. Peroxisome biogenesis in mammalian cells: The impact of genes and environment. Biochim Biophys Acta 2016;1863(5):1049–1060. DOI: 10.1016/j.bbamcr.2015.08.011.
120. Wang L, Wang C, Liu X, et al. Peroxisomal beta-oxidation regulates histone acetylation and DNA methylation in Arabidopsis. Proc Natl Acad Sci USA 2019;116(21):10576–10585. DOI: 10.1073/pnas.1904143116.
121. Rafiei S, Khodagholi F, Motamedi F, et al. Peroxisome biogenesis factor 5 controlled HDAC6 and SIRT1 expression and modulated mitochondrial biogenesis in rat dorsal hippocampus. Physiol Pharmacol 2021;25(1):341–352. DOI: 10.52547/phypha.25.4.10.
122. Pogribny IP, Tryndyak VP, Woods CG, et al. Epigenetic effects of the continuous exposure to peroxisome proliferator WY-14,643 in mouse liver are dependent upon peroxisome proliferator activated receptor alpha. Mutat Res 2007;625(1-2):62–71. DOI: 10.1016/j.mrfmmm.2007.05.004.
123. Wade H, Pan K, Zhang B, et al. Mechanistic role of long non-coding RNAs in the pathogenesis of metabolic dysfunction-associated steatotic liver disease and fibrosis. eGastroenterology 2024;2(4):e100115. DOI: 10.1136/egastro-2024-100115.
124. Yarmishyn AA, Kremenskoy M, Batagov AO, et al. Genome-wide analysis of mRNAs associated with mouse peroxisomes. BMC Genomics 2016;17(Suppl 13):1028. DOI: 10.1186/s12864-016-3330-x.
125. Zhu E, Zhang J, Li Y, et al. Long noncoding RNA Plnc1 controls adipocyte differentiation by regulating peroxisome proliferator-activated receptor gamma. FASEB J 2019;33(2):2396–2408. DOI: 10.1096/fj.201800739RRR.
126. Zhang XG, Yang LL, Feng X, et al. Maternal obesity disrupts epigenetic reprogramming via peroxisomal-dependent phospholipid-methyl uncoupling during zygotic genome activation. Nat Commun 2026;17(1). DOI: 10.1038/s41467-026-70492-2.
127. Brown LA, Baker A. Peroxisome biogenesis and the role of protein import. J Cell Mol Med 2003;7(4):388–400. DOI: 10.1111/j.1582-4934.2003.tb00241.x.
128. Mast FD, Jamakhandi A, Saleem RA, et al. Peroxins Pex30 and Pex29 Dynamically associate with reticulons to regulate peroxisome biogenesis from the endoplasmic reticulum. J Biol Chem 2016;291(30):15408–15427. DOI: 10.1074/jbc.M116.728154.
129. Williams CP, Stanley WA. Peroxin 5: A cycling receptor for protein translocation into peroxisomes. Int J Biochem Cell Biol 2010;42(11):1771–1774. DOI: 10.1016/j.biocel.2010.07.004.
130. Okumoto K, Miyata N, Fujiki Y. Identification of peroxisomal protein complexes with PTS receptors, Pex5 and Pex7, in mammalian cells. Subcell Biochem 2018;89:287–298. DOI: 10.1007/978-981-13-2233-4_12.
131. He A, Dean JM, Lodhi IJ. Peroxisomes as cellular adaptors to metabolic and environmental stress. Trends Cell Biol 2021;31(8):656–670. DOI: 10.1016/j.tcb.2021.02.005.
132. Demarquoy J, Le Borgne F. Crosstalk between mitochondria and peroxisomes. World J Biol Chem 2015;6(4):301–309. DOI: 10.4331/wjbc.v6.i4.301.
133. Chornyi S, L IJ, van Roermund CWT, et al. Peroxisomal metabolite and cofactor transport in humans. Front Cell Dev Biol 2020;8:613892. DOI: 10.3389/fcell.2020.613892.

134. Ruttermann M, Koci M, Lill P, et al. Structure of the peroxisomal Pex1/Pex6 ATPase complex bound to a substrate. Nat Commun 2023;14(1):5942. DOI: 10.1038/s41467-023-41640-9.
135. Schieferdecker A, Wendler P. Structural mapping of missense mutations in the Pex1/Pex6 complex. Int J Mol Sci 2019;20(15):3756. DOI: 10.3390/ijms20153756.
136. Lefort V, Desper R, Gascuel O. FastME 2.0: A comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol 2015;32(10):2798–2800. DOI: 10.1093/molbev/msv150.
137. Iglesias-Rivas P, Del Amparo R, Cabaleiro JA, et al. Empirical substitution models of protein evolution: Database, relationships, and modeling considerations. Database (Oxford) 2025. DOI: 10.1093/database/baaf052.
138. Whelan S, Goldman N. A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Mol Biol Evol 2001;18(5):691–699. DOI: 10.1093/oxfordjournals.molbev.a003851.
139. Jones DT, Taylor WR, Thornton JM. The rapid generation of mutation data matrices from protein sequences. Comput Appl Biosci 1992;8(3):275–282. DOI: 10.1093/bioinformatics/8.3.275.
140. Le SQ, Gascuel O. An improved general amino acid replacement matrix. Mol Biol Evol 2008;25(7):1307–1320. DOI: 10.1093/molbev/msn067.
141. Rzhetsky A, Nei M. Theoretical foundation of the minimum-evolution method of phylogenetic inference. Mol Biol Evol 1993;10(5):1073–1095. DOI: 10.1093/oxfordjournals.molbev.a040056.
142. Giribet G. Efficient tree searches with available algorithms. Evol Bioinform Online 2007;3:341–356. PMID: 19461977.
143. Luque-Fernandez MA, Schomaker M, Rachet B, et al. Targeted maximum likelihood estimation for a binary treatment: A tutorial. Stat Med 2018;37(16):2530–2546. DOI: 10.1002/sim.7628.
144. Parks SL, Goldman N. Maximum likelihood inference of small trees in the presence of long branches. Syst Biol 2014;63(5):798–811. DOI: 10.1093/sysbio/syu044.
145. Nei M. Phylogenetic analysis in molecular evolutionary genetics. Annu Rev Genet 1996;30:371–403. DOI: 10.1146/annurev.genet.30.1.371.
146. Yang Z. On the best evolutionary rate for phylogenetic analysis. Syst Biol 1998;47(1):125–133. DOI: 10.1080/106351598261067.
147. Kumar S, Stecher G, Suleski M, et al. MEGA12: Molecular evolutionary genetic analysis version 12 for adaptive and green computing. Mol Biol Evol 2024;41(12). DOI: 10.1093/molbev/msae263.
148. Langmead B, Wilks C, Antonescu V, et al. Scaling read aligners to hundreds of threads on general-purpose processors. Bioinformatics 2019;35(3):421–432. DOI: 10.1093/bioinformatics/bty648.
149. Roger AJ, Hug LA. The origin and diversification of eukaryotes: Problems with molecular phylogenetics and molecular clock estimation. Philos Trans R Soc Lond B Biol Sci 2006;361(1470):1039–1054. DOI: 10.1098/rstb.2006.1845.
150. Ishii Y. Topological analysis of vertebrate phylogenetic trees utilizing Horton's first law. J Theor Biol 2023;562:111419. DOI: 10.1016/j.jtbi.2023.111419.
151. Kashgari A. Neonate with classic Zellweger syndrome. Int J Pediatr Adolesc Med 2019;6(4):165–166. DOI: 10.1016/j.ijpam.2019.11.002.
152. Takahashi Y, Suzuki Y, Kumazaki K, et al. Epilepsy in peroxisomal diseases. Epilepsia 1997;28(2):182–188. DOI: 10.1111/j.1528-1157.1997.tb01095.x.
153. Poggi-Travert F, Fournier B, Poll-The BT, et al. Clinical approach to inherited peroxisomal disorders. J Inherit Metab Dis 1995;18(Suppl 1):1–18. DOI: 10.1007/BF00711425.
154. Berendse K, Koot BGP, Klouwer FCC, et al. Hepatic symptoms and histology in 13 patients with a Zellweger spectrum disorder. J Inherit Metab Dis 2019;42(5):955–965. DOI: 10.1002/jimd.12132.
155. Lee PR, Raymond GV. Child neurology: Zellweger syndrome. Neurology 2013;80(20):e207–e210. DOI: 10.1212/WNL.0b013e3182929f8e.
156. Berendse K, Boek M, Gijbels M, et al. Liver disease predominates in a mouse model for mild human Zellweger spectrum disorder. Biochim Biophys Acta Mol Basis Dis 2019;1865(10):2774–2787. DOI: 10.1016/j.bbadis.2019.06.013.
157. Williams JP, Secrist L, Fowler GW, et al. Roentgenographic features of the cerebrohepatorenal syndrome of Zellweger. Am J Roentgenol Radium Ther Nucl Med 1972;115(3):607–610. DOI: 10.2214/ajr.115.3.607.
158. Rush ET, Goodwin JL, Braverman NE, et al. Low bone mineral density is a common feature of Zellweger spectrum disorders. Mol Genet Metab 2016;117(1):33–37. DOI: 10.1016/j.ymgme.2015.11.009.
159. Berendse K, Engelen M, Ferdinandusse S, et al. Zellweger spectrum disorders: Clinical manifestations in patients surviving into adulthood. J Inherit Metab Dis 2016;39(1):93–106. DOI: 10.1007/s10545-015-9880-2.
160. Moser AB, Kreiter N, Bezman L, et al. Plasma very long chain fatty acids in 3,000 peroxisome disease patients and 29,000 controls. Ann Neurol 1999;45(1):100–110. DOI: 10.1002/1531-8249(199901)45:1<100::aid-art16>3.0.co;2-u.
161. Poulos A, Sharp P, Johnson D. Plasma polyenoic very-long-chain fatty acids in peroxisomal disease: Biochemical discrimination of Zellweger's syndrome from other phenotypes. Neurology 1989;39(1):44–47. DOI: 10.1212/wnl.39.1.44.
162. Steinberg S, Chen L, Wei L, et al. The PEX Gene Screen: Molecular diagnosis of peroxisome biogenesis disorders in the Zellweger syndrome spectrum. Mol Genet Metab 2004;83(3):252–263. DOI: 10.1016/j.ymgme.2004.08.008.
163. Wang XM, Yik WY, Zhang P, et al. Induced pluripotent stem cell models of Zellweger spectrum disorder show impaired peroxisome assembly and cell type-specific lipid abnormalities. Stem Cell Res Ther 2015;6:158. DOI: 10.1186/s13287-015-0149-3.
164. Diaz J, Matsumoto L, Neville JK. Prenatal diagnosis of Zellweger syndrome by fetal MRI: A case report. Radiol Case Rep 2021;16(12):3950–3954. DOI: 10.1016/j.radcr.2021.09.055.
165. Pfeifer CM, Martinot CA. Zellweger syndrome: Depiction of MRI findings in early infancy at 3.0 Tesla. Neuroradiol J 2017;30(5):442–444. DOI: 10.1177/1971400917700670.
166. Barkovich AA, Peck WW. MR of Zellweger syndrome. AJNR Am J Neuroradiol 1997;18(6):1163–1170. PMID: 9194444.
167. Nakai A, Shigematsu Y, Nishida K, et al. MRI findings of Zellweger syndrome. Pediatr Neurol 1995;13(4):346–348. DOI: 10.1016/0887-8994(95)00215-4.
168. Barth PG, Majoie CBLM, Gootjes J, et al. Neuroimaging of peroxisome biogenesis disorders (Zellweger spectrum) with prolonged survival. Neurology 2004;62(3):439–444. DOI: 10.1212/01.wnl.0000106943.40848.03.
169. Weller S, Rosewich H, Gartner J. Cerebral MRI as a valuable diagnostic tool in Zellweger spectrum patients. J Inherit Metab Dis 2008;31(2):270–280. DOI: 10.1007/s10545-008-0856-3.
170. Nitika G, Neelu D. Early Neuroimaging in Zellweger spectrum disorders. Neurol India 2022;70(1):172–173. DOI: 10.4103/0028-3886.338656.
171. Panjan DP, Meglic NP, Neubauer D. A case of Zellweger syndrome with extensive MRI abnormalities and unusual EEG findings. Clin Electroencephalogr 2001;32(1):28–31. DOI: 10.1177/155005940103200108.
172. Takenouchi T, Raju GP. Germinal matrix hemorrhage in Zellweger syndrome. J Child Neurol 2010;25(11):1398–1400. DOI: 10.1177/0883073810365545.
173. Chanraud S, Zahr N, Sullivan EV, et al. MR diffusion tensor imaging: A window into white matter integrity of the working brain. Neuropsychol Rev 2010;20(2):209–225. DOI: 10.1007/s11065-010-9129-7.
174. Groenendaal F, Van Der Grond J, De Koning TJ, et al. Cerebral proton magnetic resonance spectroscopy (1H-MRS) of a neonate with Zellweger cerebrohepatorenal syndrome 1730. Pediatr Res 1997;41(Suppl 4):291. DOI: 10.1203/00006450-199704001-01749.
175. Weerakkody Y, Jones J. Zellweger syndrome. Radiopaedia 2024;1(1):1. DOI: 10.53347/rID-9565.
176. Luisiri A, Sotelo-Avila C, Silberstein MJ, et al. Sonography of the Zellweger syndrome. J Ultrasound Med 1988;7(3):169–173. DOI: 10.7863/jum.1988.7.3.169.

177. Govaerts L, Colon E, Rotteveel J, et al. A neurophysiological study of children with the cerebro-hepato-renal syndrome of Zellweger. Neuropediatrics 1985;16(4):185–190. DOI: 10.1055/s-2008-1059534.
178. Wanders RJ, Ferdinandusse S. Peroxisomes, peroxisomal diseases, and the hepatotoxicity induced by peroxisomal metabolites. Curr Drug Metab 2012;13(10):1401–1411. DOI: 10.2174/138920012803762747.
179. Hsu M, Subhash A. Acute late-onset cirrhosis in Zellweger spectrum disorder. Case Rep Gastroenterol 2023;17(1):168–171. DOI: 10.1159/000529353.
180. Zhuo R, Rong P, Wang J, et al. The potential role of bioactive plasmalogens in lung surfactant. Front Cell Dev Biol 2021;9:618102. DOI: 10.3389/fcell.2021.618102.
181. Dhulshette SA, Thakkar H, Bansal R, et al. Prenatal and post-natal imaging of Zellweger spectrum disorder with novel prenatal sonographic findings. Int J Reprod Contracep Obs Gynecol 2024;13(11):3378–3383. DOI: 10.18203/2320-1770.ijrcog20243212.
182. Zeynelabidin S, Klouwer FCC, Meijers JCM, et al. Coagulopathy in Zellweger spectrum disorders: A role for vitamin K. J Inherit Metab Dis 2018;41(2):249–255. DOI: 10.1007/s10545-017-0113-8.
183. Mohan V. A case study through an audiological perspective on a pediatric patient diagnosed with zellweger syndrome. Indian J Otolaryngol Head Neck Surg 2023;75(Suppl 1):429–432. DOI: 10.1007/s12070-023-03485-y.
184. Setaro AM, Loi E, Micheletti S, et al. A Multidimensional and Integrated Rehabilitation Approach (A.M.I.R.A.) for Infants at risk of cerebral palsy and other neurodevelopmental disabilities. Children (Basel) 2025;12(8). DOI: 10.3390/children12081003.
185. Berendse K, Engelen M, Linthorst GE, et al. High prevalence of primary adrenal insufficiency in Zellweger spectrum disorders. Orphanet J Rare Dis 2014;9:133. DOI: 10.1186/s13023-014-0133-5.
186. Smerecnik CM, Mesters I, Verweij E, et al. A systematic review of the impact of genetic counseling on risk perception accuracy. J Genet Couns 2009;18(3):217–228. DOI: 10.1007/s10897-008-9210-z.
187. Moser HW. Genotype-phenotype correlations in disorders of peroxisome biogenesis. Mol Genet Metab 1999;68(2):316–327. DOI: 10.1006/mgme.1999.2926.
188. Shen G, Liu J, Yang H, et al. mRNA therapies: Pioneering a new era in rare genetic disease treatment. J Control Release 2024;369:696–721. DOI: 10.1016/j.jconrel.2024.03.056.

REVIEW ARTICLE

High-throughput Sequencing to Identify Errors in Deoxyribonucleic Acid Sequences and Chromosomal Abnormalities

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Abstract

High-throughput sequencing (HTS), also known as next-generation sequencing (NGS), has transformed genomic research and clinical diagnostics by enabling the rapid, parallel sequencing of millions to billions of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) fragments. Compared with conventional Sanger sequencing, HTS offers substantially greater speed, throughput, and analytical depth, permitting comprehensive investigation of genomes, transcriptomes, and microbial communities. This review provides an integrated overview of HTS technologies, including principles of nucleic acid preparation, sequencing workflows, and major biomedical applications. We summarize the use of HTS for identifying single nucleotide variants (SNVs), insertions and deletions, copy number variations (CNVs), repeat expansions, splice-site and regulatory mutations, as well as structural chromosomal abnormalities such as inversions, translocations, deletions, duplications, and complex genomic rearrangements. The biological mechanisms underlying these abnormalities, including replication errors, recombination defects, transposable element activity, DNA repair failure, and chromosomal instability, are discussed together with their relevance to human disease and genome evolution. Emerging applications in single-cell genomics, metagenomics, pathogen surveillance, cancer genomics, and precision medicine are highlighted, along with the expanding role of artificial intelligence and advanced bioinformatics in sequence interpretation. Finally, we discuss current limitations, including technical artifacts, variant interpretation, and overdiagnosis, and outline future directions that will further enhance the diagnostic accuracy, clinical utility, and research potential of HTS.

Keywords: Artificial intelligence, Bioinformatics, Cancer genomics, Complementary deoxyribonucleic acid , Chromosomal deletions, Chromosomal duplications, Chromosomal rearrangements, Chromosomal instability, Chromothripsis, Clinical bioinformatics.

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Key Points

- High-throughput sequencing (HTS), also called next-generation sequencing (NGS), refers to advanced deoxyribonucleic acid (DNA) sequencing technologies that allow millions to billions of DNA [or ribonucleic acid (RNA)] fragments to be sequenced simultaneously in a single run.
- Isolating genetic material for HTS involves extracting high-quality DNA or RNA from biological samples in a way that preserves integrity, minimizes contamination, and yields sufficient quantity for library preparation and downstream sequencing.
- Investigation of genomes using HTS has enabled comprehensive analysis of the whole DNA content of an organism at high depth and resolution.
- Abnormalities in DNA sequences include single nucleotide variants (SNVs), insertions and deletions, copy-number variations (CNVs), repeat expansions, splice-site mutations, and regulatory mutations. Structural rearrangements in chromosomes include inversions, translocations, deletions, duplications, and complex rearrangements.
- We can now detect/identify many previously unrecognized DNA sequence and chromosomal abnormalities. There is a need to be careful about technical errors, but as sequencing technology continues to evolve, integration with artificial intelligence–based analytics and large genomic databases is expected to bring forth new possibilities.

Introduction

High-throughput sequencing, also called NGS, refers to advanced DNA sequencing technologies that allow millions to billions of DNA

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(or RNA) fragments to be sequenced simultaneously in a single run.[1–3] Unlike traditional Sanger sequencing, which sequences one DNA fragment at a time, HTS processes vast numbers of fragments in parallel.[4–6] This dramatically increases speed, scale, and data output while reducing the cost per base.[7]

The term high-throughput is composed of two parts:[8]

- High refers to the size of data.
- Throughput refers to the amount of data processed in a given time, such as that measured in bases per run or per unit time.

Hence, HTS refers to processing and sequencing massive amounts of genetic material quickly and efficiently.[1,3] It can be used to analyze genetic material at a comprehensive and large scale, enabling detailed investigation of genomes, transcriptomes, and microbial communities.[7] It is widely applied in whole-genome sequencing to identify genetic variants, mutations, and structural changes, as well as in RNA sequencing (RNA-seq) to study gene expression patterns and regulatory mechanisms.[9,10] In clinical and biomedical research, HTS supports cancer genomics, rare disease diagnosis, and pathogen detection, including surveillance of emerging infectious diseases.[11,12] It is also used in metagenomics to characterize complex microbial populations in environmental or host-associated samples without the need for culturing.[13] Overall, HTS provides a powerful platform for exploring genetic diversity, evolutionary relationships, functional genomics, and precision medicine.[7,14]

High-throughput sequencing has facilitated the study of entire genomes, gene expression patterns, and microbial communities at a depth and scale that was not previously possible.[6,15] In this review, we aimed to provide a consolidated source of information about HTS. We have combined some data from our own preliminary studies with an extensive literature search in Embase, PubMed, and Scopus.[16] To avoid bias in identification of studies, keywords were short-listed *a priori* from PubMed's Medical Subject Heading thesaurus.[17]

Preparation of Genetic Material for HTS

Isolating genetic material for HTS involves extracting high-quality DNA or RNA from biological samples in a way that preserves integrity, minimizes contamination, and yields sufficient quantity for library preparation and downstream sequencing (Fig. 1).[18–20] The process typically begins with sample collection and preservation, ensuring nucleic acids are protected from degradation such as with cold storage or stabilizing reagents.[21] This is followed by cell lysis, where chemical, enzymatic, or mechanical methods break open cells to release nucleic acids.[21,22] Next, purification steps remove proteins, lipids, and other contaminants; silica column–based kits, magnetic bead systems, or phenol–chloroform extraction are used frequently to prevent these problems.[19,21–23] For RNA-seq, additional steps such as DNase treatment may be required to remove contaminating DNA.[24] The extracted genetic material is then quantified and assessed for quality using spectrophotometry, fluorometry, or electrophoresis to ensure appropriate concentration and integrity.[25] High-quality, intact nucleic acids are essential for reliable library construction, accurate sequencing, and reproducible downstream bioinformatic analysis in HTS workflows.[1,18]

Investigation of Genetic Material Using HTS

Investigation of genomes using HTS involves comprehensively analyzing the complete DNA content of an organism at high depth and resolution.[1,3] This approach enables whole-genome sequencing

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Fig. 1: High-throughput sequencing workflow and technology illustration. The figure depicts a HTS platform used for large-scale DNA sequencing and genomic analysis. The sequencing instrument processes multiple DNA fragments simultaneously, generating massive amounts of sequence data displayed as colored nucleotide reads. The visualization highlights key components of HTS, including sample processing, sequencing read generation, and digital data output for downstream bioinformatic analysis. High-throughput sequencing enables rapid, parallel sequencing of genetic material and is widely used in genome investigation, mutation detection, transcriptome analysis, and evolutionary studies

To prepare this illustration, we first drafted a legend, including the scientific content and conceptual framework. This legend was used as the initial prompt for AI-assisted image generation with ChatGPT (OpenAI; GPT-5.5, 2026), which served as an interactive design assistant to iteratively translate the written scientific concept into a visual representation.[26,27] The resulting artwork was further refined using BioRender with its curated library of biomedical illustrations and icons; Inkscape for precision vector editing, object alignment, and figure assembly; and Adobe Illustrator for graphic design, typography, and publication-quality finishing.[28–30] Approximately 10 iterative refinements were performed through successive text-based prompts to optimize the composition, symbolism, labeling, visual hierarchy, and overall presentation until the illustration achieved the intended design objectives. The final figure was critically reviewed, edited, verified, and approved by the authors, who assume full responsibility for its scientific content and accuracy

Table 1: Gene types are most prone to SNVs

Gene category	*Reason for high SNV rate*	*Examples*
Immune system genes	Rapid evolution for pathogen recognition; strong selective pressure for diversity	Immunoglobulins, T-cell receptors, defensins
Olfactory and sensory genes	Adaptive variation in sensory perception; high tolerance for sequence changes	Olfactory receptors, taste receptors
Reproductive genes	Positive selection drives variation affecting fertility or mating traits	Sperm- or egg-related genes, reproductive hormone genes
Genes in hypermutable regions	Presence of repetitive sequences or CpG islands that mutate frequently	MHC genes, promoter CpG-rich regions
Host-pathogen interaction genes	Rapid adaptation to co-evolving pathogens	Interferon-related genes, pattern recognition receptors
Non-essential or redundant genes	Less selective pressure against single-base changes	Certain gene family members
Transposable element–associated genes	Nearby mobile elements increase local mutation rates	LINE- or SINE-associated genes*

*Transposable elements. LINEs are long interspersed nuclear elements; long, about 6 kb, autonomous segments that encode the machinery for own retro transposition. Short interspersed nuclear elements are short interspersed nuclear elements; short, about 100–300 bp; non-autonomous; rely on the LINE machinery for movement. These can also insert near or within genes, affecting transcription or splicing. Genes located near or within these elements are often referred to as LINE- or SINE-associated genes. Recombination between repetitive LINE or SINE sequences can misalign chromosomes and/or associated genes, causing deletions, duplications, or inversions

to identify SNVs, insertions and deletions, CNVs, and structural rearrangements across the genome.[31–34] By generating millions to billions of short or long sequence reads in parallel, HTS allows accurate assembly, annotation, and comparison of genomes.[1,3,15] It is widely used to study genetic diversity, evolutionary relationships, inherited diseases, cancer-associated mutations, and population structure.[35,36] The large-scale data produced can be integrated with bioinformatic analyses to uncover functional elements, regulatory regions, and molecular mechanisms underlying biological traits and disease processes.[37–42]

Investigation of gene expression patterns using HTS is usually performed through RNA-seq, which enables comprehensive analysis of the transcriptome.[10] RNA is extracted from cells or tissues, converted into complementary DNA (cDNA), and then sequenced in parallel to generate millions of reads representing expressed genes.[43] This allows researchers to quantify gene expression levels, compare differential expression between conditions, and identify novel transcripts, splice variants, and non-coding RNAs.[10] High-throughput sequencing provides high sensitivity and dynamic range, making it possible to detect both abundant and rare transcripts.[3,7] It is widely used in developmental biology, cancer research, immunology, and systems biology to understand regulatory mechanisms, cellular responses, and molecular pathways underlying physiological and pathological states.[44,45]

In RNA-seq studies, messenger RNA (mRNA) is isolated from a biological sample, converted into cDNA, and sequenced in parallel to generate millions of short reads.[46] These reads are then aligned to a reference genome or transcriptome, and the number of reads mapping to each gene is counted.[46,47] As the number of reads corresponding to a gene is proportional to its abundance in the sample, gene expression levels can be quantified numerically.[48] To allow comparison across genes and samples, read counts are normalized using metrics such as transcripts per million (TPM), reads per kilobase million (RPKM), or counts per million (CPM).[39] This quantitative approach enables detection of differential gene expression between experimental conditions and provides insights into regulatory mechanisms, cellular states, and disease processes.[49,50]

In the following sections, we have described frequently seen errors in DNA sequences and chromosomal abnormalities.

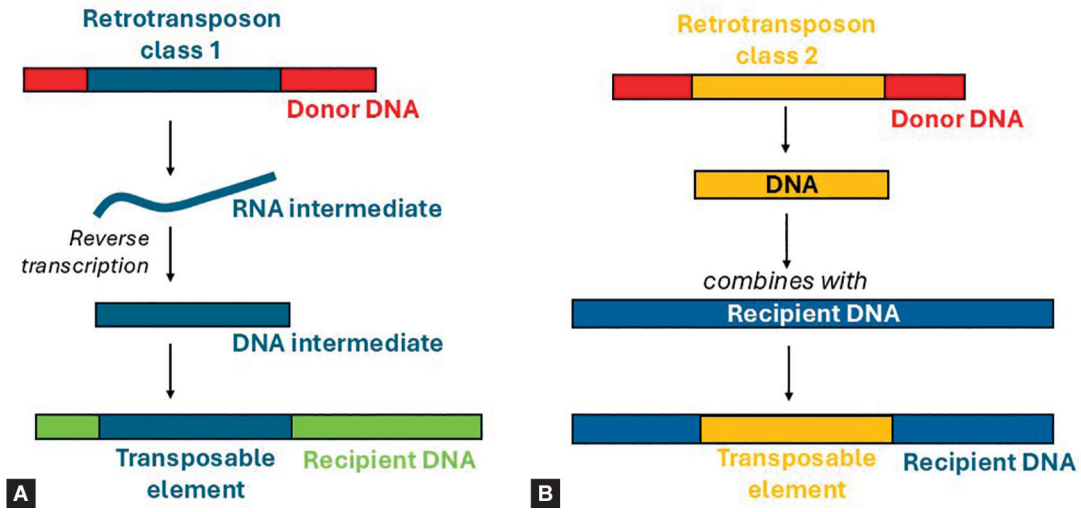
Abnormalities in DNA Sequences

SNVs

These are single base changes in DNA across the genome and can be detected with high sensitivity and accuracy.[3,51,52] In this approach, genomic DNA is extracted, fragmented, and sequenced in parallel to generate millions or billions of short reads.[2] These reads are aligned to a reference genome, and computational variant-calling algorithms detect positions where the sequenced nucleotides differ from the reference sequence.[53] Quality filtering and depth-of-coverage analysis are applied to distinguish true variants from sequencing errors.[54,55] High-throughput sequencing enables comprehensive detection of SNVs in whole genomes, exomes, or targeted regions and is widely used in population genetics, cancer genomics, rare disease diagnostics, and evolutionary studies to identify mutations associated with phenotypic traits or disease risk.[31] Table 1 shows gene categories that tend to show higher rates of SNVs.

Single nucleotide variants are frequently seen in genes regulating the immune system, olfactory and sensory system, reproductive system, genes in hypermutable regions, host-pathogen interactions, non-essential or redundant genes, and transposable elements-associated genes.[56] Host–pathogen interactions drive rapid adaptation, favoring genetic diversity that can enhance recognition and response to infectious agents. As a result, immune-related genes—particularly those involved in antigen presentation, pathogen recognition, and inflammatory signaling—often tolerate or even benefit from higher levels of nucleotide variation.[57,58] This diversity improves population-level resilience to infectious diseases, although certain variants may also increase susceptibility to autoimmune disorders or inflammatory conditions.[59] Olfactory receptor genes, in particular, belong to large gene families with functional redundancy, allowing individual receptors to accumulate nucleotide changes without severely compromising overall sensory function.[60] This tolerance facilitates diversification in odor detection and sensory perception across individuals and populations.[61]

Transposable element–associated genes are usually located near/within transposable elements, such as retrotransposons or DNA transposons (Fig. 2).[62] As transposable elements can insert into



Figs 2A and B: Schematic representation of transposable elements. (A) Retrotransposon class 1. These are mobile DNA sequences that move within the genome via a "copy-and-paste" mechanism using an RNA intermediate; these are first transcribed into RNA, which is then reverse-transcribed into DNA by the element's encoded reverse transcriptase, and the new DNA copy is inserted at a different genomic location. Because the original copy remains in place, retrotransposons can increase their copy number over time. Class 1 retrotransposons include long interspersed nuclear elements (LINEs), short interspersed nuclear elements (SINEs), and long terminal repeat (LTR) elements, and they are often found near or within genes. These can disrupt coding sequences, alter gene regulation, or create structural variation, and are therefore, important drivers of genome evolution, genetic diversity, and sometimes disease; (B) Class 2 transposable elements (DNA transposons) are mobile genetic elements that transpose directly as DNA, without an RNA intermediate. These typically encode a transposase enzyme, which recognizes terminal inverted repeat (TIR) sequences at the ends of the element, excises the transposon from its original location, and inserts it into a new genomic site. This process can generate small duplications at the target insertion site and may disrupt genes or regulatory regions. Although many DNA transposons in humans are no longer active, these have played a significant role in genome evolution by contributing to mutations, chromosomal rearrangements, and genomic diversity across species

new genomic locations, these may disrupt coding sequences, alter regulatory regions, or influence gene expression by introducing promoters, enhancers, or splice sites.[63] As a result, these genes often exhibit higher rates of mutation, structural variation, or regulatory changes.[64,65] While such insertions can sometimes contribute to genetic disorders, they have also played a significant role in genome evolution by generating diversity and novel gene regulatory patterns across species.[66]

Ribonucleic acid transposons, also known as retrotransposons (class 1 transposable elements), are mobile genetic elements that move within the genome through an RNA intermediate.[62,67] Unlike DNA transposons, RNA transposons transpose via a "copy-and-paste" mechanism in which the element is first transcribed into RNA, then reverse-transcribed back into DNA by a reverse transcriptase enzyme, and finally inserted at a new genomic location.[62,66,67] Because the original copy remains in place, each transposition event can increase the number of copies in the genome.[68] RNA transposons include long interspersed nuclear elements (LINEs), short interspersed nuclear elements (SINEs), and LTR retrotransposons.[69–71] Their activity can alter gene expression, disrupt coding sequences, or promote structural variation, making them important contributors to genome evolution, genetic diversity, and, in some cases, disease.[63]

Many DNA transposons (class 2 transposable elements) in humans are no longer active because they have accumulated mutations over millions of years that disrupt the sequences required for mobility.[72,73] Essential components such as the transposase gene or the terminal inverted repeats needed for excision and reintegration have often been truncated or degraded.[74] In addition, host genomes have evolved protective mechanisms, including DNA methylation and other epigenetic silencing processes, to suppress transposon activity and maintain genomic stability.[65,75] Since active transposition can disrupt genes or regulatory regions and reduce organismal fitness, harmful insertions are typically removed through natural selection.[74] Consequently, most DNA transposons in the human genome remain as inactive remnants of past activity, serving as molecular fossils that reflect earlier periods of genome evolution.[76]

Insertions and deletions (indels) of small or large segments of DNA can be detected that are either inserted into or deleted from the genome relative to a reference sequence.[77] In this approach, genomic DNA is fragmented and sequenced in parallel to produce millions of reads, which are then aligned to a reference genome.[2,78] Indels are identified when gaps or mismatches appear during alignment, indicating missing or additional nucleotides.[79] Specialized variant-calling algorithms analyze read depth, split reads, and paired-end mapping patterns to accurately detect and characterize these structural changes.[80,81] High sequencing coverage improves confidence in distinguishing true indels from technical artifacts.[82] This method is widely used in cancer genomics, inherited disease studies, and population genetics to identify mutations that may disrupt gene function, alter protein coding sequences, or affect regulatory regions.[83]

Gene insertions and deletions (indels) across evolution are important drivers of genomic and phenotypic diversity among species.[84] Insertions add new DNA sequences, while deletions remove existing sequences, and both can affect coding regions, regulatory elements, or non-coding regions of the genome.[85,86] Over evolutionary time, indels can arise through replication errors, unequal crossing-over, transposable element activity, or DNA repair

Table 2: Gene categories that show high rates of insertions and deletions

Gene category	*Reason for high indel rate*	*Examples*
Immune system genes	Rapid evolution for pathogen recognition; diversity favored by natural selection	Immunoglobulins, T-cell receptors, defensins
Sensory perception genes	Adaptation to environmental cues; often located in repetitive regions	Olfactory receptors, taste receptors
Genes in repetitive DNA regions	Tandem repeats or segmental duplications increase misalignment during replication/ recombination	Microsatellite-associated genes
Genes near transposable elements	Insertion events by mobile DNA elements; deletions from mis-repair	LINE or SINE–associated types of transposable elements—mobile DNA sequences that can move or copy themselves within the genome*
Rapidly evolving/adaptive genes	Positive selection allows tolerance for structural changes	Host-pathogen interaction genes, reproductive genes
Non-essential or redundant genes	Less selective pressure against structural changes	Certain gene family members with redundancy
Fragile-site–associated genes	Genomic instability at fragile sites increases breakage and rearrangements	Some tumor suppressor regions

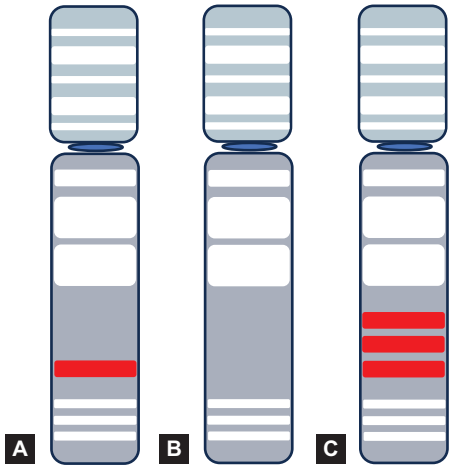
DNA, deoxyribonucleic acid

mistakes.[87,88] Beneficial indels may be retained by natural selection, leading to adaptive traits, novel gene functions, or changes in gene regulation.[84,89] Deleterious indels are often purged from populations.[90] Comparative genomic studies show that indels contribute to speciation, functional divergence of gene families, and differences in morphology, physiology, and metabolic capacity between species.[91,92] By shaping gene content and genome architecture, indels play a central role in evolutionary innovation and the emergence of species-specific traits.[93] Table 2 shows gene categories that show high rates of insertions and deletions (indels).

Copy number variations of various genes can be seen in genomic regions that are duplicated or deleted relative to a reference genome (Fig. 3).[88,94] In this approach, genomic DNA is sequenced at sufficient depth, and the number of sequencing reads mapping to specific regions is analyzed.[95] Read depth is proportional to the number of DNA copies present as regions with increased read counts suggest duplications, whereas regions with reduced read counts indicate deletions.[96,97] Computational algorithms also use paired-end mapping patterns and split-read information to improve detection accuracy.[98] High-throughput sequencing enables genome-wide, high-resolution identification of CNVs, making it a powerful tool in cancer genomics, rare disease diagnosis, developmental disorders, and population genetics to uncover structural variations that may influence gene dosage and phenotype.[99]

CNVs can occur through several mechanisms:[88]

- *Errors during DNA replication*: Slippage of DNA polymerase or unequal crossing-over during meiosis can lead to extra copies (duplications) or missing segments (deletions).
- *Recombination events*: Non-allelic homologous recombination (NAHR) between repeated sequences can cause duplication or deletion of intervening regions.
- *Deoxyribonucleic acid repair mistakes*: Improper repair of double-strand breaks, such as through non-homologous end joining, can remove or duplicate DNA segments.
- *Transposable elements*: Mobile genetic elements can insert themselves or cause nearby duplications and deletions.
- *Chromosomal instability*: Structural rearrangements, such as inversions or translocations, may indirectly lead to gains or losses of genomic regions.



