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Fragmentomics in Pediatric Genetics: A New Frontier for Non-invasive Diagnosis of Rare Congenital and Metabolic Disorders

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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

Cell-free DNA (cfDNA) circulating in plasma is fragmented non-randomly, and the resulting patterns carry information about the cells and tissues from which the molecules were released. The analysis of these patterns, termed fragmentomics, includes fragment size distributions, preferred end coordinates, end-motif frequencies, nucleosome and transcription-factor footprints, and sequencing coverage around transcription start sites. Enthusiasm for applying fragmentomics within paediatric genetics has grown alongside proposals that plasma-based assays might shorten the diagnostic pathway for rare congenital and inherited metabolic disorders. The present review examines whether the accessible evidence supports that expectation. Literature was identified through Europe PMC and Crossref Metadata Search, supplemented by targeted retrieval of works cited in recent reviews and by verification against digital object identifier landing pages, covering January 2004 to 1 June 2026. Studies were appraised for design adequacy, cohort composition, analytical validation, and the correspondence between the claim advanced and the data presented. Three observations dominate the synthesis. The mechanistic foundations of fragmentomics are comparatively secure, since nuclease activity, chromatin accessibility, and hepatic clearance jointly govern fragment length and terminal characteristics, and genome-wide association analysis has linked end-motif phenotypes to specific nuclease loci. The

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strongest clinical evidence nevertheless remains obstetric rather than paediatric, concerning fetal fraction estimation, aneuploidy screening, and placental phenotypes, where fragmentomic features operate as quantitative adjuncts rather than as primary determinants of a molecular diagnosis. Direct evidence in postnatal paediatric rare and metabolic disease is sparse, derives largely from small single-centre cohorts, and has been generated principally in graft-injury monitoring rather than in primary diagnosis. Paediatric normative fragmentomic reference data are largely absent, pre-analytical standardisation remains inconsistent, and cohort sizes attainable for individual rare disorders are incompatible with the sample requirements of the statistical learning methods commonly applied. Fragmentomics is best characterised at present as a biologically well-grounded measurement framework whose diagnostic utility in paediatric rare disease remains largely unevaluated rather than established.

Keywords: Cell-free DNA; fragmentomics; non-invasive prenatal diagnosis; inherited metabolic disorders; rare disease diagnosis; nucleosome footprint; tissue-of-origin deconvolution; paediatric genomics.

1. Introduction

Rare diseases are individually uncommon but collectively frequent, and their burden falls disproportionately on children. Analysis of the Orphanet database identified 6,172 distinct rare diseases, of which 71.9% were genetic in origin and 69.9% were exclusively of paediatric onset, with 77.3% to 80.7% of the total population burden attributable to the 4.2% of conditions occurring at a point prevalence of one to five per 10,000 (Nguengang Wakap et al., 2020). The distribution is therefore heavily skewed: a small number of comparatively common rare conditions account for most affected individuals, while the majority of disorders are encountered so infrequently that clinical familiarity is limited and diagnostic delay is routine.

Genomic sequencing has substantially altered this landscape. A prospective national study of genome sequencing in 100 critically ill infants reported a confirmed molecular diagnosis in 32% of cases, rising to a potential yield of 39% when likely diagnostic results were included, with a median turnaround time of 13 days and physician-perceived clinical utility in 81.1% of participants (Moreno et al., 2026). Rapid genome sequencing has also been proposed as an adjunct or successor to biochemical newborn screening for inherited metabolic conditions (Zheng & Yang, 2025). These figures establish an important comparator: any new diagnostic modality entering paediatric rare disease must be assessed against a sequencing-based standard that already achieves a substantial yield within a clinically actionable interval. Recent evidence syntheses likewise position genome sequencing as an early diagnostic strategy in selected rare-disease settings, reinforcing the need to assess any adjunctive assay against an established genomic comparator (Wigby et al., 2024).

Circulating cfDNA offers a different kind of information. Plasma DNA molecules are not degraded at random. Their lengths cluster around the mononucleosomal unit, their termini fall preferentially at particular genomic coordinates, and the first bases at those termini display characteristic sequence composition. Snyder et al. (2016) demonstrated that genome-wide coverage of plasma cfDNA reconstructs an *in vivo* nucleosome map whose periodicity and positioning differ according to the contributing tissue, establishing that fragmentation itself, independent of sequence variation, encodes tissue-of-origin information. Subsequent work extended the repertoire of measurable features to preferred end coordinates, four-nucleotide end motifs, orientation-aware coverage in open chromatin, and transcription-factor footprints (Jiang et al., 2020; Sun et al., 2019; Ulz et al., 2019). The collective term fragmentomics now designates this family of analyses (Hu et al., 2022; Lo et al., 2021). Recent syntheses continue to define fragmentomics through structural and epigenomic features such as fragment length, termini, and nucleosome-related patterns, while noting that translational evidence remains concentrated in oncology (Sirajee et al., 2024; Bruhm et al., 2025).

