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Early Prenatal Detection of Fetal Genetic Mutations and Epigenetic Alterations: A Critical Appraisal of Liquid Biopsy Methodologies and Paediatric Implications

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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Abstract

Circulating fetoplacental nucleic acids have transformed prenatal medicine within a single generation, and screening based on cell-free DNA (cfDNA) is now offered routinely in many health systems. The field is moving quickly from the detection of whole-chromosome aneuploidy towards earlier sampling, sub-chromosomal resolution, monogenic diagnosis and the interrogation of epigenetic marks, yet the evidence supporting these extensions is uneven and the downstream consequences for children are rarely examined. This critical narrative review evaluates the state of knowledge on early prenatal detection of fetal genetic variants and epigenetic alterations through maternal blood sampling, and appraises the paediatric implications of an expanding prenatal detection frontier. Literature was identified through structured searching of Europe PMC and MEDLINE, Crossref Metadata Search, OpenAlex, Semantic Scholar and targeted retrieval of professional society statements, supplemented

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by backward and forward citation tracking. Evidence was appraised for design adequacy, confirmatory testing, spectrum of enrolled participants, and separation of analytical from clinical validity. Three findings dominate the synthesis. First, diagnostic confidence declines sharply and predictably as the target moves from common autosomal trisomies to rare autosomal trisomies, copy number variants and single-gene conditions, and this gradient is driven more by target prevalence and by placental biology than by sequencing chemistry. Second, DNA methylation currently functions far more securely as an analytical instrument, supporting fractional quantification and tissue-of-origin deconvolution, than as a validated diagnostic target for fetal disease, and the developmental literature that motivates epigenetic prediction rests overwhelmingly on postnatal tissues rather than on prenatal plasma. Third, paediatric evidence is the weakest link in the chain: prenatal detection demonstrably alters the ascertainment and the age distribution of childhood diagnoses, but longitudinal outcome data for prenatally ascertained children remain scarce. Priorities include phenotype-linked birth cohorts of prenatally screened pregnancies, prospective validation of methylation-based classifiers against paediatric endpoints, and evaluation frameworks that treat placental discordance as clinical information rather than analytical noise.

Keywords: Cell-free DNA; non-invasive prenatal testing; DNA methylation; confined placental mosaicism; monogenic disorders; paediatric outcomes; liquid biopsy.

1. Introduction

The demonstration that DNA of fetal origin circulates in maternal plasma and serum reframed prenatal diagnosis as a problem of analytical sensitivity rather than of surgical access (Lo et al., 1997). Within little more than a decade, massively parallel sequencing of maternal plasma had been shown to reconstruct the genome-wide genetic and mutational profile of a fetus, including the inheritance of parental haplotypes at a locus responsible for a monogenic condition (Lo et al., 2010). Clinical translation followed rapidly, and screening based on cell-free DNA (cfDNA) is now embedded in publicly funded and commercial prenatal pathways across high-income settings and in a growing number of middle-income systems (Connor et al., 2025; Poulton & Hui, 2025).

The clinical achievement is real but narrow. Pooled analysis of validation and implementation studies gives a weighted detection rate of 99.7% for trisomy 21 with a false-positive rate of 0.04% in singleton pregnancies, a performance that no earlier serum or ultrasound-based strategy approached (Gil et al., 2017). Professional bodies have converged on the position that cfDNA screening is the most accurate available screen for the common autosomal trisomies and for sex chromosome aneuploidy (SCA) in singleton pregnancies, while declining to endorse routine screening for microdeletion syndromes or rare autosomal trisomies (RATs) in unselected populations on the grounds of insufficient evidence (Dungan et al., 2023; Hui et al., 2023). That distinction is the fault line along which most current controversy runs. Recent clinical syntheses continue to distinguish the well-established performance of cfDNA screening for common aneuploidies from the less mature evidence supporting broader applications, including genome-wide and single-gene testing (Knutson et al., 2024; Mito et al., 2026).

Commercial and academic development has nonetheless pushed outward along three axes at once. The first is temporal: assays incorporating fetal fraction amplification have been evaluated at 6 to 9 weeks of gestation, well before the conventional 10-week threshold (Dugoff et al., 2026). The second is resolution: genome-wide analysis detects sub-chromosomal imbalance, RATs and maternal copy number variation, generating a category of additional findings whose clinical meaning is heterogeneous (van Prooyen Schuurman et al., 2022). The third is target class: haplotype-based methods now support non-invasive prenatal diagnosis (NIPD) of autosomal recessive and X-linked conditions, including in consanguineous families previously excluded on methodological grounds, and panel-based screening for dominant single-gene disorders has entered prospective evaluation (Hanson et al., 2024; Lu et al., 2026; Zhang et al., 2026). A recent review has further situated this expansion across genetic, epigenetic, fragmentomic and transcriptomic analyses, while emphasizing that these modalities differ in clinical readiness (Stanley et al., 2025). Clinical experience with routine cfDNA screening for autosomal dominant single-gene conditions also indicates that wider use can create interpretive and counselling challenges when genotype–phenotype relationships are variable and confirmatory pathways are complex (Adams et al., 2025).

Running alongside this genetic expansion is a quieter epigenetic programme. Bisulfite sequencing of maternal plasma established that the circulating methylome can be read at genome scale and that fetoplacental molecules carry a methylation signature distinct from that of maternal blood cells (Lun et al., 2013). Methylation deconvolution subsequently allowed the tissue of origin of plasma DNA to be estimated, a principle that underpins prenatal, oncological and transplantation applications alike (Sun et al., 2015). Differential methylation has been proposed both as an analytical aid, for example in fetal fraction estimation (Ioannides et al., 2020), and as a diagnostic target in its own right, initially for trisomy 21 (Papageorgiou et al., 2011). These two roles are frequently conflated in the secondary literature, with the result that the maturity of methylation-based prenatal testing is easy to overstate.

A third strand concerns what early detection means for the child rather than for the pregnancy. Prenatal cfDNA screening has been associated with an immediate and sustained rise in sex chromosome aneuploidy diagnoses among infants, without a corresponding change among older children, indicating that the technology shifts the age at which such conditions are recognised (Kim DeLuca et al., 2026). Whether earlier recognition improves childhood outcomes depends on the condition, and the exemplar most often cited, presymptomatic initiation of therapy in spinal muscular atrophy, comes from newborn rather than prenatal ascertainment (De Vivo et al., 2019). Separately, an extensive developmental epigenetics literature links prenatal exposures to persistent methylation differences and to childhood phenotypes (Heijmans et al., 2008; Joubert et al., 2016), which has encouraged the expectation that prenatal plasma might one day yield predictive epigenetic biomarkers of paediatric disease. That expectation has not been tested in the tissue that prenatal liquid biopsy actually samples.

Existing reviews and position statements address these strands separately. Society statements concentrate on chromosomal conditions and stop short of the epigenetic literature (Dungan et al., 2023; Hui et al., 2023). Technical reviews of cell-based approaches survey methodological progress without integrating outcome evidence (Maktabi et al., 2023). Comparative implementation analyses examine policy divergence without appraising analytical validity (Connor et al., 2025). Meta-analyses address individual target classes in isolation (Acreman et al., 2023; Kónya et al., 2026; Liu et al., 2026). No recent synthesis places the genetic and epigenetic strands within a single evidential framework or follows the analytical decisions made in the first trimester through to their consequences for paediatric practice.

1.1 Scope, Research Gap and Objectives

This review examines the detection of fetal genetic variants and epigenetic alterations from maternal biological samples obtained during pregnancy, with emphasis on the first and early second trimesters, and on the implications of such detection for children after birth. Four analyte classes are considered: cell-free DNA, cell-free RNA, extracellular vesicle cargo, and intact circulating fetal cells. Target classes considered are whole-chromosome aneuploidy, sub-chromosomal imbalance, monogenic variants, and epigenetic marks including DNA methylation and fragmentation-derived signatures.