Figs 3A to C: Schematic representation of gene copy number variation (CNV) across a chromosome segment. The figure illustrates three scenarios: (A) Normal with one gene copy (red); (B) deletion with no gene copies; and (C) three gene copies. CNVs can alter gene dosage, contributing to genomic diversity, phenotypic variation, and susceptibility to genetic disorders

These duplications and deletions can affect gene dosage, regulatory regions, or genome architecture, sometimes causing phenotypic variation, disease susceptibility, or evolutionary adaptation.[100] Some CNVs are benign and contribute to normal genetic diversity, whereas others may underlie disorders such as cancer, developmental syndromes, or congenital anomalies.[88]

Certain genes are more prone to CNV than others; immune-related (immunoglobulins, T-cell receptors, and defensins), metabolic and detoxification (CYP450, amylase 1), olfactory receptors/other sensory-related gene families, or those located in regions of high genomic instability (located near fragile sites, transposable elements, or recombination hotspots) show higher CNV.[101,102] Overall, CNV is seen more frequently in genes with functional redundancy, adaptive value, or location in structurally dynamic genomic regions, whereas essential housekeeping genes are generally more stable.[103,104]

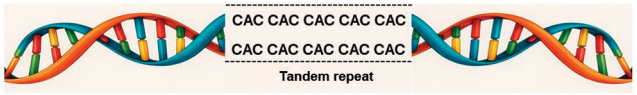


Fig. 4: Schematic representation of tandem repeats in a DNA sequence. Tandem repeats consist of short, repeated nucleotide motifs (micro-, mini-, or satellites) as described in the text. These are arranged consecutively in a head-to-tail manner within the genome. The figure illustrates a core repeat motif duplicated multiple times in direct succession, flanked by unique upstream and downstream sequences

Genes within repetitive regions such as those with tandem repeats, segmental duplications, or low-copy repeats (LCRs) are more susceptible to unequal crossing-over, and consequently, to duplication or deletion:[105]

- Tandem repeats are short nucleotide sequences that are repeated consecutively along a chromosome.[106] In these regions, the repeated units are located next to each other in tandem, not scattered throughout the genome (Fig. 4).[107] These include microsatellites (1–6 base pairs), minisatellites (10–60 base pairs), and satellites (larger repeat units).[108,109] The repeats vary among individuals as these are prone to replication slippage and unequal crossing-over during meiosis.[110] Tandem repeats contribute to genetic diversity, evolution, and genome organization, but excessive expansion of certain repeats is associated with genetic disorders, including neurological and developmental diseases.[106]
- Segmental duplications are larger, highly similar DNA sequences that are present in multiple locations within the genome.[111] These duplicated segments typically range from a few to hundreds of kilobases; the sequence identity may exceed 90%.[112] Segmental duplications arise through mechanisms such as unequal crossing-over, replication errors, or chromosomal rearrangements, and could promote gene family expansion and functional innovation.[113] However, because of their high sequence similarity, segmental duplications can promote genomic instability, increasing the risk of structural variations such as deletions, duplications, inversions, and translocations.[114,115]
- Low-copy repeats are duplications of DNA segments with a limited number of copies that share high sequence similarity.[116] These repeats are typically several kilobases long.[112] Because of their high homology, LCRs can misalign during meiosis and promote NAHR, leading to structural variations such as deletions, duplications, inversions, and translocations.[117] While LCRs contribute to genome evolution and gene family expansion, they also increase susceptibility to genomic instability and are associated with several genetic disorders when recombination occurs between inappropriate genomic regions.[118]

Progressive change in CNVs across generations refers to the accumulation or alteration of duplicated or deleted genomic segments over successive generations.[88] CNVs arise from mechanisms such as unequal crossing-over during meiosis, replication errors, or chromosomal rearrangements, and they can alter the number of copies of specific genes or regulatory regions.[88,119] When these CNVs are heritable, they can be transmitted to offspring, sometimes increasing in frequency or size over generations.[120] Progressive changes in CNVs can influence gene dosage, affecting protein expression, cellular function, and phenotypic traits.[121,122] While some CNVs are neutral or contribute to evolutionary diversity, others can predispose individuals to genetic disorders, developmental abnormalities, or susceptibility to diseases.[123] Studying CNV dynamics across generations helps in understanding genome evolution, population genetics, and inherited disease mechanisms.[88]

Evolution of gene CNVs across species refers to how duplications or deletions of genomic segments have accumulated and diverged over evolutionary time, contributing to species-specific genetic diversity.[124] CNVs can arise from mechanisms such as unequal crossing-over, replication errors, transposable element activity, or chromosomal rearrangements, and they affect the number of copies of genes or regulatory regions.[121] Over generations, beneficial CNVs may be retained by natural selection, conferring adaptive advantages such as increased enzyme activity, enhanced immunity, or novel traits, while deleterious CNVs may be purged from the population.[88] Comparative genomic studies across species reveal that CNVs can contribute to phenotypic differences, speciation, and evolutionary innovation, highlighting their role in shaping genome architecture and functional diversity in the tree of life.[125]

Repeat expansions are abnormal increases in short repetitive sequences, such as trinucleotide repeats, which can disrupt gene function.[126] These are abnormal increases in the number of short, tandemly repeated DNA sequences within a gene.[127] Increased trinucleotide repeats typically arise due to replication instability and defective DNA repair mechanisms that make short tandem repeat regions prone to expansion.[128] During DNA replication, repetitive sequences (such as CAG, CGG, or CTG repeats) can form secondary structures such as hairpins.[129] These structures may cause DNA polymerase slippage, in which the newly synthesized strand misaligns with the template and insertion of extra repeat units.[130]

Trinucleotide motifs can expand during meiotic recombination, when mispairing between repeat tracts results in unequal crossing-over.[131] In addition, deficiencies in mismatch repair or other DNA repair pathways can fail to correct slipped-strand intermediates, allowing expansions to become permanently incorporated into the genome.[132] There some differences in the molecular effects of repeat expansions involving CAG, CGG, and CTG trinucleotide motifs: (a) CAG repeats typically occur within coding regions of genes.[133] Because CAG encodes glutamine, expansion leads to elongated polyglutamine (polyQ) tracts in proteins. These expanded proteins often misfold, aggregate, and exert toxic gain-of-function effects on cells; (b) CGG repeats are commonly found in untranslated or regulatory regions, especially 5′ untranslated regions (UTRs).[134] Large CGG expansions can lead to gene silencing through DNA methylation, reducing or eliminating gene expression. In some intermediate expansions, RNA toxicity may also occur; and (c) CTG repeats are often located in noncoding regions such as 3′ UTRs or introns.[135] Expanded CTG repeats can produce toxic RNA transcripts that sequester RNA-binding proteins, disrupting normal splicing and cellular function.[136] Thus, although all three motifs expand through similar replication-slippage mechanisms, their location within the gene (coding vs noncoding), molecular consequences (protein toxicity vs RNA toxicity vs gene silencing), and clinical outcomes may differ.[137]

Gene function can get disrupted beyond once repeat numbers exceed a certain threshold, it may alter protein structure, interfere with transcription, impair RNA processing, or lead to

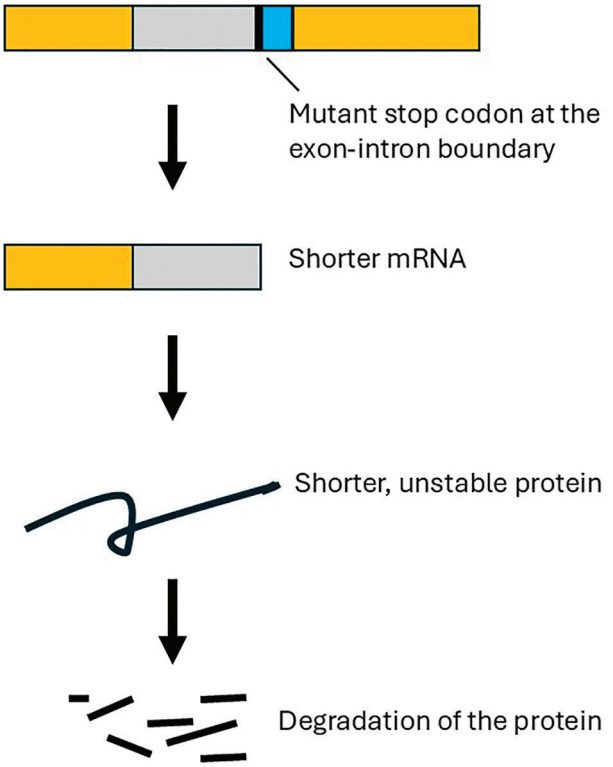


Fig. 5: Schematic illustration of the effect of a splice-site mutation on pre-mRNA processing and protein translation. In a normal pathway, accurate recognition of the 5′ donor and 3′ acceptor splice sites result in precise intron removal and proper joining of adjacent exons, generating a full-length mRNA that encodes a complete, functional protein. With splice-site mutations such as one shown in the above figure, a nucleotide change at a canonical splice site disrupts normal spliceosome recognition, leading to aberrant splicing such as exon skipping or activation of a cryptic splice site. This abnormal processing alters the reading frame or introduces a premature termination codon, producing a truncated mRNA transcript. Translation of this altered transcript results in a shortened peptide that may be unstable or have reduced/absent biological function

gene silencing.[138] Repeat expansions are often unstable across generations, meaning the repeat length can increase during meiosis, sometimes leading to earlier onset or increased severity of disease in successive generations (a phenomenon known as anticipation).[127]

Splice-site mutations affect RNA splicing, potentially producing unstable, abnormal, or truncated proteins (Fig. 5).[139] These genetic variants affect the normal process of RNA splicing, the step in which introns are removed and exons are joined to form mature mRNA. These mutations typically occur at conserved sequences located at exon–intron boundaries or within regulatory splicing elements. When disrupted, they can cause exon skipping, intron retention, or activation of cryptic splice sites, leading to abnormal mRNA transcripts.[140] The resulting protein may be truncated, structurally altered, or entirely absent, potentially contributing to genetic disorders or disease.

Increasing data suggest that different types of splice-site mutations are more likely to produce specific splicing defects, which in turn can lead to abnormal or truncated proteins.[141] Mutations at the canonical donor site (5′ splice site) or acceptor site (3′ splice site), particularly those at highly conserved nucleotides, are more likely to cause exon skipping or intron retention.[142] These changes frequently disrupt the reading frame, resulting in a premature stop codon and a truncated protein or degradation of the transcript through nonsense-mediated decay (NMD).[143]

Variants in the branch point sequence or polypyrimidine tract can weaken spliceosome assembly, increasing the likelihood of partial intron retention or activation of nearby cryptic splice sites.[144] Use of cryptic splice sites may insert or delete small portions of sequence, potentially causing frameshifts or in-frame alterations.[140] Finally, mutations within exonic or intronic splicing enhancers or silencers may subtly alter exon recognition, leading to partial exon inclusion or exclusion.[140] Depending on whether the reading frame is preserved, the resulting protein may be truncated, internally deleted, or contain inserted amino acids.[145] Thus, the specific position and nature of a splice-site mutation strongly influence the type of splicing abnormality produced and whether the outcome is a truncated protein, an altered protein, or complete loss of protein expression.[141]

Regulatory mutations are genetic alterations that occur in promoters, enhancers, silencers, or other noncoding control regions of DNA and affect how much, when, or where a gene is expressed.[146] These changes affect gene expression without altering the protein-coding sequence itself.[147] The regulatory mutations can modify transcription factor binding sites, disrupt chromatin structure, or change epigenetic regulation, leading to increased or decreased gene expression.[148,149] Although the encoded protein remains structurally unchanged, abnormal expression levels can significantly impact cellular function and may contribute to developmental disorders, complex traits, or disease.[150]

Structural Rearrangements in Chromosomes

Structural rearrangements across the genome using HTS involves detecting large-scale alterations in chromosome structure, such as inversions, translocations, deletions, duplications, and complex rearrangements.[31] These rearrangements typically arise from errors in DNA replication, repair, or recombination, such as double-strand breaks, misalignment of repetitive sequences, or replication fork stalling.[151] Mobile genetic elements can move within the genome, sometimes carrying adjacent DNA with them or causing breaks that lead to rearrangements.[152] Structural changes can disrupt gene function, remove or duplicate regulatory regions, or create novel gene fusions, leading to altered gene expression and phenotypic effects.[153] While some rearrangements may contribute to genomic diversity and evolution, others can cause genetic disorders, developmental abnormalities, or cancer.[154]

To study structural rearrangements, genomic DNA is fragmented and sequenced in parallel, generating millions of reads that are then aligned to a reference genome.[155] Structural rearrangements are identified by analyzing deviations from expected read pair orientation, insert size, split reads, and changes in read depth.[31] Sophisticated bioinformatic tools allow genome-wide detection of structural variants, providing crucial insights into cancer genomics, congenital disorders, evolutionary biology, and other conditions where chromosomal architecture plays a key role in phenotype or disease susceptibility.

Chromosome inversions occur when a segment of a chromosome breaks in two places and then reattaches in the reverse orientation (Fig. 6).[156] These alterations can be pericentric (involving

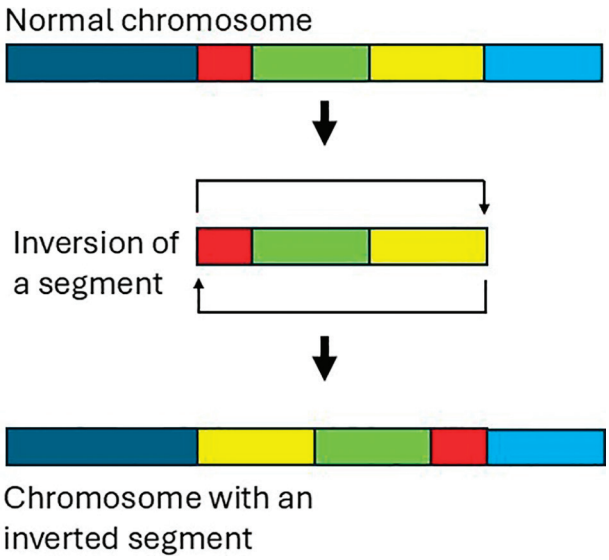


Fig. 6: Schematic illustration of chromosomal inversion. The chromosome has broken in two places and has then reattached in the reverse orientation

the centromere) or paracentric (excluding the centromere), and can affect gene function and regulation if breakpoints disrupt coding or regulatory regions.

There are several possible mechanisms:

- *Errors during meiosis*: During homologous recombination, misalignment of repeated sequences or NAHR with can cause a chromosome segment to flip, resulting in a chromosomal inversion.[151] Normally, homologous chromosomes align precisely, and recombination occurs between allelic regions, maintaining the correct sequence orientation.[157] However, when repetitive elements on non-allelic regions misalign, crossing-over can occur between these non-corresponding sites.[158] This mis-paired recombination causes the intervening segment to invert its orientation on the chromosome.[159] Such inversions can be pericentric (involving the centromere) or paracentric (excluding the centromere) and may disrupt genes or regulatory elements at the breakpoints.[160] Inversions can contribute to genetic diversity and evolution, but these can also reduce fertility in heterozygotes.[161]
- *Deoxyribonucleic acid double-strand breaks (DSBs)*: When breaks occur in a chromosome and are incorrectly repaired, the intervening segment may be lost.[162] Double-strand breaks are a severe type of DNA damage, which show a break in both strands of the DNA double helix.[163] These arise from endogenous cellular processes such as replication stress, where stalled/collapsed replication forks can generate breaks.[164] Some DSBs are induced during meiosis to facilitate genetic recombination.[165] Exogenous factors, including ionizing radiation, ultraviolet light, and certain chemical mutagens, can directly or indirectly cause DSBs.[164,166] Additionally, enzymes such as nucleases involved in DNA repair or transposases during mobile element activity can also create breaks.[167] If not accurately repaired, DSBs can lead to mutations, deletions, duplications, or chromosomal rearrangements, which is why cells rely on precise repair mechanisms like homologous recombination and non-homologous end joining.[168]
- *Transposable elements*, also known as mobile DNA elements, can facilitate chromosomal inversions when they recombine with other copies present elsewhere in the genome.[66,169] These repeated sequences get translocated and copied within the genome, and the presence at multiple sites increases the chance of misalignment.[73] During recombination, if two TEs in inverted orientation pair and undergo homologous recombination, the DNA segment between them can be flipped, producing an inversion.[170] Such inversions can alter gene orientation, disrupt coding or regulatory regions, and affect gene expression (Fig. 2).
- *Chromosomal stress or instability*: Environmental stress and genomic instability can increase the likelihood of chromosomal inversions by promoting DNA breaks and erroneous repair.[171] Radiation, chemical mutagens, reactive oxygen species, or extreme cellular stress can induce double-strand breaks in chromosomes. In cells with compromised genome maintenance or repair pathways, these breaks may be incorrectly rejoined, sometimes flipping the orientation of the intervening DNA segment and creating an inversion. Such inversions can disrupt gene coding sequences or regulatory regions, alter gene expression, and contribute to genomic instability.[170]

  Chromosomal instability is seen when the mechanisms that maintain genome integrity during DNA replication and cell division are compromised, leading to increased rates of chromosomal gains, losses, or structural alterations.[172] Key causes include defective DNA repair pathways, such as impaired homologous recombination or non-homologous end joining, which leave DNA breaks unrepaired or mis-repaired.[173] Replication stress, caused by stalled or collapsed replication forks, and telomere dysfunction, can further promote instability.[174]

Chromosome translocations occur when a segment of one chromosome breaks off and attaches to a different, non-homologous chromosome.[175] The causes include DSBs and chromosomal instability. Recombination errors and toxins also need to be considered.[176] Translocations can be reciprocal, where two chromosomes exchange segments, or non-reciprocal, where a segment moves without exchange.[175] These can disrupt genes at breakpoints, create fusion genes, or alter gene regulation.

- *Recombination errors* can often result in chromosome translocations during cell division, particularly when DNA DSBs occur in non-homologous chromosomes. Normally, homologous recombination ensures accurate exchange of genetic material between corresponding regions of chromosomes.[162] However, mis-repair of breaks or aberrant recombination between non-allelic sequences can cause segments from different chromosomes to fuse, producing reciprocal or non-reciprocal translocations.[116] These events can disrupt gene coding regions, regulatory elements, or create fusion genes with novel functions, potentially leading to altered gene expression.
- *Toxin exposure*: External mutagens such as radiation and toxins can induce breaks in chromosomes, increasing the likelihood of translocations:[177]
  - Ionizing radiation, such as X-rays or gamma rays, generates double-strand breaks (DSBs) by physically disrupting the DNA backbone.[178] When multiple breaks occur simultaneously in different chromosomes, the cellular repair machinery may mis-join DNA ends, leading to segments from one

Table 3: Toxins that commonly damage chromosomes

Toxin category	*Examples*	*Primary mechanism of chromosomal damage*	*Typical chromosomal effects*
Ionizing radiation–producing agents	X-rays, gamma rays, radon	DNA double-strand breaks; reactive oxygen species generation	Chromosome breaks, deletions, translocations
Alkylating agents	Mustard derivatives, nitrosamines, cyclophosphamide	Alkylation of DNA bases → mispairing and strand instability	Chromosomal fragmentation, mutations
Topoisomerase inhibitors	Etoposide, doxorubicin	Stabilize DNA–topoisomerase complexes → persistent double-strand breaks	Translocations, large-scale rearrangements
Heavy metals	Arsenic, cadmium, chromium, nickel	Oxidative stress; inhibition of DNA repair pathways	Chromosome aberrations, aneuploidy
Polycyclic aromatic hydrocarbons (PAHs)	Benzo[a]pyrene (cigarette smoke)	DNA adduct formation disrupting replication	Chromosomal instability, mutations
Mycotoxins	Aflatoxin B1	Bulky DNA adduct formation	DNA breaks, increased cancer risk
Reactive oxygen–generating chemicals	Hydrogen peroxide, certain pesticides	Oxidative DNA damage (base damage and strand breaks)	Single- and double-strand breaks
Spindle poisons (mitotic disruptors)	Colchicine, vincristine	Disruption of microtubules during mitosis	Chromosome mis-segregation, aneuploidy

DNA, deoxyribonucleic acid, ROS, reactive oxygen species

chromosome being aberrantly attached to another. This mis-repair can result in reciprocal or non-reciprocal translocations, which may disrupt genes or regulatory regions, create fusion genes, and alter normal gene expression.
  - Toxins can induce breaks in chromosomes and increase translocations: (i) Alkylating agents, such as cyclophosphamide and mustard gas, destabilize the double helix; (ii) topoisomerase inhibitors such as etoposide and doxorubicin interfere with DNA unwinding and create double-strand breaks; (iii) reactive oxygen species (ROS) generated by heavy metals such as arsenic, cadmium, and lead; (iv) environmental pollutants such as polycyclic aromatic hydrocarbons that can form DNA adducts to stall replication and promote breaks.[179–182] Table 3 lists some toxins that can cause chromosomal damage.

Chromosome deletions occur when a segment of a chromosome is lost, resulting in missing genetic material.[183] These deletions can vary in size, ranging from a single gene to large chromosome segments. These can disrupt gene function, remove regulatory regions, and result in genetic disorders or developmental abnormalities. There are several possible mechanisms:

- *DSBs and mis-repair*: When chromosomal breaks are incorrectly repaired, the intervening segment may be lost. Details are provided above.
- *Unequal crossing-over during meiosis*: Normally, homologous chromosomes pair precisely and exchange equivalent segments through crossing-over. However, misalignment of homologous chromosomes during recombination can result in one chromosome losing a segment (deletion) while the other gaining a segment with extra copies of a gene (duplication).[184]
- *Replication errors*: During the S phase of meiosis, DNA polymerases synthesize new strands, but errors such as slippage, replication fork collapse, and template switching can occur:[129]
  - Slippage, especially in regions with repetitive sequences, can produce insertions or deletions.[130] Replication slippage happens when the DNA polymerase temporarily dissociates from the template strand and then reanneals incorrectly, leading to misalignment of short tandem repeats. This mispairing can result in the insertion or deletion of repeat units, producing repeat expansions or contractions. Such errors are especially common in microsatellite regions and trinucleotide repeats, where secondary DNA structures can form and promote strand misalignment. If not corrected by DNA repair mechanisms, these replication errors can be propagated into gametes and contribute to heritable genetic variation or, in some cases, repeat expansion disorders.
  - Replication fork collapse during chromosome replication may occur due to damaged replication machinery, causing the replication fork to stall and break.[185] The replication fork is a Y-shaped structure where the parental DNA strands are unwound and new strands are synthesized by DNA polymerases. Collapse can be triggered by DNA damage, tightly bound proteins, unusual secondary structures, or nucleotide shortages. When a replication fork collapses, it often generates a DNA break, which, if unrepaired or mis-repaired, can lead to deletions, duplications, inversions, or other structural rearrangements. Replication fork collapse is a major source of chromosomal instability.
  - Template switching during chromosome replication is a process in which the DNA polymerase temporarily disengages from its original template strand and resumes synthesis using a different, often nearby, DNA strand as a template.[186] Template switching can lead to duplications, deletions, inversions, or complex rearrangements because the polymerase may copy segments out of order or from the wrong location. Aberrant template switching can increase genomic instability.
- *Chromosomal instability*: Stress, radiation, chemical mutagens, or defects in DNA repair pathways can increase the likelihood of deletions. Details as described above.

Chromosome duplications occur when a segment of a chromosome is copied one or more times, resulting in extra genetic material. These duplications can arise through several mechanisms:

- *Errors during DNA replication*: Replication slippage or template switching can cause a segment to be duplicated during the S phase of the cell cycle. Details as described above.
- *Unequal crossing-over during meiosis*: Misalignment of homologous chromosomes during recombination can result in

one chromosome gaining a copy of a segment while the other loses it. Details as described above.
- *Chromosomal rearrangements*: Structural events such as translocations or inversions can inadvertently duplicate segments when the DNA breaks and rejoins in non-standard ways. These events can alter gene dosage, disrupt coding or regulatory sequences, and contribute to genomic instability, disease, or evolutionary innovation. Details as described above.
- *Replication fork stalling and break-induced replication*: As mentioned above, problems in replication machinery can lead to repeated copying of a chromosomal segment.

Chromosome duplications can have significant effects. Gene dosage can lead to overexpression of genes or provide raw material for evolutionary innovation; and duplicated genes can acquire new functions.[187] Large duplications have been associated with developmental disorders and genomic instability.[118]

Complex rearrangements of chromosomes may be seen with inversions, translocations, duplications, and deletions.[188] These events often arise from major errors in DNA repair, replication, or recombination that are more intricate than simple single events. Common causes include:

- *Multiple DSBs*: As mentioned above, if several breaks occur close in time, incorrect repair can join fragments in new arrangements, producing complex patterns.[189]
- *Chromothripsis*: A phenomenon in which a chromosome gets shattered into many pieces and is then stitched back together in a random order, leading to extensive rearrangements.[190] One or a few chromosomes shatter into dozens or hundreds of fragments and are stitched back together in a random order during a single catastrophic event.[191] Its importance in genetic disorders lies in its potential to cause massive genomic rearrangements all at once, rather than gradually.[192] These rearrangements can lead to deletions, duplications, inversions, and translocations that disrupt multiple genes or regulatory regions simultaneously.[193]

  Chromothripsis has been reported in some patients with phenotypes similar to 1p36 deletion–like syndrome and some cases with Wolf–Hirschhorn–like features, although chromothripsis itself is not considered a distinct syndromic diagnosis.[194] The clinical manifestations depend primarily on which genes are disrupted during the chromothriptic event(s).[190]
- *Replication stress*: Stalled or collapsed replication forks can cause template switching or fork breakage, generating multiple rearrangements.[195] Replication stress refers to any condition that slows, stalls, or disrupts DNA replication, leading to incomplete or erroneous copying of the genome.[196] Replication stress can arise from DNA lesions, tightly-bound proteins, unusual DNA structures, or shortages of nucleotides, and it increases the likelihood of CNV, replication fork collapse, DNA DSBs, and structural rearrangements.[185] These rearrangements can dramatically alter gene structure and regulation, and even disrupt multiple genes at once.[197]

  Replication stress has been implicated in fragile X syndrome, some congenital disorders, and cancer predisposition syndromes like Bloom syndrome or Werner syndrome, where defects in DNA repair or replication exacerbate genome instability.[174] By compromising accurate genome duplication, replication stress may alter the onset and progression of many genetic diseases.[185]
- *Aberrant recombination events*: Recombination between non-allelic or repetitive sequences can produce overlapping duplications, deletions, and inversions.[158] Details as above.
- *Genomic instability*: Cells with defective DNA repair machinery, such as in many cancers, are prone to accumulating complex rearrangements over time.[105] Details as above.

Future Possibilities

With the expansion of ultra-sensitive genomic technologies, it is now becoming possible to detect/identify several previously unrecognized categories of DNA sequence and chromosomal abnormalities.[1,6,7,14,15] These include complex structural variants such as chromothriptic rearrangements, low-level mosaic mutations, and ultra-rare copy number changes that were historically below the resolution of conventional cytogenetics.[78,118] We are detecting subtle variations in chromatin sequence(s), repetitive region misalignments, and hitherto-undetected genomic instability patterns.[55,124] In addition, discoveries in single-cell genomics have highlighted cell-to-cell genomic heterogeneity, raising the possibility that some variants may represent transient developmental or somatic events rather than stable heritable mutations.[198] Continued research is required to distinguish clinically meaningful abnormalities from novel background genomic variation.

High-throughput sequencing has transformed the detection of genetic and chromosomal abnormalities by enabling rapid, comprehensive, and highly sensitive analysis of DNA sequences.[3] This technology has allowed the identification of point mutations, structural variants, copy number changes, and complex chromosomal rearrangements that were frequently missed with conventional cytogenetic methods. By improving diagnostic accuracy and expanding our understanding of genomic disorders, HTS has helped advance personalized medicine, early disease detection, and genomic research.[199] Continued refinement of sequencing technologies and bioinformatic tools will further enhance the ability to interpret genomic variation and its clinical significance.

In HTS, there remains a possibility of technical errors and over-diagnosis of genetic errors even with the most sophisticated methods.[200] Sequencing artifacts can arise from sample contamination, amplification bias, mapping errors, or limitations in bioinformatic interpretation.[201] Over-diagnosis may occur when variants of uncertain clinical significance are detected and misinterpreted as pathogenic, potentially leading to unnecessary anxiety, further testing, or unwarranted medical intervention. Sequencing results should always be interpreted in conjunction with clinical findings, confirmatory laboratory methods, and expert genetic counseling to minimize diagnostic misclassification and improve clinical utility.

High-throughput sequencing is showing rapid advancement with improvements in speed, accuracy, cost reduction, and data analysis methods.[202] Advances in short-read and long-read sequencing platforms have expanded the ability to detect single-nucleotide variants, structural rearrangements, and complex genomic abnormalities. Emerging innovations, including real-time sequencing, single-cell genomics, and enhanced bioinformatics pipelines, are improving variant interpretation and clinical diagnostic precision. Integration with artificial intelligence–based analytics and large genomic databases is expected to further enhance personalized medicine, rare disease diagnosis, and population-level genomic research.[203]

Ethical Approval

Ethics approval not needed as it is a review article.

Authors' Contribution

Both authors contributed equally.

Artificial Intelligence (AI) Disclosure

Artificial Intelligence use in Fig. 1 has been documented.

References

1. Lee JY. The principles and applications of high-throughput sequencing technologies. Dev Reprod 2023;27(1):9–24. DOI: 10.12717/DR.2023.27.1.9.
2. Satam H, Joshi K, Mangrolia U, et al. Next-generation sequencing technology: Current trends and advancements. Biology (Basel) 2023;12(7):997. DOI: 10.3390/biology12070997.
3. Churko JM, Mantalas GL, Snyder MP, et al. Overview of high throughput sequencing technologies to elucidate molecular pathways in cardiovascular diseases. Circ Res 2013;112(12):1613–1623. DOI: 10.1161/CIRCRESAHA.113.300939.
4. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 1977;74(12):5463–5467. DOI: 10.1073/pnas.74.12.5463.
5. Benz S, Mitra S. From genomics to metagenomics in the era of recent sequencing technologies. Methods Mol Biol 2023;2649:1–20. DOI: 10.1007/978-1-0716-3072-3_1.
6. Vitorino R. Transforming clinical research: The power of high-throughput omics integration. Proteomes 2024;12(3):25. DOI: 10.3390/proteomes12030025.
7. Reuter JA, Spacek DV, Snyder MP. High-throughput sequencing technologies. Mol Cell 2015;58(4):586–597. DOI: 10.1016/j.molcel.2015.05.004.
8. White CC, Embree E, Byrd WE, et al. Development of a high throughput method incorporating traditional analytical devices. J Res Natl Inst Stand Technol 2004;109(5):465–477. DOI: 10.6028/jres.109.034.
9. Bagger FO, Borgwardt L, Jespersen AS, et al. Whole genome sequencing in clinical practice. BMC Med Genomics 2024;17(1):39. DOI: 10.1186/s12920-024-01795-w.
10. Kukurba KR, Montgomery SB. RNA sequencing and analysis. Cold Spring Harb Protoc 2015;2015(11):951–969. DOI: 10.1101/pdb.top084970.
11. Tiwari S, Dhakal T, Kim BJ, et al. Genomics in epidemiology and disease surveillance: An exploratory analysis. Life (Basel) 2025;15(12):1848. DOI: 10.3390/life15121848.
12. Hasan GM, Mohammad T, Shamsi A, et al. Applications of genome sequencing in infectious diseases: From pathogen identification to precision medicine. Pharmaceuticals (Basel) 2025;18(11):1687. DOI: 10.3390/ph18111687.
13. Zhou J, He Z, Yang Y, et al. High-throughput metagenomic technologies for complex microbial community analysis: Open and closed formats. mBio 2015;6(1):e02288-14. DOI: 10.1128/mBio.02288-14.
14. Caboche S, Audebert C, Hot D. High-throughput sequencing, a versatileweapon to support genome-based diagnosis in infectious diseases: Applications to clinical bacteriology. Pathogens 2014;3(2):258–279. DOI: 10.3390/pathogens3020258.
15. Tedersoo L, Albertsen M, Anslan S, et al. Perspectives and benefits of high-throughput long-read sequencing in microbial ecology. Appl Environ Microbiol 2021;87(17):e0062621. DOI: 10.1128/AEM.00626-21.
16. Avau B, Van Remoortel H, De Buck E. Translation and validation of PubMed and Embase search filters for identification of systematic reviews, intervention studies, and observational studies in the field of first aid. J Med Libr Assoc 2021;109(4):599–608. DOI: 10.5195/jmla.2021.1219.
17. Leblanc V, Hamroun A, Bentegeac R, et al. Added value of medical subject headings terms in search strategies of systematic reviews: Comparative study. J Med Internet Res 2024;26:e53781. DOI: 10.2196/53781.
18. Kutnjak D, Tamisier L, Adams I, et al. A primer on the analysis of high-throughput sequencing data for detection of plant viruses. Microorganisms 2021;9(4):841. DOI: 10.3390/microorganisms9040841.
19. Fitzpatrick AH, Rupnik A, O'Shea H, et al. High throughput sequencing for the detection and characterization of RNA viruses. Front Microbiol 2021;12:621719. DOI: 10.3389/fmicb.2021.621719.
20. Das JK, Maheshwari A. A high-throughput analysis of gene expression in the intestine in severe neonatal anemia. Newborn (Clarksville, Md) 2025;4(4):165–177. DOI: 10.5005/jp-journals-11002-0145.
21. El-Ashram S, Al Nasr I, Suo X. Nucleic acid protocols: Extraction and optimization. Biotechnol Rep (Amst) 2016;12:33–39. DOI: 10.1016/j.btre.2016.10.001.
22. Ali N, Rampazzo RCP, Costa ADT, et al. Current nucleic acid extraction methods and their implications to point-of-care diagnostics. Biomed Res Int 2017;2017:9306564. DOI: 10.1155/2017/9306564.
23. Toni LS, Garcia AM, Jeffrey DA, et al. Optimization of phenol-chloroform RNA extraction. MethodsX 2018;5:599–608. DOI: 10.1016/j.mex.2018.05.011.
24. Green MR, Sambrook J. Removing DNA contamination from RNA samples by treatment with RNase-free DNase I. Cold Spring Harb Protoc 2019;2019(10). DOI: 10.1101/pdb.prot101725.
25. Masago K, Fujita S, Oya Y, et al. Comparison between fluorimetry (Qubit) and spectrophotometry (NanoDrop) in the quantification of DNA and RNA extracted from frozen and FFPE tissues from lung cancer patients: A real-world use of genomic tests. Medicina (Kaunas) 2021;57(12):1375. DOI: 10.3390/medicina57121375.
26. Brown TB, Mann B, Ryder N, et al. Language models are few-shot learners. Adv Neural Inf Process Syst 2020;33(1):1877–1901. DOI: 10.48550/arXiv.2005.14165.
27. Achiam J, Adler S, Agarwal S, et al. OpenAI. GPT-4 Technical Report [preprint]. Ithaca, New York, USA: Cornell University Library; 2023. Available from: https://arxiv.org/abs/2303.08774.
28. Aoki S. BioRender. Toronto, Canada: BioRender [Internet], Science Suite Inc.; 2026. Available from: https://www.biorender.com.
29. Inkscape-Project. The Inkscape Project. Boston, Massachusetts, USA; 2026. Available from: https://inkscape.org.
30. Adobe. Adobe Illustrator [computer software]. San Jose, California, USA: Adobe Inc.; 2026.
31. Ho SS, Urban AE, Mills RE. Structural variation in the sequencing era. Nat Rev Genet 2020;21(3):171–189. DOI: 10.1038/s41576-019-0180-9.
32. Simon MM, Mallon AM, Howell GR, et al. High throughput sequencing approaches to mutation discovery in the mouse. Mamm Genome 2012;23(9–10):499–513. DOI: 10.1007/s00335-012-9424-0.
33. Donda K, Torres BA, Maheshwari A. Non-coding RNAs in neonatal necrotizing enterocolitis. Newborn (Clarksville) 2022;1(1):120–130. DOI: 10.5005/jp-journals-11002-0012.
34. Donda K, Bose T, Dame C, et al. The impact of microRNAs in neonatal necrotizing enterocolitis and other inflammatory conditions of intestine: A review. Curr Pediatr Rev 2022;19(1):5–14. DOI: 10.2174/1573396318666220117102119.
35. Rego SM, Snyder MP. High throughput sequencing and assessing disease risk. Cold Spring Harb Perspect Med 2019;9:a026849. DOI: 10.1101/cshperspect.a026849.
36. Jilling T, Ambalavanan N, Cotten CM, et al. Surgical necrotizing enterocolitis in extremely premature neonates is associated with genetic variations in an intergenic region of chromosome 8. Pediatr Res 2018;83(5):943–953. DOI: 10.1038/pr.2018.33.
37. Davis-Turak J, Courtney SM, Hazard ES, et al. Genomics pipelines and data integration: Challenges and opportunities in the research setting. Expert Rev Mol Diagn 2017;17(3):225–237. DOI: 10.1080/14737159.2017.1282822.

38. Mardis ER. The impact of next-generation sequencing technology on genetics. Trends Genet 2008;24(3):133–141. DOI: 10.1016/j.tig.2007.12.007.
39. Shendure J, Mitra RD, Varma C, et al. Advanced sequencing technologies: Methods and goals. Nat Rev Genet 2004;5(5):335–344. DOI: 10.1038/nrg1325.
40. Namachivayam K, MohanKumar K, Shores DR, et al. Targeted inhibition of thrombin attenuates murine neonatal necrotizing enterocolitis. Proc Natl Acad Sci U S A 2020;117(20):10958–10969. DOI: 10.1073/pnas.1912357117.
41. Namachivayam K, Blanco CL, Frost BL, et al. Preterm human milk contains a large pool of latent TGF-beta, which can be activated by exogenous neuraminidase. Am J Physiol Gastrointest Liver Physiol 2013;304(12):G1055–G1065. DOI: 10.1152/ajpgi.00039.2013.
42. Maheshwari A. Role of platelets in neonatal necrotizing enterocolitis. Pediatr Res 2021;89(5):1087–1093. DOI: 10.1038/s41390-020-1038-8.
43. Wang Z, Gerstein M, Snyder M. RNA-seq: A revolutionary tool for transcriptomics. Nat Rev Genet 2009;10(1):57–63. DOI: 10.1038/nrg2484.
44. Hong M, Tao S, Zhang L, et al. RNA sequencing: New technologies and applications in cancer research. J Hematol Oncol 2020;13(1):166. DOI: 10.1186/s13045-020-01005-x.
45. Singh S, Maheshwari A. Epigenetics of down syndrome. Newborn (Clarksville, Md) 2024;3(4):263–280. DOI: 10.5005/jp-journals-11002-0112.
46. Deshpande D, Chhugani K, Chang Y, et al. RNA-seq data science: From raw data to effective interpretation. Front Genet 2023;14:997383. DOI: 10.3389/fgene.2023.997383.
47. Munger SC, Raghupathy N, Choi K, et al. RNA-seq alignment to individualized genomes improves transcript abundance estimates in multiparent populations. Genetics 2014;198(1):59–73. DOI: 10.1534/genetics.114.165886.
48. Lee S, Seo CH, Lim B, et al. Accurate quantification of transcriptome from RNA-seq data by effective length normalization. Nucleic Acids Res 2011;39(2):e9. DOI: 10.1093/nar/gkq1015.
49. Zhao Y, Li MC, Konate MM, et al. TPM, FPKM, or normalized counts? A comparative study of quantification measures for the analysis of RNA-seq data from the NCI patient-derived models repository. J Transl Med 2021;19(1):269. DOI: 10.1186/s12967-021-02936-w.
50. Dawadi P, Pokharel B, Shrestha A, et al. From bench to bytes: A practical guide to RNA sequencing data analysis. Front Genet 2025;16:1697922. DOI: 10.3389/fgene.2025.1697922.
51. Katsonis P, Koire A, Wilson SJ, et al. Single nucleotide variations: Biological impact and theoretical interpretation. Protein Sci 2014;23(12):1650–1666. DOI: 10.1002/pro.2552.
52. Donda KT, Torres BA, Khashu M, et al. Single nucleotide polymorphisms in neonatal necrotizing enterocolitis. Curr Pediatr Rev 2022;18(3):197–209. DOI: 10.2174/1573396318666220117091621.
53. Wu L, Yavas G, Hong H, et al. Direct comparison of performance of single nucleotide variant calling in human genome with alignment-based and assembly-based approaches. Sci Rep 2017;7(1):10963. DOI: 10.1038/s41598-017-10826-9.
54. Guo Y, Zhao S, Sheng Q, et al. The discrepancy among single nucleotide variants detected by DNA and RNA high throughput sequencing data. BMC Genomics 2017;18(Suppl 6):690. DOI: 10.1186/s12864-017-4022-x.
55. Eren KK, Cinar E, Karakurt HU, et al. Improving the filtering of false positive single nucleotide variations by combining genomic features with quality metrics. Bioinformatics 2023;39(12):btad694. DOI: 10.1093/bioinformatics/btad694.
56. Ranganathan Ganakammal S, Alexov E. An ensemble approach to predict the pathogenicity of synonymous variants. Genes (Basel) 2020;11(9):1102. DOI: 10.3390/genes11091102.
57. Glass EJ. The molecular pathways underlying host resistance and tolerance to pathogens. Front Genet 2012;3:263. DOI: 10.3389/fgene.2012.00263.
58. Maheshwari A, Lacson A, Lu W, et al. Interleukin-8/CXCL8 forms an autocrine loop in fetal intestinal mucosa. Pediatr Res 2004;56(2):240–249. DOI: 10.1203/01.PDR.0000133196.25949.98.
59. Hoang Nguyen KH, Le NV, Nguyen PH, et al. Human immune system: Exploring diversity across individuals and populations. Heliyon 2025;11(2):e41836. DOI: 10.1016/j.heliyon.2025.e41836.
60. Wang Y, Sun Y, Joseph PV. Diverse evolutionary rates and gene duplication patterns among families of functional olfactory receptor genes in humans. PLoS One 2023;18(4):e0282575. DOI: 10.1371/journal.pone.0282575.
61. Trimmer C, Keller A, Murphy NR, et al. Genetic variation across the human olfactory receptor repertoire alters odor perception. Proc Natl Acad Sci U S A 2019;116(19):9475–9480. DOI: 10.1073/pnas.1804106115.
62. Bourque G, Burns KH, Gehring M, et al. Ten things you should know about transposable elements. Genome Biol 2018;19(1):199. DOI: 10.1186/s13059-018-1577-z.
63. Dong W, Li M, Li P, et al. Transposons disrupt genomic stability and trigger cancers. Front Cell Infect Microbiol 2025;15:1704405. DOI: 10.3389/fcimb.2025.1704405.
64. Ho EKH, Bellis ES, Calkins J, et al. Engines of change: Transposable element mutation rates are high and variable within Daphnia magna. PLoS Genet 2021;17(11):e1009827. DOI: 10.1371/journal.pgen.1009827.
65. Bhat A, Ghatage T, Bhan S, et al. Role of transposable elements in genome stability: Implications for health and disease. Int J Mol Sci 2022;23(14):7802. DOI: 10.3390/ijms23147802.
66. Colonna Romano N, Fanti L. Transposable elements: Major players in shaping genomic and evolutionary patterns. Cells 2022;11(6):1048. DOI: 10.3390/cells11061048.
67. Luqman-Fatah A, Nishimori K, Amano S, et al. Retrotransposon life cycle and its impacts on cellular responses. RNA Biol 2024;21(1):11–27. DOI: 10.1080/15476286.2024.2409607.
68. Zattera ML, Bruschi DP. Transposable elements as a source of novel repetitive DNA in the eukaryote genome. Cells 2022;11(21):3373. DOI: 10.3390/cells11213373.
69. Singh DK, Rath PC. Long interspersed nuclear elements (LINEs) show tissue-specific, mosaic genome and methylation-unrestricted, widespread expression of noncoding RNAs in somatic tissues of the rat. RNA Biol 2012;9(11):1380–1396. DOI: 10.4161/rna.22402.
70. Cho J. Transposon-derived non-coding RNAs and their function in plants. Front Plant Sci 2018;9:600. DOI: 10.3389/fpls.2018.00600.
71. Meng H, Feng J, Bai T, et al. Genome-wide analysis of short interspersed nuclear elements provides insight into gene and genome evolution in citrus. DNA Res 2020;27(1):dsaa004. DOI: 10.1093/dnares/dsaa004.
72. Burns KH, Boeke JD. Human transposon tectonics. Cell 2012;149(4):740–752. DOI: 10.1016/j.cell.2012.04.019.
73. Hong Y, Liu N. Transposable elements in health and disease: Molecular basis and clinical implications. Chin Med J (Engl) 2025;138(18):2220–2233. DOI: 10.1097/CM9.0000000000003775.
74. Munoz-Lopez M, Garcia-Perez JL. DNA transposons: Nature and applications in genomics. Curr Genomics 2010;11(2):115–128. DOI: 10.2174/138920210790886871.
75. Iwasaki YW, Shoji K, Nakagwa S, et al. Transposon-host arms race: A saga of genome evolution. Trends Genet 2025;41(5):369–389. DOI: 10.1016/j.tig.2025.01.009.
76. Skipper KA, Andersen PR, Sharma N, et al. DNA transposon-based gene vehicles – Scenes from an evolutionary drive. J Biomed Sci 2013;20(1):92. DOI: 10.1186/1423-0127-20-92.
77. Fan Y, Wang W, Ma G, et al. Patterns of insertion and deletion in Mammalian genomes. Curr Genomics 2007;8(6):370–378. DOI: 10.2174/138920207783406479.
78. Giani AM, Gallo GR, Gianfranceschi L, et al. Long walk to genomics: History and current approaches to genome sequencing and assembly. Comput Struct Biotechnol J 2020;18:9–19. DOI: 10.1016/j.csbj.2019.11.002.
79. Britten RJ, Rowen L, Williams J, et al. Majority of divergence between closely related DNA samples is due to indels. Proc Natl Acad Sci U S A 2003;100(8):4661–4665. DOI: 10.1073/pnas.0330964100.