The proposition that fragmentomics might serve paediatric genetics rests on two distinct arguments. The first is quantitative: because fetal or placental DNA in maternal plasma is systematically shorter than maternal DNA, size information can be exploited to estimate or enrich the fetal contribution, thereby improving the analytical performance of prenatal assays that would otherwise fail at low fetal fraction (Chan et al., 2004; Yu et al., 2014). The second is inferential: because fragmentation patterns reflect the chromatin state and nuclease environment of the releasing cell, they might in principle report on tissue injury, cell death, or altered chromatin organisation in disorders where no circulating tumour-like clone exists to be sequenced. The first argument is supported by a substantial body of obstetric evidence. The second, applied to congenital and metabolic disease, has been advanced far more often than it has been tested.

That asymmetry is compounded by the origin of most fragmentomic methodology. Genome-wide fragmentation profiling was developed and validated principally in oncology, where a somatic clone contributes a distinguishable and often substantial fraction of plasma DNA, and where large case-control cohorts are readily assembled (Cristiano et al., 2019; Mouliere et al., 2018). The statistical architecture of those studies, in which classifiers are trained on hundreds of cases and controls, does not transfer easily to conditions affecting a handful of children per million births. Assumptions carried across from oncology therefore deserve explicit scrutiny rather than tacit adoption.

Existing reviews address adjacent territory without resolving the question posed here. Comprehensive treatments of fragmentomic biology concentrate on mechanism and on oncological application (Hu et al., 2022; Lo et al., 2021; Malki et al., 2026). Reviews of non-invasive prenatal testing for monogenic disease survey haplotype-based and targeted sequencing strategies, and identify low sensitivity for maternally inherited variants, constrained fetal DNA fraction, cost, and absent standardised validation as the principal barriers, but treat fragmentomics as peripheral rather than as a candidate solution (C. Wang et al., 2026). Reviews of cfDNA in metabolic disease focus on common adult conditions such as obesity, type 2 diabetes, and metabolic-associated fatty liver disease (Pollastri et al., 2025). Assessments of non-invasive biomarkers in primary mitochondrial disease centre on circulating proteins including fibroblast growth factor 21, growth differentiation factor 15, and neurofilament light chain, together with functional and

neuroimaging endpoints, and do not identify cfDNA fragmentation as an established modality (Arena et al., 2026; Makwikwi et al., 2026). No accessible review integrates fragmentomic biology, prenatal fragmentomic evidence, and postnatal paediatric application into a single critical assessment calibrated to what the data actually demonstrate. Consistent with this pattern, a recent review of prenatal cfDNA screening for dominant monogenic conditions centres on sequence-based detection and implementation requirements rather than on fragmentomic features as an independent diagnostic readout (Liao et al., 2025).

1.1 Scope, Research Gap and Objectives

This review is bounded to the analysis of fragmentation characteristics of cell-free DNA in human blood plasma, and to the application of those characteristics within paediatric genetics, understood to encompass prenatal diagnosis of fetal genetic conditions, neonatal and childhood diagnosis of rare congenital disorders, and diagnosis or monitoring of inherited metabolic and mitochondrial disease. Fragmentation characteristics are taken to include fragment length distribution, preferred end coordinates, end-motif composition, jagged or single-stranded ends, nucleosome and transcription-factor footprints, and coverage patterns at transcription start sites and other regulatory elements.

The knowledge gap addressed is specific. Fragmentomic features have been shown to carry tissue-of-origin and chromatin-state information, and have been incorporated into obstetric assays as quantitative adjuncts. Whether these features contribute independent diagnostic information for rare congenital and inherited metabolic disorders in children, over and above what targeted or genome-wide sequencing of the same plasma sample already provides, has not been systematically evaluated. The claim that fragmentomics constitutes a new diagnostic frontier in paediatric genetics has therefore outpaced the evidence available to support or refute it.

Several areas are deliberately excluded. Paediatric oncology, in which circulating tumour DNA analysis constitutes a distinct and comparatively mature field, is not reviewed. Cell-free RNA, extracellular vesicle cargo, circulating nucleosome immunoassays, and intact fetal cell isolation are outside scope except where they bear directly on the interpretation of fragmentomic measurements. Analyses in body fluids other than plasma are not considered. Methylation-based tissue-of-origin deconvolution is treated only where it is combined with, or directly informs the interpretation of, fragmentation data, since methylation and fragmentation are distinct measurement modalities that are frequently conflated in discussions of liquid biopsy.