The gap addressed is the absence of an integrated critical appraisal that treats genetic and epigenetic prenatal detection as one evidential problem and that carries the analysis forward into paediatric outcomes. Three specific deficiencies motivate the review: the widespread conflation of analytical validity with clinical utility as testing extends to rarer targets; the conflation of methylation as an analytical instrument with methylation as a validated diagnostic target; and the near absence of longitudinal paediatric follow-up for children ascertained prenatally by screening rather than by symptoms.

The review does not cover preimplantation genetic testing, invasive diagnostic cytogenetics except as a comparator or confirmatory standard, fetal therapy, prenatal screening for infection, or maternal cancer management beyond its intersection with prenatal screening results. Economic modelling is discussed only where it bears on implementation choices. The review is not a systematic review and does not generate pooled effect estimates.

The objectives are to characterise the biological substrate that constrains all maternal-plasma methods; to evaluate the strength of evidence supporting each target class and to explain the gradient between them; to distinguish established from hypothesised epigenetic applications; to analyse the determinants of discordance between prenatal results and fetal genotype; to appraise what is known about paediatric consequences; and to specify research priorities that would materially reduce present uncertainty.

2. Methods for Literature Selection

2.1 Review Design and Rationale

A critical narrative design was selected because the review question spans molecular biology, diagnostic test evaluation, developmental epigenetics, paediatrics and health policy, and because the constituent literatures use incommensurable outcome measures. A systematic review requires a bounded question and comparable outcome data; the present question requires the comparison of a pooled positive predictive value from a diagnostic accuracy meta-analysis with an interrupted time series of paediatric diagnoses and with epigenome-wide association findings. Quantitative synthesis across these designs would be misleading. Narrative synthesis nonetheless carries obligations of transparency and appraisal rather than exempting the author from them, and the review was conducted against explicit criteria for reporting quality and for the referencing of assertions (Baethge et al., 2019; Sukhera, 2022).

2.2 Information Sources

Bibliographic searching was performed in Europe PMC, which indexes MEDLINE and PubMed records, and in Crossref Metadata Search. OpenAlex and Semantic Scholar were used for related-record retrieval and forward citation tracking. Professional society and guideline documents were retrieved through targeted searching of publicly accessible institutional and society web pages, including those of the American College of Medical Genetics and Genomics and the International Society for Prenatal Diagnosis. Subscription-only platforms including Web of Science Core Collection, Scopus and Embase were not accessible during the preparation of this review and were not searched; no claim is made regarding their coverage. Reference lists of recent reviews, position statements and meta-analyses were examined manually, and forward citation searching was applied to the foundational reports of circulating fetal DNA and of plasma methylome analysis.

2.3 Search Strategy and Search Period

Search concepts were constructed around four axes and combined with Boolean operators. The analyte axis combined terms for cell-free DNA, cell-free fetal DNA, cell-free RNA, extracellular vesicles, circulating trophoblasts and liquid biopsy. The target axis combined terms for aneuploidy, trisomy, copy number variant, microdeletion, monogenic and single-gene disorder, mutation, DNA methylation, methylome, imprinting and fragmentomics. The setting axis combined prenatal, antenatal, fetal, maternal plasma, first trimester and placenta. The outcome axis combined sensitivity, specificity, positive predictive value, discordance, confined placental mosaicism, neurodevelopment, childhood outcome, and implementation. Representative strings included the following construction, applied with title and abstract field restriction: ("cell-free DNA" OR "cfDNA" OR "cell-free fetal DNA") AND ("prenatal" OR "fetal") AND ("trisomy" OR "copy number variant" OR "single-gene" OR "monogenic" OR "methylation"). A

parallel string replaced the analyte terms with ("circulating trophoblast" OR "fetal cell" OR "extracellular vesicle" OR "cell-free RNA"). A third string addressed downstream consequences: ("prenatal screening" OR "noninvasive prenatal testing") AND ("childhood" OR "paediatric" OR "pediatric" OR "infant" OR "neurodevelopment" OR "outcome").

The search period began in 1997, the year in which fetal DNA was first demonstrated in maternal plasma, since no earlier publication can bear directly on the review question, and foundational work in placental biology and developmental epigenetics published before that year was retrieved selectively through citation tracking where conceptually necessary. The final search date was 2 June 2026.

2.4 Eligibility Criteria

Peer-reviewed primary studies, systematic reviews, meta-analyses, methodological validations, cohort studies, registry analyses and formal professional society statements were eligible. Priority was given to studies reporting confirmatory testing against an accepted reference standard, to prospective designs, to multicentre cohorts, and to analyses reporting outcomes in the offspring. Case reports and small case series were eligible only where they documented a mechanism not otherwise represented, and were not used to support performance claims. Publications were excluded where they lacked peer review, where bibliographic identity could not be confirmed, where the design could not support the claim for which the source would have been cited, or where the report concerned paternity testing, forensic applications or non-medical sex selection, all of which fall outside the scope defined in Section 1.1. Preprints were excluded because peer-reviewed evidence was available for every theme addressed. Only publications in English were assessed, and no restriction was applied by country, funding source or sample size beyond the design requirements described above.

2.5 Screening, Duplicate Handling and Study Selection

Records retrieved from Europe PMC and Crossref were compared by digital object identifier and by title, and duplicate records arising from indexing of the same article in more than one source, or from concurrent indexing of a preprint and its published version, were resolved in favour of the peer-reviewed version of record. Titles and abstracts were assessed against the eligibility criteria, and full texts were retrieved for records that survived that stage or whose relevance could not be determined from the abstract. Where a publisher landing page and an indexing service disagreed on volume, issue or year, the publisher record was treated as authoritative. Records that could not be retrieved in full text, and records whose metadata could not be confirmed against an authoritative source, were not cited. No formal screening counts are reported, because selection was purposive and iterative rather than exhaustive, and reporting counts would imply a systematic process that was not undertaken.

2.6 Critical Appraisal and Evidence Synthesis

Studies were appraised against criteria appropriate to their design. Diagnostic accuracy studies were assessed for the completeness of reference-standard ascertainment, for whether confirmation was obtained in test-negative as well as test-positive pregnancies, for blinding of laboratory personnel to outcome, for the risk spectrum of the enrolled population, and for whether reported metrics were analytical or clinical. Cohort and registry studies were assessed for the handling of confounding, for the completeness of follow-up, and for the plausibility of the comparison group. Epigenome-wide studies were assessed for tissue provenance, cell-composition adjustment, replication in independent cohorts, and the distance between the measured tissue and the tissue of pathological interest. Formal risk-of-bias scores were not assigned, since applying a recognised instrument would require full-text data extraction of a defined study set that this design did not undertake, and reporting derived scores without that process would misrepresent the appraisal.

Themes were developed inductively from the appraised evidence and were organised around the biological substrate, the target-class gradient, the dual role of epigenetic marks, the determinants of discordance, and paediatric consequences. Where studies disagreed, explanations were sought first in population spectrum and target prevalence, second in reference-standard differences, and third in the underlying placental biology, before any conclusion of genuine effect heterogeneity was drawn.

3. Biological Foundations and Their Analytical Consequences

3.1 Origin, Size and Kinetics of Circulating Fetoplacental Nucleic Acids

Every conclusion that follows depends on a single biological fact: the nucleic acids sampled from maternal plasma are overwhelmingly of maternal haematopoietic origin, and the minority population described as fetal is in practice derived from the placental trophoblast. Fetal molecules are shorter than their maternal counterparts, and this size difference is sufficiently consistent to support diagnostic inference in its own right, allowing size-based rather than count-based analysis of maternal plasma (Yu et al., 2014). Fragment length distribution is not incidental to the assay; it is a physical trace of the chromatin state and nuclease exposure of the cell of origin, and it therefore carries information that count-based aneuploidy detection discards.