80. Jiang Y, Wang Y, Brudno M. PRISM: Pair-read informed split-read mapping for base-pair level detection of insertion, deletion and structural variants. Bioinformatics 2012;28(20):2576–2583. DOI: 10.1093/bioinformatics/bts484.
81. Trost B, Walker S, Wang Z, et al. A comprehensive workflow for read depth-based identification of copy-number variation from whole-genome sequence data. Am J Hum Genet 2018;102(1):142–155. DOI: 10.1016/j.ajhg.2017.12.007.
82. Cheng Y, Nandi SP, Culibrk L, et al. Improved mutation detection in duplex sequencing data with sample-specific error profiles. bioRxiv 2025.07.13.664565. DOI: 10.1101/2025.07.13.664565.
83. Gonzalez-Perez A, Mustonen V, Reva B, et al. Computational approaches to identify functional genetic variants in cancer genomes. Nat Methods 2013;10(8):723–729. DOI: 10.1038/nmeth.2562.
84. Barton HJ, Zeng K. The impact of natural selection on short insertion and deletion variation in the great tit genome. Genome Biol Evol 2019;11(6):1514–1524. DOI: 10.1093/gbe/evz068.
85. Taylor MS, Ponting CP, Copley RR. Occurrence and consequences of coding sequence insertions and deletions in Mammalian genomes. Genome Res 2004;14(4):555–566. DOI: 10.1101/gr.1977804.
86. Redelings BD, Holmes I, Lunter G, et al. Insertions and deletions: Computational methods, evolutionary dynamics, and biological applications. Mol Biol Evol 2024;41(9):msae177. DOI: 10.1093/molbev/msae177.
87. Savino S, Desmet T, Franceus J. Insertions and deletions in protein evolution and engineering. Biotechnol Adv 2022;60:108010. DOI: 10.1016/j.biotechadv.2022.108010.
88. Pos O, Radvanszky J, Buglyo G, et al. DNA copy number variation: Main characteristics, evolutionary significance, and pathological aspects. Biomed J 2021;44(5):548–559. DOI: 10.1016/j.bj.2021.02.003.
89. Fay JC, Wittkopp PJ. Evaluating the role of natural selection in the evolution of gene regulation. Heredity (Edinb) 2008;100(2):191–199. DOI: 10.1038/sj.hdy.6801000.
90. Orton RW, Hamilton PK, Frasier TR. Genomic evidence for the purging of deleterious genetic variation in the endangered north atlantic right whale. Evol Appl 2024;17(12):e70055. DOI: 10.1111/eva.70055.
91. Williams LE, Wernegreen JJ. Sequence context of indel mutations and their effect on protein evolution in a bacterial endosymbiont. Genome Biol Evol 2013;5(3):599–605. DOI: 10.1093/gbe/evt033.
92. Lin M, Whitmire S, Chen J, et al. Effects of short indels on protein structure and function in human genomes. Sci Rep 2017;7(1):9313. DOI: 10.1038/s41598-017-09287-x.
93. Biller P. Modeling the evolution of ultraconserved elements by indels. Mol Biol Evol 2025;42(12):msaf299. DOI: 10.1093/molbev/msaf299.
94. Nair J, Maheshwari A. Epigenetics in necrotizing enterocolitis. Curr Pediatr Rev 2021;17(3):172–184. DOI: 10.2174/1573396317666210421110608.
95. Gross AM, Ajay SS, Rajan V, et al. Copy-number variants in clinical genome sequencing: Deployment and interpretation for rare and undiagnosed disease. Genet Med 2019;21(5):1121–1130. DOI: 10.1038/s41436-018-0295-y.
96. Smith SD, Kawash JK, Grigoriev A. GROM-RD: Resolving genomic biases to improve read depth detection of copy number variants. PeerJ 2015;3:e836. DOI: 10.7717/peerj.836.
97. Yoon S, Xuan Z, Makarov V, et al. Sensitive and accurate detection of copy number variants using read depth of coverage. Genome Res 2009;19(9):1586–1592. DOI: 10.1101/gr.092981.109.
98. Zhao M, Wang Q, Wang Q, et al. Computational tools for copy number variation (CNV) detection using next-generation sequencing data: Features and perspectives. BMC Bioinformatics 2013;14(Suppl 11):S1. DOI: 10.1186/1471-2105-14-S11-S1.
99. Magi A, Benelli M, Yoon S, et al. Detecting common copy number variants in high-throughput sequencing data by using JointSLM algorithm. Nucleic Acids Res 2011;39(10):e65. DOI: 10.1093/nar/gkr068.
100. Lauer S, Gresham D. An evolving view of copy number variants. Curr Genet 2019;65(6):1287–1295. DOI: 10.1007/s00294-019-00980-0.
101. de Smith AJ, Walters RG, Froguel P, et al. Human genes involved in copy number variation: Mechanisms of origin, functional effects and implications for disease. Cytogenet Genome Res 2008;123(1–4):17–26. DOI: 10.1159/000184688.
102. Zou XZ, Hu F, Lou H, et al. Phenome-wide analysis of copy number variants in 470,727 UK Biobank genomes. Nature 2026;652:675–685. DOI: 10.1038/s41586-025-10087-x.
103. Tralamazza SM, Gluck-Thaler E, Feurtey A, et al. Copy number variation introduced by a massive mobile element facilitates global thermal adaptation in a fungal wheat pathogen. Nat Commun 2024;15(1):5728. DOI: 10.1038/s41467-024-49913-7.
104. Spealman P, de Santana C, De T, et al. Multilevel gene expression changes in lineages containing adaptive copy number variants. Mol Biol Evol 2025;42(2):msaf005. DOI: 10.1093/molbev/msaf005.
105. Oren M, Barela Hudgell MA, D'Allura B, et al. Short tandem repeats, segmental duplications, gene deletion, and genomic instability in a rapidly diversified immune gene family. BMC Genomics 2016;17(1):900. DOI: 10.1186/s12864-016-3241-x.
106. Fan H, Chu JY. A brief review of short tandem repeat mutation. Genomics Proteomics Bioinformatics 2007;5(1):7–14. DOI: 10.1016/S1672-0229(07)60009-6.
107. Liao X, Zhu W, Zhou J, et al. Repetitive DNA sequence detection and its role in the human genome. Commun Biol 2023;6(1):954. DOI: 10.1038/s42003-023-05322-y.
108. Kelkar YD, Strubczewski N, Hile SE, et al. What is a microsatellite: A computational and experimental definition based upon repeat mutational behavior at A/T and GT/AC repeats. Genome Biol Evol 2010;2:620–635. DOI: 10.1093/gbe/evq046.
109. Garrido-Ramos MA, Plohl M, Satovic-Vuksic E. Satellite DNA genomics: The ongoing story. Int J Mol Sci 2025;26(23):11291. DOI: 10.3390/ijms262311291.
110. Jeffreys AJ, Neil DL, Neumann R. Repeat instability at human minisatellites arising from meiotic recombination. EMBO J 1998;17(14):4147–4157. DOI: 10.1093/emboj/17.14.4147.
111. Ebert G, Steininger A, Weissmann R, et al. Distribution of segmental duplications in the context of higher order chromatin organisation of human chromosome 7. BMC Genomics 2014;15:537. DOI: 10.1186/1471-2164-15-537.
112. Sharp AJ, Locke DP, McGrath SD, et al. Segmental duplications and copy-number variation in the human genome. Am J Hum Genet 2005;77(1):78–88. DOI: 10.1086/431652.
113. Cheung J, Estivill X, Khaja R, et al. Genome-wide detection of segmental duplications and potential assembly errors in the human genome sequence. Genome Biol 2003;4(4):R25. DOI: 10.1186/gb-2003-4-4-r25.
114. Rudd MK, Keene J, Bunke B, et al. Segmental duplications mediate novel, clinically relevant chromosome rearrangements. Hum Mol Genet 2009;18(16):2957–2962. DOI: 10.1093/hmg/ddp233.
115. Vollger MR, Guitart X, Dishuck PC, et al. Segmental duplications and their variation in a complete human genome. Science 2022;376(6588):eabj6965. DOI: 10.1126/science.abj6965.
116. Sasaki M, Lange J, Keeney S. Genome destabilization by homologous recombination in the germ line. Nat Rev Mol Cell Biol 2010;11(3):182–195. DOI: 10.1038/nrm2849.
117. Vervoort L, Dierckxsens N, Pereboom Z, et al. 22q11.2 low copy repeats expanded in the human lineage. Front Genet 2021;12:706641. DOI: 10.3389/fgene.2021.706641.
118. Carvalho CM, Zhang F, Lupski JR. Evolution in health and medicine Sackler colloquium: Genomic disorders: A window into human gene and genome evolution. Proc Natl Acad Sci U S A 2010;107(Suppl 1):1765–1771. DOI: 10.1073/pnas.0906222107.
119. Hastings PJ, Lupski JR, Rosenberg SM, et al. Mechanisms of change in gene copy number. Nat Rev Genet 2009;10(8):551–564. DOI: 10.1038/nrg2593.
120. Yuan B, Wang L, Liu P, et al. CNVs cause autosomal recessive genetic diseases with or without involvement of SNV/indels. Genet Med 2020;22(10):1633–1641. DOI: 10.1038/s41436-020-0864-8.
121. Zhang F, Gu W, Hurles ME, et al. Copy number variation in human health, disease, and evolution. Annu Rev Genomics Hum Genet 2009;10:451–481. DOI: 10.1146/annurev.genom.9.081307.164217.

122. Auwerx C, Lepamets M, Sadler MC, et al. The individual and global impact of copy-number variants on complex human traits. Am J Hum Genet 2022;109(4):647–668. DOI: 10.1016/j.ajhg.2022.02.010.
123. Shaikh TH. Copy number variation disorders. Curr Genet Med Rep 2017;5(4):183–190. DOI: 10.1007/s40142-017-0129-2.
124. Sakina A, Alkan C, Khan A. Population-level gene copy number variations reveal distinct genetic properties of different Malus species. BMC Genomics 2025;26(1):687. DOI: 10.1186/s12864-025-11677-9.
125. Karunarathne P, Schäfer Y, Glushkevich A, et al. Copy number variation: A substrate for plant adaptation and stress response in arabidopsis. bioRxiv 2025.08.15.670553. DOI: 10.1101/2025.08.15.670553.
126. Ramakrishnan S, Gupta V. Trinucleotide Repeat Disorders. StatPearls, Florida, USA: StatPearls Publishing; 2024.
127. Paulson H. Repeat expansion diseases. Handb Clin Neurol 2018;147:105–123. DOI: 10.1016/B978-0-444-63233-3.00009-9.
128. Parniewski P, Staczek P. Molecular mechanisms of TRS instability. In: Parniewski P, Staczek P, editors. Madame Curie Bioscience Database [Internet]. Austin, Texas, USA: Landes Bioscience; 2013. pp. 1–13.
129. Polleys EJ, House NCM, Freudenreich CH. Role of recombination and replication fork restart in repeat instability. DNA Repair (Amst) 2017;56:156–165. DOI: 10.1016/j.dnarep.2017.06.018.
130. Viguera E, Canceill D, Ehrlich SD. Replication slippage involves DNA polymerase pausing and dissociation. EMBO J 2001;20(10):2587–2595. DOI: 10.1093/emboj/20.10.2587.
131. Jankowski C, Nasar F, Nag DK. Meiotic instability of CAG repeat tracts occurs by double-strand break repair in yeast. Proc Natl Acad Sci U S A 2000;97(5):2134–2139. DOI: 10.1073/pnas.040460297.
132. Guo J, Chen L, Li GM. DNA mismatch repair in trinucleotide repeat instability. Sci China Life Sci 2017;60(10):1087–1092. DOI: 10.1007/s11427-017-9186-7.
133. Khristich AN, Mirkin SM. On the wrong DNA track: Molecular mechanisms of repeat-mediated genome instability. J Biol Chem 2020;295(13):4134–4170. DOI: 10.1074/jbc.REV119.007678.
134. Takeuchi T, Nagai Y. Protein misfolding and aggregation as a therapeutic target for polyglutamine diseases. Brain Sci 2017;7(10):128. DOI: 10.3390/brainsci7100128.
135. Xi Z, Zinman L, Moreno D, et al. Hypermethylation of the CpG island near the G4C2 repeat in ALS with a C9orf72 expansion. Am J Hum Genet 2013;92(6):981–989. DOI: 10.1016/j.ajhg.2013.04.017.
136. Zhang N, Ashizawa T. RNA toxicity and foci formation in microsatellite expansion diseases. Curr Opin Genet Dev 2017;44:17–29. DOI: 10.1016/j.gde.2017.01.005.
137. Tan H, Xu Z, Jin P. Role of noncoding RNAs in trinucleotide repeat neurodegenerative disorders. Exp Neurol 2012;235(2):469–475. DOI: 10.1016/j.expneurol.2012.01.019.
138. Gene silencing in fragile X syndrome explained: Researchers pinpoint how excessive nucleotide repeats shut down protein production. Am J Med Genet A 2014;164A(6):vii–viii. DOI: 10.1002/ajmg.a.36607.
139. Singh RK, Cooper TA. Pre-mRNA splicing in disease and therapeutics. Trends Mol Med 2012;18(8):472–482. DOI: 10.1016/j.molmed.2012.06.006.
140. Anna A, Monika G. Splicing mutations in human genetic disorders: Examples, detection, and confirmation. J Appl Genet 2018;59(3):253–268. DOI: 10.1007/s13353-018-0444-7.
141. Riolo G, Cantara S, Ricci C. What's wrong in a jump? Prediction and validation of splice site variants. Methods Protoc 2021;4(3):62. DOI: 10.3390/mps4030062.
142. Weisschuh N, Buena-Atienza E, Wissinger B. Splicing mutations in inherited retinal diseases. Prog Retin Eye Res 2021;80:100874. DOI: 10.1016/j.preteyeres.2020.100874.
143. Nagar P, Islam MR, Rahman MA. Nonsense-mediated mRNA decay as a mediator of tumorigenesis. Genes (Basel) 2023;14(2):357. DOI: 10.3390/genes14020357.
144. Kadri NK, Mapel XM, Pausch H. The intronic branch point sequence is under strong evolutionary constraint in the bovine and human genome. Commun Biol 2021;4(1):1206. DOI: 10.1038/s42003-021-02725-7.
145. Bartonek L, Braun D, Zagrovic B. Frameshifting preserves key physicochemical properties of proteins. Proc Natl Acad Sci U S A 2020;117(11):5907–5912. DOI: 10.1073/pnas.1911203117.
146. Scacheri CA, Scacheri PC. Mutations in the noncoding genome. Curr Opin Pediatr 2015;27(6):659–664. DOI: 10.1097/MOP.0000000000000283.
147. Taher L, Narlikar L, Ovcharenko I. Identification and computational analysis of gene regulatory elements. Cold Spring Harb Protoc 2015;2015(1):pdb.top083642. DOI: 10.1101/pdb.top083642.
148. Song J, Yang P, Chen C, et al. Targeting epigenetic regulators as a promising avenue to overcome cancer therapy resistance. Signal Transduct Target Ther 2025;10(1):219. DOI: 10.1038/s41392-025-02266-z.
149. Santpere G. Genetic variation in transcription factor binding sites. Int J Mol Sci 2023;24(5):5038. DOI: 10.3390/ijms24055038.
150. Jia X, He X, Huang C, et al. Protein translation: Biological processes and therapeutic strategies for human diseases. Signal Transduct Target Ther 2024;9(1):44. DOI: 10.1038/s41392-024-01749-9.
151. Burssed B, Zamariolli M, Bellucco FT, et al. Mechanisms of structural chromosomal rearrangement formation. Mol Cytogenet 2022;15(1):23. DOI: 10.1186/s13039-022-00600-6.
152. Schmitz M, Querques I. DNA on the move: Mechanisms, functions and applications of transposable elements. FEBS Open Bio 2024;14(1):13–22. DOI: 10.1002/2211-5463.13743.
153. Chiang C, Scott AJ, Davis JR, et al. The impact of structural variation on human gene expression. Nat Genet 2017;49(5):692–699. DOI: 10.1038/ng.3834.
154. Pande S, Dawood M, Grochowski CM. Structural variants: Mechanisms, mapping, and interpretation in human genetics. Genes (Basel) 2025;16(8):905. DOI: 10.3390/genes16080905.
155. Abel HJ, Duncavage EJ. Detection of structural DNA variation from next generation sequencing data: A review of informatic approaches. Cancer Genet 2013;206(12):432–440. DOI: 10.1016/j.cancergen.2013.11.002.
156. Kirkpatrick M, Barton N. Chromosome inversions, local adaptation and speciation. Genetics 2006;173(1):419–434. DOI: 10.1534/genetics.105.047985.
157. Kuzminov A. Homologous recombination-experimental systems, analysis, and significance. EcoSal Plus 2011;4(2). DOI: 10.1128/ecosalplus.7.2.6.
158. Parks MM, Lawrence CE, Raphael BJ. Detecting non-allelic homologous recombination from high-throughput sequencing data. Genome Biol 2015;16(1):72. DOI: 10.1186/s13059-015-0633-1.
159. Otto SP, Payseur BA. Crossover interference: Shedding light on the evolution of recombination. Annu Rev Genet 2019;53:19–44. DOI: 10.1146/annurev-genet-040119-093957.
160. Hu H, Scheben A, Wang J, et al. Unravelling inversions: Technological advances, challenges, and potential impact on crop breeding. Plant Biotechnol J 2024;22(3):544–554. DOI: 10.1111/pbi.14224.
161. Kirkpatrick M. How and why chromosome inversions evolve. PLoS Biol 2010;8(9):e1000501. DOI: 10.1371/journal.pbio.1000501.
162. Ramsden DA, Nussenzweig A. Mechanisms driving chromosomal translocations: Lost in time and space. Oncogene 2021;40(25):4263–4270. DOI: 10.1038/s41388-021-01856-9.
163. Lieber MR. Warner-Lambert/Parke-Davis award lecture. Pathological and physiological double-strand breaks: Roles in cancer, aging, and the immune system. Am J Pathol 1998;153(5):1323–1332. DOI: 10.1016/s0002-9440(10)65716-1.
164. Cannan WJ, Pederson DS. Mechanisms and consequences of double-strand DNA break formation in chromatin. J Cell Physiol 2016;231(1):3–14. DOI: 10.1002/jcp.25048.
165. Qu W, Liu C, Xu YT, et al. The formation and repair of DNA double-strand breaks in mammalian meiosis. Asian J Androl 2021;23(6):572–579. DOI: 10.4103/aja202191.
166. Greinert R, Volkmer B, Henning S, et al. UVA-induced DNA double-strand breaks result from the repair of clustered oxidative DNA damages. Nucleic Acids Res 2012;40(20):10263–10273. DOI: 10.1093/nar/gks824.

167. Nishino T, Morikawa K. Structure and function of nucleases in DNA repair: Shape, grip and blade of the DNA scissors. Oncogene 2002;21(58):9022–9032. DOI: 10.1038/sj.onc.1206135.
168. Mao Z, Bozzella M, Seluanov A, et al. DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells. Cell Cycle 2008;7(18):2902–2906. DOI: 10.4161/cc.7.18.6679.
169. Collier LS, Largaespada DA. Transposable elements and the dynamic somatic genome. Genome Biol 2007;8(Suppl 1):S5. DOI: 10.1186/gb-2007-8-s1-s5.
170. Puig M, Casillas S, Villatoro S, et al. Human inversions and their functional consequences. Brief Funct Genomics 2015;14(5):369–379. DOI: 10.1093/bfgp/elv020.
171. Langie SA, Koppen G, Desaulniers D, et al. Causes of genome instability: The effect of low dose chemical exposures in modern society. Carcinogenesis 2015;36(Suppl 1):S61–S88. DOI: 10.1093/carcin/bgv031.
172. Hosea R, Hillary S, Naqvi S, et al. The two sides of chromosomal instability: Drivers and brakes in cancer. Signal Transduct Target Ther 2024;9(1):75. DOI: 10.1038/s41392-024-01767-7.
173. Siri SO, Martino J, Gottifredi V. Structural chromosome instability: Types, origins, consequences, and therapeutic opportunities. Cancers (Basel) 2021;13(12):3056. DOI: 10.3390/cancers13123056.
174. Wilhelm T, Said M, Naim V. DNA replication stress and chromosomal instability: Dangerous liaisons. Genes (Basel) 2020;11(6):642. DOI: 10.3390/genes11060642.
175. Canoy RJ, Shmakova A, Karpukhina A, et al. Factors that affect the formation of chromosomal translocations in cells. Cancers (Basel) 2022;14(20):5110. DOI: 10.3390/cancers14205110.
176. Nambiar M, Raghavan SC. How does DNA break during chromosomal translocations? Nucleic Acids Res 2011;39(14):5813–5825. DOI: 10.1093/nar/gkr223.
177. Agarwal S, Tafel AA, Kanaar R. DNA double-strand break repair and chromosome translocations. DNA Repair (Amst) 2006;5(9–10):1075–1081. DOI: 10.1016/j.dnarep.2006.05.029.
178. Mladenova V, Mladenov E, Stuschke M, et al. DNA damage clustering after ionizing radiation and consequences in the processing of chromatin breaks. Molecules 2022;27(5):1540. DOI: 10.3390/molecules27051540.
179. Weber GF. DNA damaging drugs. Mol Cancer Ther 2014;8(1):9–112. DOI: 10.1007/978-3-319-13278-5_2.
180. Yakkala PA, Penumallu NR, Shafi S, et al. Prospects of topoisomerase inhibitors as promising anti-cancer agents. Pharmaceuticals (Basel) 2023;16(10):1456. DOI: 10.3390/ph16101456.
181. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: Mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem 2001;1(6):529–539. DOI: 10.2174/1568026013394831.
182. Ewa B, Danuta MS. Polycyclic aromatic hydrocarbons and PAH-related DNA adducts. J Appl Genet 2017;58(3):321–330. DOI: 10.1007/s13353-016-0380-3.
183. Li X, Wu Y. Detecting genomic deletions from high-throughput sequence data with unsupervised learning. BMC Bioinformatics 2023;23(Suppl 8):568. DOI: 10.1186/s12859-023-05139-w.
184. Potapova T, Gorbsky GJ. The consequences of chromosome segregation errors in mitosis and meiosis. Biology (Basel) 2017;6(1):12. DOI: 10.3390/biology6010012.
185. Zeman MK, Cimprich KA. Causes and consequences of replication stress. Nat Cell Biol 2014;16(1):2–9. DOI: 10.1038/ncb2897.
186. Anand RP, Tsaponina O, Greenwell PW, et al. Chromosome rearrangements via template switching between diverged repeated sequences. Genes Dev 2014;28(21):2394–2406. DOI: 10.1101/gad.250258.114.
187. Birchler JA, Yang H. The multiple fates of gene duplications: Deletion, hypofunctionalization, subfunctionalization, neofunctionalization, dosage balance constraints, and neutral variation. Plant Cell 2022;34(7):2466–2474. DOI: 10.1093/plcell/koac076.
188. Maheshwari A, Jilling T. Major chromosomal abnormalities and necrotizing enterocolitis: Is there a link? Newborn (Clarksville, Md) 2022;1(2):233–237. DOI: 10.5005/jp-journals-11002-0032.
189. Rodgers K, McVey M. Error-prone repair of DNA double-strand breaks. J Cell Physiol 2016;231(1):15–24. DOI: 10.1002/jcp.25053.
190. Ejaz U, Dou Z, Yao PY, et al. Chromothripsis: An emerging crossroad from aberrant mitosis to therapeutic opportunities. J Mol Cell Biol 2024;16(4):mjae016. DOI: 10.1093/jmcb/mjae016.
191. Ly P, Cleveland DW. Rebuilding chromosomes after catastrophe: Emerging mechanisms of chromothripsis. Trends Cell Biol 2017;27(12):917–930. DOI: 10.1016/j.tcb.2017.08.005.
192. Zhang CZ, Leibowitz ML, Pellman D. Chromothripsis and beyond: Rapid genome evolution from complex chromosomal rearrangements. Genes Dev 2013;27(23):2513–2530. DOI: 10.1101/gad.229559.113.
193. Jones MJ, Jallepalli PV. Chromothripsis: Chromosomes in crisis. Dev Cell 2012;23(5):908–917. DOI: 10.1016/j.devcel.2012.10.010.
194. Gamba BF, Richieri-Costa A, Costa S, et al. Chromothripsis with at least 12 breaks at 1p36.33–p35.3 in a boy with multiple congenital anomalies. Mol Genet Genomics 2015;290(6):2213–2216. DOI: 10.1007/s00438-015-1072-0.
195. Kondratick CM, Washington MT, Spies M. Making choices: DNA replication fork recovery mechanisms. Semin Cell Dev Biol 2021;113:27–37. DOI: 10.1016/j.semcdb.2020.10.001.
196. Saxena S, Zou L. Hallmarks of DNA replication stress. Mol Cell 2022;82(12):2298–2314. DOI: 10.1016/j.molcel.2022.05.004.
197. Kim C, Hong S, Ma SH, et al. Replication stress-induced nuclear hypertrophy alters chromatin topology and impacts cancer cell fitness. Proc Natl Acad Sci U S A 2025;122(37):e2424709122. DOI: 10.1073/pnas.2424709122.
198. Shao DD, Kriz AJ, Snellings DA, et al. Advances in single-cell DNA sequencing enable insights into human somatic mosaicism. Nat Rev Genet 2025;26(11):761–774. DOI: 10.1038/s41576-025-00832-3.
199. Alterovitz G, Dean D, Goble C, et al. Enabling precision medicine via standard communication of HTS provenance, analysis, and results. PLoS Biol 2018;16(12):e3000099. DOI: 10.1371/journal.pbio.3000099.
200. Donohue KE, Gooch C, Katz A, et al. Pitfalls and challenges in genetic test interpretation: An exploration of genetic professionals experience with interpretation of results. Clin Genet 2021;99(5):638–649. DOI: 10.1111/cge.13917.
201. Das S, Biswas NK, Basu A. Mapinsights: Deep exploration of quality issues and error profiles in high-throughput sequence data. Nucleic Acids Res 2023;51(14):e75. DOI: 10.1093/nar/gkad539.
202. Szymanski P, Markowicz M, Mikiciuk-Olasik E. Adaptation of high-throughput screening in drug discovery-toxicological screening tests. Int J Mol Sci 2012;13(1):427–452. DOI: 10.3390/ijms13010427.
203. Maheshwari A, Huisman TAGM. Artificial intelligence in newborn medicine. Newborn (Clarksville, Md) 2024;3(2):96–110. DOI: 10.5005/jp-journals-11002-0095.

REVIEW ARTICLE

The Multimodal Imperative: Reengineering Patient and Family Communication for Safety, Equity, and Understanding

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Abstract

Background: Effective patient and family–clinician communication is fundamental to safe, high-quality healthcare. Communication failures contribute to preventable harm, non-adherence, avoidable healthcare utilization, and medical liability. Yet clinical communication continues to rely heavily on brief verbal exchanges and text-based materials that may be poorly matched to differences in health literacy, language, cognitive load, sensory abilities, and the capacity of patients and families to process information during illness. This limitation is particularly important when patients cannot independently receive, interpret, or act upon medical information and families or caregivers become essential communication partners. A multimodal approach that communicates information through complementary verbal, written, visual, audiovisual, interactive, and digitally enabled formats may provide a more adaptable model for patient- and family-centered clinical communication.

Objective: This review examines the mechanisms and consequences of communication failure in contemporary healthcare and evaluates multimodal strategies for improving comprehension, information retention, participation in care, adherence, clinical safety, health equity, and healthcare efficiency.

Methods: We synthesized evidence from patient-safety research, malpractice analyses, health-economic studies, cognitive science, health-literacy research, and digital-health literature. Evidence included randomized controlled trials (RCTs), systematic reviews, meta-analyses, observational studies, and legal datasets examining communication and communication-related interventions across inpatient and outpatient settings.

Results: Communication failures are associated with adverse events, medication and treatment errors, non-adherence, avoidable readmissions, prolonged healthcare utilization, and malpractice claims. Limited health literacy, cognitive overload, linguistic and cultural differences, sensory and accessibility barriers, and disparities in digital access can further compromise comprehension. Established approaches, including plain language, teach-back, professional interpretation, accessible written materials, and shared decision-making, provide a clinical foundation for effective communication. Visual aids, pictograms, infographics, and audiovisual education can complement these approaches and improve comprehension, recall, engagement, and selected behavioral and clinical outcomes. Emerging technologies, including generative artificial intelligence (AI), conversational systems, digital human avatars, and immersive technologies, may further enable personalization, scalability, linguistic adaptation, and interactive communication, although their clinical effectiveness, safety, accessibility, and economic value require continued evaluation.

Conclusion: Patient- and family-centered communication should be regarded as a clinical and patient-safety function rather than an ancillary component of care. We propose a Multimodal Communication Principle in which the communication strategy is matched to the patient or family, the principal communication barrier, the clinical context, and the informational task, with complementary modalities combined when a single approach is insufficient. Thoughtful integration of established and emerging modalities may improve comprehension, safety, equity, and healthcare efficiency, provided that technological innovation remains clinically validated, accessible, and centered on human communication.

Keywords: Artificial intelligence Avatar communication, Digital health, Health equity, Health literacy, Healthcare communication, Infographics, Multimodal communication, Patient communication, Visual communication.

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Key Points

1. Orientation: Effective patient–clinician communication is a core component of safe, high-quality healthcare, yet conventional verbal and text-based approaches may not adequately meet the diverse informational needs of patients.
2. Clinical Premise: Communication effectiveness is shaped by health literacy, language, cognitive load, sensory abilities, digital access, illness-related stress, and the complexity of the information being conveyed.
3. Conceptual Advancement: We propose a *Multimodal Communication Principle*, in which effective communication depends on (a) identifying the principal communication barrier(s), (b) matching the communication modality to the patient, clinical context, and informational task, and (c) combining complementary modalities when a single approach is insufficient.

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4. Mechanistic Explanation: Visual, audiovisual, interactive, and digitally enabled approaches can complement verbal and written communication by reducing cognitive demands, reinforcing information through multiple channels, enabling repetition, and adapting information to individual needs.
5. Clinical Relevance: Appropriately selected multimodal strategies may strengthen medication instruction, discharge education, informed consent, risk communication, chronic disease self-management, and other information-intensive clinical encounters.
6. Translational Implications: The future of patient communication should be patient-first and modality-appropriate rather than digital-first, video-first, or artificial intelligence (AI)-first, integrating established and emerging approaches while preserving accessibility, equity, privacy, clinical accuracy, and meaningful human interaction.

Introduction

Effective patient and family–clinician communication is strongly associated with understanding, participation in care, decision-making, satisfaction, and psychosocial well-being and, particularly in neonatal care, may also influence infant and family outcomes.[1–3] In the neonatal intensive care unit (NICU), where the patient cannot participate directly in communication, parents and families become essential recipients, interpreters, and participants in clinical information exchange and decision-making. The traditional clinical encounter is a brief, time-constrained verbal exchange followed by text-heavy documentation. Such clinician-directed communication may implicitly assume that patients or families can receive, comprehend, retain, and act upon complex information despite illness, stress, and cognitive burden. Many parents misunderstand medication instructions at discharge. This paternalistic model assumes a passive, stress-free patient/patient family who fully understands instructions. Many parents misunderstand medication instructions at discharge.[4] Poor understanding of perioperative instructions delays complication recognition and increases readmissions.[5] Hence, there is a growing view that medical communication needs to evolve from static formats to dynamic, multimodal approaches using visuals, video, and AI.[6] This review analyzes communication failures, evaluates modern interventions, and proposes a framework for multimodal strategies.

Quantifying the Adverse Impact

Adverse events affect approximately 1 in 20 patients, often resulting in harm.[7] Safety incidents range from planning errors to death.[8–10] The Joint Commission reported that poor communication contributes to over 60% of all hospital adverse events.[9] Poor communication is linked to a 19% higher risk of non-adherence to treatment and contributes to 24% of safety incidents.[11–13] These include omission, commission, and transmission errors. Collectively, these failures contribute to misdiagnosis, delays, medication errors, and wrong-site surgery.[10]

The Malpractice Lens: Legal and Forensic Evidence

Communication failures frequently result in litigation. Two landmark reports of over 46,000 malpractice cases found that 30% were cited for miscommunication failures, with almost 2,000 preventable deaths.[11] A more recent analysis reported that this had risen to 49%.[13] Malpractice data provide an additional perspective on where communication failures occur, although claims data should not be interpreted as a direct measure of the prevalence or causation of communication-related harm.

The Joint Commission found that 80% of serious medical errors were the result of miscommunication during patient handover.[12] Patient handover is a high-risk moment where critical information is often lost. These failures occur across all disciplines, reflecting a systemic issue.

The Swiss Cheese Model of Communication

The Swiss Cheese Model is a systems-based framework for understanding how errors and adverse events occur despite the presence of multiple safeguards.[14,15] The model conceptualizes a healthcare system as a series of defensive layers, analogous to slices of Swiss cheese. Each layer provides protection but also contains vulnerabilities – the metaphorical "holes" created by human limitations, organizational conditions, process failures, or other weaknesses.[16] An adverse event can occur when vulnerabilities in several layers temporarily align, creating a pathway through which an error can pass from its origin to the patient. Importantly, the model shifts attention away from searching for a single point of failure and toward understanding how multiple, individually incomplete defenses interact within a complex system.[17,18]

Applied to patient communication, these defensive layers can be considered at the system, provider, communication-modality, and patient levels. At the system level, workload, time pressure, staffing constraints, fragmented workflows, poorly integrated information systems, and organizational hierarchies can restrict the time and opportunity available for effective communication.[1,19] At the provider level, differences in communication skills, inadequate training, inconsistent terminology, reliance on medical jargon, assumptions regarding patient understanding, and the absence of standardized communication processes can create additional gaps.[12,20,21] At the communication-modality level, dependence on a single format, such as a brief verbal explanation or complex written instructions, may fail when that format is poorly matched to the information being conveyed or to the patient's needs.[22,23] At the patient level, limited health literacy, language differences, sensory or cognitive limitations, illness-related stress, fatigue, pain, and unfamiliarity with medical concepts can impair comprehension, recall, and the ability to act on information.[24,25]

These vulnerabilities may interact rather than operate independently. A time-pressured clinician may, for example, provide a complex verbal explanation to a distressed patient with limited health literacy without professional interpretation, visual reinforcement, written information, or confirmation of understanding. Each vulnerability alone may be insufficient to cause harm; when several align, however, clinically important information may be misunderstood, forgotten, or acted upon incorrectly.[1]

Multimodal communication can be understood within this framework as the deliberate creation of complementary defensive layers.[26] Plain-language explanation, professional interpretation, written or visual reinforcement, audiovisual instruction, and teach-back provide partially independent opportunities for information

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to be conveyed, understood, reinforced, and verified. If one communication layer is ineffective, another may interrupt the pathway toward misunderstanding. Multimodal communication, therefore, is not simply the use of multiple media; it can function as a communication-safety architecture designed to reduce the likelihood that vulnerabilities in any single layer will propagate into patient harm.

The Economic Architecture of Failure

Ineffective communication increases costs, prolongs hospital stays, and drives malpractice expenses. Hurtig et al. showed higher mean total cost for malpractice for litigations involving communication breakdown (US$237,600 vs US$154,100).[27]

Direct Costs of Inefficiency

Hospitals in the United States waste over $12 billion annually as a direct result of communication inefficiency among care providers.[28] Even 30 to 50 minutes wasted per patient due to poor communication translates to approximately $728,000 of annual labor cost per hospital.[29] Much of this burden is driven by extended hospitalizations and delayed care.[28]

The Cost of Litigation and Liability

The 7,000 communication-related lawsuits identified by Controlled Risk Insurance Company (CRICO) resulted in an estimated $1.7 billion in malpractice costs.[12]

The Economics of Readmission and Non-adherence

Ineffective communication increases readmissions and non-adherence (~38%).[25] The annual adjusted disease-specific economic cost of non-adherence per person is $949 to $44,190.[30] Poor physician communication increases risk of patient non-adherence by 19%, bearing a higher direct and indirect financial cost burden.[1,25] Ineffective communication is a key factor in financial penalties under contemporary value-based care models like the Hospital Readmissions Reduction Program (HRRP).[31] In the UK, unused medicines cost over £300 million annually due to misunderstanding.[32]

The Physiology of Comprehension: Health Literacy and Cognitive Load

Health literacy is defined as the capacity to obtain, process, and use health information to make appropriate health decisions. It extends beyond reading to applying complex health information. Cognitive load is high during illness, limiting comprehension.

The Prevalence of Limited Health Literacy

Only 23–50% have adequate health literacy across varying units, including general medicine and emergency.[33–35] Age plays a more complex role: Older age reduces intentional non-adherence but increases cognitive barriers.[32] Lower socioeconomic groups demonstrate higher odds of intentional non-adherence (OR = 2.32).[36] In a study of patients with chronic kidney disease, only 27.2% demonstrated adequate health literacy.[37]

The Link Between Literacy and Outcomes

Health literacy affects surgical outcomes, including complications and readmissions.[38] Shahid et al. demonstrated a threefold increased odds of emergency revisits within 90 days in those with inadequate health literacy.[32] Low literacy leads to unintentional non-adherence and medication errors. Patients are more likely to have medication errors (47.7 vs 20.5%) compared to adequate literacy groups.[39]

The Digital Divide as a Barrier

Limited digital access further worsens health-literacy challenges. A systematic review revealed poor digital-health literacy levels among adults using an eHealth Literacy Scale.[34] Older age, lower educational attainment, reduced internet usage, and poor perceived health are determinants of lower digital-health literacy.[33] In one study, only 9.7% of elderly individuals with low health literacy used the internet for health info, compared to 31.9% of those with adequate literacy. Older adults face barriers including low digital skills and security concerns.[35]

The digital divide highlights a central paradox of contemporary patient communication: technologies designed to improve access to information can themselves become barriers when they are poorly matched to the needs, capabilities, or circumstances of the patient.[40,41] Effective communication consequently depends not on technological sophistication, but on selecting and combining modalities that patients can access, understand, and use.[42] This principle provides the foundation for a patient-first, multimodal approach to communication.