Four objectives follow. The first is to establish which fragmentomic features have secure mechanistic foundations and which remain descriptive correlations, since only the former can support inference in conditions where large validation cohorts are unobtainable. The second is to appraise the obstetric evidence base on its own terms, distinguishing demonstrated analytical contributions from claims of diagnostic capability. The third is to assemble and evaluate the direct paediatric evidence, prenatal and postnatal, and to characterise its methodological quality rather than merely its existence. The fourth is to specify the conditions under which fragmentomic measurement could plausibly add diagnostic value in paediatric rare disease, and the study designs capable of testing that proposition.

2. Methods for Literature Selection

2.1 Review Design and Rationale

A critical narrative design was selected because the review question is conceptual and evaluative rather than quantitative. The literature spans molecular biology of nuclease-mediated DNA degradation, obstetric screening performance, paediatric transplantation monitoring, and computational methodology, and the studies within these domains differ in population, comparator, outcome definition, and analytical platform to an extent that precludes meaningful pooling. A systematic review with a single answerable question would exclude the mechanistic literature that is essential to interpreting the clinical findings, while a meta-analysis would require an outcome homogeneity that does not exist. The narrative form permits evaluation of whether inferences drawn in one domain are legitimately transferable to another, which is the central problem addressed.

Narrative synthesis does not remove the obligation of methodological transparency. The reporting of this review was structured around the domains specified in the Scale for the Assessment of Narrative Review Articles, which addresses justification of importance, statement of aims, description of literature search, referencing, scientific reasoning, and appropriate presentation of data (Baethge et al., 2019). The search strategy, information sources, eligibility criteria, and appraisal approach are reported below so that the basis of selection can be examined and challenged.

2.2 Information Sources

The primary bibliographic source was Europe PMC, searched programmatically through its representational state transfer interface, which provides access to MEDLINE-indexed records together with additional life-science literature. Bibliographic metadata were retrieved and cross-checked through Crossref Metadata Search. Digital object identifiers were resolved through the International DOI Foundation resolver, and publisher landing pages were consulted directly where metadata from the two indexes disagreed. Supplementary strategies comprised examination of the reference lists of recent reviews retrieved during searching, targeted title-level retrieval of foundational works cited within those reviews, and related-record searching within Europe PMC.

Subscription-restricted databases, including Web of Science Core Collection, Scopus, and Embase, were not accessible during preparation of this review, and no search of those resources was performed. No exported database records were supplied. The consequences of this restriction for coverage are addressed in the Limitations section.

2.3 Search Strategy and Search Period

Search concepts were derived from the review question and combined using Boolean operators. The first concept covered the analytical modality, expressed as fragmentomics, cell-free DNA, cfDNA, plasma DNA, and liquid biopsy. The second covered the fragmentation features themselves, expressed as fragment size, fragment length, size distribution, end motif, preferred end, jagged end, nucleosome footprint, transcription start site coverage, and open chromatin. The third covered the biological determinants, expressed as *DNASE1L3*, *DNASE1*, *DFFB*, nuclease, chromatin accessibility, and clearance. The fourth covered the clinical domain, expressed as prenatal, fetal, neonatal, paediatric, pediatric, congenital, monogenic, single-gene, rare disease, inborn errors of metabolism, and mitochondrial disease. The fifth covered evaluative and translational terms, expressed as diagnostic yield, validation, reproducibility, pre-analytical, reference interval, and implementation.

Representative strings included the combination of fragmentomics with cell-free DNA; the combination of cell-free fetal DNA with fragment size and fetal fraction; the combination of end motif with cell-free DNA and *DNASE1L3*; the combination of cell-free DNA with paediatric or pediatric and rare disease; the combination of cell-free DNA with inborn errors of metabolism; the combination of non-invasive prenatal with single gene and relative haplotype dosage; and the combination of pre-analytical with cell-free DNA and fragment size. Field-restricted title searches were used to retrieve specific foundational works identified through citation examination.

The search period extended from 1 January 2004 to 1 June 2026. The starting year corresponds to the first systematic characterisation of the size distribution of fetal and maternal DNA in maternal plasma, which established fragment length as an analysable property of circulating DNA and constitutes the intellectual origin of the field (Chan et al., 2004). Publications preceding this date were considered only where necessary to explain conceptual antecedents. The final search date was 1 June 2026.