The proportion of circulating cfDNA attributable to the fetoplacental unit, conventionally termed the fetal fraction, rises with gestational age and falls with maternal body mass. Both relationships have direct operational consequences. Evaluation of an assay incorporating fetal fraction amplification reported a median fetal fraction of 9.0% for samples drawn between 6 weeks 0 days and 9 weeks 6 days of gestation and 18.5% at 10 weeks 0 days or later, with the corresponding early-gestation median falling to 7.2% among participants with a body mass index of 30 or above (Dugoff et al., 2026). Two inferences follow. Earlier sampling is

technically feasible, and the populations for whom early sampling would be most valuable are the same populations in whom the fetal signal is weakest.

3.2 The Placental Methylome as the Epigenetic Substrate

Placental DNA methylation differs systematically from that of maternal leukocytes, and this difference is what makes fetoplacental molecules identifiable within maternal plasma at all. Genome-wide bisulfite sequencing of maternal plasma established that the circulating methylome is readable at scale and that placental molecules carry a recognisable hypomethylated signature (Lun et al., 2013). Methylation deconvolution formalised this observation into a general method for estimating the tissue contributions to a plasma DNA pool (Sun et al., 2015).

The placenta is not a single tissue for these purposes. Cell-specific characterisation of the placental methylome has shown that trophoblast subtypes, stromal cells and endothelium carry distinguishable methylation profiles, and that whole-villus measurements represent a composite whose interpretation depends on cellular proportions (Yuan et al., 2021). This matters for two reasons. Reference atlases used in deconvolution inherit whatever cellular composition the reference samples had, and a shift in the cellular source of circulating molecules during pregnancy complications may register as an apparent change in fetal fraction or in methylation profile without any change in fetal genotype.

3.3 Why the Substrate Limits what Can be Claimed

The consequence of a placental rather than fetal signal is that maternal plasma reports on the placenta and infers the fetus. That inference is usually correct and occasionally wrong, and the circumstances in which it fails are biological rather than technical. Tissue-specific mosaicism has been documented as a mechanism of false-negative results in the presence of chromosomal structural abnormality, showing that the failure mode operates in both directions and is not confined to false positives (Huang et al., 2026). No improvement in sequencing depth or in bioinformatic calling can correct a discrepancy that exists between the placenta and the fetus.

Table 1 sets out the principal circulating analyte classes, the biological compartment each interrogates, and the analytical claim each can currently support. The distinctions drawn there are used throughout the remainder of the review, and the table is intended to make explicit a separation that is frequently blurred: interrogating a placental analyte is not the same as interrogating the fetus, and interrogating an intact fetal-derived cell is not the same as interrogating a fragmented plasma pool. The rightmost column of the table is deliberately conservative, and reflects claims supported by validation studies rather than claims that are mechanistically plausible.

Table 1. Circulating analyte classes used in prenatal liquid biopsy, the biological compartment interrogated, and the level of claim currently supported by validation evidence

Analyte class	Biological compartment interrogated	Principal analytical strengths	Principal constraints	Level of claim currently supported	Supporting references
Cell-free DNA fragments	Placental trophoblast within a predominantly maternal haematopoietic pool	Genome-wide coverage; fragment length informative; established at scale	Fetal fraction dependent; placental rather than fetal provenance	Screening for common aneuploidy; diagnosis for defined monogenic indications	Gil et al. (2017); Lo et al. (1997); Yu et al. (2014)
Plasma DNA methylation	Tissue-of-origin composition of the plasma pool	Enables fractional quantification and tissue deconvolution independent of fetal sex	Reference atlas dependent; placental cellular composition varies	Analytical support and tissue attribution; not a validated fetal disease target	Ioannides et al. (2020); Lun et al. (2013); Sun et al. (2015); Yuan et al. (2021)
Cell-free RNA	Transcriptional activity of placenta, maternal tissues and immune cells	Reports on physiological state rather than genotype	Labile analyte; standardisation immature	Risk prediction for placental syndromes under evaluation	Castillo-Marco et al. (2025); Moufarrej et al. (2022)
Extracellular vesicles and cargo	Vesicle-secreting cells including syncytiotrophoblast	Compartmentalised, potentially cell-type resolved sampling	Isolation methods heterogeneous; limited clinical validation	Exploratory	Buca et al. (2022)
Intact circulating trophoblasts	Single fetal-derived cells	Pure fetal genome without maternal background; single-cell resolution	Very low yield; recovery affected by maternal body mass index and gestational age	Proof of principle for aneuploidy and copy number variant detection	Crovetti et al. (2022); Panchalee et al. (2020); Vossaert et al. (2019)

4. Detection of Fetal Genetic Variation: A Gradient of Confidence

4.1 Common Autosomal Trisomies and Sex Chromosome Aneuploidy

The evidence for common autosomal trisomy screening is the strongest in the field and is the appropriate benchmark against which every extension should be judged. Pooled estimates derived from validation and implementation studies with outcome ascertainment in more than 85% of participants gave a detection rate of 99.7% for trisomy 21 against a false-positive rate of 0.04% (Gil et al., 2017). Guidance from the American College of Medical Genetics and Genomics recommends cfDNA screening for the common trisomies and for sex chromosome aneuploidy in singleton pregnancies on the basis of high-certainty evidence, while treating screening for 22q11.2 deletion as a conditional suggestion supported by moderate certainty (Dungan et al., 2023). The International Society for Prenatal Diagnosis reached compatible conclusions and declined to recommend routine screening for microdeletion syndromes or RATs in unselected populations (Hui et al., 2023).

Two qualifications temper this picture. Performance in twin pregnancies is supported by a smaller evidence base, and pooled estimates there rest on fewer affected cases with wider confidence intervals (Judah et al., 2021). Sex chromosome aneuploidy occupies an unusual position: analytical detection is good, but the conditions detected span a wide phenotypic range, many affected individuals historically escaped diagnosis, and the counselling problem created by detection is qualitatively different from that created by trisomy 21.

4.2 Sub-chromosomal Imbalance, Rare Autosomal Trisomies and Additional Findings

Extension to sub-chromosomal targets produces a sharp fall in positive predictive value that is largely explained by prevalence rather than by assay quality. Prospective multicentre enrolment of pregnancies undergoing single-nucleotide polymorphism-based screening for 22q11.2 deletion syndrome, with confirmatory chromosomal microarray sought in all cases, provided the first adequately powered assessment of this target and confirmed that feasibility studies had overstated the clinical position (Dar et al., 2022). For RATs the position is weaker still. Meta-analysis of 31 studies encompassing 1703 women gave a pooled positive predictive value of 11.46%, with high statistical heterogeneity (Acreman et al., 2023). A pooled value of that magnitude means that most positive results do not reflect a fetal trisomy, which is a statement about the population rather than a criticism of the laboratory.

Genome-wide screening in a national programme provides the most informative account of what additional findings actually represent. In the TRIDENT-2 cohort, additional findings were detected in 402 of 110,739 pregnancies, and among the 358 cases in which origin was established, 22.1% were fetal, 52.8% were attributed to confined placental mosaicism (CPM), and 25.1% were maternal in origin (van Prooyen Schuurman et al., 2022). Most fetal aberrations in that series were pathogenic and associated with severe phenotypes, which is the strongest available argument for genome-wide analysis. The majority of positive results, though, pointed to the placenta or to the pregnant person rather than to the fetus. A subsequent systematic review and meta-analysis of discordant genome-wide results reinforced the distinction between single-chromosome discordance, typically placental, and complex multichromosomal profiles, which more often indicate maternal malignancy (Kónya et al., 2026).