Foundational Patient-communication Strategies

Multimodal communication should not be equated with high-technology communication. Plain language, teach-back, professional interpretation and language access, shared decision-making, and accessible written communication form the clinical foundation upon which visual, audiovisual, interactive, and AI-enabled approaches should be added.[43,44] These strategies address different barriers: Plain language reduces unnecessary linguistic complexity; teach-back assesses whether information has been understood; professional interpretation supports communication across language differences; shared decision-making aligns information with patient values and preferences; and accessible formats accommodate sensory, cognitive, and literacy-related needs.[24]

The choice of modality should therefore follow the communication problem rather than the novelty of the technology. When a single modality is insufficient, complementary modalities can be combined. For example, a plain-language verbal explanation can be reinforced by pictograms, a professionally interpreted discussion followed by translated written instructions, or a video supplemented by teach-back.[22] This patient-first approach provides the conceptual bridge from established communication practices to emerging digital technologies (Fig. 1).

Visual Clarity: Infographics and Pictograms

Evidence shows that visual aids, infographics, and pictograms can meaningfully improve patient comprehension, recall, and engagement compared with text-only communication. Visuals enhance pattern recognition and processing. The "picture superiority effect" explains improved recall of images.[45] It has

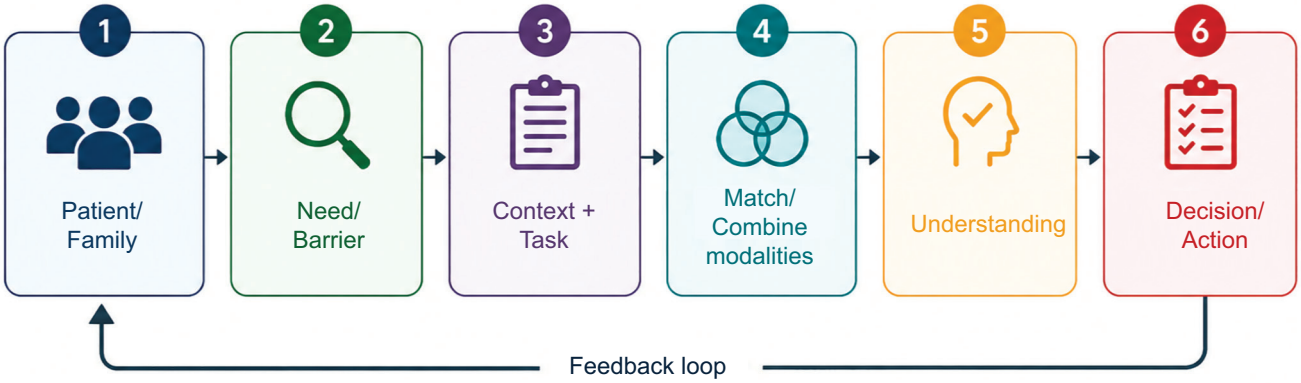

Fig. 1: The Multimodal Communication Principle. We view effective clinical communication as a six-stage process: (1) identifying the patient, family, or caregiver receiving and acting upon information; (2) defining the principal communication need or barrier; (3) considering the clinical context and informational task; (4) selecting or combining complementary communication modalities; (5) confirming understanding, including through teach-back when appropriate; and (6) translating understanding into informed decisions and actions. Communication modalities may include verbal, written, visual, audiovisual, interactive, and AI-enabled approaches, selected according to individual needs and circumstances rather than technological novelty. The feedback loop emphasizes continuous reassessment and adaptation as communication needs, understanding, and clinical circumstances change. The framework prioritizes patient- and family-centered, modality-appropriate communication rather than any single digital, video, or AI-based approach

demonstrated efficacy in mitigating adverse events and reducing associated costs.[27]

The Science of Visual Analogy

Infographics combine text and visuals to explain complex ideas, while pictograms convey single concepts rapidly. They reduce cognitive load by shifting from text processing to visual recognition, improving understanding under stress.[46] Visuals improve risk perception and reduce misinterpretation.[47]

Efficacy in Patient Education

Videos and structured visuals significantly enhance understanding of health information.[48] Sutthiworapon et al. demonstrated superior comprehension metrics in type 2 diabetics using direct-to-mobile visuals compared with traditional pamphlets, highlighting improvements even with chronic disease.[49]

Infographics improve retention, engagement, and satisfaction, with sustained knowledge gains.[50] User satisfaction (4.4/5) and ease of understanding (4.7/5) were high, and 83% would engage with the infographic voluntarily, underscoring the intrinsic motivational value of visual communication. Complementing these findings, both creation and consumption of infographics are associated with enhanced professional skill development, increased learner motivation, and higher overall satisfaction among health sciences trainees.[51]

Bridging the Language Gap

For patients who do not communicate primarily in English, visual communication can be a useful tool that can complement professional interpretation and appropriately translated materials. Pictograms have demonstrated potential to improve the communication of health information and self-management behavior to low-literacy populations across diverse racial and ethnic groups.[52] In transitional care contexts, particularly at hospital discharge, the integration of icons with simplified text has been identified as the most efficient strategy for improving comprehension among older adults.[53] Cultural adaptation is required to ensure accurate interpretation.

Transforming Informed Consent

Informed consent represents one of the most legally and ethically critical aspects of clinical care. Traditional consent forms are often verbose and full of jargon, resulting in poor comprehension. Infographic-based consent tools are emerging as a promising solution. In complex clinical trial settings, patients have co-designed infographics to convey key concepts, including randomization procedures, benefits, and risks.[54] Supplementing or replacing conventional consent documents with simplified fact sheets and infographics can significantly enhance patient understanding and reduce decisional uncertainty.[55]

Video-based Communication

Videos can provide standardized, repeatable information that complements clinician–patient dialogue and allow patients or caregivers to revisit instructions after an encounter. The value is context-dependent: Videos may improve comprehension and retention for selected tasks, but these should not be assumed to be superior to written or verbal communication for every patient or informational need.

Video Discharge Instructions

Discharge is a high-stress period with poor information retention. Video discharge instructions can be a useful take-home expert.

Randomized clinical evidence supports their efficacy, with higher knowledge scores with video discharge instructions compared to written in a military hospital setting.[56] Video improved objective patient comprehension without diminishing the perceived helpfulness of clinical staff.[57] A meta-analysis involving almost 4,000 patients showed that multimedia reduces readmissions (31%), improves adherence (24%), and increases satisfaction (41%).[58]

Multimedia for Medication Adherence

Video is effective for medication instruction and remote adherence monitoring. Direct observation via video is now a viable alternative to in-person supervision. In HIV prevention (PrEP) and tuberculosis treatment, patients used smartphones to record themselves taking medication (Video Directly Observed Therapy, VDOT), achieving comparable adherence rates (87%) to traditional observation.[59]

Educational videos are beneficial in chronic disease self-management. Randomized controlled trials in asthma care showed that video education prevented attacks through self-care ($p = 0.03$) and managing environmental triggers ($p < 0.001$). Printed material is still more effective for rapid recall tasks.[60]

Return on Investment (ROI) and Operational Impact

Our current evidence for the economic and operational effects of video-based patient communication is less mature than that for selected comprehension and educational outcomes. Improvements in engagement or workflow may create operational efficiencies in some settings, but marketing conversion metrics and local implementation reports should not be interpreted yet as proof of healthcare cost savings or ROI.[61]

AI Avatars and Digital Humans: Emerging Communication Technologies

At the present time, AI avatars and digital humans can be viewed as emerging-to-experimental approaches to patient communication. These systems combine synthetic visual or conversational interfaces with generated speech and, in some applications, large language models (LLMs). Their potential advantages include repeatability, scalability, multilingual delivery, and interactive presentation; however, evidence for clinically meaningful improvements in comprehension, trust, safety, equity, or outcomes remains limited and application-dependent.

Human Factors, Trust, and the Uncanny Valley

Early digital avatars sometimes elicited discomfort or mistrust associated with the "uncanny valley," in which near-human appearance or behavior can be perceived as unnatural.[62,63] Human-factor research therefore remains important when evaluating avatar appearance, voice, transparency, and patient acceptance. Some reports that suggest that users may perceive AI-generated advice as trustworthy should be interpreted cautiously, because perceived trustworthiness does not establish clinical accuracy or safety.[64]

Personalization, Voice, and Clinician Likeness

Some avatar systems can synthesize speech, translate content, or reproduce elements of an individual clinician's voice or likeness. These capabilities could support continuity and linguistic adaptation, but clinician replication should not be treated as an established communication intervention. Voice cloning and likeness synthesis introduce additional questions concerning informed consent, authenticity, privacy, data governance, unauthorized reuse, responsibility for generated statements, and the possibility that patients may incorrectly believe they are interacting directly with their clinician.[65]

Accordingly, clinician digital twins and voice-cloned avatars should presently be regarded as emerging-to-experimental technologies requiring prospective clinical validation, explicit governance, transparent disclosure of synthetic content, human oversight, and safeguards against misleading or unauthorized use before routine deployment.

Generative AI and LLMs

Beneath the visual interface of digital avatars lies the cognitive engine of LLMs such as ChatGPT, Claude, and Gemini. These emerging tools have been useful for complementing human instruction and helping simplify, personalize, translate, and enhance interactive explanations of medical information to our patient families. However, the systems are still maturing; our current tools have to be viewed as complementary to clinician communication rather than autonomous, particularly when generated content could influence clinical decisions.

The Power of Simplification

Clinicians often need to translate complex concepts into plain language. Large language models can reduce the reading level and linguistic complexity of patient-facing materials, but improved readability does not by itself establish factual accuracy, completeness, or clinical appropriateness. A study conducted at New York University (NYU) Langone evaluated the ability of multiple LLMs to simplify patient education materials from the American Heart Association, originally written at an 11th-grade reading level. These can easily reduce readability from 11th-grade to ~6th-grade levels.[66]

The Risk of Hallucination

Despite the transformative potential, current LLMs still carry a risk of generating incorrect information ("hallucinations"). One contributing factor is "algorithmic brittleness," where minor changes in the phrasing of a prompt can produce substantially different outputs.[67]

Retrieval-augmented generation (RAG), source-constrained generation, cross-verification, human review, and governance can reduce some risks but do not eliminate them. Clinical deployment therefore requires validation for the intended use, transparency regarding AI involvement, protection of patient information, monitoring for bias and error, and clearly defined human accountability. Regulatory requirements will depend on the function performed and the degree to which an AI system influences clinical care.[68]

Immersive Communication: Virtual Reality (VR)

Within a patient-communication framework, VR is most relevant when it helps patients understand, anticipate, or rehearse a clinical experience. Immersive environments can support procedural preparation, visualization, and selected educational applications, although evidence varies by indication and the technology remains less accessible than conventional communication modalities.

Procedural Preparation and Patient Education

Virtual reality has been evaluated for preprocedural preparation and for reducing anxiety in selected clinical settings. It is viewed as useful in allowing patients to experience or visualize aspects of a procedure or care environment before the event. At Stanford

Health Care, VR is used to transport patients from hospital environments to calming virtual landscapes. This application uses the gate control theory of pain, in which immersive distraction modulates neural pain signals and reduces subjective discomfort. This is very effective with children's burn dressings using the "Smiley Scope" VR device.[69]

Virtual reality has also been utilized in preoperative preparation to reduce anxiety and improve psychological readiness. Patients exposed to virtual walkthroughs of surgical procedures report decreased fear and improved confidence in their care. In broader clinical training applications, VR has demonstrated the ability to enhance surgical performance, with a Harvard Business Review study reporting up to 230% improvement in objective measures of surgical proficiency among trainees.[70,71] However, even though most colleagues at our hospital view the use of VR as promising for reducing the need for analgesic and anxiolytic medications, rehabilitation tools, or surgical-training technology, they still viewed it as outside the principal scope of patient communication considered in this review.

The Information Ecosystem: Social Media and Influencers

Patient communication increasingly occurs within a broader health-information ecosystem in which social media, online communities, influencers, healthcare organizations, and clinicians compete for attention. These channels are not substitutes for patient–clinician communication, but they shape the information, expectations, and misconceptions that patients may bring to clinical encounters.

The Changing Information Environment

Social media has altered how patients encounter and evaluate health information, particularly among younger populations. Influencers and other online sources may acquire substantial perceived credibility and reach, although trust, engagement, and clinical accuracy are distinct constructs. Surveys indicate that 82% of patients are likely to follow health advice from influencers, and 63% report trusting health information sourced from social media personalities.[72] This shift reflects the rise of parasocial relationships–one-sided emotional connections in which followers feel personally connected to influencers. Content disseminated through these channels also demonstrates significantly higher engagement than institutional content, with a reported 45% increase in interaction rates.

A Double-edged Sword

A systematic review on social media influencers' effects on health outcomes found variable effects. Exposure to idealized body imagery significantly negatively impacts body image and mood; however, influencer-driven public health campaigns can promote positive behavior (e.g., hygiene messaging in influenza and COVID-19 contexts).[73] Influencer-delivered flu vaccination reached millions of users and generated overwhelmingly positive engagement (≈94% positive reactions).[74]

Clinicians in the Digital Information Ecosystem

In response to these dynamics, healthcare professionals are increasingly adopting the role of content creators popularly referred to as "Medfluencers." They exemplify clinicians who successfully translate evidence-based information into compelling, accessible social media content.

Clinicians and health systems can use social platforms to disseminate accessible, evidence-based information and respond to misinformation, provided that professional standards, transparency, privacy, and the distinction between general education and individualized medical advice are maintained.[72] The objective should be to improve the quality of the information environment rather than to compete for authority or engagement alone.

Platform Landscape and Integration

The successful deployment of multimodal communication relies on robust digital infrastructure, primarily embedded patient portals. Relevant functions include electronic health record (EHR)-integrated patient portals and specialized patient engagement platforms (PEPs) or learning management systems (LMSs). There is increasing confidence in their use in omnichannel messaging, conversational interfaces, language-support tools, accessibility features, and as mechanisms for delivering validated content at appropriate points in the care pathway.

Emerging Tools

Electronic health record vendors such as Epic (MyChart) and Cerner (Oracle) are key gatekeepers. Epic's research shows that patients with active MyChart accounts were 21.5% less likely to miss appointments.[75] Beyond EHRs, specialized platforms include:

- Simbi.ai: Focuses on voice-based AI for patients with limited literacy;[76]
- TeleVox: Provides omnichannel communication (text, e-mail, voice) for appointment reminders;[77]
- HealthStream and Relias: Function as healthcare-focused LMSs for standardized education.[78]

These solutions reflect a shift toward modular tools that complement the core EHR system.

Integration and Interoperability

Communication tools are more likely to become clinically useful when they are integrated into existing workflows rather than deployed as isolated applications. Bidirectional interoperability, identity management, content governance, accessibility, documentation, and the ability to update or withdraw outdated information are therefore important implementation considerations. Standards such as Fast Healthcare Interoperability Resources (FHIR) may facilitate exchange between systems, but technical interoperability alone does not establish clinical effectiveness or equity.

Strategic Framework and Conclusion

The evidence reviewed supports a transition from communication models that assume uniform patient comprehension toward a patient-first, modality-appropriate approach. The central question is not which technology should replace conventional communication, but whether one particular modality or a combination of modalities will better address communication barriers for families in explaining the clinical context and with specific informational tasks.

Implementation should also reflect the maturity of the supporting evidence. We therefore propose three pragmatic evidence tiers: Established, emerging, and experimental. These categories are not permanent labels; technologies and strategies

Table 1: Evidence maturity and clinical role of multimodal communication strategies

Communication strategy	*Evidence maturity*	*Principal role*	*Important limitations*
Plain language	Established	Reduces linguistic complexity	Must preserve accuracy and essential detail
Teach-back	Established	Confirms understanding and identifies gaps	Requires time and appropriate implementation
Professional interpretation	Established	Addresses language barriers	Availability and workflow integration
Accessible written materials	Established	Reinforces information and permits later review	Literacy, language, vision, and cognitive barriers
Visual aids and pictograms	Established	Reinforce comprehension and recall	Cultural interpretation and design quality
Video and audiovisual education	Established for selected uses	Standardized, repeatable explanation	Not universally superior to other modalities
Patient portals and interactive tools	Established to emerging	Access, reinforcement, and ongoing engagement	Digital access and digital literacy
Generative AI and LLMs	Emerging	Simplification, personalization, translation, interaction	Accuracy, hallucination, bias, privacy, governance
Conversational AI and avatars	Emerging	Interactive and potentially scalable communication	Clinical validation, trust, transparency, privacy
Clinician digital twins and voice cloning	Experimental	Personalized synthetic communication	Consent, authenticity, unauthorized use, accountability
VR	Emerging for selected applications	Procedural preparation and immersive education	Cost, accessibility, tolerability, limited indications

AI, artificial intelligence; LLMs, large language models; VR, virtual reality

may move between tiers as evidence accumulates, and the appropriate classification may vary by clinical use case (Table 1).

Strategic Recommendations for Health Systems

Patient communication must be treated as a core clinical, system-wide function. Key strategies include:

- Mandate Multimodal Communication: Adopt a "video-first" standard as the default to ensure consistent, high-quality information regardless of staffing or time constraints.
- Invest in Clinician Digital Twins: Leverage AI avatar technology to scale the expertise and communication skills of effective clinicians, enabling personalized, high-touch education for large patient populations at minimal marginal cost.
- Redesign Informed Consent: Prioritize comprehension alongside legal protection by integrating infographics, animations, and explanatory videos into workflows, transforming consent into a meaningful ethical exchange.
- Utilize Social Media Strategically: Recognize social media as a clinical information environment. Invest in evidence-based clinician influencers ("medfluencers") to counter misinformation, shape health behaviors, and reestablish institutional credibility.
- Inclusive Design: Ensure all digital communication tools are usable by patients with the lowest levels of health literacy, digital familiarity, and physical ability. Co-design is foundational to equity and safety.

Final Thoughts

Patient communication is a critical clinical intervention, not merely a soft skill. It is the direct lever for measurable improvements in safety, adherence, and outcomes. Embracing a full spectrum of multimodal strategies from simple infographics to AI-driven digital humans will finally close the dangerous gap between what is prescribed and what is truly understood, allowing real healthcare improvement.

Use of AI Systems

The authors did not intentionally use generative AI or AI-assisted technologies to generate or materially modify the scientific content, analysis, interpretation, references, or illustrations in this manuscript, except as specifically disclosed. Routine use of AI-enabled software for word processing, formatting, language checking, reference management, or similar technical functions is not considered substantive AI assistance. The authors reviewed the final manuscript and remain fully responsible for its accuracy, integrity, originality, and content.

Ethics Approval

Ethics approval not required this is a review paper.

Informed Consent Statement

Informed consent was not required, as the study did not involve human participants.

Clinical Trial Registration

Not applicable.

Data Availability

Not applicable.

Artificial Intelligence (AI) Disclosure

During the preparation of this manuscript, the authors used Claude to help with prompting a search literature search strategy

in Perplexity to provide a check literature search for latest research papers relevant to the paper. Gemini was also used solely to improve readability and language and was not used to replace essential researcher tasks or produce scientific insights. Following its use, the authors critically reviewed, verified, and edited all generated content. The authors take full responsibility for the accuracy and integrity of the content of this manuscript.

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Authors' Contributions

Bhaveshkumar Patel: Conceptualization, Investigation, Writing – review and editing, Supervision.
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References

1. Zolnierek KB, Dimatteo MR. Physician communication and patient adherence to treatment: A meta-analysis. Med Care 2009;47(8): 826–834. DOI: 10.1097/MLR.0b013e31819a5acc.
2. Labrie NHM, van Veenendaal NR, Ludolph RA, et al. Effects of parent-provider communication during infant hospitalization in the NICU on parents: A systematic review with meta-synthesis and narrative synthesis. Patient Educ Couns 2021;104(7):1526–1552. DOI: 10.1016/j.pec.2021.04.023.
3. Wreesmann WW, Lorie ES, van Veenendaal NR, et al. The functions of adequate communication in the neonatal care unit: A systematic review and meta-synthesis of qualitative research. Patient Educ Couns 2021;104(7):1505–1517. DOI: 10.1016/j.pec.2020.11.029.
4. Kripalani S, LeFevre F, Phillips CO, et al. Deficits in communication and information transfer between hospital-based and primary care physicians: Implications for patient safety and continuity of care. JAMA 2007;297(8):831–841. DOI: 10.1001/jama.297.8.831.
5. Jencks SF, Williams MV, Coleman EA. Rehospitalizations among patients in the Medicare fee-for-service program. N Engl J Med 2009;360(14):1418–1428. DOI: 10.1056/NEJMsa0803563.
6. McCarthy ML, Zeger SL, Ding R, et al. Crowding delays treatment and lengthens emergency department length of stay, even among high-acuity patients. Ann Emerg Med 2009;54(4):492–503 e4. DOI: 10.1016/j.annemergmed.2009.03.006.
7. Wong A, Su T. Poor communication closely associated with patient safety incidents. Boston, Massachusetts, USA: 2minutemedicine; 2025. Available from: https://www.2minutemedicine.com/poor-communication-closely-associated-with-patient-safety-incidents/. Last accessed on: 11 December 2025.
8. Kohn LT, Corrigan JM, Donaldson MS. To Err is Human: Building a Safer Health System. Health CoQo, Care in America IoM, editors. Washington (DC): National Academies Press; 2000.
9. Chartis. Physical Environment Solutions. McLean, Virginia, USA: Chartis Healthcare Consulting; 2026. Available from: https://www.chartis.com/expertise/physical-environment-solutions. Last accessed on: 15 December 2025.
10. Howick J, Bennett-Weston A, Solomon J, et al. How does communication affect patient safety? Protocol for a systematic review and logic model. BMJ Open 2024;14(5):e085312. DOI: 10.1136/bmjopen-2024-085312.
11. Diaz B. 30% of Malpractice Complaints Involved Communication Failure. Cranbury, New Jersey, USA: The American Journal of Managed Care; 2016. Available from: https://www.ajmc.com/view/30-of-malpractice-complaints-involved-communication-failure?utm. Last accessed on: 15 December 2025.
12. Alder S. Consequences of Poor Communication in Healthcare. Dallas, Texas, USA: The HIPAA Journal; 2022. Available from: https://www.hipaajournal.com/effects-of-poor-communication-in-healthcare/. Last accessed on: 11 December 2025.
13. Humphrey KE, Sundberg M, Milliren CE, et al. Frequency and Nature of Communication and Handoff Failures in Medical Malpractice Claims. J Patient Saf 2022;18(2):130–137. DOI: 10.1097/PTS.0000000000000937.
14. Reason J. Human error: Models and management. BMJ 2000;320(7237):768–770. DOI: 10.1136/bmj.320.7237.768.
15. Stein JE, Heiss K. The Swiss cheese model of adverse event occurrence–Closing the holes. Semin Pediatr Surg 2015;24(6): 278–282. DOI: 10.1053/j.sempedsurg.2015.08.003.
16. Perneger TV. The Swiss cheese model of safety incidents: Are there holes in the metaphor? BMC Health Serv Res 2005;5:71. DOI: 10.1186/1472-6963-5-71.
17. van Beuzekom M, Boer F, Akerboom S, et al. Patient safety: Latent risk factors. Br J Anaesth 2010;105(1):52–59. DOI: 10.1093/bja/aeq135.
18. Nolan TW. System changes to improve patient safety. BMJ 2000;320(7237):771–773. DOI: 10.1136/bmj.320.7237.771.
19. Tipping MD, Forth VE, O'Leary KJ, et al. Where did the day go? A time-motion study of hospitalists. J Hosp Med 2010;5(6):323–328. DOI: 10.1002/jhm.790.
20. Rao JK, Anderson LA, Inui TS, et al. Communication interventions make a difference in conversations between physicians and patients: A systematic review of the evidence. Med Care 2007;45(4):340–349. DOI: 10.1097/01.mlr.0000254516.04961.d5.
21. Schillinger D, Piette J, Grumbach K, et al. Closing the loop: Physician communication with diabetic patients who have low health literacy. Arch Intern Med 2003;163(1):83–90. DOI: 10.1001/archinte.163.1.83.
22. Houts PS, Doak CC, Doak LG, et al. The role of pictures in improving health communication: A review of research on attention, comprehension, recall, and adherence. Patient Educ Couns 2006;61(2):173–190. DOI: 10.1016/j.pec.2005.05.004.
23. Kessels RP. Patients' memory for medical information. J R Soc Med 2003;96(5):219–222. DOI: 10.1177/014107680309600504.
24. Karliner LS, Jacobs EA, Chen AH, et al. Do professional interpreters improve clinical care for patients with limited English proficiency? A systematic review of the literature. Health Serv Res 2007;42(2): 727–754. DOI: 10.1111/j.1475-6773.2006.00629.x.
25. Achterbosch M, Aksoy N, Obeng GD, et al. Clinical and economic consequences of medication nonadherence: A review of systematic reviews. Front Pharmacol 2025;16:1570359. DOI: 10.3389/fphar.2025.1570359.
26. Guo X. Multimodality in language education: Implications of a multimodal affective perspective in foreign language teaching. Front Psychol 2023;14:1283625. DOI: 10.3389/fpsyg.2023.1283625.
27. Hurtig RR, Alper RM, Berkowitz B. The cost of not addressing the communication barriers faced by hospitalized patients. Perspect ASHA Spec Interest Groups 2018;3(12):99–112. DOI: 10.1044/persp3.SIG12.99.
28. Agarwal R, Sands DZ, Schneider JD. Quantifying the economic impact of communication inefficiencies in U.S. hospitals. J Healthc Manag 2010;55(4):265–281; discussion 281–282. PMID: 20812527.
29. Ponemon Institute. Advancing Responsible Information Management. Traverse City, Michigan, USA: Ponemon Institute; 2026. Available from: https://www.ponemon.org/. Last accessed on: 17 December 2025.
30. Cutler RL, Fernandez-Llimos F, Frommer M, et al. Economic impact of medication non-adherence by disease groups: A systematic review. BMJ Open 2018;8(1):e016982. DOI: 10.1136/bmjopen-2017-016982.
31. Psotka MA, Fonarow GC, Allen LA, et al. The hospital readmissions reduction program: Nationwide perspectives and recommendations: A JACC: Heart Failure Position Paper. JACC Heart Fail 2020;8(1):1–11. DOI: 10.1016/j.jchf.2019.07.012.

32. Shahid R, Shoker M, Chu LM, et al. Impact of low health literacy on patients' health outcomes: A multicenter cohort study. BMC Health Serv Res 2022;22(1):1148. DOI: 10.1186/s12913-022-08527-9.
33. Qiu CS, Lunova T, Greenfield G, et al. Determinants of digital health literacy: International cross-sectional study. J Med Internet Res 2025;27:e66631. DOI: 10.2196/66631.
34. Wiener RC, Abuhalimeh BJ. Evaluating adult digital health literacy, 2020–2025: A systematic review. PLOS Digit Health 2025;4(11):e0001075. DOI: 10.1371/journal.pdig.0001075.
35. Emmesjö L, Hallgren J, Gillsjö C. Older adults' digital technology experiences: A qualitative study. BMC Digital Health 2025;3(1):24. DOI: 10.1186/s44247-025-00163-7.
36. Fan ZY, Yang Y, Zhang F. Association between health literacy and mortality: A systematic review and meta-analysis. Arch Public Health 2021;79(1):119. DOI: 10.1186/s13690-021-00648-7.
37. Shi Z, Du X, Li J, et al. Factors influencing digital health literacy among older adults: A scoping review. Front Public Health 2024;12:1447747. DOI: 10.3389/fpubh.2024.1447747.
38. Fan JH, Lyons SA, Goodman MS, et al. Relationship between health literacy and unintentional and intentional medication nonadherence in medically underserved patients with type 2 diabetes. Diabetes Educ 2016;42(2):199–208. DOI: 10.1177/0145721715624969.
39. Lindquist LA, Go L, Fleisher J, et al. Relationship of health literacy to intentional and unintentional non-adherence of hospital discharge medications. J Gen Intern Med 2012;27(2):173–178. DOI: 10.1007/s11606-011-1886-3.
40. Sarkar U, Karter AJ, Liu JY, et al. The literacy divide: Health literacy and the use of an internet-based patient portal in an integrated health system-results from the diabetes study of northern California (DISTANCE). J Health Commun 2010;15 Suppl 2(Suppl 2):183–196. DOI: 10.1080/10810730.2010.499988.
41. Sarkar U, Karter AJ, Liu JY, et al. Social disparities in internet patient portal use in diabetes: Evidence that the digital divide extends beyond access. J Am Med Inform Assoc 2011;18(3):318–321. DOI: 10.1136/jamia.2010.006015.
42. Wilson S, Tolley C, Mc Ardle R, et al. Recommendations to advance digital health equity: A systematic review of qualitative studies. NPJ Digit Med 2024;7(1):173. DOI: 10.1038/s41746-024-01177-7.
43. Okuhara T, Furukawa E, Okada H, et al. Readability of written information for patients across 30 years: A systematic review of systematic reviews. Patient Educ Couns 2025;135:108656. DOI: 10.1016/j.pec.2025.108656.
44. Dunnett J, Holkham J, Trebacz A, et al. Effectiveness and acceptability of interventions to improve readability of patient healthcare materials: A narrative systematic review. Public Health 2025;248:105937. DOI: 10.1016/j.puhe.2025.105937.
45. Curran T, Doyle J. Picture superiority doubly dissociates the ERP correlates of recollection and familiarity. J Cogn Neurosci 2011;23(5):1247–1262. DOI: 10.1162/jocn.2010.21464.
46. Kelly S, Gaytan J. Book Review: The power of infographics: Using pictures to communicate and connect with your audiences by Smiciklas, M. Bus Prof Commun Q 2014;77(2):240–242. DOI: 10.1177/2329490614527821.
47. He S, Chen Y, Xia Y, et al. Visual Harmony: Text-Visual Interplay in Circular Infographics. arXiv [Internet] 2024;1(1):2402.05798. DOI: 10.48550/arXiv.2402.05798
48. Galmarini E, Marciano L, Schulz PJ. The effectiveness of visual-based interventions on health literacy in health care: A systematic review and meta-analysis. BMC Health Serv Res 2024;24(1):718. DOI: 10.1186/s12913-024-11138-1.
49. Sutthiworapon S, Vichitkunakorn P, Choomalee K, et al. Effect of online infographics for enhancing health literacy among patients with type 2 diabetes in primary care unit during the COVID-19 pandemic: A randomized controlled trial. BMC Prim Care 2024;25(1):87. DOI: 10.1186/s12875-024-02335-2.
50. Coyne P, Ely FO, Fiala J, et al. Effectiveness of infographics at disseminating health information during the COVID-19 pandemic. Health Fit J Can 2021;14(4):3–9. DOI: 10.14288/hfjc.v14i4.367.
51. Navarrete-Munoz EM, Valera-Gran D, Garcia-Campos J, et al. Enhancing Evidence-Based Practice Competence and Professional Skills Using Infographics as a Pedagogical Strategy in Health Science Students: Insights from the InfoHealth Project. Eur J Investig Health Psychol Educ 2024;14(4):929–940. DOI: 10.3390/ejihpe14040060.
52. Wang T, Voss JG. Effectiveness of pictographs in improving patient education outcomes: A systematic review. Health Educ Res 2021;36(1):9–40. DOI: 10.1093/her/cyaa046.
53. Choi J. Literature review: Using pictographs in discharge instructions for older adults with low-literacy skills. J Clin Nurs 2011;20 (21–22):2984–2996. DOI: 10.1111/j.1365-2702.2011.03814.x.
54. O'Grady HK, Bhimani Z, Dalziel S, et al. Co-designing and pilot testing an infographic to support patients/families through the REMAP-CAP consent process: A mixed-methods study protocol. Pilot Feasibility Stud 2023;9(1):58. DOI: 10.1186/s40814-023-01290-6.
55. Taylor HA, Washington D, Wang NY, et al. Randomized comparison of two interventions to enhance understanding during the informed consent process for research. Clin Trials 2021;18(4):466–476. DOI: 10.1177/17407745211009529.
56. Wilkin ZL. Effects of video discharge instructions on patient understanding: A prospective, randomized trial. Adv Emerg Nurs J 2020;42(1):71–78. DOI: 10.1097/TME.0000000000000279.
57. Maffei JB, Song JJ, Saffati G, et al. Video discharge instructions increase patient understanding for office-based procedures: A randomized controlled study. Transl Androl Urol 2025;14(3):678–684. DOI: 10.21037/tau-24-501.
58. Hoek AE, Anker SCP, van Beeck EF, et al. Patient Discharge Instructions in the Emergency Department and Their Effects on Comprehension and Recall of Discharge Instructions: A Systematic Review and Meta-analysis. Ann Emerg Med 2020;75(3):435–444. DOI: 10.1016/j.annemergmed.2019.06.008.
59. M MAD, A JA, A MA, et al. Use of Smartphone-Based Video Directly Observed Therapy to Increase Tuberculosis Medication Adherence: An Interventional Study. Galen Med J 2023;12:e3067. DOI: 10.31661/gmj.v12i.3067.
60. Sekandi JN, Buregyeya E, Zalwango S, et al. Video directly observed therapy for supporting and monitoring adherence to tuberculosis treatment in Uganda: A pilot cohort study. ERJ Open Res 2020;6(1). DOI: 10.1183/23120541.00175-2019.
61. Bruce G. 'The ROI is absolutely there': Smart rooms at Valley Health System. Chicago, Illinois, USA: Becker's Health IT; 2025. Available from: https://www.beckershospitalreview.com/healthcare-information-technology/innovation/the-roi-is-absolutely-there-smart-rooms-at-valley-health-system/. Last accessed on: 11 December 2025.
62. Haider SA, Prabha S, Gomez-Cabello CA, et al. Artificial intelligence physician avatars for patient education: A pilot study. J Clin Med 2025;14(23). DOI: 10.3390/jcm14238595.
63. Ashrafi N, Graf P, Marquardt M, et al. Avatar Appearance Beyond Pixels - User Ratings and Avatar Preferences Within Health Applications. In: Duffy VG, editor. Digital Human Modeling and Applications in Health, Safety, Ergonomics and Risk Management HCII 2025 Lecture Notes in Computer Science, vol 15793. Cham, Switzerland: Springer Publications; 2025.
64. Shekar S, Pataranutaporn P, Sarabu C, et al. People over trust AI-generated medical responses and view them to be as valid as doctors, despite low accuracy. ArXiv 2024(1):1–24. DOI: 10.48550/arXiv.2408.15266.
65. Port t. How AI Avatars Are Revolutionizing Patient Health Education. Newark, Delaware, USA: 5th Port; 2025. Available from: https://

www.5thport.com/how-ai-powered-avatars-are-revolutionizing-patient-health-education/. Last accessed on: 11 December 2025.
66. Will J, Gupta M, Zaretsky J, et al. Enhancing the readability of online patient education materials using large language models: Cross-sectional study. J Med Internet Res 2025;27:e69955. DOI: 10.2196/69955.
67. Draelos RL, Afreen S, Blasko B, et al. Large language models provide unsafe answers to patient-posed medical questions. NPJ Digit Med 2026;9(1). DOI: 10.1038/s41746-026-02428-5.
68. Jenko S, Papadopoulou E, Kumar V, et al. Artificial Intelligence in Healthcare: How to Develop and Implement Safe, Ethical and Trustworthy AI Systems. AI 2025;6(6):116. DOI: 10.3390/ai6060116.
69. Storey K, Dimanopoulos TA, Plummer K, et al. Acceptability and usability of smiley scope virtual reality for paediatric pain management during burn procedures: Perspectives of patients, carers and clinicians. J Adv Nurs 2025;81(3):1568–1582. DOI: 10.1111/jan.16417.
70. Teh JJ, Pascoe DJ, Hafeji S, et al. Efficacy of virtual reality for pain relief in medical procedures: A systematic review and meta-analysis. BMC Med 2024;22(1):64. DOI: 10.1186/s12916-024-03266-6.
71. Lassen KL, Hermander K, Jildenstal P, et al. Virtual reality is safe and can reduce in-hospital anxiety and pain: A systematic review with meta-analyses and trial sequence analyses. Eur J Pain 2025;29(10):e70165. DOI: 10.1002/ejp.70165.
72. Bill. How Social Media Influencers Shape Health Behaviors in 2025. Admiralty, Hong Kong Island, Hong Kong: Scrumball; 2025. Available from: https://www.scrumball.com/blog/social-media-influencers-health-impact-2025. Last accessed on: 11 December 2025.
73. Powell J, Pring T. The impact of social media influencers on health outcomes: Systematic review. Soc Sci Med 2024;340:116472. DOI: 10.1016/j.socscimed.2023.116472.
74. Bonnevie E, Smith SM, Kummeth C, et al. Social media influencers can be used to deliver positive information about the flu vaccine: Findings from a multi-year study. Health Educ Res 2021;36(3):286–294. DOI: 10.1093/her/cyab018.
75. Bartelt K, Sandberg N, Gantman D, et al. Patient Portal Use Associated with 21 Million Fewer Visit No-Shows in 2024. Verona, Wisconsin, USA: Epic Health Research Network; 2025. Available from: https://www.epicresearch.org/articles/patient-portal-use-associated-with-21-million-fewer-visit-no-shows-in-2024/. Last accessed on: 11 December 2025.
76. Sharma P. AI-Powered Patient Education: How Voice Agents Improve Health Literacy and Outcomes. New York, USA: Simbie.ai; 2026. Available from: https://www.simbie.ai/ai-powered-patient-education-voice-agents/. Last accessed on: 15 March 2026.
77. Televox.com. Make Every Patient Interaction Actually Matter. Omaha, Nebraska, USA: Televox-WestCX Orchestrate; 2026. Available from: https://televox.com/. Last accessed on: 11 December 2025.
78. Gates M. Relias vs. HealthStream: Picking a Workforce Partner. Morrisville, North Carolina, USA: Relias LLC; 2026. Available from: https://www.relias.com/blog/relias-vs-healthstream-picking-the-right-healthcare-workforce-partner. Last accessed on: 12 January 2026.

EXPERT VIEW/REVIEW PAPER

Newborn Feeding and Human Milk: The Evolving Role of Specialized Lactation Consultants—An Expert Review

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Abstract

Background: Breastfeeding and human-milk feeding are fundamental components of newborn nutrition and provide important benefits for infants and mothers. The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) recommend exclusive breastfeeding for the first 6 months of life, followed by continued breastfeeding with appropriate complementary foods up to 2 years of age or beyond. Despite these recommendations, establishing and sustaining breastfeeding can be limited by maternal, infant, psychological, clinical, and health-system barriers.

Objective: This Expert Review examines newborn feeding and human-milk feeding with particular emphasis on the role of International Board-certified Lactation Consultants and other specialized lactation professionals in establishing, protecting, and sustaining breastfeeding across the maternal–infant care continuum.

Methods: A narrative review of international guidance and peer-reviewed literature was undertaken, including publications from the WHO, UNICEF, the Academy of Breastfeeding Medicine, and clinical and health-economic studies. Published evidence was integrated with clinical and institutional experience to examine practical approaches to specialized lactation care and its implementation within healthcare systems.

Results: Successful breastfeeding and human-milk feeding require assessment of the mother–infant dyad and timely recognition of maternal and infant feeding difficulties. Specialized lactation consultants provide evidence-based assessment and management of ineffective latch and milk transfer, nipple trauma, engorgement, the mastitis spectrum, perceived or actual low milk supply, and infant feeding dysfunction. Professional lactation support can improve breastfeeding initiation, exclusivity, and duration, and strengthen maternal breastfeeding self-efficacy. Integration of specialized lactation care across maternity, neonatal, pediatric, and primary care settings can facilitate continuity, multidisciplinary management, and family-centered feeding support.

Conclusion: Newborn feeding should be supported as a continuum of care rather than as an isolated maternal or infant intervention. Specialized lactation consultants provide important expertise within this continuum, particularly when breastfeeding difficulties are complex or persistent. Equitable access, effective referral pathways, continuity of support, telelactation, and integration of lactation expertise into healthcare systems may strengthen breastfeeding and human-milk feeding and improve maternal and infant outcomes.

Keywords: Baby-friendly hospital initiative, Breastfeeding, Cost-effectiveness, International Board-certified Lactation consultant, Lactation consultant, Lactation medicine, Lactation science, Telehealth, Telelactation, Value-based care.