2.4 Eligibility Criteria

Peer-reviewed original research articles and peer-reviewed reviews published in English were eligible. Eligible study designs comprised experimental molecular investigations in human samples or defined animal models, diagnostic accuracy studies, prospective and retrospective clinical cohorts, methodological and computational development studies with empirical evaluation, and genetic association studies. Records were included when they reported empirical data on cfDNA fragmentation characteristics, on the biological mechanisms generating those characteristics, or on the clinical application of cfDNA analysis within the defined paediatric and prenatal domains. Records addressing comparator diagnostic modalities, rare disease epidemiology, or implementation and counselling considerations were included where required to situate fragmentomic findings within the diagnostic pathway.

Records were excluded when they addressed cfDNA exclusively in adult oncology without transferable methodological content, when they concerned body fluids other than plasma, when they were conference abstracts, editorials without primary data, commercial materials, or preprints for which peer-reviewed evidence on the same question was available, and when bibliographic details could not be confirmed against at least one authoritative index. Publications reporting only cell-free RNA, extracellular vesicle, or intact cell analyses were excluded. Retracted articles were excluded, and retrieved records were checked for corrections and expressions of concern.

2.5 Screening, Duplicate Handling and Study Selection

Retrieved records were screened by title and abstract against the eligibility criteria. Records passing this stage were assessed through the full abstract and, where accessible, the full text or the publisher landing page. Duplicate records arising from the retrieval of the same work through multiple search strings, or from the coexistence of preprint and published versions, were identified by digital object identifier and by title matching, and were resolved in favour of the peer-reviewed version of record. Where an index recorded a preprint and a journal article with distinct identifiers, only the journal article was retained. Language was restricted to English, and no translation was undertaken. Records whose bibliographic identity could not be confirmed, or whose full text was inaccessible and whose abstract was insufficient to verify the claim for which the record would be cited, were not used as supporting evidence. Formal screening counts are not reported, because the selection process was purposive and iterative rather than systematic, and reporting counts would imply a procedural rigour that was not applied.

2.6 Critical Appraisal and Evidence Synthesis

Included studies were appraised against criteria appropriate to their design rather than through a single instrument. Mechanistic studies were assessed for the adequacy of controls, the specificity of the perturbation employed, and the extent to which conclusions rested on correlation rather than manipulation. Clinical studies were assessed for cohort size and composition, prospective or retrospective ascertainment, the presence and adequacy of a reference standard, the handling of indeterminate results, the reporting of confidence intervals, single-centre or multi-centre design, and geographical concentration of the evidence. Computational studies were assessed for the separation of training and evaluation data, the presence of independent validation, sequencing depth requirements, and the plausibility of the sample sizes used given the number of features modelled. No formal risk-of-bias scoring was applied, since the heterogeneity of designs precluded the consistent application of any single recognised tool and the information required for such scoring was frequently unavailable.

Themes were developed inductively from the retrieved evidence, organised around the biological determinants of fragmentation, the obstetric evidence base, the extrapolation to congenital disorders, the postnatal paediatric evidence, and the methodological

constraints on translation. Where studies disagreed, the disagreement is reported and the likely sources are examined, including differences in sequencing depth, platform, cohort composition, and pre-analytical handling. Where evidence for a proposition was absent rather than negative, this distinction is stated explicitly, since absence of evaluation is frequently presented in this field as though it were evidence of promise.

3. Biological Determinants of Cell-Free DNA Fragmentation Relevant to Paediatric Genetics

3.1 Nuclease-Mediated Fragmentation and Chromatin Architecture

The interpretability of any fragmentomic measurement depends on whether the process generating it is understood. For cfDNA, the mechanistic account has become progressively more secure and rests on the combined action of intracellular and extracellular nucleases operating on chromatin. Han et al. (2020) delineated distinct and partially complementary contributions from DNA fragmentation factor subunit beta, which acts intracellularly during apoptosis, deoxyribonuclease 1-like 3, which is secreted and capable of digesting DNA within chromatin, and deoxyribonuclease 1. The consequence is that the size distribution and terminal characteristics of plasma DNA are not incidental properties of degradation but the recorded output of a defined enzymatic system.

Direct manipulation supports this account. Deletion of *Dnase1l3* in mice produced an increase in molecules shorter than 120 base pairs, which correlated with anti-DNA antibody levels, together with an increase in long multinucleosomal molecules and a reduction in the frequencies of the most common end motifs observed in plasma DNA (Serpas et al., 2019). The last of these changes was independent of the anti-DNA response, indicating a primary consequence of enzyme loss rather than a secondary effect of autoimmunity. This is among the few instances in the field where a fragmentomic phenotype has been tied to a defined molecular cause by genetic ablation rather than by association, and it carries particular weight for paediatric genetics because it establishes that a single-gene defect can produce a measurable and mechanistically explicable change in the plasma fragmentome.