Independent cohort data on RATs and segmental imbalances detected through cfDNA screening are consistent with this interpretation and show that confirmatory pathways rather than screening performance determine clinical value (Raymond et al., 2022). The disagreement between enthusiasts and sceptics of genome-wide screening is therefore not primarily empirical. Both sides accept the same numbers. They differ on whether a low positive predictive value is acceptable when the small proportion of true fetal findings is severe, and on how the burden of confirmatory testing and parental uncertainty should be weighed.

4.3 Monogenic Conditions

Non-invasive detection of single-gene conditions divides into three technically distinct problems. Detection of a paternally inherited or de novo variant absent from the maternal genome is the simplest, since any signal in maternal plasma must be fetal. Determination of maternal allele inheritance is the hardest, because the fetal contribution must be distinguished against a large maternal background of the same sequence, and this is the problem that relative haplotype dosage analysis was designed to solve (Lo et al., 2010).

Clinical services built on relative haplotype dosage have progressively removed the constraints that limited early implementations. Extension of the method to consanguineous couples addressed a limitation that had excluded families in whom shared parental haplotypes prevented phasing, a group with a materially elevated prior risk of autosomal recessive disease (Hanson et al., 2024). Haplotype-based approaches using large targeted single-nucleotide polymorphism panels have been developed with the aim of making the method gene-agnostic rather than requiring bespoke design for each locus, and have been evaluated in families affected by monogenic disease alongside samples with known fetal karyotypes (Zhang et al., 2026). These are diagnostic rather than screening applications, applied to pregnancies with a defined prior risk, and their performance should not be generalised to unselected populations.

Panel-based screening for dominant single-gene disorders occupies a different evidential position, and here the recent literature is genuinely informative. Meta-analysis of ten studies comprising 12,577 cases reported a pooled positivity rate of 2.2% and a pooled positive predictive value of 93.8%, with pooled sensitivity of 94.5% and specificity of 99.7%; subgroup analysis showed positivity rates of 0.3% in low-risk populations, 1.2% in mixed-risk populations and 6.0% in high-risk populations (Liu et al., 2026). A prospective cohort of 2000 pregnancies at intermediate risk in China, using a panel targeting 155 genes associated with 202 dominant conditions with confirmation by amniocentesis and Sanger sequencing or by newborn exome sequencing, reported

sensitivity of 100%, specificity of 99.8% and a positive predictive value of 77.8%, with no false negatives among confirmed cases (Lu et al., 2026). The apparent divergence in positive predictive value between the pooled estimate and the prospective cohort is explained by the risk composition of the populations rather than by assay differences, which is precisely the pattern that a prevalence-driven gradient predicts.

Two cautions attach to this literature. Confirmatory testing in test-negative pregnancies is incomplete in most series, so reported sensitivity is bounded by ascertainment; the prospective cohort described above obtained genetic validation in roughly half of its participants. The conditions on such panels are individually rare and phenotypically variable, so a positive result frequently identifies a variant whose expressivity in a given fetus cannot be predicted.

4.4 Established Single-locus Applications and Cell-Based Approaches

Fetal *RHD* genotyping is the one single-locus application that has achieved routine use in several health systems, and it is instructive precisely because it is simple. A large United States clinical validation of a next-generation sequencing-based cfDNA test for fetal *RHD* in 655 pregnancies with serological confirmation reported no false-negative results, with concordance in all 356 fetuses identified as *RHD*-positive (Gilstrop Thompson et al., 2025). The target is a single well-characterised locus with a binary clinical decision attached and an unambiguous postnatal reference standard, and the resulting evidence is correspondingly clean.

Cell-based non-invasive prenatal testing offers the theoretical advantage of a pure fetal genome without maternal background. Validation work using magnetic-activated cell sorting and single-cell low-coverage whole-genome sequencing detected aneuploidies and copy number variants at approximately 1 Mb resolution, identifying on average 0.20 putative trophoblasts per millilitre of maternal blood, of which 55% were of sufficient quality to score (Vossaert et al., 2019). That yield is the central obstacle. Recovery is further reduced by maternal body mass index and varies with gestational age (Panchalee et al., 2020), and circulating trophoblast numbers themselves appear to vary with pregnancy complications, which suggests that yield is a biological variable rather than a fixed technical parameter (Crovetti et al., 2022). Reviews of the field have accurately characterised the position as promising but not yet clinically validated (Maktabi et al., 2023).

Table 2 summarises the target classes discussed in this section, the strongest available evidence for each, and the confidence that can reasonably be placed in current conclusions. Reading the table by row makes the gradient explicit: the fall in confidence from common trisomies to RATs is not a fall in analytical performance but a consequence of prevalence acting on positive predictive value, while the recovery of confidence at *RHD* and at haplotype-based diagnosis reflects the return to defined-risk populations with unambiguous reference standards. The table also identifies where the reference standard itself is incomplete, which is the most common unstated limitation in this literature.

5. Epigenetic Alterations: Analytical Instrument or Diagnostic Target

5.1 Methylation as an Analytical Instrument

The secure applications of methylation in prenatal liquid biopsy are analytical. Estimating the fetal fraction requires a marker that distinguishes fetoplacental from maternal molecules independently of fetal sex and of the presence of paternally inherited polymorphisms, and differential methylation supplies exactly that. A multiplex droplet digital polymerase chain reaction assay based on differentially methylated regions has been developed for this purpose and demonstrates that a small marker set suffices for fractional quantification (Ioannides et al., 2020). This application is unglamorous and consequential, because fetal fraction governs whether a result can be issued at all.

Tissue-of-origin deconvolution is the second secure application. Methylation-based mapping of the plasma DNA pool distinguishes placental, haematopoietic, hepatic and other contributions, and the same principle serves prenatal, oncological and transplantation contexts (Sun et al., 2015). The clinical value of this capability has now been demonstrated within prenatal screening itself. A methylation and aneuploidy-aware pipeline applied to enzymatically converted plasma cfDNA identified placental and cancer-derived aneuploidies with accuracy comparable to conventional analysis, and methylation-based deconvolution correctly identified the tumour of origin in 91.67% of the profiles examined (Tuveri et al., 2026). This is the clearest current demonstration that methylation earns its place in the prenatal assay: not by detecting fetal disease, but by resolving the ambiguity created when a copy number aberration is found and its source is unknown.

5.2 Methylation as a Diagnostic Target

The proposition that fetal disease can be diagnosed directly from methylation marks in maternal plasma has a longer history and a thinner evidence base. A fetal-specific methylation ratio was shown to permit non-invasive detection of trisomy 21, establishing feasibility (Papageorgiou et al., 2011). Counting-based and single-nucleotide polymorphism-based approaches nonetheless became the clinical standard, and the reason is instructive. Methylation-based aneuploidy detection depends on the stability of methylation at the selected loci across gestational age, placental cellular composition and pregnancy pathology, and the demonstration that trophoblast subtypes carry distinct methylation profiles indicates that this stability cannot be assumed (Yuan et al., 2021). A method that must be recalibrated whenever placental composition shifts is at a structural disadvantage against a method that counts molecules.