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Key Points

- Breastfeeding and human-milk feeding provide important nutritional, immunological, developmental, and maternal benefits, but successful initiation and continuation may be limited by maternal, infant, clinical, and health-system barriers.
- Newborn feeding requires individualized assessment of the mother–infant dyad, including maternal breast health and milk production, infant oral and feeding function, milk transfer, growth and hydration, and family feeding goals.
- International Board-certified Lactation Consultants (IBCLCs) provide specialized, evidence-based care for breastfeeding and human-milk feeding challenges, including nipple pain, low milk supply, mastitis, breast engorgement, ineffective milk transfer, and infant feeding dysfunction.
- Integration of specialized lactation care within multidisciplinary maternity, neonatal, and primary care teams, together with continuity of support through in-person care, telelactation, and family-centered approaches, can strengthen maternal confidence and support breastfeeding exclusivity and duration.
- Equitable access to specialized lactation care, supported by healthcare system integration, appropriate referral pathways, professional education, and health policy, can strengthen newborn feeding and improve maternal and infant outcomes.

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Introduction

Breastfeeding is widely recognized as the biological standard for infant nutrition and one of the most effective interventions for improving maternal and child health worldwide.[1–3] The World Alliance for Breastfeeding Action established World Breastfeeding Week in 1992 to commemorate the 1990 Innocenti Declaration and mobilize global action.[4] WHO and UNICEF recommend

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breastfeeding initiation within the first hour after birth, exclusive breastfeeding for 6 months, and continued breastfeeding with complementary foods for up to 2 years or beyond.[5–9] Despite these recommendations, global and national surveillance indicates that exclusive breastfeeding remains below established targets in many settings.[5,10,11]

Although most mothers intend to breastfeed, establishing and sustaining breastfeeding and human-milk feeding can be challenging. Maternal and infant factors—including poor infant latch, nipple pain, perceived or actual low milk supply, engorgement, mastitis, ineffective milk transfer, and concerns about infant growth or intake—may complicate feeding during the early postnatal period.[2,12–14] Breastfeeding outcomes are also influenced by interacting family, health care, workplace, and societal factors.[2,12,15] Effective support, therefore, requires attention to the mother–infant dyad, timely identification of feeding difficulties, and individualized management within a coordinated continuum of care.

Specialized lactation care represents one component of this broader approach to newborn feeding. International Board-certified Lactation Consultants hold an internationally recognized credential in clinical lactation management.[16,17] Following lactation-specific education, supervised clinical experience, and successful completion of an international certification examination, IBCLCs can contribute specialized expertise in the assessment and management of breastfeeding and human lactation challenges across healthcare settings.[16–22] In our practice, they have made a major contribution to the assessment of maternal and infant factors, evaluation of feeding and milk transfer, individualized care planning, education and counseling, interdisciplinary collaboration, and longitudinal follow-up.[16,18–22]

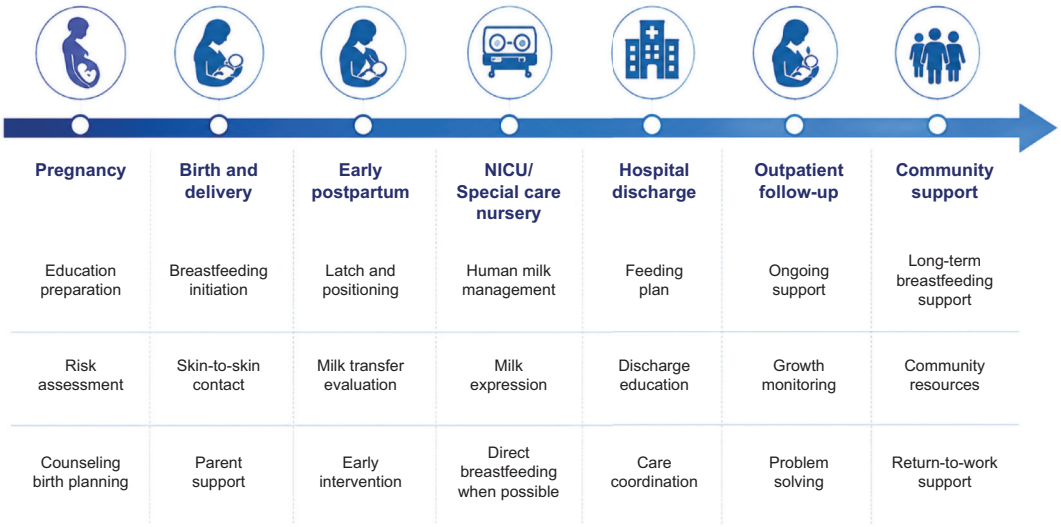
Over the past 3 decades, research has evaluated IBCLC and lactation-consultant interventions in maternity, neonatal, outpatient, primary care, and community settings.[16,23–28] In this Expert Review, we examine breastfeeding and human-milk feeding within the broader context of newborn care, with particular attention to the contribution of specialized lactation consultants. We synthesize current evidence, consider clinical and health-system implications, integrate relevant institutional experience, and examine approaches for incorporating specialized lactation care into multidisciplinary maternal and newborn services.

The IBCLC in Contemporary Maternal and Infant Care

International Board-certified Lactation Consultants are among the healthcare professionals who provide specialized support for breastfeeding and human lactation within multidisciplinary maternal and newborn care.[16,17,21] Through advanced education, supervised clinical training, and certification, they contribute expertise in the assessment and management of breastfeeding and lactation challenges. Depending on clinical needs and the care setting, their contributions may include assessment of the mother–infant dyad, individualized management of feeding difficulties, education and counselling, interdisciplinary collaboration, and longitudinal support.[16,17,21]

Specialized lactation support can show a major impact in the broader framework of coordinated, multidisciplinary care across the maternal–infant continuum.[15,16,23,29] International Board-certified Lactation Consultants can complement the care provided by physicians, nurses, midwives, dietitians, feeding specialists, and other healthcare professionals, particularly when breastfeeding or human-milk feeding challenges require additional expertise.

When available and clinically indicated, specialized lactation support may extend across pregnancy, birth hospitalization, neonatal care, discharge, and community follow-up (Fig. 1).[15,21,25,28–30]



IBCLC: International Board Certified Lactation Conultant; NICU: Neonatal Intensive Care Unit.

Fig. 1: The role of the IBCLC across the continuum of maternal and infant care. International Board-certified Lactation Consultants provide evidence-based lactation care throughout the maternal–infant continuum, from pregnancy through community follow-up, integrating education, clinical assessment, individualized management, interdisciplinary collaboration, and ongoing breastfeeding support

Antenatally, IBCLCs may provide education and help identify factors that could affect lactation. After delivery, they may support breastfeeding initiation, positioning and latch, assessment of milk transfer, and management of feeding difficulties. In neonatal intensive care and special-care settings, they may contribute to multidisciplinary efforts to establish and maintain maternal milk production, support human-milk feeding for preterm or medically complex newborns, and facilitate transition to direct breastfeeding when appropriate.[21,25,30,31] After discharge, they may provide outpatient follow-up, individualized counseling, and coordination with community resources.[12,28]

Evidence from Published Studies

An expanding body of research has evaluated lactation-consultant interventions in maternity hospitals, neonatal units, outpatient clinics, and primary-care practices.[23–28] Overall, access to specialized lactation care is associated with better initiation, exclusivity, duration, and maternal confidence, although effects vary by intervention timing, intensity, population, and outcome.[23,24,27,28] In neonatal settings, dedicated support improves access and early provision of mother's own milk, while evidence for sustained effects through discharge remains less certain.[25]

Study designs, populations, interventions, and outcome measures vary, so individual findings should be interpreted in context. The 2025 systematic review and meta-analysis nevertheless found that lactation-consultant interventions improved exclusive and any breastfeeding compared with usual care.[23] Table 1 summarizes the representative studies across care settings.[24–28]

Institutional Experience

Published evidence provides the scientific foundation for specialized lactation care, while institutional implementation shows how these principles can be translated into routine practice.[15,16,23,32] Successful integration requires multidisciplinary collaboration, administrative support, staff education, standardized pathways, and continuous quality improvement.

Table 2 summarizes the quality indicators selected for institutional monitoring and the intended direction of improvement. Because this narrative report does not present a formal pre–post dataset, no numerical effect estimates are claimed.

Implementing an IBCLC Program

The evidence supporting IBCLC services has important implications for health systems seeking to improve breastfeeding and maternal–newborn care.[16,23] Successful implementation requires

Table 1: Representative studies evaluating the impact of IBCLC services

Study	*Setting*	*Study design*	*Population*	*IBCLC/LC intervention*	*Principal outcome*
Bonuck et al.[24]	Prenatal and postpartum care	Randomized-controlled trial	Low-income mother–infant dyads	Individual prenatal and postnatal LC contacts	Higher cumulative breastfeeding intensity; effects varied across time points
Gray et al.[26]	Maternity hospital	Retrospective cohort	7,370 term newborns	Early IBCLC-assisted feed before formula	Exclusive human-milk feeding was 80% with early IBCLC assistance versus 40% with non-IBCLC assistance
Broom et al.[25]	Neonatal unit	Pre/postimplementation audit	Premature and sick newborns	Permanent unit-based lactation consultant	LC access increased from 15 to 80% and mother's own milk at 12 hours from 46 to 61%; later differences were not sustained
Griffin et al.[27]	Postpartum hospital	Secondary cohort analysis	517 women with gestational diabetes	Inpatient IBCLC consultation	Higher odds of any breastfeeding at discharge and 3 months; no difference in exclusive breastfeeding
Witt et al.[28]	Pediatric primary care	Retrospective pre-/post-chart review	350 consecutive newborns	Routine LC-led first postpartum visit	Non-formula feeding improved, and the difference was sustained through 9 months

IBCLC, International Board-certified Lactation Consultant; LC, lactation consultant

Table 2: Institutional quality indicators for an IBCLC program

Domain	*Program indicator*	*Observed direction**
Breastfeeding	Initiation and exclusive breastfeeding at discharge[12,23,24,26]	Increase (50%)
Human milk/NICU	Mother's own milk use[25]	Increase (70%)
Access	IBCLC consultation before discharge[25,26]	Increase (90%)
Patient experience	Breastfeeding self-efficacy and satisfaction[10,23,33]	Improved (80%)
Quality and safety	Unnecessary supplementation and feeding-related readmissions[15,29,34]	Decrease (30%)

*Data summated from quality-of-care meetings in our NICU. IBCLC, International Board-certified Lactation Consultant; NICU, neonatal intensive care unit

organizational commitment, multidisciplinary collaboration, standardized clinical pathways, ongoing education, and outcome measurement.[15,16,32,35]

Effective IBCLC programs extend beyond individual encounters by establishing sustainable systems of care across the maternal–infant continuum.[15,16,32] Core components include leadership support, referral pathways, evidence-based protocols, staff education, patient education, quality improvement, and routine monitoring.

Implementation strategies should be adapted to local resources and patient populations, while preserving core elements of coordinated, evidence-based care.[15,16,32] The principal organizational components used in our institutional program are summarized in Table 3. These experience-based elements are presented as a practical implementation framework rather than as comparative effectiveness data.

Clinical Assessment and Management of Breastfeeding Challenges

Successful breastfeeding support requires a systematic, individualized assessment of the mother–infant dyad, including maternal history and breast health, infant growth and hydration, oral function, milk transfer, feeding observation, and family goals (Fig. 2).[16,21,30,36,37]

Breastfeeding challenges vary across dyads. Common concerns include ineffective latch, nipple pain or trauma, delayed secretory activation, engorgement, perceived or actual low milk supply, poor infant weight gain, mastitis-spectrum conditions, and feeding difficulty in premature or medically complex infants.[13,14,30,31,36–39]

Early recognition and targeted intervention can support continued human-milk feeding and reduce avoidable complications.[12–14,23,30,31,36–38] Table 4 links common presentations to focused assessment and evidence-based intervention.

Clinical Case Study: Evidence-based Management of the Mastitis Spectrum

The following illustrative composite case shows how coordinated medical and lactation care can alter the course of an acute lactation complication and help prevent early weaning:[37,38]

A 28-year-old primiparous woman presented 24 days postpartum with severe pain, expanding erythema, and localized swelling of the left breast, accompanied by a 24-hour history of systemic malaise and a documented temperature of 38.6°C.[38]

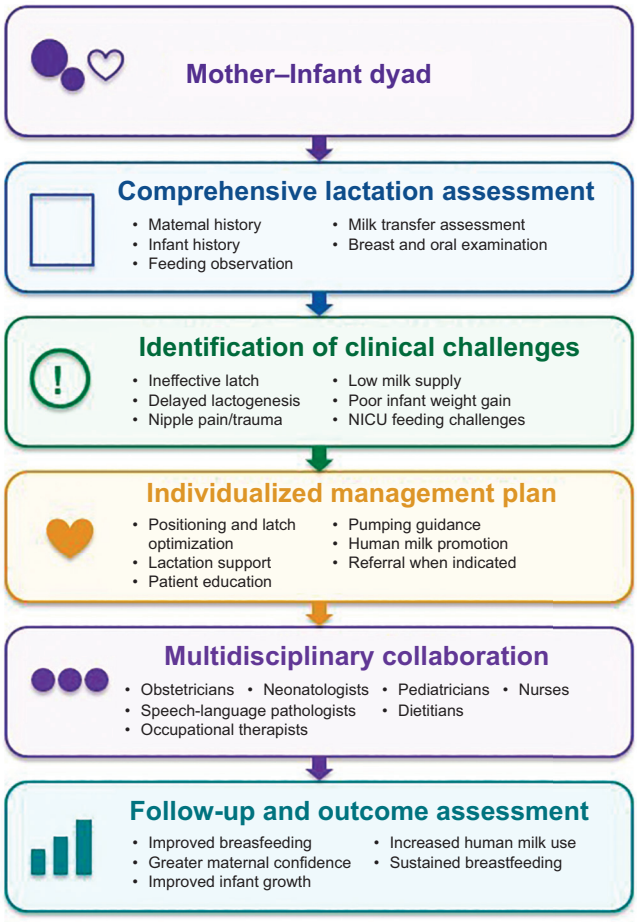


Fig. 2: Clinical assessment and management of breastfeeding challenges by IBCLCs. International Board-certified Lactation Consultants typically use a structured, evidence-based approach that begins with a comprehensive assessment of the mother–infant dyad and proceeds through identification of feeding challenges, individualized management, multidisciplinary collaboration, and longitudinal follow-up. This integrated process combines maternal and infant assessment with targeted clinical interventions, education and counseling, and ongoing evaluation of feeding outcomes to support effective breastfeeding and promote maternal and infant health

Table 3: Implementation of an IBCLC program: Our experience

Program component	*Implementation strategy*	*Observed benefits*
Leadership support	Administrative commitment and resource allocation[15,16,32]	Program sustainability and accountability
Multidisciplinary collaboration	Integration with physicians, nurses, dietitians, therapists, and other professionals[16,29,40]	Coordinated, patient-centered care
Standardized clinical pathways	Evidence-based breastfeeding protocols and referral criteria[15,30,38]	Consistent clinical practice
Staff education	Ongoing professional education and competency development[15,21,40]	Improved provider knowledge and confidence
Patient education	Individualized prenatal, inpatient, and postpartum counseling[12,23,24]	Improved confidence and breastfeeding self-efficacy
Quality improvement	Continuous monitoring of breastfeeding indicators[15,32]	Identification of improvement opportunities
Outcome measurement	Monitoring breastfeeding outcomes, patient experience, and program performance[23–28]	Demonstration of clinical effectiveness and value

Table 4: Common breastfeeding challenges and evidence-based IBCLC interventions

Clinical challenge	*IBCLC assessment*	*Evidence-based intervention*	*Expected outcome*
Ineffective latch	Maternal and infant feeding assessment[36,37]	Positioning and latch optimization[13,36]	Improved milk transfer
Delayed lactogenesis	Maternal history and milk-production assessment[14,21,31]	Frequent, effective milk removal and follow-up[14,31]	Increased milk production
Nipple pain/trauma	Breast examination and observed feed[13,36]	Correct underlying latch or pump mechanics; individualized care[13,36]	Reduced pain and continued breastfeeding
Perceived low milk supply	Milk-transfer and infant-growth assessment[14,30,31,37]	Education, reassurance, and a pumping plan when indicated[14,31]	Improved confidence and protected milk supply
Poor infant weight gain	Growth, hydration, and milk-transfer assessment[14,30,37]	Individualized feeding plan, supplementation when indicated, and close follow-up[14,30]	Safe growth while protecting breastfeeding
NICU feeding challenges	Developmental feeding and maternal milk-production assessment[21,25,30,31]	Human-milk support, pumping guidance, and transition to direct breastfeeding[21,25,31]	Increased access to mother's own milk and breastfeeding support

IBCLC, International Board-certified Lactation Consultant; NICU, neonatal intensive care unit

The patient reported escalating nipple pain since hospital discharge.[13] She noted that the infant spent long periods at the breast but remained unsettled after feeds.[36] To address her worsening discomfort and ensure her breast was "completely empty," she began mechanically pumping after every breastfeeding session.[38,41]

Clinical Findings

Physical examination revealed localized erythema and induration across the upper outer quadrant of the left breast, with no clinical signs of fluctuance.[38] Superficial epidermal trauma and linear fissuring were noted on the left nipple.[13] Direct observation of a breastfeeding session revealed a shallow infant latch, poor lower lip flanging, and minimal audible swallowing, indicating inefficient milk transfer.[36,37]

International Board-certified Lactation Consultant Assessment

The clinical evaluation identified a multifactorial presentation:

- Secondary inflammatory mastitis driven by mechanical milk stasis.[38,41]
- Ineffective milk transfer due to a shallow latch and mild infant laryngomalacia affecting suction mechanics.[36,37]
- Secondary hyperlactation and worsening tissue edema caused by aggressive post-feed pumping.[38,41]
- High maternal anxiety leading to excessive manual manipulation of the breast tissue.[10,38]
- No clinical evidence of a localized breast abscess.[38]

Individualized Management Plan

The intervention strategy focused on restoring normal physiology, optimizing milk transfer, and reducing tissue inflammation:[10,33,38]

- *Latch optimization*: Coached the mother in positional adjustments, shifting to a laid-back biological nurturing position to maximize infant mouth opening and achieve a deep, asymmetric latch.[13,36]
- *Inflammation control*: Discontinued post-feed pumping and manual manipulation.[38,41] Prescribed scheduled cold compresses for 15 minutes after feeds alongside appropriate anti-inflammatory analgesia.[38]
- *Clinical coordination*: Collaborated with the primary care physician to initiate a targeted course of antibiotics given the systemic symptoms and fever, while ensuring the continuity of exclusive breastfeeding.[38,40]
- *Supportive measures*: Advised on increased maternal fluid intake and complete physical rest.[37] Scheduled a mandatory clinical review within 48 hours.[30,38]

Clinical Outcome

At the 48-hour follow-up, the patient reported significant improvement.[38] Her fever had resolved, the localized erythema had visibly decreased, and breast pain had diminished.[38] Direct clinical observation confirmed shorter, more efficient feeding sessions characterized by consistent, rhythmic swallowing and a settled infant post-feed.[36,37] A follow-up at 2 weeks confirmed complete resolution of the mastitis episode with no recurrence, and the dyad successfully maintained exclusive breastfeeding.[12,23,38]

This case illustrates how acute lactation complications often arise from interacting maternal, infant, and feeding-related factors rather than from a single isolated cause, underscoring the value of coordinated, individualized management. Beyond resolving the immediate clinical problem, successful care must also restore the mother's confidence in her ability to breastfeed—a dimension captured by the concept of maternal breastfeeding self-efficacy.

Maternal Breastfeeding Self-efficacy

Long-term breastfeeding success depends on clinical capability as well as maternal psychological confidence.[10,33] Dennis's breastfeeding self-efficacy theory highlights that a mother's confidence in her ability to breastfeed predicts initiation, exclusivity, and overall duration (Fig. 3).[10,33] Conversely, low self-efficacy correlates strongly with early formula introduction and unplanned weaning.[10,33]

Breastfeeding self-efficacy is a modifiable psychological construct that develops through successful performance accomplishments, vicarious experiences, verbal persuasion from healthcare providers, and physiological stabilization.[10,33] International Board-certified Lactation Consultants act as clinical coaches rather than passive instructors.[10,17] By reframing normal infant behaviors, teaching practical problem-solving skills, and providing objective feedback, they transition the mother from a state of dependence on external validation to independent clinical competence.[10,33]

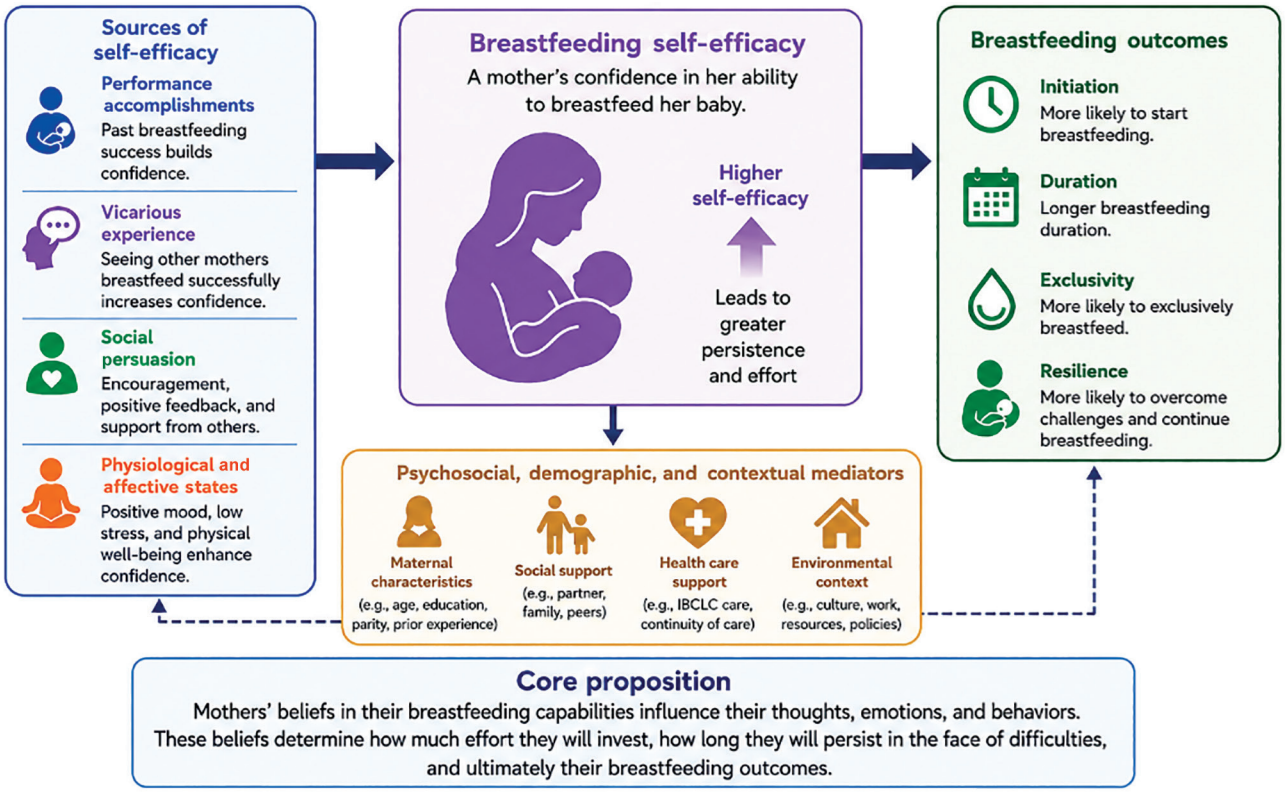


Fig. 3: Dennis's breastfeeding self-efficacy theory. Four principal sources—performance accomplishments, vicarious experience, verbal persuasion, and physiological or affective states—shape breastfeeding confidence and persistence. Adapted from Dennis

Source: Adapted from Dennis CL. Theoretical underpinnings of breastfeeding confidence: a self-efficacy framework. J Hum Lact. 1999;15(3): 195–201.

Addressing Maternal Anxiety and Perceived Low Milk Supply

The primary psychological barrier to sustained exclusive breastfeeding is a mother's anxiety regarding her milk volume.[14,33] Normal newborn behavior, including cluster feeding, frequent night waking, transient crying, and brief sleep intervals, is frequently misinterpreted by families as clinical indicators of lactation insufficiency, even when these behaviors are developmentally appropriate.[14,33]

Normal newborn behaviors, including cluster feeding, frequent night waking, transient crying, and short sleep intervals, may be interpreted by families as signs that milk intake is inadequate.[14,33] When perceived low milk supply is not distinguished from true lactation insufficiency through careful assessment of the mother–infant dyad, families may introduce formula supplementation that is not clinically indicated. If supplementation replaces feeding at the breast or milk expression without adequate breast stimulation, reduced milk removal may diminish the physiological signals that maintain milk production and contribute to a subsequent decline in maternal milk supply.[14,31] Specialized lactation consultants, including IBCLCs, can help distinguish perceived from actual low milk supply by assessing feeding effectiveness and milk transfer, observing swallowing, evaluating infant growth and hydration, and reviewing feeding and elimination patterns.[10,14,33,37] Objective assessment, individualized counseling, and appropriate reassurance can reduce feeding-related uncertainty and support continued breastfeeding while ensuring that infants with true inadequate intake are identified and managed appropriately.[10,23,33]

Psychosocial Benefits of Continuity of Care

The transition from maternity care to the home is a vulnerable period for establishing and sustaining breastfeeding and human-milk feeding.[12,30] Families may encounter conflicting advice from relatives, popular media, and different healthcare professionals, potentially undermining maternal confidence and creating uncertainty about feeding.[2,12,33]

Continuity of feeding support can provide reassurance, reinforce consistent guidance, and facilitate early recognition and management of emerging breastfeeding difficulties. Specialized lactation consultants, including IBCLCs, can play an important role in ensuring continuity across the transition from hospital to home.[12,23] Systematic reviews demonstrate that isolated, single-point educational sessions are less effective than longitudinal support extending across the perinatal continuum.[12,42] Models combining prenatal education, inpatient clinical support, and planned post-discharge follow-up have been associated with improved breastfeeding outcomes, including higher rates of exclusive breastfeeding.[12,23,42]

Family-centered Breastfeeding Support

Lactation is a shared family journey rather than an isolated maternal activity.[2] Infant feeding choices are heavily influenced by partners, grandparents, and immediate support networks.[2] Well-meaning

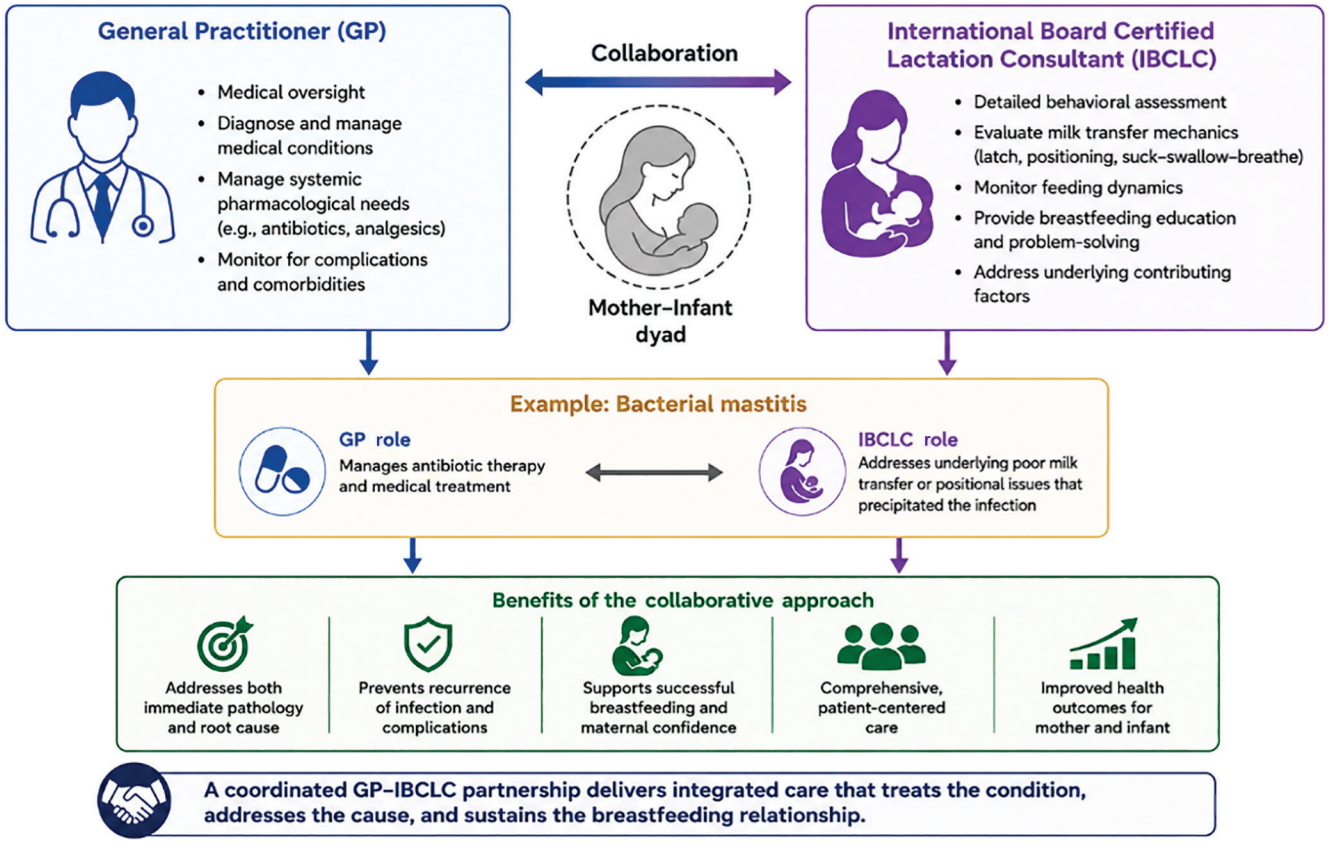


Fig. 4: Collaborative practice model integrating GPs and IBCLCs in breastfeeding care. A collaborative care model pairs the complementary expertise of the GP and the IBCLC to provide comprehensive, patient-centered breastfeeding support. The GP provides medical evaluation, pharmacologic management, and treatment of maternal comorbidities, while the IBCLC performs detailed lactation assessment, evaluates milk transfer and feeding mechanics, identifies contributing breastfeeding problems, and develops individualized management strategies. For instance, if a mother develops bacterial mastitis, the GP manages antibiotic therapy and medical complications, while the IBCLC addresses the underlying causes, such as, ineffective milk transfer or suboptimal positioning, thereby reducing the risk of recurrence and supporting continuation of breastfeeding. This integrated model combines treatment of the immediate clinical condition with correction of its underlying causes to improve maternal and infant outcomes

but misinformed advice from a partner regarding infant settling behaviors can inadvertently undermine exclusive breastfeeding.[2,33]

International Board-certified Lactation Consultants use a family-centered approach to counseling, actively engaging partners and support networks in the clinical plan.[2,21] Education focuses on normal newborn behavioral cues, realistic infant sleep biology, and practical ways partners can provide support, such as, managing responsive settling and sharing household responsibilities.[2,43] Aligning the family unit reduces conflicting advice and builds a supportive home environment.[2]

Health System Integration, Economic Impact, and Digital Health

Establishing and sustaining breastfeeding and human-milk feeding requires coordinated support across the healthcare continuum.[2,15] Fragmented postpartum care can disrupt continuity of feeding support and contribute to earlier breastfeeding cessation.[15,40] International guidance, therefore, emphasizes continuity of care across maternity, newborn, community, and primary-care settings. Specialized lactation consultants, including IBCLCs, can provide an important component of multidisciplinary perinatal teams alongside obstetricians, midwives, neonatologists, pediatricians, and primary care physicians.[15,29,40]

International Board-certified Lactation Consultants bring specialized expertise in human lactation science, assessment of the mother–infant dyad, management of breastfeeding and milk-transfer difficulties, and therapeutic counselling to the care team.[17,21,23] Integration of IBCLC services within inpatient maternity and neonatal intensive care unit (NICU) has been associated with improved breastfeeding outcomes, including higher exclusive breastfeeding rates at discharge and reduced formula supplementation, although reported effects vary according to population, intervention, and setting.[12,23]

Collaborative Models in Primary Care

Following hospital discharge, primary care clinics serve the first line of defense for managing lactation concerns.[36] General practitioners (GPs) regularly manage presentations involving severe nipple pain, infant weight stalling, neonatal jaundice, and mastitis.[13,38,40] While primary care physicians are skilled in medical diagnostics, many reports limited formal training in advanced lactation medicine.[40]

A collaborative practice model pairing GPs with IBCLCs offers a highly effective approach to care (Fig. 4).[40] Within this framework, the physician provides medical oversight and manages systemic pharmacological needs, while the IBCLC conducts detailed behavioral assessments, evaluates milk transfer mechanics, and

monitors feeding dynamics.[37,40] For instance, in managing bacterial mastitis, the GP manages antibiotic therapy, while the IBCLC addresses the underlying poor milk transfer or positional issues that precipitated the infection.[38,40] This collaborative approach addresses both the immediate pathology and its root cause, preventing recurrence and supporting the breastfeeding relationship.[12,38,40]

Public Health and Economic Impact

Sustained exclusive breastfeeding is an effective, evidence-based preventive health measure for the mother–infant dyad (Table 5).[2,3,34] Higher national breastfeeding rates correlate with a lower incidence of acute otitis media, gastroenteritis, severe respiratory tract infections, necrotizing enterocolitis, sudden infant death syndrome, childhood obesity, and Type 2 diabetes in infants.[3,34,43,44] For the mother, extended lactation reduces lifetime rates of breast cancer, ovarian cancer, cardiovascular disease, and metabolic syndrome.[3,45,46]

The economic benefits of breastfeeding extend beyond treatment costs to reduced illness-related healthcare use and parental work absence.[2,34] Modeling studies estimate substantial costs from suboptimal breastfeeding.[2,34] These findings support investment in lactation services, although direct program-specific cost-effectiveness evidence for IBCLC models remains limited.[16,23]

Telelactation and Digital Health Delivery

The integration of digital health delivery models has expanded access to postpartum care.[47,48] Telelactation consultations allow IBCLCs to observe feeding sessions, positioning, and infant behaviors, which can help triage clinical concerns virtually and reduce the need for families to travel in the immediate postpartum period.[47,48]

Telelactation offers several institutional and public health benefits:

- *Improved equity*: Provides specialized clinical access for rural, isolated, or underserved communities.[47,48]
- *Reduced barriers*: Minimizes physical strain and logistical challenges for individuals recovering from cesarean births or managing multiple children.[47,48]
- *Early clinical triage*: Facilitates early detection of feeding issues before they require emergency care or lead to premature weaning.[47,48]
- *Enhanced continuity*: Offers flexible, accessible follow-up options during the critical weeks following hospital discharge.[12,47,48]

Hybrid care models comprised of hands-on inpatient care with scheduled virtual follow-ups can increase family satisfaction and improve exclusive breastfeeding rates.[47,48] Although virtual care cannot replace an in-person clinical examination when accurate infant weight tracking or detailed oral palpatory examinations are required, it serves as an excellent tool for continuous clinical support.[47,48]

Health Policy Recommendations

To advance national and international breastfeeding goals, health systems should implement evidence-based policies that protect breastfeeding and human-milk feeding, facilitate timely identification and management of feeding difficulties, and promote equitable access to specialized lactation care:[5,6,15,43]

- Integrate specialized lactation consultants, including IBCLCs, within maternity services, newborn nurseries, and NICUs, with access determined by clinical and institutional needs;[15,23,29]
- Establish standardized assessment of breastfeeding and human-milk feeding for mother–infant dyads before hospital discharge, with mechanisms for identifying feeding difficulties that require additional evaluation or support;[15,30]
- Develop structured referral pathways connecting maternity and neonatal services with primary care, community-based services, and specialized lactation consultants to promote continuity of feeding support after discharge;[23,40]
- Support appropriate insurance coverage and public funding for specialized lactation services, including in-person and telelactation consultations, to reduce financial barriers to care;[34,47]
- Expand telelactation infrastructure and other accessible models of lactation support, particularly for rural and resource-constrained communities;[47,48]
- Strengthen education in breastfeeding, human lactation, and the recognition and management of common feeding difficulties within undergraduate and postgraduate curricula for physicians, midwives, nurses, and other professionals involved in maternal and newborn care;[21,40] and
- Strengthen institutional implementation of the WHO/UNICEF baby-friendly hospital initiative and adherence to the International Code of Marketing of Breast-milk Substitutes and relevant subsequent resolutions.[15,32,35,49]

Future Research

Despite substantial advances, important evidence gaps remain. Future research should determine how breastfeeding and human-milk feeding can be most effectively established, supported, and sustained across diverse maternal–infant populations and healthcare settings. Priorities include multicenter cost-effectiveness studies; prospective evaluation of long-term maternal and child outcomes; validated quality indicators; clinical pathways for preterm and medically complex infants; and rigorous assessment of telelactation, digital monitoring, and decision-support tools.[16,23,47,48]

Table 5: Health and economic impacts of exclusive breastfeeding supported by specialized lactation care

Infant health benefits. Reduced risk of:	*Maternal health benefits. Breastfeeding can reduce:*	*Institutional benefits*
Acute otitis media[3,34]	Breast cancer[3,46]	Shorter hospital stays[34]
Gastroenteritis[3,34]	Ovarian cancer[3,46]	Fewer readmissions[34]
Respiratory infections[3,34]	Cardiovascular disease[45,46]	Lower medication costs[34]
Necrotizing enterocolitis[34]	Type 2 diabetes[3,46]	Reduced parental absenteeism[2,34]
Sudden infant death[34,43,44]	–	–
Long-term risk of obesity[34,43,44]	–	–

Conclusion

Breastfeeding and human-milk feeding are important components of newborn care, yet their successful establishment and continuation may be limited by maternal, infant, clinical, psychological, and health-system barriers.[3,5,6,12] Specialized lactation consultants, including IBCLCs, can help identify and address these barriers through comprehensive assessment of the mother–infant dyad, evidence-based management of feeding difficulties, maternal education, and structured continuity of care.[12,17,21,23]

Current evidence indicates that specialized lactation support can improve exclusive and any breastfeeding outcomes, although effects vary according to the timing, intensity, population, and setting of the intervention.[12,16,23–28] Integration of lactation expertise within maternity, neonatal, pediatric, and primary care services can strengthen coordinated and family-centered feeding support across the continuum of care. Embedding IBCLCs within maternity, neonatal, and primary care workflows can strengthen coordinated, preventive care.[15,16,29,40] Equitable access to specialized lactation care, supported by effective referral pathways and continuity of care, represents an important strategy for protecting breastfeeding and human-milk feeding and improving maternal and infant outcomes.[2,3,16,34]

Declarations

Data Availability Statement

No new datasets were generated or analyzed for this narrative review. All information discussed is derived from publicly available published literature cited in the article.

Protocol Registration

Not applicable. This article is a narrative expert review and was not prospectively registered.

Artificial Intelligence (AI) Disclosure

No generative AI tools were used in the preparation, writing, or editing of this manuscript.

Acknowledgment

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Authors' Contributions

Monika Kaushal and Sarah Ahmed contributed to the conception and design of the review, literature evaluation, interpretation of evidence, drafting of the manuscript, critical revision for important intellectual content, and approval of the final manuscript.