A second determinant is chromatin architecture. Nucleosome positioning constrains where nucleases can cut, so coverage of the plasma cfDNA population reconstructs nucleosome occupancy genome-wide, and differences in occupancy between tissues generate differences in coverage that can be used to infer contributing tissue (Snyder et al., 2016). Orientation-aware analysis in open chromatin regions refined this inference by exploiting the asymmetry of fragment ends relative to nucleosome-depleted regions (Sun et al., 2019), and footprints at transcription-factor binding sites have been used to infer regulatory state (Ulz et al., 2019). The same principle underlies the recovery of nucleosome footprints informative of gene activity in critically ill adults (Cano-Gamez et al., 2025). Subsequent methodological work has emphasised that fragment ends should be characterised holistically rather than through any single summary statistic, since end position, end motif, and single-stranded overhang structure capture partially independent aspects of the cutting process (Jiang et al., 2026).

An important qualification applies to the placental setting that dominates prenatal application. Sun et al. (2018) found that fetal and maternal preferred ends were generated at different positions relative to the nucleosome, with fetal DNA cut more frequently within the nucleosome core and maternal DNA within the linker region, and attributed this to higher nucleosome accessibility in placental cells than in white blood cells. This provides a chromatin-level explanation for the shortness of fetal DNA that does not depend on deoxyribonuclease 1-like 3 activity, consistent with the observation that the fetal-maternal size difference persisted in *Dnase1l3*-deficient mice (Serpas et al., 2019). Two mechanisms therefore operate in parallel, and the relative contribution of each in any given clinical context is not fully resolved.

3.2 Genetic Control of Fragmentomic Phenotypes

For paediatric genetics, the most consequential recent development is the demonstration that fragmentomic phenotypes are themselves heritable traits under identifiable genetic control. A genome-wide association study of cfDNA end-motif frequencies in 28,016 pregnant women identified 15 study-wide significant loci, including the previously implicated nuclease genes *DFFB* and *DNASE1L3* alongside candidates such as *PANX1* and *DNASE1L1*, with findings supported by three independent association analyses and by experimental work in knockout mice and cell lines; causal analysis further implicated leukocytes, and neutrophils in particular, in shaping cfDNA characteristics (Zhu et al., 2026).

Two implications follow, and they point in opposite directions. The constructive implication is that germline variation in nuclease genes produces measurable fragmentomic consequences, which establishes in principle that a monogenic defect affecting DNA degradation or chromatin structure could be detectable through plasma fragment analysis. The cautionary implication is that common germline variation contributes to inter-individual variance in exactly the features that diagnostic classifiers use. A classifier trained on a modest case-control cohort may therefore capture ancestry-correlated or genotype-correlated variance rather than disease-related signal, an error that is difficult to detect without genotype data on the training cohort and that would generalise poorly across populations.

Clinical illustration of the constructive implication exists but is limited. Deficiency of deoxyribonuclease 1-like 3 causes early-onset monogenic systemic lupus erythematosus, including an overlap phenotype with antineutrophil cytoplasmic antibody-associated vasculitis, and constitutes a paediatric-onset Mendelian condition in which the fragmentomic consequence of the genetic defect is mechanistically predictable (Triaille et al., 2025). The disorder is nonetheless exceedingly rare, and the available reports are individual cases rather than cohorts with systematic fragmentomic characterisation. The inference from mouse ablation to human diagnostic application remains, at present, an argument from mechanism rather than a demonstrated diagnostic capability.

3.3 Clearance, Concentration and the Confounding of Fragmentomic Signals

A recurrent interpretive error treats a change in the plasma fragmentome as evidence of a change in cell death. Evidence from severe infection argues against this equivalence. Profiling of cfDNA in sepsis demonstrated a 41-fold increase in concentration during disease, yet methylation-based deconvolution revealed cellular compositions similar to those of controls, indicating that the accumulation reflected impaired hepatic clearance rather than excess cell death; fragmentation and end-motif patterns were consistent with prolonged exposure of cfDNA to circulating nucleases (Cano-Gamez et al., 2025). The fragmentome in that setting reported on the disposal system rather than on the tissue of origin.

Analysis in health points to the same conclusion from a different direction. Across 862 healthy individuals with cfDNA concentrations ranging from 1.61 to 41.01 ng/mL, higher concentrations were accompanied by an increase in large fragments of 231 to 600 base pairs, decreased frequencies of shorter fragments of 20 to 160 base pairs, and an increased frequency of guanine-initiated end motifs, with end-motif deconvolution indicating reduced contributions from deoxyribonuclease 1-like 3 and DNA fragmentation factor subunit beta at higher concentrations; the five individuals with the highest concentrations displayed aberrantly reduced plasma protein levels of deoxyribonuclease 1-like 3 (Malki et al., 2025). Fragment profile and cfDNA concentration are therefore not independent variables, and analyses that adjust for one without accounting for the other risk attributing to disease what belongs to nuclease availability.