Imprinting disorders represent the target class for which a methylation-based prenatal test would be most valuable, because the causal lesion is frequently epigenetic and therefore invisible to sequence-based analysis. Evidence that these disorders occur more

often after assisted reproductive technology (ART) is reasonably consistent across designs. Meta-analysis of studies relating ART to imprinting disorders reported summary odds ratios of 4.7 for Angelman syndrome, 5.8 for Beckwith-Wiedemann syndrome, 2.2 for Prader-Willi syndrome and 11.3 for Silver-Russell syndrome (Cortessis et al., 2018). Register-based analysis of more than two million Swedish liveborn singletons identified 1044 children with the disorders of interest, of whom 52 were conceived after ART, providing a population-level estimate against a very large denominator (Ye et al., 2024). Mechanistic work distinguishes epimutation-mediated from uniparental disomy-mediated pathways and links the latter to aneuploid gametes and parental age (Hara-Isono et al., 2023). The absolute risks remain small, and the case for prenatal epigenetic testing in ART-conceived pregnancies has not been made. No prospective study has demonstrated detection of an imprinting disorder from maternal plasma methylation at a performance level approaching that achieved for aneuploidy.

Table 2. Target classes for non-invasive detection of fetal genetic variation, strongest available evidence, and appraised confidence

Target class	Strongest available evidence	Reported performance	Principal methodological limitation	Appraised confidence	Supporting references
Trisomy 21, 18 and 13, singleton	Meta-analysis of validation and implementation studies	Detection rate 99.7% and false-positive rate 0.04% for trisomy 21	Outcome ascertainment incomplete in a minority of pregnancies	High	Dungan et al. (2023); Gil et al. (2017)
Sex chromosome aneuploidy	Guideline evidence review and population implementation data	Recommended on high-certainty evidence	Wide phenotypic spectrum limits interpretive value of a positive result	Moderate to high for detection; low for prognostication	Dungan et al. (2023); Kim DeLuca et al. (2026)
22q11.2 deletion syndrome	Prospective multicentre cohort with microarray confirmation	Adequately powered estimates; guideline support conditional	Low prevalence constrains positive predictive value	Moderate	Dar et al. (2022); Dungan et al. (2023)
Rare autosomal trisomies	Systematic review and meta-analysis of 31 studies, 1703 women	Pooled positive predictive value 11.46%, high heterogeneity	Most positives reflect placental rather than fetal aneuploidy	Low	Acreman et al. (2023); Raymond et al. (2022)
Genome-wide additional findings	National implementation cohort with outcome follow-up	402 findings in 110,739 pregnancies; 22.1% fetal, 52.8% placental, 25.1% maternal	Interpretation requires extensive confirmatory pathway	Moderate for detection; contested for clinical utility	Kónya et al. (2026); van Prooyen Schuurman et al. (2022)
Dominant single-gene disorder panels	Meta-analysis of 10 studies, 12,577 cases; prospective cohort of 2000 pregnancies	Pooled positive predictive value 93.8%; prospective positive predictive value 77.8%	Confirmation in test-negative pregnancies incomplete	Moderate	Liu et al. (2026); Lu et al. (2026)
Recessive and X-linked monogenic diagnosis	Accredited service data and haplotype-based method development	Diagnostic application in defined-risk families, including consanguineous couples	Requires parental and proband samples for phasing	Moderate to high within indication	Hanson et al. (2024); Zhang et al. (2026)
Fetal *RHD* genotyping	Large clinical validation with serological confirmation	No false negatives in 655 pregnancies; 356 of 356 concordant positives	Single locus; not generalisable to other targets	High	Gilstrop Thompson et al. (2025)
Cell-based testing on circulating trophoblasts	Single-cell validation studies	Aneuploidy and copy number variants at approximately 1 Mb; 0.20 cells per millilitre	Yield varies with body mass index, gestational age and pregnancy complications	Low, proof of principle	Crovetti et al. (2022); Maktabi et al. (2023); Panchalee et al. (2020); Vossaert et al. (2019)

5.3 Fragmentation and Transcriptional Readouts

Fragmentomics occupies an intermediate position between the genetic and epigenetic domains, since fragment length and end position are physical consequences of chromatin organisation. The diagnostic utility of size-based analysis was established early (Yu et al., 2014), and recent work has applied fragmentomic features to preeclampsia risk assessment rather than to fetal genotype,

which is a change of purpose rather than of technique (Xu et al., 2026). Cell-free RNA follows a similar logic. Prediction of preeclampsia from circulating cell-free RNA before clinical onset has been reported, with subsequent work extending the approach to distinguish early-onset from late-onset disease across gestation (Castillo-Marco et al., 2025; Moufarrej et al., 2022). Extracellular vesicles have been proposed as a compartmentalised source of placental material, though isolation methods remain heterogeneous and clinical validation is limited (Buca et al., 2022).

These three lines share a feature that separates them from genetic testing and that is often lost in summary: they predict a maternal or placental syndrome, not a fetal genotype. Their appropriate comparator is existing clinical risk prediction, not karyotyping, and their evaluation requires calibration and net benefit analysis rather than sensitivity and specificity alone.

Table 3 separates the epigenetic applications discussed in this section into those supported by validation evidence and those that remain hypothesised, and states the evidence required to move each hypothesised application into the first category. The distinction is the central analytical claim of this section: methylation is presently an instrument of considerable and demonstrated value and a diagnostic target of largely unrealised promise, and reviews that report these two roles under a single heading systematically overstate the maturity of the field.

Table 3. Epigenetic and fragmentation-based applications in prenatal liquid biopsy, separated by evidential status

Application	Epigenetic feature used	Evidential status	Evidence required to advance status	Supporting references
Fetal fraction estimation	Differentially methylated regions between placenta and maternal blood	Established analytical application	Multi-platform comparability studies	Ioannides et al. (2020)
Tissue-of-origin deconvolution of plasma DNA	Genome-wide methylation atlas deconvolution	Established analytical application	Reference atlases spanning placental cell types and gestational ages	Sun et al. (2015); Yuan et al. (2021)
Resolving the source of aneuploidy detected in screening	Methylation-based tumour typing with aneuploidy-aware calling	Demonstrated in a prenatal screening pipeline; tumour origin correct in 91.67% of profiles	Prospective evaluation within routine screening programmes	Tuveri et al. (2026)
Aneuploidy detection by methylation ratio	Fetal-specific methylation at selected loci	Feasibility demonstrated; superseded in practice	Demonstration of stability across gestation and placental composition	Papageorgiou et al. (2011); Yuan et al. (2021)
Prenatal detection of imprinting disorders	Locus-specific methylation at imprinted control regions	Hypothesised; no validated plasma-based assay	Prospective cohorts in defined-risk pregnancies with postnatal confirmation	Cortessis et al. (2018); Hara-Isono et al. (2023); Ye et al. (2024)
Preeclampsia risk assessment from fragmentomics	Fragment length and end-position signatures	Emerging; single-cohort development stage	External validation with calibration and net benefit analysis	Xu et al. (2026); Yu et al. (2014)
Preeclampsia prediction from cell-free RNA	Transcript abundance reflecting placental and maternal state	Emerging; replication across cohorts reported	Prospective evaluation against established risk models	Castillo-Marco et al. (2025); Moufarrej et al. (2022)
Prediction of childhood outcomes from prenatal plasma epigenetics	Methylation signatures associated with later phenotype	Hypothesised; developmental evidence derives from postnatal tissues	Birth cohorts linking archived maternal plasma to childhood phenotype	Heijmans et al. (2008); Joubert et al. (2016); Knight et al. (2016)

6. Determinants of Discordance Between Prenatal Result and Fetal Genotype

6.1 Fetal Fraction as a Clinical Variable

Fetal fraction has traditionally been treated as a quality control threshold, with results withheld below approximately 4%. Evidence from large cohorts indicates that it is also a clinical variable. In a Japanese multicentre retrospective cohort of 40,716 singleton pregnancies with negative screening results, women in the lowest fetal fraction quartile had elevated adjusted odds of fetal demise, preterm birth, fetal growth restriction and hypertensive disorders of pregnancy, with no significant association for gestational diabetes or placental abruption (Yamamoto et al., 2025). A nationwide Dutch cohort reported a comparable direction of association between fetal fraction and adverse pregnancy outcomes (Becking et al., 2025). The consistency across two health systems with different screening architectures strengthens the inference, although both analyses are retrospective and residual confounding by maternal body mass index and gestational age at sampling cannot be excluded.