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References

1. Binns C, Lee M, Low WY. The long-term public health benefits of breastfeeding. Asia Pac J Public Health 2016;28(1):7–14. DOI: 10.1177/1010539515624964.
2. Rollins NC, Bhandari N, Hajeebhoy N, et al. Why invest, and what it will take to improve breastfeeding practices? Lancet 2016;387(10017): 491–504. DOI: 10.1016/S0140-6736(15)01044-2.
3. Victora CG, Bahl R, Barros AJ, et al. Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. Lancet 2016;387(10017):475–490. DOI: 10.1016/S0140-6736(15)01024-7.
4. WABA. World breastfeeding week [Internet]. Penang, Malaysia: World Alliance for Breastfeeding Action; 2026. Available from: https://waba.org.my/wbw/.
5. UNICEF, WHO. Global Breastfeeding Scorecard 2023. New York, USA: United Nations Children's Fund; 2023. Available from: https://www.unicef.org/documents/global-breastfeeding-scorecard-2023.
6. WHO. Infant and young child feeding [Internet]. Geneva, Switzerland: World Health Organization; 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding.
7. WHO. Exclusive breastfeeding for optimal growth, development and health of infants [Internet]. Geneva, Switzerland: World Health Organization; 2026.
8. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. Cochrane Database Syst Rev 2012(8):CD003517. DOI: 10.1002/14651858.CD003517.pub2.
9. WHO. Global strategy for infant and young child feeding. Geneva, Switzerland: World Health Organization; 2003. Available from: https://www.who.int/publications/i/item/9241562218.
10. Dennis CL. Theoretical underpinnings of breastfeeding confidence: A self-efficacy framework. J Hum Lact 1999;15(3):195–201. DOI: 10.1177/089033449901500303.
11. UNICEF. Breastfeeding: A mother's gift, for every child. New York, USA: United Nations Children's Fund; 2018. Available from: https://data.unicef.org/resources/breastfeeding-a-mothers-gift-for-every-child/.
12. Gavine A, Shinwell SC, Buchanan P, et al. Support for healthy breastfeeding mothers with healthy term babies. Cochrane Database Syst Rev 2022;10(10):CD001141. DOI: 10.1002/14651858.CD001141.pub6.
13. Berens P, Eglash A, Malloy M, et al. ABM clinical protocol #26: Persistent pain with breastfeeding. Breastfeed Med 2016;11(2):46–53. DOI: 10.1089/bfm.2016.29002.pjb.
14. Kellams A, Harrel C, Omage S, et al. ABM clinical protocol #3: Supplementary feedings in the healthy term breastfed neonate, revised 2017. Breastfeed Med 2017;12:188–198. DOI: 10.1089/bfm.2017.29038.ajk.
15. WHO. Guideline: Protecting, promoting and supporting breastfeeding in facilities providing maternity and newborn services. Executive summary. Geneva, Switzerland: World Health Organization; 2017. Available from: https://www.ncbi.nlm.nih.gov/books/NBK487814/.
16. Ahmed AH, Rojjanasrirat W, Malik S, et al. The role and impact of the IBCLC: Updated position paper. J Hum Lact 2026:8903344261434475. DOI: 10.1177/08903344261434475.
17. IBLCE. Candidate information guide [Internet]. Fairfax, Virginia: International Board of Lactation Consultant Examiners; 2025. Available from: https://ibclc-commission.org/ibclc-information/candidate-information-guide/.
18. Hale TW, Krutsch K. Hale's Medications & Mothers' Milk 2025-2026: A Manual of Lactational Pharmacology, 21st edition. Manhattan, New York: Springer Publishing Company; 2026. p. 758.
19. Lawrence RA, Lawrence RM. Breastfeeding, 9th edition. Amsterdam, the Netherlands: Elsevier; 2022. p. 1088.
20. Riordan J, Auerbach KG. Breastfeeding and Human Lactation, 2nd edition. Burlington, Massachusetts: Jones & Bartlett Learning; 2016. p. 704.
21. Wambach K, Spencer B. Breastfeeding and Human Lactation, 6th edition. Burlington, Massachusetts: Jones & Bartlett Learning; 2021. pp. 1–622.
22. ILCA. Standards of Practice for International Board Certified Lactation Consultants 2013:[5 p.]. Available from: https://ilca.org/wp-content/uploads/2021/05/Standards-of-Practice-for-International-Board-Certified-Lactation-Consultants-newlogo.pdf.

23. D'Hollander CJ, McCredie VA, Uleryk EM, et al. Breastfeeding support provided by lactation consultants: A systematic review and meta-analysis. JAMA Pediatr 2025;179(5):508–520. DOI: 10.1001/jamapediatrics.2024.6810.
24. Bonuck KA, Trombley M, Freeman K, et al. Randomized, controlled trial of a prenatal and postnatal lactation consultant intervention on duration and intensity of breastfeeding up to 12 months. Pediatrics 2005;116(6):1413–1426. DOI: 10.1542/peds.2005-0435.
25. Broom M, Youseman ME, Kent AL. Impact of introducing a lactation consultant into a neonatal unit. J Paediatr Child Health 2022;58(4):636–640. DOI: 10.1111/jpc.15799.
26. Gray KD, Hannon EA, Erickson E, et al. Influence of early lactation assistance on inpatient exclusive breastfeeding rates. J Hum Lact 2021;37(3):556–565. DOI: 10.1177/0890334420957967.
27. Griffin LB, Ding JJ, Has P, et al. Lactation consultation by an International Board Certified Lactation Consultant improves breastfeeding rates for mothers with gestational diabetes mellitus. J Hum Lact 2022;38(1):141–147. DOI: 10.1177/08903344211018622.
28. Witt AM, Smith S, Mason MJ, et al. Integrating routine lactation consultant support into a pediatric practice. Breastfeed Med 2012;7(1):38–42. DOI: 10.1089/bfm.2011.0003.
29. Meek JY, Noble L, Section on B. policy statement: Breastfeeding and the use of human milk. Pediatrics 2022;150(1):e2022057988. DOI: 10.1542/peds.2022-057988.
30. Hoyt-Austin AE, Kair LR, Larson IA, et al. Academy of breastfeeding medicine clinical protocol #2: Guidelines for birth hospitalization discharge of breastfeeding dyads, revised 2022. Breastfeed Med 2022;17(3):197–206. DOI: 10.1089/bfm.2022.29203.aeh.
31. Kent JC, Prime DK, Garbin CP. Principles for maintaining or increasing breast milk production. J Obstet Gynecol Neonatal Nurs 2012;41(1):114–121. DOI: 10.1111/j.1552-6909.2011.01313.x.
32. Perez-Escamilla R, Martinez JL, Segura-Perez S. Impact of the baby-friendly hospital initiative on breastfeeding and child health outcomes: A systematic review. Matern Child Nutr 2016;12(3):402–417. DOI: 10.1111/mcn.12294.
33. Dennis CL. Identifying predictors of breastfeeding self-efficacy in the immediate postpartum period. Res Nurs Health 2006;29(4):256–268. DOI: 10.1002/nur.20140.
34. Bartick MC, Schwarz EB, Green BD, et al. Suboptimal breastfeeding in the United States: Maternal and pediatric health outcomes and costs. Matern Child Nutr 2017;13(1):e12366. DOI: 10.1111/mcn.12366.
35. Hernandez-Aguilar MT, Bartick M, Schreck P, et al. ABM clinical protocol #7: Model maternity policy supportive of breastfeeding. Breastfeed Med 2018;13(9):559–574. DOI: 10.1089/bfm.2018.29110.mha.
36. Elad D, Kozlovsky P, Blum O, et al. Biomechanics of milk extraction during breast-feeding. Proc Natl Acad Sci USA 2014;111(14):5230–5235. DOI: 10.1073/pnas.1319798111.
37. Perrella SL, Lai CT, Geddes DT. Case studies in breastfeeding assessment and management. Breastfeed Med 2018;13(10):689–693.
38. Mitchell KB, Johnson HM, Rodriguez JM, et al. Academy of breastfeeding medicine clinical protocol #36: The mastitis spectrum, revised 2022. Breastfeed Med 2022;17(5):360–376. DOI: 10.1089/bfm.2022.29207.kbm.
39. Thomas J, Bunik M, Holmes A, et al. Identification and management of ankyloglossia and its effect on breastfeeding in infants: Clinical report. Pediatrics 2024;154(2):e2024067605. DOI: 10.1542/peds.2024-067605.
40. ACOG. Committee opinion no. 756: Optimizing support for breastfeeding as part of obstetric practice. Obstet Gynecol 2018;132(4):e187–e196. DOI: 10.1097/AOG.0000000000002890.
41. Douglas P. Re-thinking benign inflammation of the lactating breast: Classification, prevention, and management. Womens Health (Lond) 2022;18:17455057221091349. DOI: 10.1177/17455057221091349.
42. Renfrew MJ, McCormick FM, Wade A, et al. Support for healthy breastfeeding mothers with healthy term babies. Cochrane Database Syst Rev 2012;5(5):CD001141. DOI: 10.1002/14651858.CD001141.pub4.
43. Feldman-Winter L, Van T, Varadi D, et al. Academy of breastfeeding medicine position statement: Breastfeeding as a basic human right. Breastfeed Med 2022;17(8):633–634. DOI: 10.1089/bfm.2022.29216.abm.
44. Horta BL, Cesar G. Victora CG. Long-term effects of breastfeeding: A systematic review. Geneva, Switzerland: World Health Organization; 2013. Available from: https://iris.who.int/server/api/core/bitstreams/c517e728-aabb-4b47-9b1c-a11abb5a6355/content.
45. Tschiderer L, Seekircher L, Kunutsor SK, et al. Breastfeeding is associated with a reduced maternal cardiovascular risk: Systematic review and meta-analysis involving data from 8 studies and 1 192 700 parous women. J Am Heart Assoc 2022;11(2):e022746. DOI: 10.1161/JAHA.121.022746.
46. Chowdhury R, Sinha B, Sankar MJ, et al. Breastfeeding and maternal health outcomes: A systematic review and meta-analysis. Acta Paediatr 2015;104(467):96–113. DOI: 10.1111/apa.13102.
47. Chua CMS, Mathews J, Ong MSB, et al. Use of telelactation interventions to improve breastfeeding outcomes among mothers: A mixed-studies systematic review. Women Birth 2023;36(3):247–256. DOI: 10.1016/j.wombi.2022.06.011.
48. Iamchareon T, Maneesriwongul W. The effectiveness of real-time telelactation intervention on breastfeeding outcomes among employed mothers: A systematic review and meta-analysis. BMC Pregnancy Childbirth 2025;25(1):341. DOI: 10.1186/s12884-025-07440-3.
49. WHO. International Code of Marketing of Breast-Milk Substitutes. Geneva, Switzerland: World Health Organization; 1981. Available from: https://www.who.int/publications/i/item/9241541601.

EXPERT VIEW/REVIEW PAPER

Maternal Immunization: Optimizing Passive Immunity in the Newborn

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Abstract

Maternal immunization has emerged as an important strategy for preventing infectious disease in pregnant mothers and newborn infants by extending immune protection across pregnancy, birth, and early infancy. Vaccination during pregnancy augments naturally occurring maternal passive immunity, increasing pathogen-specific immunoglobulin G (IgG) available for neonatal Fc receptor-mediated placental transfer and complementing postnatal mucosal protection provided by secretory immunoglobulin A and other immune factors in human milk. Maternal vaccination against tetanus, influenza, pertussis, coronavirus disease 2019 (COVID-19), and respiratory syncytial virus has demonstrated that an intervention administered to the mother can support healthy pregnancies and reduce serious disease, morbidity, and mortality in young infants before direct infant vaccination provides effective protection. Maternal immunization should, therefore, be considered a safe preventive medicine, in which pregnancy becomes an opportunity to modify newborn immune protection early. This approach may be particularly important for premature infants because shortened gestation interrupts the rapid acquisition of maternal IgG during the third trimester, leaving these infants with lower passive antibody concentrations at birth during a period of developmental immune vulnerability. Neonatologists can contribute to maternal immunization through counseling families, supporting clinical investigation, interpreting maternal vaccination history in neonatal care, and collaborating with obstetric and public health teams. The success of existing programs provides a foundation for expanding maternal vaccines against pathogens, including group B *Streptococcus* and cytomegalovirus. Advances in structural biology, computational protein design, systems immunology, vaccine platforms, and artificial intelligence may enable precision maternal vaccinology, with vaccine selection and gestational timing increasingly optimized according to maternal immunity, placental functions, gestational age, pathogen exposure, geography, and epidemiological risk. Maternal immunization represents protection for the maternal–newborn dyad. It bridges fetal and infant immunity, expanding prenatal prevention against neonatal diseases and strengthening preparedness against emerging infections and future pandemics.

Keywords: Immunoglobulin G, Maternal immunization, Maternal vaccination, Neonatal immunity, Neonatologists, Pandemic preparedness, Passive immunity, Placental antibody transfer, Precision vaccinology, Pregnancy vaccines, Respiratory syncytial virus, Secretory immunoglobulin A.

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Key Points

- Orientation. Maternal immunization has become an important component of perinatal care by protecting pregnant individuals and enhancing the transfer of pathogen-specific antibodies across the placenta before birth and through human milk after birth.
- Clinical Premise. Maternal vaccination against tetanus, influenza, pertussis, coronavirus disease 2019 (COVID-19), and respiratory syncytial virus can reduce maternal and early infant morbidity; selected programs have reduced severe neonatal or early infant disease and mortality.
- Conceptual Advancement. Maternal immunization can be viewed as prenatal neonatal preventive medicine: An intervention delivered to the mother that modifies the newborn's immune protection before direct infant vaccination can provide effective immunity.
- Mechanistic Explanations. Neonatal protection is mediated principally through vaccine-induced maternal antibodies, neonatal Fc receptor (FcRn)-dependent placental immunoglobulin G (IgG) transfer, and complementary postnatal mucosal protection from human milk, with the magnitude of protection influenced by gestational timing, placental function, maternal immunity, and gestation. Maternal protection by vaccination reduces rates of stillbirth and premature labor.
- Neonatal Relevance. Premature infants are especially vulnerable because shortened gestation limits third-trimester IgG acquisition; maternal immunization, therefore, has particular potential to bridge the interval between birth and effective infant immune responses.
- Translational Implications. Future maternal vaccinology may combine new vaccine platforms, precision antigen design, individualized timing, epidemiologic risk assessment, and pandemic preparedness to extend passive immune protection to a broader range of neonatal infections.

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Introduction

Preventing neonatal disease through interventions delivered before birth has a long and highly successful record.[1–4] Periconceptional folic acid supplementation substantially reduces the risk of neural

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tube defects, and antenatal vitamin D supplementation improves maternal vitamin D status and may influence selected pregnancy and neonatal outcomes.[1,5,6] Population vaccination against rubella has transformed the prevention of congenital rubella syndrome, demonstrating that immunization before or around the reproductive period can prevent devastating fetal and neonatal disease.[3] Routine antenatal ultrasonography has likewise become integral to pregnancy care by improving gestational dating and earlier recognition of multiple pregnancy and fetal abnormalities.[4] These advances established a broader principle: Neonatal disease can often be prevented most effectively and safely by intervening before birth. Maternal immunization extends this principle by harnessing the maternal immune system to protect both the pregnant individual and the developing fetus and newborn.[7–13]

Vaccinating during pregnancy developed from trials directed at supporting maternal health. Pregnancy involves dynamic immune adaptations to support fetal tolerance while preserving antimicrobial defenses; these changes are not adequately described by a simple state of generalized immunosuppression.[7–12] Maternal immunity changes across gestation through coordinated alterations in innate and adaptive responses at systemic and maternal–fetal interfaces. As one consequence of this progression, mothers become more liable to severe infections, particularly from respiratory viruses. Fatal pneumonias were well documented during influenza pandemics in pregnant women, for instance. It became apparent that early influenza vaccines did not compromise pregnancy, but did protect mothers from severe respiratory disease while reducing influenza-related illness among young infants.[14–18]

These observations supported a new strategy, ethically and practically, to manage the lethal condition of infant tetanus. It was recommended to vaccinate pregnant mothers in specific high-risk areas against tetanus, with the intention of protecting their infants. Maternal tetanus immunization has dramatically reduced neonatal tetanus worldwide and remains one of the most successful public health interventions in maternal–child health.[19–27] Maternal pertussis vaccination provides high concentrations of anti-pertussis antibodies that protect newborns before completion of the primary infant vaccination series.[28–32] More recently, maternal COVID-19 vaccination demonstrated that vaccination during pregnancy can safely generate protective antibodies that cross the placenta and reduce the risk of severe disease in both mothers and young infants.[33–42]

Extending maternal and neonatal protection from fetal life with vaccines has added a new layer to antenatal management.[7,8,43] Vaccinations against influenza, pertussis and tetanus (Tdap), COVID-19, and even more recently, respiratory syncytial virus (RSV) are now routinely recommended during pregnancy by health authorities.[7–10,44–48] Maternal passive immunity, or the transfer of protective maternal antibodies, happens before birth via the placenta and after birth through breast milk. This immune protection persists in the infant through early infancy.[11–53] By augmenting the physiological transfer of pathogen-specific maternal IgG antibodies across the placenta, vaccination during pregnancy enhances passive immunity to the developing fetus, extending through the vulnerable first months of life. This is particularly important because immune responses in early life differ developmentally from those of older children and adults, and protective responses to direct infant vaccination require time to develop. Infants can be difficult to reach geographically to deliver a vaccine schedule.[11–52,54] Maternal immunization transforms pregnancy into an opportunity to improve the health of two immunologically vulnerable patients simultaneously—the pregnant mother and the developing fetus.[7,8] Vaccination programs against tetanus, influenza, pertussis, COVID-19, and RSV have all reduced morbidity and mortality in newborn infants safely, without increasing complications to pregnancies or newborns.[44–48,55–66] Pregnancy has become a unique developmental window during which neonatal health, including growth and neurological development, can be favorably influenced.[7,8,43]

Vaccination during pregnancy is known to enhance several immunological events. First, it increases the placental transfer of vaccine-specific IgG antibodies.[11–52] Second, after birth, breast milk provides specific secretory immunoglobulin A (sIgA), which predominates over immunoglobulin M and IgG and complements placentally acquired IgG by protecting infant gastrointestinal and respiratory mucosal surfaces.[50–53] Together, placental IgG and breast milk-derived sIgA provide continuous passive immune protection that bridges pregnancy, birth, and early infancy. These effects are evident from epidemiology.[11–53] What is not known in detail, however, is how maternal vaccination changes innate and adaptive cellular repertoires in the fetus, the placenta, or the mother.[54–66] It is not known, for instance, how these vaccines might influence the cellular microchimerism, a component of the maternal–fetal immunological relationship that involves both the placenta and breast. This scientific frontier is not a clinical concern because epidemiology demonstrates a strong safety profile for maternal vaccination.[67]

Maternal Immunization as a New Strategy in Neonatal Prevention

Biological Basis of Maternal Immunization

Maternal immunization builds upon a physiological adaptation of pregnancy: The transfer of maternal immunity from mother to fetus.[44–47] It has become a powerful strategy for protecting newborn infants during the earliest and most vulnerable stages of life.[19–77] By increasing maternal levels of pathogen-specific IgG antibodies, maternal immunization augments the quantity of protective antibodies available for transplacental transfer, thereby extending passive immune protection throughout the neonatal period when infants remain particularly vulnerable to severe infectious diseases.[44–47,19–77]

The efficiency of transplacental antibody transfer depends upon many factors, including gestational age, maternal antibody concentrations, placental integrity, antibody subclass, and the timing of maternal immunization.[44–47] Active transport is mediated by the FcRn, which selectively transfers IgG across the syncytiotrophoblast into the fetal circulation.[44–47] This process accelerates during the third trimester, allowing healthy term infants to acquire substantial passive immunity before birth.[44–47] Consequently, appropriately timed maternal immunization can maximize neonatal antibody concentrations during the first months of life, when endogenous immune responses remain immature and routine infant immunization schedules are only beginning.[11–50,44–47]

Maternal passive immunity extends beyond placental antibody transfer. Following birth, human milk provides complementary protection through sIgA, the predominant immunoglobulin in breast milk.[48–82] Whereas transplacentally acquired IgG provides systemic protection against invasive infection, milk-derived sIgA protects the gastrointestinal and respiratory mucosal surfaces by

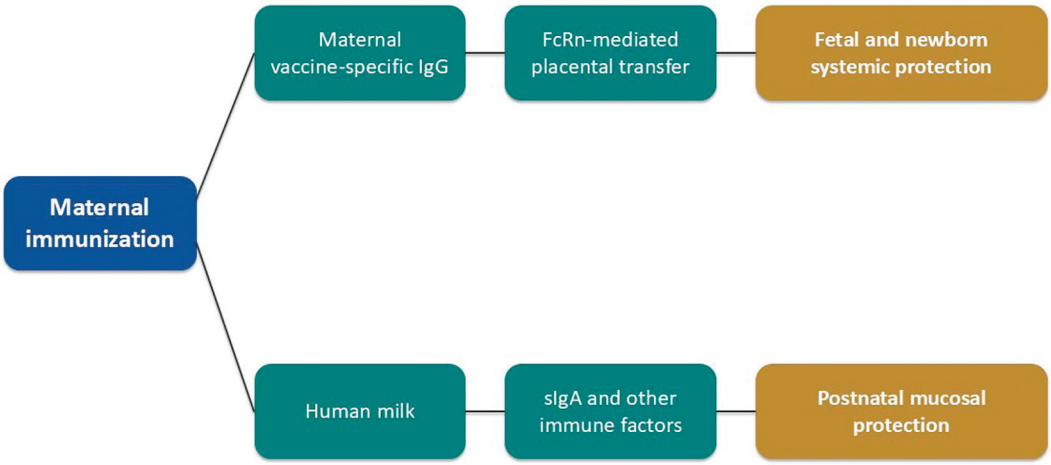

Fig. 1: Continuum of maternal passive immunity

neutralizing pathogens, limiting microbial adherence, and reducing inflammation without activating complement.[48–82] Together, placentally transferred IgG and breast milk-derived sIgA provide complementary systemic and mucosal protection during the critical interval before the infant's own immune system reaches functional maturity.[11–82] This biological continuum is particularly important for infants born prematurely, who receive substantially reduced third-trimester transplacental IgG transfer and therefore derive even greater benefit from the immunological protection provided by human milk (Fig. 1).[44–47,54–82]

Maternal immunization can protect the newborn through complementary prenatal and postnatal pathways. Vaccine-specific maternal IgG is transferred across the placenta through FcRn-mediated mechanisms to provide systemic protection before and after birth, whereas sIgA and other immune factors in human milk contribute additional mucosal protection after birth.

Design Principles for Maternal Immunization

One important principle of maternal immunization is to induce a maternal immune response that results in efficient transplacental transfer of protective antibodies. Protection of the newborn, therefore, depends primarily on passive immunity acquired from the mother rather than activation of the fetal immune system.[44–47,19–77] This distinction has guided vaccine development, clinical evaluation, and regulatory approval of immunization during pregnancy.[19–77,83–97]

Vaccines recommended during pregnancy rely on non-live vaccines with favorable maternal and fetal safety profiles.[19–77,83–97] These include inactivated vaccines, protein subunit vaccines, recombinant vaccines, polysaccharide-conjugate vaccines, and, more recently, messenger RNA (mRNA) vaccines.[19–77] These technologies stimulate maternal antibody production without introducing replicating microorganisms that might infect a fetus.[19–77]

The successful implementation of maternal RSV vaccination illustrates these principles. Although RSV is a naturally occurring respiratory virus and one of the leading causes of hospitalization among infants worldwide, the currently approved maternal RSV vaccine is based on a recombinant prefusion F protein. This generates a robust maternal neutralizing antibody response that transfers effectively across the placenta. These antibodies provide neonatal protection throughout the early months of life while maintaining the highest standards of maternal and fetal safety.[19–77,83–97]

Maternal vaccine development requires rigorous evaluation of maternal immune responses, fetal development, pregnancy outcomes, and neonatal health.[44–47,98–101] Strategies should maximize neonatal protection while maintaining the highest standards of safety for both members of the maternal–fetal dyad.[19–77,83–97] Comparisons of vaccines may also inform future maternal vaccine development. In a prospective cohort study conducted during the COVID-19 pandemic, mRNA vaccination during pregnancy elicited more rapid and robust humoral immune responses, higher antibody titers and neutralizing capacity, and stronger antigen-specific CD4+ T-cell responses than adenoviral vector vaccination.[102] These findings suggest that mRNA vaccine platforms may have particular value when rapid and robust maternal respiratory immunity is required, including during future pandemic responses, in which respiratory viruses are likely threats.[102]

Premature Infants are More Likely to Benefit from Maternal Immunization

Premature infants, in some respects, are more likely than term ones to benefit from maternal immunization. Because they are born before maximal third-trimester transplacental transfer of maternal IgG has occurred, they enter extrauterine life with substantially lower concentrations of passively acquired antibodies than term infants.[44–47,54–57] At the same time, both innate and adaptive immune responses remain developmentally immature, placing these infants at the highest risk for severe respiratory, bacterial, and other invasive infections during the first weeks and months of life.[11–50,54–60] This combination of reduced passive immunity and immature endogenous immunity creates a period of exceptional vulnerability immediately after birth (Fig. 2).[11–47]

Preterm birth interrupts the period of rapid transplacental IgG acquisition that occurs during late gestation. Reduced third-trimester antibody transfer can, therefore, result in lower passive antibody stores at birth and greater vulnerability to infection during early infancy, providing a biological rationale for optimizing maternal immunization strategies for pregnancies at risk of preterm delivery.



Fig. 2: Prematurity and the passive immunity gap



Fig. 3: Maternal immunization as prenatal neonatal prevention

This vulnerability is further compounded by the limitations of active immunization during early life. Although most routine infant vaccines are administered according to chronological age rather than birth weight or gestational age, protective immune responses require time to develop after vaccination.[7–10] Most routine vaccines for clinically stable preterm infants are administered according to chronological age, although specific vaccines and clinical circumstances may require individualized timing.[83–97] Consequently, the immediate postnatal period represents a window of profound susceptibility during which maternally acquired passive immunity provides the infant's principal defense against serious infectious diseases.[11–43]

Maternal immunization is uniquely positioned to bridge this critical period of vulnerability. By augmenting maternal passive immunity before birth, vaccination during pregnancy provides protection at a time when premature infants are least capable of mounting effective immune responses and before routine childhood immunization can provide sustained immunity.[19–77] Rather than replacing infant immunization, maternal immunization complements it by extending immune protection across the interval between birth and maturation of the infant's own adaptive immune system.[11–43] For premature infants, maternal immunization functions as the earliest form of neonatal immune protection, beginning before delivery and continuing throughout the first months of life.[7–10,19–77]

Maternal immunization may also protect pregnancy indirectly when prevention of maternal infection reduces infection-associated maternal or fetal complications. This potential benefit is important and warrants further pathogen-specific investigation. Future vaccines targeting selected reproductive tract pathogens could potentially reduce infection-associated adverse pregnancy outcomes, but this remains an investigational objective rather than an established clinical benefit.

Respiratory Syncytial Virus: An Example of Prenatal Neonatal Protection

Respiratory syncytial virus has become one of the clearest demonstrations of the potential of maternal immunization to prevent neonatal and early infant disease. Respiratory syncytial virus is the leading cause of lower respiratory tract infection and one of the most common reasons for hospitalization during infancy worldwide.[33–77] Although nearly all children are infected within the first 2 years of life, the greatest burden of severe disease occurs during the first 6 months, when immune defenses remain immature and infants are too young to derive full protection from active immunization.[11–50,33–77] For decades, prevention relied primarily on supportive care and passive monoclonal antibody administration for selected high-risk infants, underscoring the need for broader preventive strategies.[33–77]

Maternal immunization against RSV aimed to prevent a leading cause of hospitalization among young infants.[33–77] Respiratory syncytial virus provides compelling evidence that maternal immunization can be intentionally designed as an infant health intervention, using pregnancy as the window for disease prevention.[33–77]

Vaccination during pregnancy induces high concentrations of RSV-specific neutralizing antibodies that are actively transferred across the placenta before birth, providing immediate passive protection during the period of greatest susceptibility.[33–77] Clinical trials have demonstrated significant reductions in severe RSV-associated lower respiratory tract disease, hospitalization, and healthcare utilization during early infancy.[33–77] These findings establish that meaningful neonatal protection can be achieved without directly vaccinating the infant, instead leveraging the normal physiology of maternal antibody transfer (Fig. 3).[44–47]

Maternal immunization extends neonatal preventive care into pregnancy by generating maternal immune responses that can provide passive protection before birth and during the early postnatal period. This protection can bridge a period of heightened vulnerability until the infant develops more effective endogenous immunity and receives direct immunization.[16,78–83]

The success of maternal RSV immunization illustrates too the importance of collaboration across obstetrics, maternal–fetal medicine, neonatology, pediatrics, infectious diseases, immunology, and public health.[83–97] Achieving optimal neonatal protection depends not only on vaccine efficacy, but also on appropriate gestational timing, patient selection, vaccine acceptance, equitable access, and seamless integration into prenatal care.[83–97]

Evolving Roles of Neonatologists in Antenatal Care and Pandemic Preparedness

Neonatologists have an increasingly important role in supporting maternal immunization programs, educating families about the neonatal benefits of vaccination during pregnancy, and partnering with obstetric colleagues to maximize protection during the earliest stages of life.[83–97] As prenatal interventions become increasingly capable of reducing disease during the earliest stages of life, the responsibilities of neonatologists might extend to include strategies that optimize neonatal health during pregnancy.[103–128] Once delivered, knowledge of maternal vaccination status provides valuable clinical context when evaluating newborn infants with

respiratory disease, suspected infection, or other conditions for which maternally derived passive immunity may influence disease severity, diagnostic evaluation, or preventive management.[11–43]

Education represents a further important responsibility for neonatologists. Families frequently seek guidance regarding the safety and benefits of vaccination during pregnancy, particularly when recommendations involve recently introduced vaccines.[83–97] Although prenatal counseling is primarily provided by obstetric care teams, neonatologists are uniquely positioned to explain how maternal immunization protects newborn infants during the period of greatest vulnerability.[11–43,19–77] By communicating the neonatal benefits of vaccination in clear, evidence-based language, neonatologists can reinforce public confidence, address vaccine hesitancy, and support informed decision-making among expectant families.[83–97]

Beyond clinical practice, neonatologists also have important responsibilities in research, quality improvement, and advocacy. Future investigations should continue to define the effectiveness, durability, optimal timing, and implementation of maternal immunization across diverse populations.[19–77,83–97] Quality improvement initiatives can strengthen vaccine uptake through integrated maternal–newborn care pathways, whereas advocacy efforts can promote equitable access to clinical trials and maternal vaccines.[83–97] Because newborn infants are the principal beneficiaries of these interventions, neonatologists have an important voice in shaping research priorities, clinical guidelines, public health policy, and healthcare systems to support maternal immunization.[83–97]

Potential Risks, Safety Monitoring, Research Inputs

Although maternal immunization has demonstrated substantial benefits for both mothers and newborn infants, careful evaluation of vaccine safety remains fundamental to its clinical implementation.[19–77,83–97] Vaccines currently recommended during pregnancy have demonstrated favorable maternal and fetal safety profiles in randomized clinical trials, post-licensure surveillance, pregnancy registries, and large observational studies.[19–77,83–97] As with all vaccines, transient local and systemic reactions—including injection-site pain, fatigue, headache, myalgia, and low-grade fever—may occur but are generally mild and self-limited.[83–97] Serious vaccine-related adverse events are uncommon, and current evidence does not indicate that recommended maternal vaccines increase the overall risk of adverse pregnancy outcomes, maternal autoimmune disease, or impaired neonatal immune development.[19–77,83–97]

The benefit-risk profile of maternal immunization should always be considered within the context of the substantial morbidity associated with vaccine-preventable infections during pregnancy and early infancy.[19–77] This assessment may vary according to the specific vaccine, the pathogen being targeted, maternal health status, gestational age, and regional disease epidemiology.[83–97] Live attenuated vaccines are generally avoided during pregnancy because of their theoretical potential to infect the fetus, whereas the inactivated, protein-based, recombinant, and mRNA vaccines currently recommended for maternal immunization have demonstrated favorable safety profiles.[19–77,83–97] Pregnant individuals with autoimmune disorders, immunodeficiency, or other complex medical conditions may benefit from individualized assessment of vaccination timing and expected immune responses.[83–97]

Transparent communication regarding both the demonstrated benefits and the potential risks of maternal immunization is valuable for informed decision-making and maintaining public confidence.[83–97] Continued pharmacovigilance, pregnancy registries, long-term maternal and infant follow-up, and ongoing evaluation of vaccines remain important as additional maternal vaccines are introduced.[83–97] Equally important is clear, evidence-based communication with mothers and families that enables healthcare professionals to discuss vaccine safety, address misconceptions, and support informed decisions throughout pregnancy.[83–97]

For neonatologists, vaccine safety should be considered and discussed alongside the substantial burden of vaccine-preventable disease during early infancy.[19–77] The risks associated with currently recommended maternal vaccines are generally uncommon and well characterized, whereas the consequences of severe infections, such as, pertussis, influenza, COVID-19, RSV, and neonatal tetanus, can be profound.[19–77] New vaccine trials, continued surveillance, rigorous scientific evaluation, and transparent reporting will remain essential as maternal immunization continues to evolve.[83–97]

Future Directions

The next phase of maternal immunization will extend beyond established vaccines toward a broader, more precise, and more adaptable strategy for neonatal prevention.[98–116] Future progress can be considered across four complementary priorities: Expansion of the maternal vaccine portfolio, precision vaccine design, personalization of maternal immunization, and adaptation to geographic and challenging infectious risks.[117–129]

Expanding the Maternal Vaccine Portfolio

The success of maternal immunization against tetanus, influenza, pertussis, COVID-19, and RSV has established proof of principle that vaccination during pregnancy can substantially reduce neonatal infectious morbidity.[19–77] Multiple maternal vaccines targeting pathogens of particular importance to newborn infants—including group B *Streptococcus* (GBS), cytomegalovirus (CMV), and additional respiratory, enteric, and congenital pathogens—are currently under investigation.[130–149] Among these priorities, maternal vaccination against GBS has the potential to transform the prevention of early onset neonatal sepsis.[130–139] Despite widespread intrapartum antibiotic prophylaxis, GBS remains a leading cause of early onset infection worldwide, and current antibiotic-based strategies provide limited protection against late-onset disease.[130–139] Maternal vaccination offers the opportunity to prevent both early- and late-onset GBS disease through transplacental antibody transfer while potentially reducing maternal colonization and dependence on intrapartum antibiotics.[130–139] Similarly, maternal vaccines against CMV have the potential to reduce congenital infection, one of the leading causes of sensorineural hearing loss and neurodevelopmental impairment in children.[140–149] As this portfolio expands, maternal immunization has the potential to become a routine and safe method for preventing a broad spectrum of neonatal infectious diseases.[150–153]

Beyond GBS, future maternal immunization strategies may also seek to reduce the burden of late-onset neonatal infections, particularly among premature and critically ill infants. Whereas

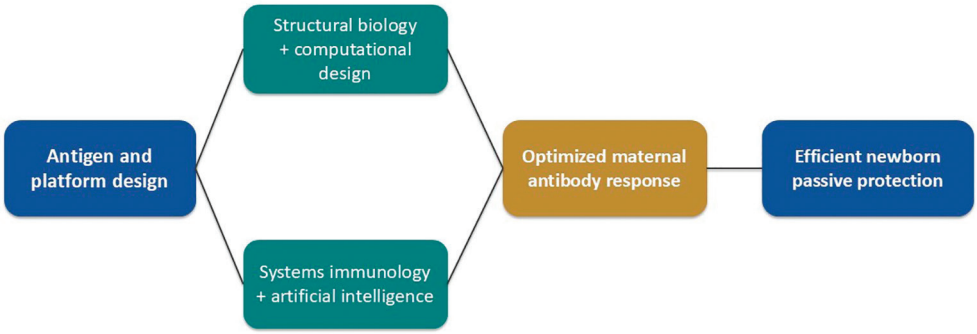

Fig. 4: Precision maternal vaccinology

early onset sepsis is typically associated with vertically transmitted pathogens, such as, GBS, late-onset sepsis is more commonly caused by opportunistic bacteria, including coagulase-negative *Staphylococcus* species, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Acinetobacter* species, and *Pseudomonas* species. Vaccine development against these organisms remains challenging because of their biological diversity and antigenic variability. However, continued advances in vaccine science may expand maternal passive immunity to protect newborn infants against a broader spectrum of neonatal infections.[154–173]

Precision Maternal Vaccinology

An important advantage of maternal immunization is that many vaccines administered during pregnancy function primarily as boosters rather than primary immunizations. Because most pregnant individuals have previously acquired immunological memory through childhood vaccination, prior immunization, or natural infection, maternal vaccination rapidly reactivates memory B cells, producing high-affinity antibodies—predominantly IgG1—that are efficiently transferred across the placenta by the FcRn.[44–47,127] This rapid anamnestic response is particularly advantageous during pregnancy when the interval available to achieve optimal antibody concentrations before delivery may be limited. Next-generation maternal vaccines could be designed to maximize the speed, magnitude, durability, and placental transfer efficiency of these recall responses, further enhancing neonatal protection.[163–173]

Advances in structural biology have demonstrated that preservation of key neutralizing epitopes can dramatically improve vaccine performance, as exemplified by the prefusion RSV F protein vaccine.[33–77] Similar structure-guided approaches, combined with computational protein design, systems immunology, predictive immunology, and artificial intelligence, are expected to accelerate antigen discovery and enable increasingly precise maternal vaccine development.[154–173] Future advances may also improve vaccine adjuvants, optimize antigen presentation, enhance placental antibody transfer, and refine the induction of long-lasting functional antibody responses, further strengthening maternal passive immunity and neonatal protection (Fig. 4).[154–173]

Advances in structural biology, computational protein design, systems immunology, and artificial intelligence may enable increasingly precise selection of antigens and vaccines for use during pregnancy. These approaches seek to optimize the magnitude and quality of maternal antibody responses, thereby strengthening passive immune protection in the newborn.

Toward Personalized Maternal Immunization

Increasing recognition of the biological diversity among pregnant individuals suggests that maternal immunization will become increasingly personalized. Maternal age, underlying medical conditions, nutritional status, immune history, placental function, gestational age, and the maternal microbiome all influence vaccine immunogenicity and the efficiency of transplacental antibody transfer.[98–123] Future approaches may integrate maternal biomarkers, placental assessment, systems immunology, predictive computational models, and artificial intelligence to individualize vaccine selection and optimize the timing of immunization for each pregnancy.[154–173] Such "smarter" precision strategies could identify pregnancies at risk for suboptimal vaccine responses, guide booster immunization when appropriate, maximize maternal antibody generation, enhance placental antibody transfer, and ultimately provide more effective and durable passive protection for newborn infants.

Risk-based Maternal Immunization: Geography and Pandemics

Future maternal immunization strategies may extend beyond standardized vaccination schedules to incorporate individualized epidemiologic risk. Geographic location, international travel, occupational exposures, humanitarian deployments, and infectious disease outbreaks all influence maternal susceptibility to specific pathogens and the corresponding risk to the fetus and newborn. As global mobility increases and infectious diseases evolve, maternal immunization is likely to become progressively tailored to anticipated environmental exposures in addition to routine recommendations.[83–97] Maternal vaccine readiness is an important public health consideration for many low- and middle-income countries. Future pandemics are highly likely; this approach allows new pathogens to be blocked during pregnancy and infancy. Experience with COVID-19 vaccination in the Gambia demonstrated both the feasibility of including pregnant women in emergency vaccine programs and the importance of coordinated guidance, community engagement, healthcare-worker preparedness, and integration with maternal health services.[152]

Future maternal vaccines targeting regionally important pathogens—such as malaria, dengue, chikungunya, Zika virus, and emerging or challenging infections—may further expand opportunities to protect mothers and newborn infants in high-risk settings.[154–173] Together, these approaches have the potential to extend the benefits of maternal passive immunity to populations facing diverse infectious threats, further strengthening neonatal protection on a global scale (Fig. 5).



Fig. 5: Future directions in maternal immunization

Future maternal immunization strategies can be organized across three complementary domains: Expanding and optimizing core vaccination approaches, developing precision and individualized immunization strategies, and integrating prenatal systemic immunity with postnatal mucosal protection. Together, these approaches may extend the breadth, duration, and effectiveness of passive immune protection during the period of greatest neonatal and early infant vulnerability.

Realizing this vision will require continued investment in vaccine research, implementation science, and global health infrastructure. Pregnancy registries, effective diagnostic systems, and standardized frameworks for assessing maternal, fetal, and neonatal outcomes, including the Global Alignment of Immunisation Safety Assessment in Pregnancy, will be important components of this infrastructure.[129] Equitable access to maternal vaccines will remain an international priority, particularly in low- and middle-income countries where neonatal infectious diseases account for a disproportionate share of infant mortality.[83–97] Effective public health communication, healthcare provider education, robust surveillance systems, and policies that promote vaccine confidence will also be essential as maternal immunization expands.[83–97,150,151,153]

From Maternal Immunization to Prenatal Preventive Medicine

The Developmental Origins of Health and Disease framework established that exposures during fetal life can influence physiology, metabolism, immune function, and susceptibility to disease beyond birth.[103–110] Parallel advances in fetal immunology have demonstrated that immune development begins early in gestation through coordinated developmental programs that establish tolerance, permit maternal–fetal coexistence, and prepare the fetus for postnatal microbial exposures.[112–119] Together, these observations position pregnancy as an important period during which interventions directed toward maternal health may also influence the biological environment in which neonatal immunity develops.