Physiological variation compounds the difficulty. Longitudinal profiling of 432 plasma samples from 160 healthy individuals identified circadian patterns in both concentration and fragment size, with morning samples showing 63% higher cfDNA concentrations and a 7% increase in mononucleosomal fragments relative to afternoon and evening samples, alongside cell-type-specific rhythms in natural killer cells, monocytes, and hepatocytes; contributions to cfDNA were more consistent within individuals than between them (Aerden et al., 2026). Clinical and demographic variables have been argued to exert sufficient influence on fragmentomic measurements to require explicit control in study design rather than *post hoc* adjustment (Malki & Lo, 2026). For paediatric application, where sampling times are dictated by clinical convenience and where cohorts are necessarily small, these effect sizes are not negligible relative to the differences that diagnostic classifiers seek to detect.

The features discussed above differ substantially in the security of their mechanistic foundations and in the extent to which their behaviour in children is characterised. Table 1 sets out the principal feature classes, the biological process each is understood to report, the interpretive cautions applicable in a paediatric context, and the evidence supporting each entry. The table makes explicit that the features with the strongest mechanistic grounding, namely fragment length and end motif, are also those most sensitive to clearance and to nuclease availability, whereas the features that would be most informative for tissue-specific diagnosis, namely footprint and transcription start site coverage, carry the heaviest sequencing depth requirements and the least paediatric characterisation.

Table 1. Principal classes of cell-free DNA fragmentomic feature, their biological basis, and interpretive constraints in paediatric application

Feature class	Biological process reported	Interpretive constraints in paediatric application	Supporting references
Fragment length distribution	Nucleosomal protection combined with nuclease digestion; placental chromatin accessibility exceeds that of leucocytes	Sensitive to plasma cfDNA concentration and to hepatic clearance; diurnal variation in mononucleosomal proportion; no established paediatric reference intervals	Aerden et al. (2026); Cano-Gamez et al. (2025); Chan et al. (2004); Malki et al. (2025); Sun et al. (2018)
Preferred end coordinates	Position-specific cleavage determined by nucleosome positioning and chromatin accessibility	Requires substantial sequencing depth; fetal and maternal ends arise from different intranucleosomal positions, so tissue attribution is not transferable across contexts	Chan et al. (2016); Jiang et al. (2026); Sun et al. (2018, 2019)
End-motif frequencies	Sequence preference of the cleaving nuclease, principally deoxyribonuclease 1-like 3 and DNA fragmentation factor subunit beta	Under partial germline genetic control; shifts with cfDNA concentration and with nuclease protein availability independently of disease	Jiang et al. (2020); Malki et al. (2025); Serpas et al. (2019); Zhu et al. (2026)
Nucleosome and transcription-factor footprints	Chromatin occupancy and regulatory activity of contributing cells	High depth requirement limits application in small paediatric cohorts; inference of gene activity remains indirect	Cano-Gamez et al. (2025); Snyder et al. (2016); Ulz et al. (2019)
Transcription start site coverage and coverage-derived indices	Promoter chromatin state of contributing tissues	Metric stability shown to require very deep whole-genome sequencing; correction for guanine-cytosine bias is fragment-length dependent	Rahman et al. (2025); J. Wang et al. (2026); Xu et al. (2026)
Genome-wide fragmentation profiles	Aggregate short-to-long fragment ratios across genomic windows	Developed in adult oncology with large case-control cohorts; sample sizes attainable in individual rare disorders are far smaller	Cristiano et al. (2019); Mouliere et al. (2018)

Note. cfDNA = cell-free DNA. Feature classes are not mutually exclusive, and several published assays combine two or more. Entries describe constraints identified in the cited sources and, where indicated, the absence of paediatric-specific evaluation

4. Prenatal Fragmentomics as the Established Evidential Base

4.1 Fragment Size as a Quantitative Proxy for Fetal Fraction

The single best-supported clinical proposition in fragmentomics is that fetal DNA in maternal plasma is shorter than maternal DNA and that this difference is analytically exploitable. Quantitative polymerase chain reaction assays with amplicons of differing length established the phenomenon in 34 non-pregnant and 31 pregnant women, with median proportions of plasma DNA exceeding 201 base pairs of 57% and 14% respectively, and with fetal-derived molecules exceeding 193 base pairs constituting 20% and molecules exceeding 313 base pairs constituting none of the fetal fraction (Chan et al., 2004). The design was small, and the fetal measurement relied on *SRY* targeting and therefore on male pregnancies, but the effect size was large and the finding has been reproduced consistently since.