The practical implication is that a low fetal fraction is a finding rather than a failure. A result reported only as inconclusive discards information about placental function that two independent national datasets suggest is prognostically meaningful. Whether acting on that information improves outcomes has not been tested, and the absence of an intervention trial is the principal reason that this observation has not entered guidance.

6.2 Confined Placental Mosaicism

CPM is the dominant biological explanation for discordance between prenatal cfDNA results and fetal genotype. National implementation data attributed 52.8% of additional findings with an established origin to CPM (van Prooyen Schuurman et al., 2022), and that series also reported markedly increased frequencies of preeclampsia and of birth weight below the 2.3rd percentile among CPM cases relative to the general obstetric population. A prospective matched-cohort study in Melbourne, in which four biopsies were taken from each placenta in pregnancies with false-positive cfDNA results and in matched controls, was designed specifically to establish the prevalence of CPM among false positives and its obstetric consequences (Raymond et al., 2026). Designs of this kind, which sample the placenta directly rather than inferring its composition, are what the field requires to convert a recognised nuisance into interpretable clinical information.

The parental experience of such results has been documented and is not incidental to their evaluation. Qualitative work with parents receiving a prenatal diagnosis of CPM describes an absence of reliable prognostic information at the point of disclosure (Farø et al., 2026). This is a direct consequence of an evidence base built around analytical performance rather than around the counselling situations that positive results create.

6.3 Maternal Contributions and Incidental Malignancy

A quarter of additional findings with established origin in the TRIDENT-2 series were maternal (van Prooyen Schuurman et al., 2022), and the most consequential maternal finding is occult malignancy. Retrospective analysis of more than two million single-nucleotide polymorphism-based screening samples identified 38 with patterns suspicious for maternal malignancy, a frequency of approximately 1 in 52,748, and malignancy or suspected malignancy was identified in 20 of the 30 patients for whom clinical follow-up was available (Goldring et al., 2023). Prospective evaluation with a uniform screening protocol found cancer in 52 of 107 participants who had received unusual or non-reportable cfDNA results, with whole-body magnetic resonance imaging achieving sensitivity of 98.0% and specificity of 88.5% for occult cancer, while physical examination and routine laboratory tests contributed little (Turriff et al., 2024). Complementary work has examined how such results distribute across cancer stage (Zhao et al., 2025), and the clinical literature on this phenomenon has been synthesised for the laboratory audience (Goldlust & Bianchi, 2025).

Three points follow. The prior probability of malignancy given an unusual cfDNA result is high enough to justify a structured diagnostic pathway rather than watchful waiting. Imaging rather than clinical examination is the effective investigation. Methylation-based tissue attribution offers a route to shortening that pathway, since identifying the tumour of origin from the same plasma sample would reduce the interval between an anomalous screening result and directed investigation (Tuveri et al., 2026).

Table 4 organises the principal sources of discordance, their relative frequency where this has been quantified, and the investigative response each warrants. Presenting these mechanisms together makes clear that discordance is not a single phenomenon requiring a single fix: placental, maternal and technical causes have different frequencies, different clinical implications and different appropriate responses, and pathways that treat all positive results identically will over-investigate some pregnancies and under-investigate others.

Table 4. Sources of discordance between prenatal cell-free DNA results and fetal genotype, with quantification where available and appropriate investigative response

Source of discordance	Direction	Reported frequency or magnitude	Clinical implication	Appropriate investigative response	Supporting references
Confined placental mosaicism	False positive; occasionally false negative	52.8% of additional findings of established origin	Associated with preeclampsia and low birth weight	Diagnostic amniocentesis; consider placental cytogenetics; enhanced growth surveillance	Raymond et al. (2026); van Prooyen Schuurman et al. (2022)
Low fetal fraction	Non-reportable result	Lowest quartile associated with fetal demise, preterm birth, growth restriction and hypertensive disorders	Prognostic information currently discarded	Redraw with attention to gestational age; consider surveillance rather than reassurance	Becking et al. (2025); Yamamoto et al. (2025)
Maternal copy number variation	False positive	25.1% of additional findings of established origin were maternal	Usually benign but requires explanation	Maternal chromosomal microarray	van Prooyen Schuurman et al. (2022)
Occult maternal malignancy	False positive, often multichromosomal	Approximately 1 in 52,748 screening samples; cancer in 52 of	Time-critical maternal diagnosis	Whole-body magnetic resonance imaging;	Goldring et al. (2023); Kónya et al. (2026);

Source of discordance	Direction	Reported frequency or magnitude	Clinical implication	Appropriate investigative response	Supporting references
		107 in prospective evaluation		methylation-based tissue attribution	Turriff et al. (2024); Tuveri et al. (2026)
Tissue-specific mosaicism with structural abnormality	False negative	Documented mechanism; frequency not established	False reassurance	Ultrasound remains an independent screening modality	Huang et al. (2026)
Low target prevalence	Reduced positive predictive value	Pooled positive predictive value 11.46% for rare autosomal trisomies	Most positives are not fetal	Pre-test counselling calibrated to target prevalence	Acreman et al. (2023); Hui et al. (2023)
Maternal body mass index and early gestational age	Reduced fetal fraction	Median fetal fraction 7.2% at 6 to 9 weeks with body mass index of 30 or above	Differential access to early testing	Gestational-age-specific and body-mass-specific reporting thresholds	Dugoff et al. (2026); Panchalee et al. (2020)

7. Paediatric Implications

7.1 Prenatal Screening Changes who is Diagnosed and when

The most direct paediatric consequence of prenatal cfDNA screening is a change in ascertainment. A controlled interrupted time series using United States claims data for 2008 to 2019 found that the introduction of prenatal cfDNA screening was associated with an immediate increase in sex chromosome aneuploidy diagnosis rates among infants, from 3.55 to 6.56 cases per 100,000 persons, followed by a sustained upward trend, with no corresponding change among children aged 2 to 18 years (Kim DeLuca et al., 2026). The design, with an internal control group unaffected by prenatal screening, supports causal interpretation more strongly than a simple before-and-after comparison.

The implication is that the population of children carrying a diagnosis of sex chromosome aneuploidy is changing in composition, not only in size. Historically such diagnoses were made after presentation with a clinical concern; increasingly they are made before any phenotype exists. Prognostic literature derived from clinically ascertained cohorts is therefore systematically biased towards more severe presentation when applied to prenatally ascertained children. This is a general property of screening-based ascertainment rather than a defect of any particular assay, and it applies with equal force to prenatally detected copy number variants and to variants identified through single-gene panels.

7.2 Early Detection and Time-Critical Intervention

The strongest paediatric argument for early genetic detection is that some interventions lose effectiveness with delay. Presymptomatic initiation of nusinersen in infants genetically diagnosed with spinal muscular atrophy produced motor outcomes substantially better than those historically observed in symptomatic cohorts (De Vivo et al., 2019). The example is important, and its limits should be stated plainly. Ascertainment in that study was neonatal rather than prenatal, and no comparable evidence demonstrates that moving diagnosis from the neonatal period into the first trimester further improves outcomes for that condition.