The placenta is central to this relationship. In addition to mediating nutrient exchange and FcRn-dependent transfer of maternal IgG, the placenta participates in inflammatory signaling, cytokine networks, cellular communication, and immunological interactions at the maternal–fetal interface.[98–101] Maternal antibodies represent one component of a broader biological dialogue between mother, placenta, and fetus. Maternal nutrition, metabolism, infection, inflammation, immunization, and placental function may each contribute to the environment in which fetal immune development occurs.

This developmental continuum does not end at birth. Breastfeeding, establishment of the neonatal microbiome, environmental exposures, and infant immunization contribute to continuing immune maturation during infancy.[120–125] Maternal immunization can therefore be understood not simply as vaccination during pregnancy, but as one component of a broader strategy of prenatal neonatal prevention. By intentionally strengthening maternal immunity to protect the fetus and newborn, it demonstrates that neonatal preventive medicine can begin before birth. This principle provides the foundation for a broader concept of prenatal preventive medicine: interventions during pregnancy designed not only to protect maternal health, but also to reduce predictable vulnerabilities of the newborn.

Conclusions

Maternal immunization augments physiological immunological pathways to protect pregnant individuals and newborn infants before direct infant immunization can provide effective protection. Its established successes provide a strong foundation for expanding the maternal vaccine portfolio, refining gestational timing, and integrating prenatal and postnatal preventive strategies. Neonatologists can contribute to this evolving field through clinical counseling, research, surveillance, implementation, and collaboration across maternal–newborn care, including preparedness for emerging infections and future pandemics.

References

1. MRC Vitamin Study Research Group. Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study. Lancet 1991;338(8760):131–137. PMID: 1677062.
2. Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 1992;327(26):1832–1835. DOI: 10.1056/NEJM199212243272602.
3. Ou AC, Zimmerman LA, Alexander JP Jr, et al. Progress toward rubella and congenital rubella syndrome elimination—worldwide, 2012-2022. MMWR Morb Mortal Wkly Rep 2024;73(8):162–167. DOI: 10.15585/mmwr.mm7308a2.
4. Whitworth M, Bricker L, Mullan C. Ultrasound for fetal assessment in early pregnancy. Cochrane Database Syst Rev 2015;(7):CD007058. DOI: 10.1002/14651858.CD007058.pub3.
5. Roth DE, Leung M, Mesfin E, et al. Vitamin D supplementation during pregnancy: State of the evidence from a systematic review of randomised trials. BMJ 2017;359:j5237. DOI: 10.1136/bmj.j5237.

6. Yang WC, Chitale R, O'Callaghan KM, et al. The effects of vitamin D supplementation during pregnancy on maternal, neonatal, and infant health: A systematic review and meta-analysis. Nutr Rev 2025;83(3):e892–e903. DOI: 10.1093/nutrit/nuae065.
7. Munoz FM, Jamieson DJ. Maternal immunization. Obstet Gynecol 2019;133(4):739–753. DOI: 10.1097/AOG.0000000000003161.
8. Marchant A, Sadarangani M, Garand M, et al. Maternal immunisation: Collaborating with mother nature. Lancet Infect Dis 2017;17(7):e197–e208. DOI: 10.1016/S1473-3099(17)30229-3.
9. Abu-Raya B, Maertens K, Edwards KM, et al. Global perspectives on immunization during pregnancy and priorities for future research and development. Front Immunol 2020;11:1282. DOI: 10.3389/fimmu.2020.01282.
10. Swamy GK, Heine RP. Vaccinations for pregnant women. Obstet Gynecol 2015;125(1):212–226. DOI: 10.1097/AOG.0000000000000581.
11. Giles ML, Krishnaswamy S, Wallace EM. Maternal immunisation: What have been the gains? Where are the gaps? What does the future hold? F1000Res 2018;7:F1000 Faculty Rev-1733. DOI: 10.12688/f1000research.15475.1.
12. Healy CM. Vaccines in pregnant women and research initiatives. Clin Obstet Gynecol 2012;55(2):474–486. DOI: 10.1097/GRF.0b013e31824f3acb.
13. Jennewein MF, Abu-Raya B, Jiang Y, et al. Transfer of maternal immunity and programming of the newborn immune system. Semin Immunopathol 2017;39(6):605–613. DOI: 10.1007/s00281-017-0653-x.
14. Amirthalingam G, Andrews N, Campbell H, et al. Effectiveness of maternal pertussis vaccination in England: An observational study. Lancet 2014;384(9953):1521–1528. DOI: 10.1016/S0140-6736(14)60686-3.
15. Dabrera G, Amirthalingam G, Andrews N, et al. A case-control study to estimate the effectiveness of maternal pertussis vaccination in protecting newborn infants in England and Wales, 2012–2013. Clin Infect Dis 2015;60(3):333–337. DOI: 10.1093/cid/ciu821.
16. Baxter R, Bartlett J, Fireman B, et al. Effectiveness of vaccination during pregnancy to prevent infant pertussis. Pediatrics 2017;139(5):e20164091. DOI: 10.1542/peds.2016-4091.
17. Abu Raya B, Srugo I, Kessel A, et al. The effect of timing of maternal tetanus, diphtheria, and acellular pertussis immunization during pregnancy on newborn pertussis antibody levels—A prospective study. Vaccine 2014;32(44):5787–5793. DOI: 10.1016/j.vaccine.2014.08.038.
18. Eberhardt CS, Blanchard-Rohner G, Lemaître B, et al. Maternal immunization earlier in pregnancy maximizes antibody transfer and expected infant seropositivity against pertussis. Clin Infect Dis 2016;62(7):829–836. DOI: 10.1093/cid/ciw027.
19. Zaman K, Roy E, Arifeen SE, et al. Effectiveness of maternal influenza immunization in mothers and infants. N Engl J Med 2008;359(15):1555–1564. DOI: 10.1056/NEJMoa0708630.
20. Madhi SA, Cutland CL, Kuwanda L, et al. Influenza vaccination of pregnant women and protection of their infants. N Engl J Med 2014;371(10):918–931. DOI: 10.1056/NEJMoa1401480.
21. Steinhoff MC, Katz J, Englund JA, et al. Year-round influenza immunisation during pregnancy in Nepal: A phase 4, randomised, placebo-controlled trial. Lancet Infect Dis 2017;17(9):981–989. DOI: 10.1016/S1473-3099(17)30252-9.
22. Tapia MD, Sow SO, Tamboura B, et al. Maternal immunisation with trivalent inactivated influenza vaccine for prevention of influenza in infants in Mali: A prospective, active-controlled, observer-blind, randomised phase 4 trial. Lancet Infect Dis 2016;16(9):1026–1035. DOI: 10.1016/S1473-3099(16)30054-8.
23. Omer SB, Richards JL, Madhi SA, et al. Three randomized trials of maternal influenza immunization in Mali, Nepal, and South Africa: Methods and expectations. Vaccine 2015;33(32):3801–3812. DOI: 10.1016/j.vaccine.2015.05.077.
24. Nunes MC, Cutland CL, Jones S, et al. Efficacy of maternal influenza vaccination against all-cause lower respiratory tract infection hospitalizations in young infants: Results from a randomized controlled trial. Clin Infect Dis 2017;65(7):1066–1071. DOI: 10.1093/cid/cix497.
25. Benowitz I, Esposito DB, Gracey KD, et al. Influenza vaccine given to pregnant women reduces hospitalization due to influenza in their infants. Clin Infect Dis 2010;51(12):1355–1361. DOI: 10.1086/657309.
26. Poehling KA, Szilagyi PG, Staat MA, et al. Impact of maternal immunization on influenza hospitalizations in infants. Am J Obstet Gynecol 2011;204(6 Suppl 1):S141–S148. DOI: 10.1016/j.ajog.2011.02.042.
27. Nunes MC, Madhi SA. Influenza vaccination during pregnancy for prevention of influenza confirmed illness in the infants: A systematic review and meta-analysis. Hum Vaccin Immunother 2018;14(3):758–766. DOI: 10.1080/21645515.2017.1345385.
28. Amirthalingam G, Campbell H, Ribeiro S, et al. Sustained effectiveness of the maternal pertussis immunization program in England 3 years following introduction. Clin Infect Dis 2016;63(Suppl 4):S236–S243. DOI: 10.1093/cid/ciw559.
29. Winter K, Nickell S, Powell M, et al. Effectiveness of prenatal versus postpartum tetanus, diphtheria, and acellular pertussis vaccination in preventing infant pertussis. Clin Infect Dis 2017;64(1):3–8. DOI: 10.1093/cid/ciw634.
30. Saul N, Wang K, Bag S, et al. Effectiveness of maternal pertussis vaccination in preventing infection and disease in infants: The NSW Public Health Network case-control study. Vaccine 2018;36(14):1887–1892. DOI: 10.1016/j.vaccine.2018.02.047.
31. Gkentzi D, Katsakiori P, Marangos M, et al. Maternal vaccination against pertussis: a systematic review of the recent literature. Arch Dis Child Fetal Neonatal Ed 2017;102(5):F456–F463. DOI: 10.1136/archdischild-2016-312341.
32. Maertens K, Caboré RN, Huygen K, et al. Pertussis vaccination during pregnancy in Belgium: Results of a prospective controlled cohort study. Vaccine 2016;34(1):142–150. DOI: 10.1016/j.vaccine.2015.10.100.
33. Gray KJ, Bordt EA, Atyeo C, et al. Coronavirus disease 2019 vaccine response in pregnant and lactating women: A cohort study. Am J Obstet Gynecol 2021;225(3):303.e1–303.e17. DOI: 10.1016/j.ajog.2021.03.023.
34. Collier AY, McMahan K, Yu J, et al. Immunogenicity of COVID-19 mRNA vaccines in pregnant and lactating women. JAMA 2021;325(23):2370–2380. DOI: 10.1001/jama.2021.7563.
35. Rottenstreich A, Zarbiv G, Oiknine-Djian E, et al. Efficient maternofetal transplacental transfer of anti-SARS-CoV-2 spike antibodies after antenatal SARS-CoV-2 BNT162b2 mRNA vaccination. Clin Infect Dis 2021;73(11):1909–1912. DOI: 10.1093/cid/ciab266.
36. Mithal LB, Otero S, Shanes ED, et al. Cord blood antibodies following maternal COVID-19 vaccination during pregnancy. Am J Obstet Gynecol 2021;225(2):192–194. DOI: 10.1016/j.ajog.2021.03.035.
37. Halasa NB, Olson SM, Staat MA, et al.; Overcoming COVID-19 Investigators. Effectiveness of maternal vaccination with mRNA COVID-19 vaccine during pregnancy against COVID-19-associated hospitalization in infants aged <6 months. N Engl J Med 2022;387(2):109–119. DOI: 10.1056/NEJMoa2204399.
38. Halasa NB, Olson SM, Staat MA, et al. Maternal vaccination and risk of hospitalization for Covid-19 among Infants. N Engl J Med 2022;387(2):109–119. DOI: 10.1056/NEJMoa2204399.
39. Rasmussen SA, Jamieson DJ. Covid-19 Vaccination during Pregnancy - Two for the Price of One. N Engl J Med 2022;387(2):178–179. DOI: 10.1056/NEJMe2206730.
40. Shimabukuro TT, Kim SY, Myers TR, et al. Preliminary findings of mRNA COVID-19 vaccine safety in pregnant persons. N Engl J Med 2021;384(24):2273–2282. DOI: 10.1056/NEJMoa2104983.
41. Kharbanda EO, Haapala J, DeSilva M, et al. Spontaneous abortion following COVID-19 vaccination during pregnancy. JAMA 2021;326(16):1629–1631. DOI: 10.1001/jama.2021.15494.
42. Prasad S, Kalafat E, Blakeway H, et al. Systematic review and meta-analysis of the effectiveness and perinatal outcomes of COVID-19 vaccination during pregnancy. Nat Commun 2022;13:2414. DOI: 10.1038/s41467-022-30052-w.

43. Lönnerdal B. Bioactive proteins in human milk: mechanisms of action. J Pediatr 2010;156(2 Suppl):S26–S30. DOI: 10.1016/j.jpeds.2009.11.017.
44. Simister NE. Placental transport of immunoglobulin G. Vaccine 2003;21(24):3365–3369. DOI: 10.1016/S0264-410X(03)00334-7.
45. Roopenian DC, Akilesh S. FcRn: The neonatal Fc receptor comes of age. Nat Rev Immunol 2007;7(9):715–725. DOI: 10.1038/nri2155.
46. Palmeira P, Quinello C, Silveira-Lessa AL, et al. IgG placental transfer in healthy and pathological pregnancies. Clin Dev Immunol 2012;2012:985646. DOI: 10.1155/2012/985646.
47. Malek A, Sager R, Kuhn P, et al. Evolution of maternofetal transport of immunoglobulins during human pregnancy. Am J Reprod Immunol 1996;36(5):248–255. DOI: 10.1111/j.1600-0897.1996.tb00172.x.
48. Ballard O, Morrow AL. Human milk composition: Nutrients and bioactive factors. Pediatr Clin North Am 2013;60(1):49–74. DOI: 10.1016/j.pcl.2012.10.002.
49. Fouda GG, Martinez DR, Swamy GK, et al. The impact of IgG transplacental transfer on early life immunity. Immunohorizons 2018;2(1):14–25. DOI: 10.4049/immunohorizons.1700057.
50. Kollmann TR, Kampmann B, Mazmanian SK, et al. Protecting the newborn and young infant from infectious diseases: Lessons from immune ontogeny. Immunity 2017;46(3):350–363. DOI: 10.1016/j.immuni.2017.03.009.
51. Levy O. Innate immunity of the newborn: Basic mechanisms and clinical correlates. Nat Rev Immunol 2007;7(5):379–390. DOI: 10.1038/nri2075.
52. Adkins B, Leclerc C, Marshall-Clarke S. Neonatal adaptive immunity comes of age. Nat Rev Immunol 2004;4(7):553–564. DOI: 10.1038/nri1394.
53. PrabhuDas M, Adkins B, Gans H, et al. Challenges in infant immunity: Implications for responses to infection and vaccines. Nat Immunol 2011;12(3):189–194. DOI: 10.1038/ni0311-189.
54. Newburg DS. Innate immunity and human milk. J Nutr 2005;135(5):1308–1312. DOI: 10.1093/jn/135.5.1308.
55. Le Doare K, Holder B, Bassett A, et al. Mother's milk: A purposeful contribution to the development of the infant microbiota and immunity. Front Immunol 2018;9:361. DOI: 10.3389/fimmu.2018.00361.
56. Donovan SM, Comstock SS. Human milk oligosaccharides influence neonatal mucosal and systemic immunity. Ann Nutr Metab 2016;69(Suppl 2):42–51. DOI: 10.1159/000452818.
57. Walker WA. Initial intestinal colonization in the human infant and immune homeostasis. Ann Nutr Metab 2013;63(Suppl 2):8–15. DOI: 10.1159/000354907.
58. Garofalo RP. Cytokines in human milk. J Pediatr 2010;156(2 Suppl):S36–S40. DOI: 10.1016/j.jpeds.2009.11.019.
59. van den Berg JP, Westerbeek EAM, van der Klis FRM, et al. Transplacental transport of IgG antibodies to preterm infants: A review of the literature. Early Hum Dev 2011;87(2):67–72. DOI: 10.1016/j.earlhumdev.2010.11.003.
60. Palmeira P, Carneiro-Sampaio M. Immunology of breast milk. Rev Assoc Med Bras (1992) 2016;62(6):584–593. DOI: 10.1590/1806-9282.62.06.584.
61. Marchant A, Goldman M. T cell-mediated immune responses in human newborns: Ready to learn? Clin Exp Immunol 2005;141(1):10–18. DOI: 10.1111/j.1365-2249.2005.02799.x.
62. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. Proc Biol Sci 2015;282(1821):20143085. DOI: 10.1098/rspb.2014.3085.
63. Darmstadt GL, Bhutta ZA, Cousens S, et al. Evidence-based, cost-effective interventions: How many newborn babies can we save? Lancet 2005;365(9463):977–988. DOI: 10.1016/S0140-6736(05)71088-6.
64. Bhutta ZA, Das JK, Bahl R, et al. Can available interventions end preventable deaths in mothers, newborn babies, and stillbirths, and at what cost? Lancet 2014;384(9940):347–370. DOI: 10.1016/S0140-6736(14)60792-3.
65. Lawn JE, Blencowe H, Oza S, et al. Every Newborn: Progress, priorities, and potential beyond survival. Lancet 2014;384(9938):189–205. DOI: 10.1016/S0140-6736(14)60496-7.
66. Munoz FM. Current challenges and achievements in maternal immunization research. Front Immunol 2018;9:436. DOI: 10.3389/fimmu.2018.00436.
67. Nelson JL, Lambert NC. The when, what, and where of naturally-acquired microchimerism. Semin Immunopathol 2025;47(1):20. DOI: 10.1007/s00281-024-01029-2.
68. Kampmann B, Madhi SA, Munjal I, et al.; MATISSE Study Group. Bivalent prefusion F vaccine in pregnancy to prevent RSV illness in infants. N Engl J Med 2023;388(16):1451–1464. DOI: 10.1056/NEJMoa2216480.
69. Madhi SA, Polack FP, Piedra PA, et al. Respiratory syncytial virus vaccination during pregnancy and effects in infants. N Engl J Med 2020;383(5):426–439. DOI: 10.1056/NEJMoa1908380.
70. Almeida IR, de Vita Silva MF, de Brito Cavalcante T, et al. Maternal immunization against respiratory syncytial virus: An integrative review of efficacy, safety, and implementation. Vaccines (Basel) 2026;14(7). DOI: 10.3390/vaccines14070624.
71. Simoes EAF, Pahud BA, Madhi SA, et al. Efficacy, safety, and immunogenicity of the MATISSE (Maternal Immunization Study for Safety and Efficacy) maternal respiratory syncytial virus prefusion F protein vaccine trial. Obstet Gynecol 2025;145(2):157–167. DOI: 10.1097/AOG.0000000000005816.
72. PATH. RSV vaccine and mAb snapshot Seattle, Washington, USA: Path.org; 2026. Available from: https://www.path.org/our-impact/resources/rsv-vaccine-and-mab-snapshot/.
73. Modjarrad K, Giersing B, Kaslow DC, et al. WHO consultation on respiratory syncytial virus vaccine development. Vaccine 2016;34(2):190–197. DOI: 10.1016/j.vaccine.2015.05.093.
74. Graham BS. Vaccine development for respiratory syncytial virus. Curr Opin Virol 2017;23:107–112. DOI: 10.1016/j.coviro.2017.03.012.
75. McLellan JS, Chen M, Joyce MG, et al. Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. Science 2013;342(6158):592–598. DOI: 10.1126/science.1243283.
76. Graham BS. Immunological goals for RSV vaccine development. Curr Opin Immunol 2019;59:57–64. DOI: 10.1016/j.coi.2019.03.005.
77. Anderson LJ, Dormitzer PR, Nokes DJ, et al. Strategic priorities for respiratory syncytial virus vaccine development. Vaccine 2013;31(Suppl 2):B209–B215. DOI: 10.1016/j.vaccine.2012.11.106.
78. Andreas NJ, Kampmann B, Le Doare KM. Human breast milk: A review on its composition and bioactivity. Early Hum Dev 2015;91(11):629–635. DOI: 10.1016/j.earlhumdev.2015.08.013.
79. Field CJ. The immunological components of human milk and their effect on immune development in infants. J Nutr 2005;135(1):1–4. DOI: 10.1093/jn/135.1.1.
80. Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. Lancet 2016;387(10017):475–490. DOI: 10.1016/S0140-6736(15)01024-7.
81. Bode L. Human milk oligosaccharides: Every baby needs a sugar mama. Glycobiology 2012;22(9):1147–1162. DOI: 10.1093/glycob/cws074.
82. Chu HY, Englund JA. Maternal immunization. Clin Infect Dis 2014;59(4):560–568. DOI: 10.1093/cid/ciu327.
83. Shimabukuro TT, Kim SY, Myers TR, et al. Preliminary findings of mRNA COVID-19 vaccine safety in pregnant persons. N Engl J Med 2021;384(24):2273–2282. DOI: 10.1056/NEJMoa2104983.
84. Kharbanda EO, Haapala J, DeSilva M, et al. Spontaneous abortion following COVID-19 vaccination during pregnancy. JAMA 2021;326(16):1629–1631. DOI: 10.1001/jama.2021.15494.
85. DeSilva M, Haapala J, Vazquez-Benitez G, et al. Evaluation of acute adverse events after Covid-19 vaccination during pregnancy. N Engl J Med 2022;387(2):187–189. DOI: 10.1056/NEJMc2205276.
86. Moro PL, McNeil MM, Sukumaran L, et al. The Centers for Disease Control and Prevention's public health response to monitoring Tdap safety in pregnant women in the United States. Hum Vaccin Immunother 2015;11(12):2872–2879. DOI: 10.1080/21645515.2015.1072664.
87. Sukumaran L, McCarthy NL, Kharbanda EO, et al. Safety of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccination during pregnancy. Obstet Gynecol 2015;126(5):1069–1074. DOI: 10.1097/AOG.0000000000001066.

88. Wilson RJ, Paterson P, Larson HJ. Strategies to improve maternal vaccination acceptance. Vaccine 2019;37(32):4846–4853. DOI: 10.1186/s12889-019-6655-y.
89. Danchin M, Biezen R, Manski-Nankervis JA, et al. Preparing the public for COVID-19 vaccines: How can general practitioners build vaccine confidence? BMJ 2020;369:m2514. DOI: 10.31128/AJGP-08-20-5559.
90. Paterson P, Meurice F, Stanberry LR, et al. Vaccine hesitancy and healthcare providers. Vaccine 2016;34(52):6700–6706. DOI: 10.1016/j.vaccine.2016.10.042.
91. World Health Organization. Vaccines and immunization Geneva, Switzerland: World Health Organization; 2026. Available from: https://www.who.int/health-topics/vaccines-and-immunization#tab=tab_1.
92. Centers for Disease control guidelines for vaccinating pregnant women atlanta, georgia, USA: US centers for disease control and prevention; 2025.Available from: https://www.cdc.gov/vaccines-pregnancy/hcp/vaccination-guidelines/index.html.
93. American College of Obstetricians and Gynecologists. Maternal Immunizations. ACOG Committee Statement [Internet]. 2026; 147(5):e123–128. Available from: https://www.acog.org/-/media/project/acog/acogorg/clinical/files/committee-statement/articles/2026/02/maternal-immunizations.pdf?rev=18c3aef1d22240d4a2f21323481d0e8d&hash=1F85003A8C2C1C60B9D4BE48B9296785.
94. Wodi AP, Issa AN, Moser CA, et al. Advisory committee on immunization practices recommended immunization schedule for adults aged 19 years or older - United States, 2025. MMWR Morb Mortal Wkly Rep 2025;74(2):30–33. DOI: 10.15585/mmwr.mm7402a3.
95. Plotkin SA. Vaccines: Past, present and future. Nat Med 2005; 11(4 Suppl):S5–S11. DOI: 10.1038/nm1209.
96. Rappuoli R, Black S, Bloom DE. Vaccines and global health: In search of a sustainable model for vaccine development and delivery. Sci Transl Med 2019;11(497):eaaw2888. DOI: 10.1126/scitranslmed.aaw2888.
97. Plotkin SA, Orenstein WA, Offit PA, et al., editors. Plotkin's Vaccines, 8th edition. Philadelphia: Elsevier; 2024. pp. 1–1840.
98. Burton GJ, Fowden AL. The placenta: A multifaceted, transient organ. Philos Trans R Soc Lond B Biol Sci 2015;370(1663):20140066. DOI: 10.1098/rstb.2014.0066.
99. Ander SE, Diamond MS, Coyne CB. Immune responses at the maternal-fetal interface. Sci Immunol 2019;4(31):eaat6114. DOI: 10.1126/sciimmunol.aat6114.
100. Mor G, Cardenas I. The immune system in pregnancy: A unique complexity. Am J Reprod Immunol 2010;63(6):425–433. DOI: 10.1111/j.1600-0897.2010.00836.x.
101. Erlebacher A. Immunology of the maternal-fetal interface. Annu Rev Immunol 2013;31:387–411. DOI: 10.1146/annurev-immunol-032712-100003.
102. De Brabandere L, Herzog SA, Desombere I, et al. Enhanced immune responses to mRNA compared with adenoviral vector COVID-19 vaccines during pregnancy: Implications for pandemic preparedness. Vaccine 2026;73:128153. DOI: 10.1016/j.vaccine.2025.128153.
103. Barker DJP. The developmental origins of adult disease. J Am Coll Nutr 2004;23(6 Suppl):588S–595S. DOI: 10.1080/07315724.2004.10719428.
104. Barker DJP. In utero programming of chronic disease. Clin Sci (Lond) 1998;95(2):115–128. DOI: 10.1042/CS19980019.
105. Gluckman PD, Hanson MA. Living with the past: Evolution, development, and patterns of disease. Science 2004;305(5691):1733–1736. DOI: 10.1126/science.1095292.
106. Gluckman PD, Hanson MA, Cooper C, et al. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 2008;359(1):61–73. DOI: 10.1056/NEJMra0708473.
107. Hanson MA, Gluckman PD. Developmental origins of health and disease: Moving from biological concepts to interventions and policy. Int J Gynaecol Obstet 2011;115(Suppl 1):S3–S5. DOI: 10.1016/S0020-7292(11)60003-9.
108. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: Physiology or pathophysiology? Physiol Rev 2014;94(4):1027–1076. DOI: 10.1152/physrev.00029.2013.
109. Prescott SL. Early-life environmental determinants of allergic diseases and the wider pandemic of inflammatory noncommunicable diseases. J Allergy Clin Immunol 2013;131(1):23–30. DOI: 10.1016/j.jaci.2012.11.019.
110. Godfrey KM, Gluckman PD, Hanson MA. Developmental origins of metabolic disease: Life course and intergenerational perspectives. Trends Endocrinol Metab 2010;21(4):199–205. DOI: 10.1016/j.tem.2009.12.008.
111. Levy O, Wynn JL. A prime time for trained immunity: Innate immune memory in newborns and infants. Neonatology 2014;105(2):136–141. DOI: 10.1159/000356035.
112. Mold JE, Michaëlsson J, Burt TD, et al. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. Science 2008;322(5907):1562–1565. DOI: 10.1126/science.1164511.
113. McGovern N, Shin A, Low G, et al. Human fetal dendritic cells promote prenatal T-cell immune suppression through arginase-2. Nature 2017;546(7660):662–666. DOI: 10.1038/nature22795.
114. Burt TD. Fetal regulatory T cells and peripheral immune tolerance *in utero*: Implications for development and disease. Am J Reprod Immunol 2013;69(4):346–358. DOI: 10.1111/aji.12083.
115. Elahi S. Neglected cells: immunomodulatory roles of CD71+ erythroid cells. Trends Immunol 2019;40(3):181–185. DOI: 10.1016/j.it.2019.01.003.
116. Ygberg S, Nilsson A. The developing immune system—from foetus to toddler. Acta Paediatr 2012;101(2):120–127. DOI: 10.1111/j.1651-2227.2011.02494.x.
117. Bilbo SD, Block CL, Bolton JL, et al. Beyond infection—maternal immune activation by environmental factors, microglial development, and relevance for autism spectrum disorders. Exp Neurol 2018;299(Pt A):241–251. DOI: 10.1016/j.expneurol.2017.07.002.
118. Olin A, Henckel E, Chen Y, et al. Stereotypic immune system development in newborn children. Cell 2018;174(5):1277–1292.e14. DOI: 10.1016/j.cell.2018.06.045.
119. Gollwitzer ES, Marsland BJ. Impact of early-life exposures on immune maturation and susceptibility to disease. Trends Immunol 2015;36(11):684–696. DOI: 10.1016/j.it.2015.09.009.
120. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 2010;107(26):11971–11975. DOI: 10.1073/pnas.1002601107.
121. Arrieta MC, Stiemsma LT, Amenyogbe N, et al. The intestinal microbiome in early life: health and disease. Front Immunol 2014;5:427. DOI: 10.3389/fimmu.2014.00427.
122. Tamburini S, Shen N, Wu HC, et al. The microbiome in early life: Implications for health outcomes. Nat Med 2016;22(7):713–722. DOI: 10.1038/nm.4142.
123. Robertson RC, Manges AR, Finlay BB, et al. The human microbiome and child growth—first 1000 days and beyond. Trends Microbiol 2019;27(2):131–147. DOI: 10.1016/j.tim.2018.09.008.
124. Ben-Shlomo Y, Kuh D. A life course approach to chronic disease epidemiology: Conceptual models, empirical challenges and interdisciplinary perspectives. Int J Epidemiol 2002;31(2):285–293. DOI: 10.1093/ije/31.2.285.
125. Halfon N, Forrest CB, Lerner RM, et al., editors. Handbook of Life Course Health Development. Cham: Springer; 2018. pp. 1–667.
126. Brambell FWR. The Transmission of Passive Immunity from Mother to Young. Amsterdam: North-Holland Publishing Company; 1970. pp. 1–385.
127. Netea MG, Domínguez-Andrés J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. Nat Rev Immunol 2020;20(6):375–388. DOI: 10.1038/s41577-020-0285-6.
128. Dowling DJ, Levy O. Ontogeny of early life immunity. Trends Immunol 2014;35(7):299–310. DOI: 10.1016/j.it.2014.04.007.
129. Bonhoeffer J, Kochhar S, Hirschfeld S, et al.; GAIA project participants. Global alignment of immunization safety assessment in pregnancy: The GAIA project. Vaccine 2016;34(49):5993–5997. DOI: 10.1016/j.vaccine.2016.07.006.

130. Baker CJ, Rench MA, McInnes P, et al. Immunization of pregnant women with a group B streptococcal type III capsular polysaccharide-tetanus toxoid conjugate vaccine. Vaccine 2003;21(24):3468–3472. DOI: 10.1016/S0264-410X(03)00353-0.
131. Madhi SA, Dangor Z, Heath PT, et al. Considerations for a phase III trial to evaluate a maternal group B streptococcus polysaccharide-protein conjugate vaccine. Vaccine 2013;31(Suppl 4):D52–D57. DOI: 10.1016/j.vaccine.2013.02.029.
132. Kobayashi M, Schrag SJ, Alderson MR, et al. WHO consultation on group B *Streptococcus* vaccine development: Report from a meeting held on 27–28 April 2016. Vaccine 2019;37(50):7307–7314. DOI: 10.1016/j.vaccine.2016.12.029.
133. Nuccitelli A, Rinaudo CD, Maione D. Group B Streptococcus vaccine: State of the art. Ther Adv Vaccines 2015;3(3-4):76–90. DOI: 10.1177/2051013615579869.
134. Heath PT. Status of vaccine research and development of vaccines for GBS. Vaccine 2016;34(26):2876–2879. DOI: 10.1016/j.vaccine.2015.12.072.
135. Plotkin SA. The history of vaccination against cytomegalovirus. Med Microbiol Immunol 2015;204(3):247–254. DOI: 10.1007/s00430-015-0388-z.
136. Krause PR, Bialek SR, Boppana SB, et al. Priorities for CMV vaccine development. Vaccine 2013;32(1):4–10. DOI: 10.1016/j.vaccine.2013.09.042.
137. Permar SR, Schleiss MR, Plotkin SA. Advancing our understanding of protective maternal immunity as a guide for development of vaccines to reduce congenital cytomegalovirus infections. J Virol 2018;92(7):e00030-18. DOI: 10.1128/JVI.00030-18.
138. Schleiss MR, Fernandez-Alarcon C, Bierle CJ, et al. Replication-deficient whole-virus vaccines against cytomegalovirus induce protective immunity in a guinea pig congenital infection model. J Virol 2025;99(7):e0020725. DOI: 10.1128/jvi.00207-25.
139. Schleiss MR. Cytomegalovirus vaccines under clinical development. J Virus Erad 2016;2(4):198–207. DOI: 10.1016/S2055-6640(20)30872-4.
140. Munoz FM. Maternal immunization: An update for pediatricians. Pediatr Ann 2013;42(8):153–158. DOI: 10.3928/00904481-20130723-09.
141. Omer SB. Maternal Immunization. N Engl J Med 2017;376(13):1256–1267. DOI: 10.1056/NEJMra1509044.
142. Nunes MC, Aqil AR, Omer SB, et al. The effects of influenza vaccination during pregnancy on birth outcomes: A systematic review and meta-analysis. Am J Perinatol 2016;33(11):1104–1114. DOI: 10.1055/s-0036-1586101.
143. Madhi SA, Cutland CL, Jose L, et al. Safety and immunogenicity of an investigational maternal trivalent group B streptococcus glycoconjugate vaccine in pregnant women and their infants: A randomised phase 2 trial. Lancet Infect Dis 2016;16(8):923–934. DOI: 10.1016/S1473-3099(16)00152-3.
144. WHO. Preferred product characteristics for group B Streptococcus vaccines. Geneva: World Health Organization; 2021. Available from: https://iris.who.int/server/api/core/bitstreams/9f0bb34a-c5cd-429a-b400-2548f707a99e/content.
145. PATH. Advancing maternal immunization for group B streptococcus. Seattle (WA): PATH; 2022. Available from: https://www.path.org/our-impact/resources/one-shot-stop-group-b-streptococcus/.
146. Englund JA. Introduction. Maternal immunization - Promises and concerns. Vaccine 2015;33(47):6372–6373. DOI: 10.1016/j.vaccine.2015.07.084.
147. Maertens K, Orije MRP, Van Damme P, et al. Vaccination during pregnancy: Current and possible future recommendations. Eur J Pediatr 2020;179(2):235–242. DOI: 10.1007/s00431-019-03563-w.
148. Vekemans J, Moorthy V, Friede M, et al. Maternal immunization against Group B streptococcus: World Health Organization research and development technological roadmap and preferred product characteristics. Vaccine 2019;37(50):7391–7393. DOI: 10.1016/j.vaccine.2017.09.087.
149. Heath PT, Culley FJ, Jones CE, et al. Group B streptococcus and respiratory syncytial virus immunisation during pregnancy: A landscape analysis. Lancet Infect Dis 2017;17(7):e223–e234. DOI: 10.1016/S1473-3099(17)30232-3.
150. Turner LH, Sun J, Brady AE, et al. Learning immunology from mothers and babies. Mucosal Immunol 2026;19(4):100353. DOI: 10.1016/j.mucimm.2026.100353.
151. Davies HG, Rukundo G, Komugisha C, et al. Baseline adverse birth outcomes in a prospective pregnancy cohort in Uganda: Evidence to inform maternal vaccine readiness. Vaccine 2026;90:128952. DOI: 10.1016/j.vaccine.2026.128952.
152. Nkereuwem O, Jallow AO, Ogbebor A, et al. How were pregnant women included in emergency COVID-19 vaccine delivery in the Gambia? A qualitative study of perspectives of healthcare workers and policy influencers. BMJ Open 2026;16(8):e118873. DOI: 10.1136/bmjopen-2026-118873.
153. Perin J, Mulick A, Yeung D, et al. Global, regional, and national causes of under-5 mortality in 2000-19: An updated systematic analysis with implications for the Sustainable Development Goals. Lancet Child Adolesc Health 2022;6(2):106–115. DOI: 10.1016/S2352-4642(21)00311-4.
154. Huttner A, Harbarth S, Carlet J, et al. Antimicrobial resistance: A global view from the 2013 World Healthcare-Associated Infections Forum. Antimicrob Resist Infect Control 2013;2:31. DOI: 10.1186/2047-2994-2-31.
155. Rappuoli R, De Gregorio E, Costantino P. On the mechanisms of conjugate vaccines. Proc Natl Acad Sci U S A 2019;116(1):14–16. DOI: 10.1073/pnas.1819612116.
156. Micoli F, Bagnoli F, Rappuoli R, et al. The role of vaccines in combatting antimicrobial resistance. Nat Rev Microbiol 2021;19(5):287–302. DOI: 10.1038/s41579-020-00506-3.
157. Feldman MF, Mayer Bridwell AE, Scott NE, et al. A promising bioconjugate vaccine against hypervirulent Klebsiella pneumoniae. Proc Natl Acad Sci U S A 2019;116(37):18655–18663. DOI: 10.1073/pnas.1907833116.
158. Cryz SJ Jr, Sadoff JC, Furer E, et al. Pseudomonas aeruginosa polysaccharide-tetanus toxoid conjugate vaccine: Safety and immunogenicity in humans. J Infect Dis 1986;154(4):682–688. DOI: 10.1093/infdis/154.4.682.
159. Rogerson SJ. Management of malaria in pregnancy. Indian J Med Res 2017;146(3):328–333. DOI: 10.4103/ijmr.IJMR_1304_17.
160. Duffy PE, Fried M. Malaria in the pregnant woman. Curr Top Microbiol Immunol 2005;295:169–200. DOI: 10.1007/3-540-29088-5_7.
161. Poland GA, Kennedy RB, Ovsyannikova IG, et al. Development of vaccines against Zika virus. Lancet Infect Dis 2018;18(7):e211–e219. DOI: 10.1016/S1473-3099(18)30063-X.
162. Wilder-Smith A. Dengue vaccine development: Status and future. Curr Opin Infect Dis 2020;33(5):397–404. DOI: 10.1097/QCO.0000000000000675.
163. Hsieh CL, Goldsmith JA, Schaub JM, et al. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. Science 2020;369(6510):1501–1505. DOI: 10.1126/science.abd0826.
164. Graham BS. Vaccine development for respiratory syncytial virus. Curr Opin Virol 2017;23:107–112. DOI: 10.1016/j.coviro.2017.03.012.
165. Correia BE, Bates JT, Loomis RJ, et al. Proof of principle for epitope-focused vaccine design. Nature 2014;507(7491):201–206. DOI: 10.1038/nature12966.
166. Kwong PD, Mascola JR, Nabel GJ. Rational design of vaccines to elicit broadly neutralizing antibodies. Cold Spring Harb Perspect Med 2011;1(1):a007278. DOI: 10.1101/cshperspect.a007278.
167. Pulendran B. Systems vaccinology: Probing humanity's diverse immune systems with vaccines. Proc Natl Acad Sci U S A 2014;111(34):12300–12306. DOI: 10.1073/pnas.1400476111.
168. Hagan T, Pulendran B. Will systems biology deliver its promise and contribute to the development of new or improved vaccines? From data to understanding through systems biology. Cold Spring Harb Perspect Biol 2018;10(8):a028894. DOI: 10.1101/cshperspect.a028894.
169. Nakaya HI, Pulendran B. Systems vaccinology: Its promise and challenge for HIV vaccine development. Curr Opin HIV AIDS 2012;7(1):24–31. DOI: 10.1097/COH.0b013e32834dc37b.

170. Rahman M, Adeli M, Schellhorn HE, et al. Editorial: Precision vaccinology for infectious diseases. Front Immunol 2024;15:1400443. DOI: 10.3389/fimmu.2024.1400443.
171. Dowling DJ, Levy O. Pediatric vaccine adjuvants: Components of the modern vaccinologist's toolbox. Pediatr Infect Dis J 2015;34(12): 1395–1398. DOI: 10.1097/INF.0000000000000893.
172. He Y, Rappuoli R, De Groot AS, et al. Emerging vaccine informatics. J Biomed Biotechnol 2010;2010:218590. DOI: 10.1155/2010/218590.
173. Topol EJ. High-performance medicine: The convergence of human and artificial intelligence. Nat Med 2019;25(1):44–56. DOI: 10.1038/s41591-018-0300-7.

CASE REPORT

Bilaterally Enlarged Echogenic and Cystic Fetal Kidneys: *HNF1B*-Related Disease in the Differential Diagnosis

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Abstract

Bilaterally enlarged hyperechogenic fetal kidneys with visible cysts represent a diagnostically challenging phenotype that may occur in hepatocyte nuclear factor 1 homeobox B (*HNF1B*)-related disease, autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive polycystic kidney disease (ARPKD), renal dysplasia, and other developmental or cystic kidney disorders. We describe a fetus identified at 22 weeks' gestation with progressive bilateral nephromegaly, markedly increased renal echogenicity, reduced corticomedullary differentiation, and multiple small cortical/subcapsular cysts, with preserved bladder filling, amniotic fluid volume, fetal growth, and no major extrarenal abnormalities. Chromosomal microarray analysis (CMA) demonstrated no pathogenic copy-number abnormality, including deletion of the *HNF1B*-containing 17q12 region. Subsequent trio genomic sequencing identified a heterozygous pathogenic *HNF1B* nonsense variant, NM_000458.4:c.826C>T (p.Arg276Ter). The same variant was unexpectedly identified in the clinically asymptomatic mother, whose subsequent evaluation revealed subtle renal abnormalities and hypomagnesemia, illustrating marked intrafamilial variable expressivity. The infant was delivered at term with preserved urine output; postnatal imaging reproduced the prenatal renal phenotype, and follow-up demonstrated mild renal dysfunction and hypomagnesemia. Importantly, visible fetal renal cysts are a morphologic finding rather than a molecular diagnosis: Cysts may occur in *HNF1B*-related disease, ADPKD, ARPKD, and renal dysplasia, although conspicuous macrocysts are not typically the dominant early prenatal feature of classic ARPKD. The subsequent behavior of cysts in *HNF1B*-related disease also should not be assumed to follow the progressive cyst-expansion paradigm of ADPKD, because cyst burden may remain stable in many affected children and renal morphology may evolve variably over time. This case demonstrates the complementary roles of detailed fetal phenotyping, CMA, sequence analysis, parental testing, and longitudinal surveillance and emphasizes that molecular diagnosis establishes etiology but does not reliably predict renal progression or overall phenotypic severity.