Massively parallel paired-end sequencing subsequently converted this observation into a diagnostic parameter. Yu et al. (2014) demonstrated that fetal DNA fraction could be deduced from the overall size distribution of maternal plasma DNA, and that aneuploidy could be detected from an aberrant proportion of short fragments derived from the affected chromosome, reporting sensitivity and specificity of 100% for trisomy 21 in 36 affected and 88 unaffected pregnancies and for trisomy 18 in 27 affected and 97 unaffected pregnancies, sensitivity of 95.2% and specificity of 99% for trisomy 13, and complete concordance for monosomy X in 10 affected and 8 unaffected cases. The trisomy 13 and monosomy X figures rest on very small numbers, and the confidence intervals implied by such denominators are wide; the study establishes principle rather than population-level performance.

Size information has since been deployed to address the practical constraint that limits prenatal cfDNA screening, namely insufficient fetal fraction. Factors influencing fetal fraction, including gestational age, maternal body mass, and specimen handling, have been catalogued in review (Deng & Liu, 2022). Physical enrichment through gel-based size selection improved fetal fraction in samples collected in ethylenediaminetetraacetic acid gel tubes (Daryabari et al., 2022), and *in vitro* enrichment permitted screening from eight weeks of gestation in a cohort of 435 women recruited between seven weeks zero days and nine weeks six days, of whom 371 provided a paired later sample; sex determination was fully concordant with sex at birth, and 5.9% of 170 male pregnancies retained a fetal fraction below 4% after enrichment (Daryabari et al., 2026). The same study recorded 4.4% spontaneous abortion between the early and later sampling points, and detected seven aneuploidies early against eight at twelve weeks or later with one false positive, which illustrates that earlier sampling shifts the population tested as well as the analytical performance achieved.

Computational rather than physical size selection has produced a comparable effect with an additional analytical benefit. A pipeline that filtered longer fragments *in silico* and compared the filtered with the unfiltered data in 204 pregnancies enabled all 37 samples previously failing for low fetal fraction to be analysed, and identified 56 of 131 initially positive samples as false positives attributable predominantly to maternal abnormalities, with no false negatives observed (Yang et al., 2026). This is the clearest demonstration in the reviewed literature that fragment size contributes information beyond simple enrichment, since the comparison between size-filtered and unfiltered signal permitted attribution of an abnormality to fetal or maternal origin. The study was retrospective and single-centre, the reference standard for the reclassified positives is not fully specified, and independent replication has not been reported.

The convergent conclusion across these studies is that fragment size functions as a robust quantitative adjunct within prenatal screening. The evidence is strongest for fetal fraction estimation and enrichment, and for the attribution of signal origin. It does not demonstrate that size information alone identifies a specific genetic condition, and none of the studies claims that it does.

4.2 Preferred Ends, End Motifs and Chromatin Accessibility in Placental DNA

Beyond length, the positions and sequence composition of fragment termini carry tissue-specific information. Deep sequencing of maternal plasma to 270-fold haploid genome coverage revealed that particular genomic locations were preferentially represented at fragment ends, and that a subset of these preferred ends showed selectivity for fetal or maternal origin, with the ratio of molecules bearing fetal to maternal preferred ends correlating with fetal DNA fraction (Chan et al., 2016). Combining preferred end analysis with size stratification improved both fetal fraction estimation and trisomy 21 detection, and revealed that fetal and maternal ends arise from different intranucleosomal positions (Sun et al., 2018). End-motif profiles were subsequently shown to cluster by tissue of origin across liver, placenta, and haematopoietic cells, supporting their use as tissue-informative markers in prenatal testing as well as in oncology and transplantation (Jiang et al., 2020).

The methodological cost of these features is high and is frequently understated. The 270-fold coverage employed by Chan et al. (2016) is far beyond routine screening depth. Analysis of coverage-derived indices in a preeclampsia cohort found that stable transcription start site coverage, transcription start site scores, and Gini coefficients required 600 million whole-genome sequencing reads per sample (Xu et al., 2026). Correction for guanine-cytosine composition bias, which distorts coverage-based measurements, has itself been shown to require fragment-length-specific treatment rather than a single global adjustment (Rahman et al., 2025). A feature that is informative at research depth may therefore be uninformative, or systematically biased, at the depth achievable within a diagnostic budget.