For most targets currently detectable in maternal plasma, no time-critical intervention exists. Detection of a common autosomal trisomy informs reproductive decision-making and delivery planning rather than treatment. Detection of a dominant single-gene variant on a screening panel identifies a condition whose expressivity is frequently unpredictable, and prospective work in this area has begun to include postnatal physical examination of infants in both test-positive and test-negative groups, which is the correct design and remains uncommon (Lu et al., 2026). Prenatal exome sequencing after invasive sampling in fetuses with structural anomalies established that a molecular diagnosis can be reached in a clinically meaningful minority of such pregnancies (Lord et al., 2019; Petrovski et al., 2019), and these studies define the diagnostic ceiling that non-invasive methods aspire to reach. Neither trial was designed to measure paediatric outcomes, and the interval between diagnostic yield and demonstrated child benefit remains unbridged for prenatal genomics as a whole.

7.3 Prenatal Epigenetic Marks and Childhood Phenotype

The developmental origins literature supplies the strongest motivation for prenatal epigenetic testing and simultaneously the strongest reason for caution. Persistent methylation differences were demonstrated in adults exposed periconceptionally to famine, establishing that the prenatal period leaves durable epigenetic traces (Heijmans et al., 2008). Consortium meta-analysis of newborn cord blood identified widespread and reproducible differential methylation associated with maternal smoking in pregnancy, providing the clearest evidence that a specific prenatal exposure produces a detectable and replicable neonatal epigenetic signature (Joubert et al., 2016). Methylation-based estimation of gestational age from neonatal blood demonstrates that developmentally relevant information is encoded with sufficient fidelity to support quantitative prediction (Knight et al., 2016). Placental methylation networks have been related both to maternal chemical exposure and to child neurodevelopmental measures, which is the closest existing approximation to the tissue that prenatal liquid biopsy samples (Mouat et al., 2023).

Four constraints prevent this literature from supporting a prenatal predictive test at present. The measured tissue is cord blood, adult blood or delivered placenta, not first-trimester maternal plasma, and no study has demonstrated that the relevant signatures are recoverable from the diluted, fragmented and predominantly maternal circulating pool. Reported effect sizes at individual loci are small and support population-level inference rather than individual prediction. Confounding by maternal genotype, cell composition and postnatal environment is difficult to exclude in observational designs. The causal status of the methylation differences is generally unresolved, so a mark may be a durable record of exposure rather than a mediator of outcome, and prediction and mechanism are not the same claim.

7.4 Downstream Paediatric Services and the Follow-Up Deficit

Prenatal detection creates paediatric obligations that are rarely costed. A child identified before birth as carrying a chromosomal or monogenic variant enters postnatal care with a label and, frequently, without a phenotype, which generates surveillance, specialist referral and parental anxiety of uncertain duration. Follow-up of pregnancies with additional findings has documented perinatal outcomes but not childhood trajectories (van Prooyen Schuurman et al., 2022). Qualitative evidence indicates that parents receiving results with uncertain fetal implications are left without the prognostic information they need (Farø et al., 2026), and evidence on the psychological consequences of additional findings detected through genome-wide screening shows measurable impact on those who receive them (Bakkeren et al., 2024). No prospective cohort has followed children ascertained through prenatal cfDNA screening into school age with standardised developmental assessment, and this absence is the single largest gap in the evidence reviewed here.

8. Implementation, Access and Ethical Considerations

Implementation patterns differ substantially across health systems, and comparative analysis of Japan, the Netherlands and the United States shows that the same technology has been introduced under markedly different regulatory, funding and professional arrangements (Connor et al., 2025). These differences are not incidental to evidence interpretation. Performance metrics derived from a commercial laboratory serving a self-selected population cannot be transferred without adjustment to a national programme with universal offer, because positive predictive value depends on the prevalence in the tested population, and the tested populations differ.

Cost-effectiveness analyses have generally supported cfDNA screening for common trisomies, with results sensitive to whether screening is offered universally or contingent on a prior risk assessment (Xie et al., 2020). Comparison of published models demonstrates that structural choices in model construction materially change the conclusions reached, which means that economic evidence in this field should be read as conditional on modelling assumptions rather than as settled (Salisbury et al., 2024). Extending screening to targets with low positive predictive value alters the economic calculation through the cost of confirmatory testing and follow-up rather than through the cost of the screen itself.

Informed choice is more demanding for cfDNA screening than the routine nature of the blood draw suggests. Work examining the knowledge required for an informed decision about non-invasive prenatal screening indicates that the concepts patients must grasp, including the screening rather than diagnostic status of the test and the dependence of predictive value on prevalence, are not consistently conveyed (Griffin et al., 2023). Qualitative research with clinicians in Lebanon and Quebec identified implementation challenges relating to counselling capacity, commercial provision and equity of access that recur across settings (Haidar et al., 2020). Access is further stratified by the biology described in Section 3, since reduced fetal fraction at earlier gestations and higher body mass indices means that the earliest testing is least reliable for some of the groups most likely to face barriers to later care (Dugoff et al., 2026; Panchalee et al., 2020).

The disclosure of incidental maternal findings raises a distinct set of obligations. Where an anomalous result carries a substantial probability of occult malignancy, the argument for a structured disclosure and investigation pathway is strong, and prospective data support it (Turriff et al., 2024). Consent processes designed around fetal aneuploidy do not straightforwardly extend to a test that may reveal maternal cancer, and this mismatch between what is consented to and what may be found is not resolved by adding a sentence to a form.

9. Future Directions

The most consequential unresolved problem is the absence of paediatric outcome data for prenatally ascertained children. Present evidence establishes that prenatal screening changes the age and manner of diagnosis (Kim DeLuca et al., 2026) but not whether children so identified fare better than they otherwise would have. The appropriate design is a prospective birth cohort recruiting from an existing national screening programme, linking prenatal results, confirmatory testing and placental cytogenetics where obtained to standardised developmental, educational and health service outcomes to at least school age, with a comparison group of children with the same conditions ascertained postnatally. Sample sizes for rare targets will require multi-programme collaboration and harmonised phenotyping. Without such data, the clinical utility of expanded prenatal detection will continue to be argued from diagnostic yield, which is a proxy and not an outcome.

A second priority is the conversion of placental discordance from an analytical nuisance into interpretable clinical information. The prospective matched-cohort approach of sampling multiple placental biopsies in pregnancies with false-positive results (Raymond et al., 2026) should be extended to determine whether the location and extent of mosaicism predict fetal growth restriction and hypertensive disease with sufficient accuracy to guide surveillance. The parallel question, whether reporting fetal fraction as a graded risk indicator rather than a binary quality threshold improves outcomes, is answerable by a pragmatic randomised trial

comparing standard reporting with reporting that triggers enhanced growth surveillance, using perinatal morbidity as the primary outcome (Becking et al., 2025; Yamamoto et al., 2025).

A third priority concerns the epigenetic programme. The immediate opportunity is not fetal disease prediction but source attribution, where methylation-based deconvolution has already demonstrated value within prenatal screening pipelines (Tuveri et al., 2026). Prospective evaluation should determine whether integrating tissue attribution into primary screening shortens the interval from anomalous result to maternal diagnosis and reduces unnecessary invasive testing. The longer-term question, whether prenatal plasma methylation can predict childhood phenotype, requires a preparatory step that has not been taken: demonstrating that signatures identified in cord blood and delivered placenta (Joubert et al., 2016; Mouat et al., 2023) are recoverable at all from first-trimester maternal plasma. Nested case-control analyses within existing birth cohorts holding archived maternal plasma would answer this at modest cost and should precede any prospective biomarker development.

A fourth priority is the evaluation of single-gene screening on its own terms. Existing panel studies report positive predictive value against confirmatory testing in test-positive pregnancies, with incomplete confirmation in test-negative pregnancies (Liu et al., 2026; Lu et al., 2026). Prospective cohorts with universal postnatal genomic confirmation, or with linkage to newborn screening and paediatric records, are required to establish negative predictive value and to quantify the frequency with which a prenatally detected variant is followed by a corresponding childhood phenotype. Studies of this kind would also generate the expressivity data that counselling currently lacks.