Keywords: Autosomal dominant polycystic kidney disease, Autosomal recessive polycystic kidney disease, Case report, Echogenic kidney, Fetal kidney, Genomic sequencing, hepatocyte nuclear factor 1-beta, Nephromegaly, Prenatal diagnosis, Renal cysts, Renal dysplasia.

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Key Points

- Hepatocyte nuclear factor 1-beta homeobox B (HNF1B)-related disease should be considered early in the differential diagnosis of bilaterally enlarged echogenic and cystic fetal kidneys, alongside autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive polycystic kidney disease (ARPKD), renal dysplasia, and other cystic renal disorders.
- Visible fetal renal cysts do not establish a specific molecular diagnosis; classic ARPKD more often presents with diffusely enlarged echogenic kidneys without conspicuous macrocysts early in gestation, whereas cysts may be observed in HNF1B-related disease, ADPKD, and renal dysplasia.
- Stepwise genomic evaluation can distinguish HNF1B-related disease from phenotypically overlapping disorders: Chromosomal microarray analysis (CMA) can identify the recurrent 17q12 deletion, whereas sequencing can detect intragenic pathogenic HNF1B variants.
- HNF1B-related disease demonstrates marked variable expressivity, illustrated in this case by striking prenatal renal abnormalities in the infant and previously unrecognized mild disease in the mother carrying the same pathogenic variant.
- Identification of a pathogenic HNF1B variant has lifelong implications, including renal and multisystem surveillance, assessment for later-emerging manifestations, and counseling regarding autosomal dominant inheritance and future reproductive options.

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Introduction

Bilateral hyperechogenic fetal kidneys represent a sonographic phenotype with a broad and genetically heterogeneous differential diagnosis, ranging from developmental renal abnormalities to ADPKD, ADPKD, renal ciliopathies, and syndromic disorders.[1–4] Kidney size, visibility and distribution of cysts, corticomedullary differentiation, gestational age at presentation, amniotic fluid

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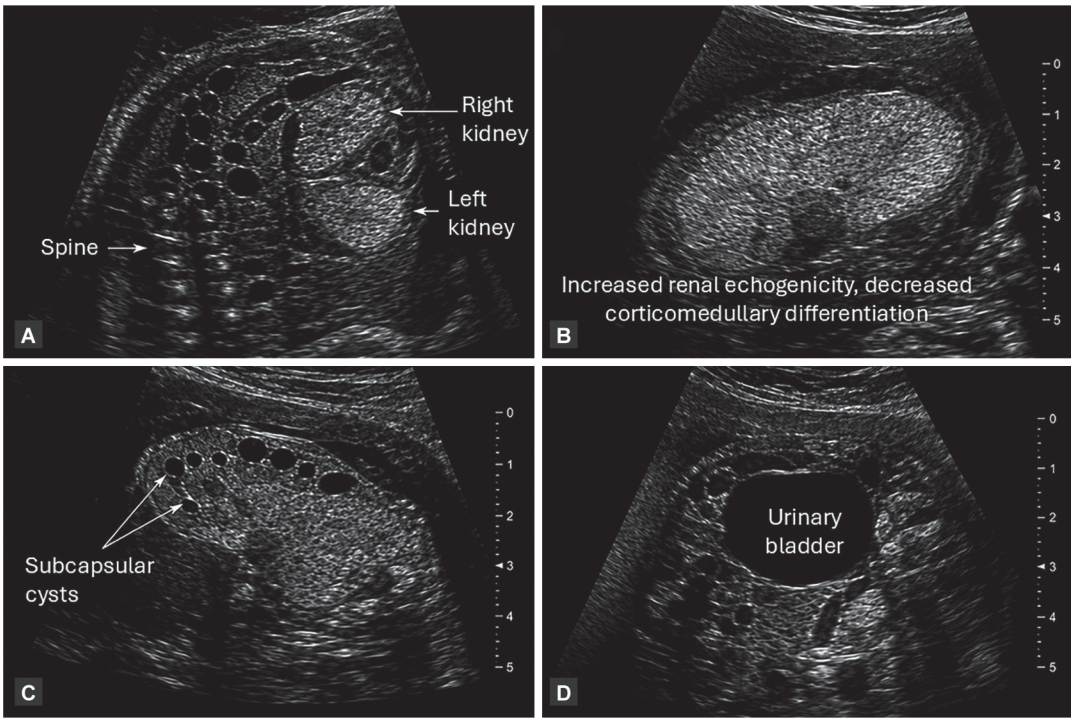


Figs 1A to D: Prenatal sonographic features of bilateral echogenic nephromegaly associated with *HNF1B*-related renal disease. (A) Coronal oblique view of the fetal abdomen at 22 + 3 weeks' gestation demonstrating bilateral enlarged, hyperechogenic kidneys in relation to the fetal spine; (B) Longitudinal view of the right kidney showing diffuse increased parenchymal echogenicity and reduced corticomedullary differentiation; (C) High-resolution oblique view of the left kidney demonstrating multiple small cortical and subcapsular cysts; (D) Normally filled fetal urinary bladder with preserved amniotic fluid volume, consistent with continued fetal urine production despite the structural renal abnormalities

volume, and associated extrarenal abnormalities can help refine the diagnostic possibilities and prognosis.[1–4] The presence of visible cysts is particularly informative but is not disease-specific; cysts may occur prenatally in ADPKD, ARPKD, renal dysplasia, and developmental disorders including *HNF1B*-related disease.[1,3–8] Here, we present an infant with bilaterally enlarged, hyperechogenic, and cystic kidneys identified prenatally and subsequently diagnosed with *HNF1B*-related disease caused by a pathogenic intragenic nonsense variant.

Hepatocyte nuclear factor 1-beta homeobox B–related disease is an important consideration in fetuses with hyperechogenic, cystic, or dysplastic renal abnormalities, and pathogenic *HNF1B* copy-number and sequence variants are well-established causes of bilateral fetal hyperechogenic kidneys.[1,8–10] Prenatal imaging alone, however, cannot establish the molecular diagnosis because substantial phenotypic overlap exists among these disorders.[1,3,8–10] This case illustrates how systematic fetal phenotyping followed by stepwise genomic investigation can distinguish *HNF1B*-related disease from phenotypically overlapping polycystic and dysplastic kidney disorders, guide neonatal surveillance, and unexpectedly reveal previously unrecognized familial disease.

Case Description

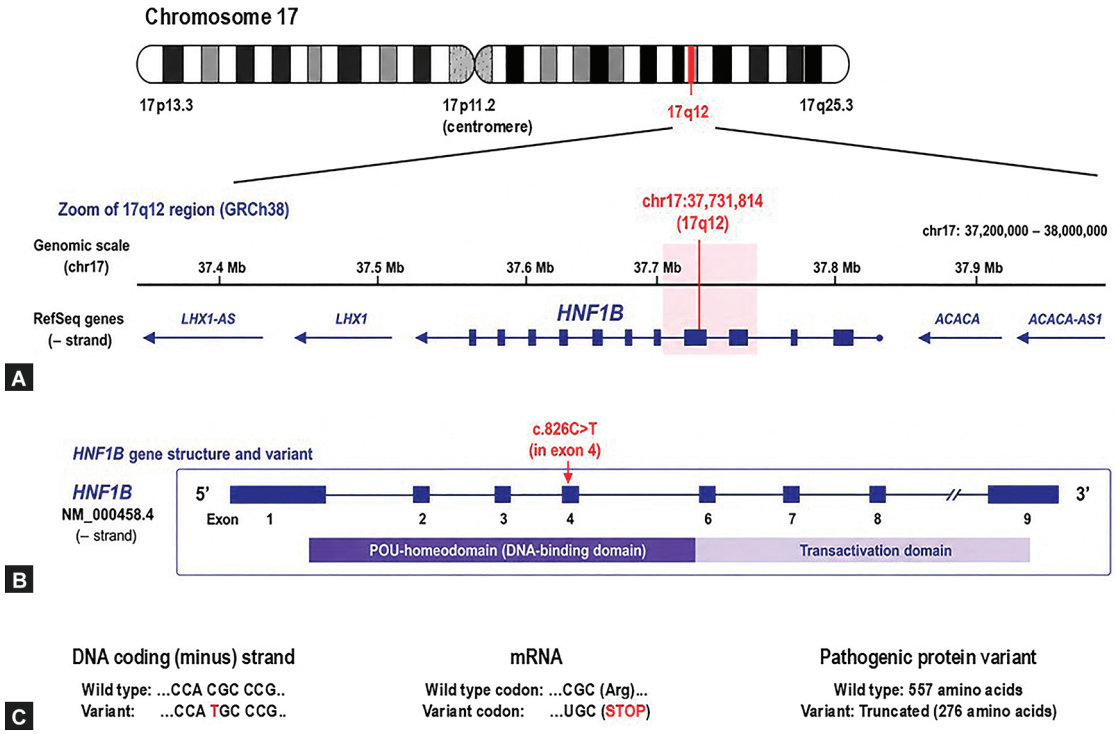
Maternal History

A 31-year-old primigravida was referred to maternal–fetal medicine at 22 weeks' gestation after routine fetal ultrasonography demonstrated bilateral renal enlargement and increased renal echogenicity. The pregnancy had otherwise been uncomplicated, with low-risk first-trimester screening for common aneuploidies and fetal growth appropriate for gestational age. There was no known parental consanguinity or family history of polycystic kidney disease, congenital anomalies of the kidney and urinary tract, childhood renal failure, unexplained perinatal death, or known hereditary renal disease. The mother had no known history of renal disease, diabetes, electrolyte abnormalities, or other significant medical illness.

Evaluation

Targeted fetal ultrasonography at 22 weeks confirmed symmetric bilateral nephromegaly with markedly hyperechogenic renal parenchyma, reduced corticomedullary differentiation, and several small cortical/subcapsular cysts (Fig. 1). There was no hydronephrosis or ureteral dilatation. Despite the striking renal abnormalities, the urinary bladder demonstrated normal filling and emptying, amniotic fluid volume was normal, and fetal growth and Doppler studies were appropriate for gestational age, suggesting preserved fetal urine production.

Detailed anatomic evaluation demonstrated none of the major extrarenal findings that can suggest a syndromic ciliopathy, including postaxial polydactyly, occipital encephalocele, posterior fossa or cerebellar abnormalities, skeletal shortening, short ribs or a narrow thorax, or other major structural malformations. Serial ultrasonography at 26 and 30 weeks demonstrated progressive bilateral renal enlargement and persistent parenchymal hyperechogenicity, whereas bladder filling, amniotic fluid volume, fetal growth, and Doppler findings remained reassuring.



Figs 2A to C: Chromosomal localization and molecular consequence of the pathogenic *HNF1B* variant. (A) Ideogram of human chromosome 17 showing the *HNF1B* locus localized to 17q12. An expanded schematic of the 17q12 region, constructed using the Genome Reference Consortium Human Build 38 (GRCh38), shows the *HNF1B* gene on the minus (–) strand and localizes the variant to chr17:37,731,814; (B) Schematic of the *HNF1B* transcript showing the exon structure, POU-homeodomain DNA-binding and transactivation regions, and localization of the heterozygous pathogenic variant c.826C>T to exon 4; (C) Deoxyribonucleic acid (DNA), messenger ribonucleic acid (mRNA), and protein-level consequences of the variant. The coding DNA sequence change produces a premature termination codon in the corresponding mRNA, resulting in p.Arg276Ter (p.Arg276*). The normal HNF1B isoform 1 contains 557 amino acids; the variant introduces termination at residue 276, resulting in a truncated protein and loss of normal HNF1B function. Because *HNF1B* is located on the negative strand, genomic and transcript-level sequence representations reflect complementary strand orientations

Differential Diagnosis

The combination of bilateral echogenic nephromegaly, visible cortical cysts, reduced corticomedullary differentiation, preserved bladder filling and amniotic fluid volume, and absence of major extrarenal malformations suggested a developmental or cystic renal disorder and guided the genomic differential.[1–4] Autosomal dominant polycystic kidney disease, ARPKD, renal dysplasia, renal ciliopathies, and other monogenic cystic kidney disorders were considered.[1–7]

Prenatal ADPKD may manifest with enlarged hyperechogenic kidneys, variable corticomedullary differentiation, and with or without sonographically visible cysts.[3,5,6] Discrete renal cysts are not invariably present prenatally; in a multicenter series of 27 fetuses with ADPKD, prenatal cysts were identified in only four fetuses.[6] Thus, increased renal size and echogenicity may precede the appearance of conspicuous cysts.[5,6]

Classic fetal ARPKD more typically produces bilaterally and symmetrically enlarged, diffusely hyperechogenic kidneys with reduced or absent corticomedullary differentiation.[1,3,4,7] The cystic abnormality predominantly reflects collecting-duct dilatation, which may remain microscopic or below conventional sonographic resolution, particularly earlier in gestation.[3,7] Nevertheless, visible cysts do not exclude ARPKD; improved imaging resolution and contemporary fetal series demonstrate that small cysts may sometimes be visualized, with their conspicuity varying according to gestational age and disease expression.[3,4] Thus, conspicuous discrete macroscopic cysts are not required for, and historically have not been the dominant early sonographic feature of, classic ARPKD.[3,7] Renal dysplasia can likewise produce abnormal renal size, increased parenchymal echogenicity, impaired corticomedullary differentiation, architectural distortion, and cysts of variable number and distribution.[1,8] Consequently, the presence of visible cysts in enlarged echogenic fetal kidneys does not by itself indicate the cause, such as ADPKD, ARPKD, renal dysplasia, or other genetic disorders.[1,3,5–10]

Management and Genetic Diagnosis

Following multidisciplinary genetic counseling, amniocentesis was performed at 23 weeks' gestation, and fetal DNA obtained from amniotic fluid cells underwent CMA. The results were inconclusive. Subsequent genomic sequencing showed a heterozygous pathogenic nonsense variant, NM_000458.4:c.826C>T (p.Arg276Ter) in the *HNF1B* gene (Fig. 2).[1,9,10] This substitution introduces a premature termination codon at amino-acid position 276 and is expected to disrupt normal HNF1B function through a loss-of-function mechanism.[11] In our index case, preserved amniotic fluid and bladder filling, small predominantly cortical/subcapsular cysts, reduced corticomedullary differentiation, and absence of a recognizable syndromic phenotype raised *HNF1B*-related disease as a plausible cause.[1,9,10]

Parental testing demonstrated the same *HNF1B* variant in the clinically asymptomatic mother. This unexpected finding prompted phenotype-directed maternal evaluation, which identified previously unrecognized subtle renal abnormalities and hypomagnesemia, with subsequent assessment for abnormalities of glucose metabolism. The markedly different maternal and fetal presentations illustrated the variable expressivity and intrafamilial phenotypic heterogeneity characteristic of *HNF1B*-related disease.[12–14]

Pregnancy Course and Delivery

Because fetal growth, bladder filling, amniotic fluid volume, and Doppler findings remained reassuring, the pregnancy was managed expectantly with serial ultrasonographic surveillance and multidisciplinary planning involving maternal–fetal medicine, neonatology, pediatric nephrology, and clinical genetics. The parents were counseled that identification of the pathogenic *HNF1B* variant established the molecular diagnosis but could not precisely predict the severity of postnatal renal disease, particularly given the substantial phenotypic variability demonstrated within the family.[12–14] The pregnancy progressed without oligohydramnios or fetal growth restriction, and the infant was delivered at term in good condition, with preserved urine output and no obvious extrarenal congenital abnormalities.

Neonatal Evaluation and Follow-up

Postnatal renal ultrasonography confirmed bilateral nephromegaly, diffuse increased renal echogenicity, reduced corticomedullary differentiation, and multiple small cortical cysts, closely reproducing the prenatal renal phenotype. Renal function was monitored serially; mild renal dysfunction became apparent despite preserved urine output, and laboratory evaluation demonstrated hypomagnesemia, providing an additional phenotypic correlate of *HNF1B*-related disease.[15,16] Longitudinal follow-up was established to monitor renal function, serum electrolytes, blood pressure, growth, and glucose metabolism, with additional surveillance guided by the evolving phenotype.[12–16]

Discussion

The prenatal finding of bilaterally enlarged hyperechogenic and cystic kidneys has a broad differential diagnosis that includes ADPKD, ARPKD, renal ciliopathies, renal dysplasia, chromosomal abnormalities, and developmental disorders caused by *HNF1B* variants.[1–10] The present case illustrates why visible fetal renal cysts should be interpreted as a morphologic finding rather than as evidence of a particular polycystic kidney disease.[1,3,5–10] Prenatal ADPKD may produce enlarged echogenic kidneys with or without visible cysts, and renal enlargement or increased echogenicity may precede their sonographic appearance.[5,6] Classic fetal ARPKD more commonly produces symmetrically enlarged, diffusely hyperechogenic kidneys with poor corticomedullary differentiation; its underlying collecting-duct dilatations may remain below conventional imaging resolution, although small visible cysts can occur.[3,4,7] Renal dysplasia can similarly produce abnormal kidney size, increased echogenicity, architectural distortion, and cysts of variable size and distribution.[1,8] Thus, neither the presence nor absence of discrete cysts reliably separates ADPKD, ARPKD, renal dysplasia, and *HNF1B*-related disease.[1,3,5–10] In this fetus, the combination of small cortical/subcapsular cysts, reduced corticomedullary differentiation, preserved bladder filling and amniotic fluid volume, and absence of a recognizable syndromic phenotype was consistent but not specific for *HNF1B*-related disease.[8–10]

Hepatocyte nuclear factor 1-beta homeobox B encodes hepatocyte nuclear factor 1-beta (HNF1B), a transcription factor with critical roles in nephrogenesis, nephron differentiation, tubular epithelial function, cellular polarity, ion transport, and regulation of kidney-specific developmental programs.[12,17,18] The heterozygous nonsense variant NM_000458.4:c.826C>T (p.Arg276Ter) identified in this infant introduces a premature termination codon (Fig. 2) and is expected to result in loss of normal HNF1B function.[11] Hepatocyte nuclear factor 1-beta also regulates developmental networks that intersect with cystogenesis and primary-cilium biology, including genes implicated in cystic kidney disease.[17,19,20] Experimental disruption of HNF1B signaling alters renal developmental programs and promotes renal cyst formation.[19,20] Hepatocyte nuclear factor 1-beta should therefore be regarded not as a structural ciliary protein but as an upstream transcriptional regulator of developmental pathways that include cilia-associated proteins; this helps explain why *HNF1B*-related renal disease can resemble inherited renal ciliopathies without constituting a primary structural ciliopathy.[17,19,20] Whether HNF1B deficiency itself produces a clinically important primary defect of ciliogenesis remains uncertain.[17,18]

Hepatocyte nuclear factor 1-beta homeobox B–related disease is a multisystem disorder historically associated with renal cysts and diabetes (RCAD) syndrome and maturity-onset diabetes of the young type 5 (MODY5).[12,17] Its spectrum includes renal dysplasia and other congenital renal abnormalities, hypomagnesemia, hyperuricemia, diabetes, pancreatic abnormalities, genital-tract malformations, and hepatobiliary abnormalities.[12,17] Renal manifestations may precede metabolic manifestations by many years and may dominate the phenotype during fetal life and childhood.[8–10,12–14] Hepatocyte nuclear factor 1-beta homeobox B–related disease caused by an intragenic pathogenic variant should also be distinguished from recurrent 17q12 deletion syndrome, in which *HNF1B* and neighboring genes are deleted, and the resulting phenotype may be broader.[12,21,22] In this case, the absence of a 17q12 deletion on CMA followed by identification of an intragenic *HNF1B* nonsense variant supports *HNF1B*-related disease within the RCAD/MODY5 spectrum rather than 17q12 recurrent deletion syndrome.[11,12,21,22] The postnatal hypomagnesemia provides additional genotype–phenotype coherence: Renal magnesium wasting is a recognized manifestation of *HNF1B*-related disease, and HNF1B-mediated regulation of *FXYD2* provides a mechanistic connection between the molecular defect and abnormal tubular magnesium handling.[15,16]

The natural history of renal cysts in *HNF1B*-related disease appears to differ from a simple model of progressive cyst expansion. In the German Multicenter HNF1B Childhood Registry, bilateral cysts were present in 74% and unilateral cysts in 16% of affected children; among 47 children with longitudinal imaging, cyst development was non-progressive in 34 (72%) and progressive in 13 (28%).[23] These numerical observations come directly from the original longitudinal cohort. An increase in cyst number was associated with a greater mean decline in glomerular filtration rate, suggesting that cyst evolution may carry prognostic information without constituting a reliable morphologic staging system.[23] Renal morphology may also occasionally become less conspicuous with age; in a small series of children with fetal-onset renal abnormalities associated with 17q12 deletions encompassing *HNF1B*, renal parenchymal abnormalities normalized in four of five children during follow-up.[24]

Other prenatal observations suggest that cysts may become more apparent after birth, further emphasizing that renal morphology can evolve in more than one direction.[8,25] These observations argue against interpreting cyst number or size alone as a disease stage or surrogate for future renal function. Rather, *HNF1B* nephropathy is better viewed as a developmental and dysplastic renal disorder in which cysts constitute one component of a heterogeneous and dynamically evolving phenotype.[8,23–25]

The markedly different manifestations in the infant and mother illustrate the substantial variable expressivity of *HNF1B*-related disease.[12–14,23] Renal disease can range from conspicuous prenatal abnormalities to mild or slowly progressive disease recognized much later in life.[12–14,23] Because the disorder is autosomal dominant, a heterozygous affected parent has a 50% probability of transmitting the pathogenic allele in each pregnancy; however, inheritance of the allele does not permit reliable prediction of disease severity.[12–14] The German HNF1B Childhood Registry reported maternal inheritance in 81% of inherited dominant cases, raising the possibility of parent-of-origin or epigenetic influences; however, this cohort observation does not establish that maternal transmission produces more severe disease.[23] The present family illustrates the broader principle particularly well: The same pathogenic variant produced striking prenatal renal abnormalities in the fetus but remained clinically unrecognized in the mother until targeted evaluation.

Several mechanisms may contribute to such variable expressivity. Genome-wide methylomic analysis has demonstrated a shared DNA-methylation signature in individuals with intragenic *HNF1B* variants and 17q12 deletions, providing evidence that HNF1B haploinsufficiency can influence the epigenome.[26] DNA methylation, histone modifications, chromatin remodeling, regulatory non-coding RNAs, modifier genes, regulatory variants, and somatic or tissue-specific mosaicism may modify gene expression or developmental responses, although their individual contributions to clinical variability remain uncertain.[17,26] Hepatocyte nuclear factor 1-beta has also been linked experimentally to mitochondrial regulation and cellular bioenergetics; inhibition of HNF1B in renal tubular cells reduces the expression of the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*) gene and alters mitochondrial morphology and respiration.[27] Whether epigenetic or mitochondrial mechanisms contribute directly to prenatal cystogenesis or explain intrafamilial differences in severity remains unknown. Parent-of-origin mechanisms, including uniparental disomy, have not been established as determinants of *HNF1B*-related disease and should remain hypotheses for future study rather than recognized mechanisms. Gonadal mosaicism is principally relevant to recurrence assessment in apparently *de novo* disease and is not required to explain transmission in this family because the pathogenic variant was identified in maternal somatic DNA. Integrated genomic, epigenomic, transcriptomic, metabolomic, mitochondrial, and developmental studies may ultimately clarify why the same pathogenic allele produces markedly different phenotypes.[17,23,26,27]

The short-term prognosis in this infant appears cautiously favorable because fetal urine production and amniotic fluid volume remained preserved, major extrarenal structural abnormalities were absent, postnatal urine output was maintained, and early renal dysfunction was mild. Nevertheless, long-term renal prognosis remains guarded because *HNF1B*-related nephropathy can progress to chronic kidney disease (CKD) and, in a subset of children, kidney failure requiring kidney replacement therapy or transplantation.[12–14,23,28] The German Childhood Registry demonstrated a generally slow-progressive course in most children but identified a subgroup with severe very-early-onset kidney failure.[23] A large international cohort subsequently demonstrated substantial heterogeneity in kidney survival and renal and extrarenal phenotypes, further supporting the inability to infer an individual patient's prognosis simply from the presence or burden of cysts.[28] Pediatric nephrology follow-up should therefore integrate renal morphology with growth and nutrition, blood pressure, serum creatinine and age-appropriate estimated glomerular filtration rate (eGFR), proteinuria/albuminuria, and electrolyte and acid–base homeostasis, particularly magnesium.[12–16,23,28] Serial renal ultrasonography remains useful for documenting evolving morphology, but changes in cyst number or size should be interpreted alongside functional measures rather than used in isolation to define progression.[23,28] Surveillance should intensify with declining kidney function, increasing proteinuria, hypertension, impaired growth, or complications of CKD. If progressive renal dysfunction develops, preparation for kidney replacement therapy and transplantation can begin before kidney failure.[23,28] When counseling the parents, it is reasonable to explain that kidney transplantation may eventually become necessary in a subset of severely affected children, but the need and timing cannot presently be predicted for this infant.[23,28]

Longitudinal pediatric care should extend beyond renal surveillance. Neurodevelopment should be followed through developmental milestones, motor and language development, cognition, learning and school performance, behavior, and seizures.[21,29] Neurodevelopmental abnormalities are considerably more strongly associated with recurrent 17q12 deletions than with isolated intragenic *HNF1B* variants, making the negative CMA in this infant clinically meaningful, although routine developmental surveillance remains appropriate.[21,29] Developmental, learning, behavioral, or neurological concerns should prompt formal assessment and appropriate early-intervention or educational support, with neuroimaging or electroencephalography guided by clinical indications.[21,29] Pulmonary status can likewise be followed clinically, with further evaluation for persistent or recurrent respiratory abnormalities rather than routine intensive surveillance in an asymptomatic child, because *HNF1B*-related disease is not established as a primary motile ciliopathy.[17–20] As the child grows, pediatric follow-up should also remain attentive to later-emerging abnormalities of glucose metabolism and pancreatic function, hepatobiliary abnormalities, hyperuricemia, and genital or reproductive tract abnormalities.[12,17,23] Thus, neonatal renal disease may represent the earliest manifestation of a lifelong multisystem condition.[12,17]

The autosomal dominant inheritance and unpredictable expressivity of *HNF1B*-related disease have important implications for future reproductive planning.[12–14] Because the mother carries the familial pathogenic variant, each future pregnancy has a 50% probability of inheriting it.[12,17] Preconception genetic counseling may include natural conception with targeted prenatal diagnosis or *in vitro* fertilization (IVF) with preimplantation genetic testing for monogenic disease (PGT-M), presented as reproductive options within nondirective counseling.[30,31] Because the familial c.826C>T (p.Arg276Ter) variant is known, definitive prenatal testing can be offered using chorionic villus sampling (CVS) or amniocentesis without waiting for development of an abnormal

renal phenotype.[30,31] If echogenic or cystic renal abnormalities are identified, CMA and, when indicated, broader genomic testing may remain appropriate because the sonographic phenotype is not specific for *HNF1B*-related disease.[1,8–10] Importantly, identification of the familial variant would establish inheritance but would not reliably predict severity; longitudinal fetal imaging would therefore remain essential for phenotypic assessment, counseling, and perinatal planning.[8–10,12–14]

This case also demonstrates the complementary roles of fetal phenotyping, CMA, and sequence analysis. Recognition of enlarged echogenic and cystic kidneys established a rational genomic differential but could not distinguish *HNF1B*-related disease from ADPKD, ARPKD, renal dysplasia, or other monogenic disorders.[1–10] Because *HNF1B* lies within the recurrent 17q12 deletion region, CMA appropriately evaluated a copy-number abnormality; when CMA was negative, sequencing identified an intragenic pathogenic variant that morphology and copy-number analysis alone could not establish.[8–10,21,22] Contemporary studies of fetal hyperechogenic kidneys support the complementary use of chromosomal microarray and sequencing, particularly when copy-number analysis is nondiagnostic and a monogenic disorder remains suspected.[1,9,10]

Expert ultrasonography remains the primary modality for characterization and longitudinal evaluation of fetal renal disease, whereas fetal magnetic resonance imaging (MRI) may provide complementary anatomical information when sonographic characterization is limited or inconclusive.[32,33] Emerging quantitative and functional MRI techniques may eventually provide additional information about fetal renal tissue characteristics or function, but their clinical role remains under development.[32] Analysis of cell-free fetal/placental DNA in maternal plasma may ultimately provide another noninvasive route to monogenic prenatal diagnosis.[34–36] For maternally inherited variants, however, fetal inheritance must be distinguished from the maternal cell-free DNA background, making diagnosis technically more challenging than detection of a paternally inherited or *de novo* allele.[34–36] Consequently, simple detection of the maternal *HNF1B* variant in maternal plasma would not by itself establish fetal inheritance.[34–36] In phenotype-positive/genotype-negative cases, future investigation may appropriately extend beyond conventional sequence analysis to regulatory and epigenetic abnormalities, mosaicism, modifier genes, cilia-associated pathways, and metabolic or mitochondrial mechanisms.[1–22,26,27]

Overall, this case demonstrates that bilaterally enlarged echogenic and cystic fetal kidneys constitute a sonographic phenotype rather than a molecular diagnosis.[1–10] The presence of visible cysts appropriately raises ADPKD and dysplastic kidney disorders in the differential, whereas classic early ARPKD more commonly manifests as diffusely enlarged echogenic kidneys without conspicuous macrocysts, although small visible cysts can occur.[3–7] The natural history after an *HNF1B* diagnosis should likewise not be assumed to follow the progressive cyst-expansion paradigm of ADPKD: Cyst burden remains stable in most affected children, may increase in a subset, and renal parenchymal abnormalities may occasionally become less conspicuous with age.[23–25] Careful characterization of cyst distribution, renal size and echogenicity, corticomedullary differentiation, fetal renal function, amniotic fluid volume, temporal evolution, family phenotype, and extrarenal anatomy can narrow the differential, but molecular testing is often required for definitive diagnosis.[1–10] Identification of a pathogenic variant need not mark the end of molecular inquiry; in disorders characterized by substantial variable expressivity, understanding the genetic, regulatory, epigenetic, ciliary, mitochondrial, metabolic, and developmental factors that modify its consequences may ultimately be equally important.[12–14,17–20,23–28] Parental testing may extend the significance of the diagnosis beyond the fetus by identifying previously unrecognized disease, directing surveillance, informing recurrence counseling, and providing reproductive options for future pregnancies.[12–14,30,31] In this case, the bright fetal kidneys illuminated more than a renal abnormality: They directed the molecular diagnosis, defined a multisystem disorder requiring longitudinal surveillance, shaped neonatal care, and uncovered a previously unrecognized familial disorder.

Declarations

Ethics Waiver/Approval

The local ethics committee provided a waiver.

Artificial Intelligence (AI) Disclosure

No AI methods were used.

Authors' Contributions

The authors contributed equally to the preparation of this case report, including clinical data collection and interpretation, review of the relevant literature, manuscript preparation, and critical revision of the final version.

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References

1. Hertenstein CB, Miller KA, Estroff JA, et al. Fetal hyperechoic kidneys: Diagnostic considerations and genetic testing strategies. Prenat Diagn 2024;44(2):222–236. DOI: 10.1002/pd.6517.
2. Shuster S, Keunen J, Shannon P, et al. Prenatal detection of isolated bilateral hyperechogenic kidneys: Etiologies and outcomes. Prenat Diagn 2019;39(9):693–700. DOI: 10.1002/pd.5418.
3. Yang W, Zu S, Jin Q, et al. Fetal hyperechoic kidney cohort study and a meta-analysis. Front Genet 2023;14:1237912. DOI: 10.3389/fgene.2023.1237912.
4. Simonini C, Froschen EM, Nadal J, et al. Prenatal ultrasound in fetuses with polycystic kidney appearance – expanding the diagnostic algorithm. Arch Gynecol Obstet 2023;308(4):1287–1300. DOI: 10.1007/s00404-022-06814-8.
5. Journel H, Guyot C, Barc RM, et al. Unexpected ultrasonographic prenatal diagnosis of autosomal dominant polycystic kidney disease. Prenat Diagn 1989;9(9):663–671. DOI: 10.1002/pd.1970090910.
6. Brun M, Maugey-Laulom B, Eurin D, et al. Prenatal sonographic patterns in autosomal dominant polycystic kidney disease: A multicenter study. Ultrasound Obstet Gynecol 2004;24(1):55–61. DOI: 10.1002/uog.1098.
7. Wisser J, Hebisch G, Froster U, et al. Prenatal sonographic diagnosis of autosomal recessive polycystic kidney disease during the early second trimester. Prenat Diagn 1995;15(9):868–871. DOI: 10.1002/pd.1970150914.

8. Decramer S, Parant O, Beaufils S, et al. Anomalies of the TCF2 gene are the main cause of fetal bilateral hyperechogenic kidneys. J Am Soc Nephrol 2007;18(3):923–933. DOI: 10.1681/ASN.2006091057.
9. Yu QX, Jing XY, Li DZ. Prenatal diagnosis of intragenic *HNF1B* variant-associated renal disease by exome sequencing. Mol Syndromol 2023;14(1):59–64. DOI: 10.1159/000526394.
10. Deng L, Liu Y, Yuan M, et al. Prenatal diagnosis and outcome of fetal hyperechogenic kidneys in the era of antenatal next-generation sequencing. Clin Chim Acta 2022;528:16–28. DOI: 10.1016/j.cca.2022.01.012.
11. National Center for Biotechnology Information. ClinVar. NM_000458.4(*HNF1B*):c.826C>T (p.Arg276Ter), Variation ID 12640.
12. Bockenhauer D, Jaureguiberry G. *HNF1B*-associated clinical phenotypes: The kidney and beyond. Pediatr Nephrol 2016;31(5):707–714. DOI: 10.1007/s00467-015-3142-2.
13. Izzi C, Dordoni C, Econimo L, et al. Variable expressivity of *HNF1B* nephropathy, from renal cysts and diabetes to medullary sponge kidney through tubulo-interstitial kidney disease. Kidney Int Rep 2020;5(12):2341–2350. DOI: 10.1016/j.ekir.2020.09.042.
14. Heidet L, Decramer S, Pawtowski A, et al. Spectrum of *HNF1B* mutations in a large cohort of patients who harbor renal diseases. Clin J Am Soc Nephrol 2010;5(6):1079–1090. DOI: 10.2215/CJN.06810909.
15. Adalat S, Woolf AS, Johnstone KA, et al. *HNF1B* mutations associate with hypomagnesemia and renal magnesium wasting. J Am Soc Nephrol 2009;20(5):1123–1131. DOI: 10.1681/ASN.2008060633.
16. Kołbuc M, Leßmeier L, Salamon-Słowińska D, et al. Hypomagnesemia is underestimated in children with *HNF1B* mutations. Pediatr Nephrol 2020. DOI: 10.1007/s00467-020-04576-6.
17. Ferrè S, Igarashi P. New insights into the role of HNF-1β in kidney (patho)physiology. Pediatr Nephrol 2019;34(8):1325–1335. DOI: 10.1007/s00467-018-3990-7.
18. Shao A, Chan SC, Igarashi P. Role of transcription factor hepatocyte nuclear factor-1β in polycystic kidney disease. Cell Signal 2020;71:109568. DOI: 10.1016/j.cellsig.2020.109568.
19. Hiesberger T, Bai Y, Shao X, et al. Mutation of hepatocyte nuclear factor-1β inhibits *Pkhd1* gene expression and produces renal cysts in mice. J Clin Invest 2004;113(6):814–825. DOI: 10.1172/JCI20083.
20. Hiesberger T, Shao X, Gourley E, et al. Role of the hepatocyte nuclear factor-1β C-terminal domain in *Pkhd1* gene transcription and renal cystogenesis. J Biol Chem 2005;280(11):10578–10586. DOI: 10.1074/jbc.M414121200.
21. Clissold RL, Shaw-Smith C, Turnpenny P, et al. Chromosome 17q12 microdeletions but not intragenic *HNF1B* mutations link developmental kidney disease and psychiatric disorder. Kidney Int 2016;90(1):203–211. DOI: 10.1016/j.kint.2016.03.027.
22. Clissold RL, Hamilton AJ, Hattersley AT, et al. *HNF1B*-associated renal and extra-renal disease—An expanding clinical spectrum. Nat Rev Nephrol 2015;11(2):102–112. DOI: 10.1038/nrneph.2014.232.
23. Okorn C, Goertz A, Vester U, et al. *HNF1B* nephropathy has a slow-progressive phenotype in childhood—with the exception of very early onset cases: Results of the German Multicenter *HNF1B* Childhood Registry. Pediatr Nephrol 2019;34(6):1065–1075. DOI: 10.1007/s00467-018-4188-8.
24. Cleper R, Reches A, Shapira D, et al. Improving renal phenotype and evolving extra-renal features of 17q12 deletion encompassing the *HNF1B* gene. Transl Pediatr 2021;10(12):3130–3139. DOI: 10.21037/tp-21-386.
25. Verscaj CP, Velez-Bartolomei F, Bodle E, et al. Characterization of the prenatal renal phenotype associated with 17q12 *HNF1B* microdeletions. Prenat Diagn 2024;44(2):237–246. DOI: 10.1002/pd.6424.
26. Clissold RL, Ashfield B, Burrage J, et al. Genome-wide methylomic analysis in individuals with *HNF1B* intragenic mutation and 17q12 microdeletion. Clin Epigenetics 2018;10:97. DOI: 10.1186/s13148-018-0530-z.
27. Casemayou A, Fournel A, Bagattin A, et al. Hepatocyte nuclear factor-1β controls mitochondrial respiration in renal tubular cells. J Am Soc Nephrol 2017;28(11):3205–3217. DOI: 10.1681/ASN.2016050508.
28. Buffin-Meyer B, Richard J, Guigonis V, et al. Renal and extrarenal phenotypes in patients with *HNF1B* variants and chromosome 17q12 microdeletions. Kidney Int Rep 2024;9(8):2514–2526. DOI: 10.1016/j.ekir.2024.05.007.
29. Nittel CM, Dobelke F, König J, et al. Review of neurodevelopmental disorders in patients with *HNF1B* gene variations. Front Pediatr 2023;11:1149875. DOI: 10.3389/fped.2023.1149875.
30. Practice Committee and Genetic Counseling Professional Group of the American Society for Reproductive Medicine. Indications and management of preimplantation genetic testing for monogenic conditions: A committee opinion. Fertil Steril 2023;120(1):61–71. DOI: 10.1016/j.fertnstert.2023.03.003.
31. Bunnell ME, Dobson LJ, Lanes A, et al. Use of preimplantation genetic testing for monogenic disorders and subsequent prenatal care and diagnostic testing. Prenat Diagn 2022;42(8):1022–1030. DOI: 10.1002/pd.6189.
32. Chalouhi GE, Millischer AE, Mahallati H, et al. The use of fetal MRI for renal and urogenital tract anomalies. Prenat Diagn 2020;40(1):100–109. DOI: 10.1002/pd.5610.
33. Gupta P, Kumar S, Sharma R, et al. The role of magnetic resonance imaging in fetal renal anomalies. Int J Gynaecol Obstet 2010;111(3):209–212. DOI: 10.1016/j.ijgo.2010.07.024.
34. Zhang J, Li J, Saucier JB, et al. Non-invasive prenatal sequencing for multiple Mendelian monogenic disorders using circulating cell-free fetal DNA. Nat Med 2019;25(3):439–447. DOI: 10.1038/s41591-018-0334-x.
35. Scotchman E, Shaw J, Paternoster B, et al. Non-invasive prenatal diagnosis and screening for monogenic disorders. Eur J Obstet Gynecol Reprod Biol 2020;253:320–327. DOI: 10.1016/j.ejogrb.2020.08.001.
36. Vermeulen C, Geeven G, de Wit E, et al. Sensitive monogenic noninvasive prenatal diagnosis by targeted haplotyping. Am J Hum Genet 2017;101(3):326–339. DOI: 10.1016/j.ajhg.2017.07.012.