A fifth priority concerns equity and generalisability. The performance evidence reviewed here derives predominantly from North America, Western Europe, East Asia and Australia. Population allele frequencies, consanguinity rates, maternal body mass distributions and the prevalence of haemoglobinopathies differ substantially elsewhere, and each of these affects test performance directly. Evaluation in under-represented settings, including validation of haplotype-based diagnosis in populations with high consanguinity where the method has the greatest potential value (Hanson et al., 2024), is a scientific requirement rather than a matter of representation alone.

Table 5 sets out these priorities with the study design most likely to resolve each and an indicative timescale. Immediate priorities are those answerable with existing cohorts and archived samples; longer-term priorities require new prospective recruitment. The ordering reflects the principle that questions about clinical utility should be resolved before further extension of analytical scope, since the field has repeatedly demonstrated the capacity to detect more targets than it can interpret.

Table 5. Research priorities, unresolved problems, appropriate designs and indicative timescales

Priority	Unresolved problem	Why current evidence is inadequate	Appropriate design	Indicative timescale	Supporting references
Paediatric outcomes of prenatal ascertainment	Whether prenatally identified children fare better than postnatally identified children	Existing data establish changed ascertainment, not changed outcome	Prospective birth cohort nested in a national screening programme with postnatally ascertained comparison group	Long term	Kim DeLuca et al. (2026); van Prooyen Schuurman et al. (2022)
Clinical meaning of placental mosaicism	Whether extent and location of mosaicism predict adverse obstetric outcome	Placental sampling studies are small and single-centre	Multicentre matched-cohort study with standardised multi-site placental biopsy	Immediate to medium term	Raymond et al. (2026); van Prooyen Schuurman et al. (2022)
Fetal fraction as a graded risk indicator	Whether acting on low fetal fraction improves perinatal outcome	Associations are retrospective with residual confounding	Pragmatic randomised trial of enhanced surveillance versus standard reporting	Medium term	Becking et al. (2025); Yamamoto et al. (2025)
Methylation-based source attribution in screening	Whether integrated tissue attribution shortens diagnostic pathways	Demonstrated analytically; not evaluated prospectively in service	Prospective implementation study within an established screening programme	Immediate	Tuveri et al. (2026)
Recoverability of developmental epigenetic signatures	Whether signatures found in cord blood and placenta exist in first-trimester maternal plasma	No study has attempted recovery from prenatal plasma	Nested case-control analysis using archived plasma from existing birth cohorts	Immediate	Joubert et al. (2016); Mouat et al. (2023)
Negative predictive value of single-gene panels	Frequency of missed variants and of phenotype among test-negative pregnancies	Confirmation is incomplete in test-negative pregnancies	Prospective cohort with universal postnatal genomic confirmation or record linkage	Medium term	Liu et al. (2026); Lu et al. (2026)
Performance in	Whether reported	Evidence	Multicentre validation	Medium term	Connor et al.

Priority	Unresolved problem	Why current evidence is inadequate	Appropriate design	Indicative timescale	Supporting references
under-represented populations	performance transfers across populations	concentrated in a small number of high-income regions	in populations with differing consanguinity, allele frequency and body mass distributions		(2025); Hanson et al. (2024)

10. Conclusions

Prenatal liquid biopsy has produced one of the clearest translational successes in contemporary genomics and one of the clearest illustrations of how quickly the evidential foundation of a technology can be outpaced by its extension. Screening for the common autosomal trisomies in singleton pregnancies rests on evidence that is strong by any standard, and this position is reflected consistently in independent professional guidance. Beyond that core, confidence declines along a predictable gradient governed by the prevalence of the target and by the biology of the placenta rather than by the capability of the sequencing platform. For rare autosomal trisomies the majority of positive results do not reflect a fetal abnormality, and this is a structural property of screening for rare conditions rather than a deficiency that better chemistry will correct.

The distinction between analytical validity and clinical utility organises most of the disagreement in this field. Where the target class returns to a defined-risk population with an unambiguous reference standard, as in fetal red cell genotyping or in haplotype-based diagnosis of monogenic conditions in families with a known variant, performance is again strong and the evidence is correspondingly clean. The gradient is therefore not evidence of technological immaturity but of the mathematics of predictive value operating on populations of differing prior risk, and pre-test counselling that fails to convey this cannot support informed choice.

The epigenetic strand requires a sharper separation than it usually receives. Methylation currently functions as a powerful analytical instrument, supporting fetal fraction estimation, tissue-of-origin deconvolution and, most recently, attribution of the source of aneuploidies detected during screening. As a direct diagnostic target for fetal disease it remains largely unrealised, and the developmental epigenetics literature that motivates enthusiasm for prenatal epigenetic prediction rests on cord blood, adult blood and delivered placenta rather than on first-trimester maternal plasma. Whether the relevant signatures are recoverable from the circulating pool at all is an open empirical question, and it should be answered before predictive claims are advanced.

The paediatric evidence is the weakest element of the chain and the one on which the ultimate justification of the field depends. Prenatal screening demonstrably alters who is diagnosed and when, and it is changing the composition of the childhood populations carrying particular genetic labels. Whether this improves the lives of those children is not established. Prognostic information derived from clinically ascertained cohorts does not transfer to children identified before any phenotype exists, and the resulting counselling deficit is experienced directly by parents. Detection has advanced considerably faster than the capacity to say what detection means for a child, and closing that interval, rather than extending the range of detectable targets, is the task that should now organise the field.

11. Limitations

This review is a critical narrative synthesis and carries the limitations intrinsic to that design. Study identification was purposive rather than exhaustive, and although searching was structured and criteria were stated in advance, selection and interpretive bias cannot be excluded. A different author working from the same literature might reasonably weight the constituent evidence differently, particularly in judging whether the clinical benefit of genome-wide screening outweighs the burden of low positive predictive value. No formal risk-of-bias instrument was applied, and appraisal judgements are therefore transparent in their criteria but not reproducible in the manner of a systematic review.

Access to subscription databases was incomplete. Web of Science Core Collection, Scopus and Embase could not be searched, and while the sources that were searched provide broad coverage of the biomedical literature relevant to this topic, records indexed exclusively in those platforms may have been missed. Only publications in English were assessed, which is likely to have reduced representation of work from regions where prenatal screening is expanding rapidly and where publication in other languages is common. The evidence base itself is geographically concentrated in North America, Western Europe, East Asia and Australia, and conclusions about test performance should not be assumed to transfer to populations with different allele frequencies, consanguinity rates or body mass distributions.

The constituent literatures are heterogeneous in design and outcome measurement to a degree that precludes quantitative synthesis. Diagnostic accuracy studies, national implementation cohorts, interrupted time series, epigenome-wide association studies and qualitative research cannot be pooled, and comparisons drawn between them in this review are necessarily interpretive. Confirmatory testing is incomplete in most diagnostic accuracy studies, particularly among test-negative pregnancies, so reported sensitivity and negative predictive value are bounded by ascertainment rather than by biology. Several key associations, including those between fetal fraction and adverse pregnancy outcome, derive from retrospective analyses in which residual confounding cannot be excluded.

Evidence concerning paediatric outcomes is scarce, and the review is correspondingly more confident in describing what is not known than in characterising effects. Long-term follow-up of prenatally ascertained children is essentially absent from the literature, and statements about paediatric implications are therefore inferential. The field is developing rapidly, and applications described

here as emerging may have been evaluated further since the search was completed. Publication bias is likely in the diagnostic technology literature, where favourable validation results are more readily published than negative ones, and commercial involvement in the development and evaluation of several assays discussed here is common, which is disclosed in the source publications but cannot be adjusted for in a narrative synthesis.

Consent

It is not applicable.

Ethical Approval

It is not applicable.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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