A further constraint concerns the biology of the source tissue. Cell-free DNA in maternal plasma originates predominantly from the placenta rather than from the fetus proper, and the basic biology of this material, including its precise cellular origins and the determinants of its release, remains incompletely characterised (Yuen et al., 2024). Fragmentomic features attributed to the fetus in

fact report on trophoblast chromatin and trophoblast turnover. For conditions in which the placental and fetal genomes are concordant this distinction is of limited practical consequence, but for conditions involving confined placental mosaicism, or for disorders whose pathology resides in fetal tissues not contributing materially to plasma cfDNA, the inference from placental fragmentome to fetal phenotype is not automatically warranted.

4.3 Fragmentomics as an Adjunct Rather than a Primary Modality for Monogenic Disorders

Non-invasive prenatal diagnosis of single-gene disorders has advanced considerably, but the advances have come from sequence-based rather than fragment-based analysis. Relative haplotype dosage implemented as a routine clinical service in 152 pregnancies for spinal muscular atrophy, Duchenne and Becker muscular dystrophies, and cystic fibrosis achieved a mean reporting time of 11 calendar days, a failure rate of 4% with no technical failures, and complete concordance with confirmatory testing where audit follow-up was available (Young et al., 2020). Targeted panel sequencing for dominant monogenic disorders in 422 pregnancies produced 20 true-positive and 127 true-negative results with no false positives or negatives among cases with available outcome data (Zhang et al., 2019). A proband-independent haplotyping approach for spinal muscular atrophy constructed haplotypes in 75 of 81 eligible families from parental samples and in the remaining six from grandparental samples, returned results in 76 families, and achieved successful diagnosis at a minimum fetal fraction of 1.9% and as early as seven weeks and three days of gestation (H. Li et al., 2026).

In each case, the diagnostic determination derives from allelic or haplotypic dosage, and fragmentation characteristics enter only as a means of estimating or enriching fetal fraction. The distinction matters because the literature frequently describes these successes under the general heading of fragmentomics, which obscures the fact that the diagnostic information is sequence-based. A recent synthesis of non-invasive prenatal testing for monogenic disease identifies low sensitivity for maternally inherited variants, limited fetal DNA fraction, cost, absent standardised clinical validation, and counselling complexity as the principal barriers, and does not identify fragment analysis as a solution to any of them (C. Wang et al., 2026).

Fragmentomic analysis has, however, been applied directly to obstetric phenotypes with results that are instructive about both potential and limits. Coverage-derived fragmentomic metrics in 1,058 pregnant women distinguished pregnancies that subsequently developed preeclampsia, and models integrating these metrics with maternal risk factors achieved mean areas under the curve of 0.903 for early-onset and 0.850 for late-onset disease across two independent test sets, with sensitivities of 0.731 and 0.607 respectively at a 10% false positive rate, including in samples collected at or before 16 weeks of gestation (Xu et al., 2026). Promoter coverage analysis in 788 singleton pregnancies comprising 282 with fetal growth restriction and 506 matched controls, drawn from four hospitals, identified 198 differentially covered transcription start site features and achieved areas under the curve of 0.70 and 0.69 for logistic regression and support vector machine models respectively in validation (J. Wang et al., 2026). Mechanistic accounts linking placental hypoxia and toll-like receptor 9 mediated inflammation to fragmentomic change have been proposed (Guo et al., 2025).

The contrast between these two studies is the more informative result. Both applied coverage-based fragmentomics to a placental phenotype in cohorts of comparable order, yet discrimination differed markedly. Plausible contributors include sequencing depth, since the preeclampsia study established and met a demanding read requirement while the growth restriction study did not report an equivalent determination; the inclusion of maternal clinical risk factors in the preeclampsia models, which contributes independent predictive information; differences in outcome definition and in the timing of sampling relative to disease onset; and differences in the separation between cases and controls in the underlying biology. The comparison suggests that reported discrimination in coverage-based fragmentomics is strongly dependent on analytical and design choices rather than being an intrinsic property of the feature class. For paediatric rare disease, where cohorts will be smaller and sequencing budgets no larger, this dependence is a material concern.

The prenatal evidence base is heterogeneous in design, cohort size, and the strength of the claim each study supports. Table 2 summarises representative studies across the principal application areas, identifying the fragmentomic feature employed, the population studied, the principal finding, and the methodological limitation that most constrains interpretation. The table is intended to make visible a pattern that narrative description tends to obscure: the studies with the largest cohorts address obstetric risk prediction rather than genetic diagnosis, whereas the studies addressing genetic diagnosis use fragmentomic features only in a supporting quantitative role.

Table 2. Representative prenatal studies employing cell-free DNA fragmentomic features, by application area

Application area	Fragmentomic feature	Population and design	Principal finding and constraint	Supporting references
Characterisation of fetal fragment size	Fragment length by amplicon-specific quantitative polymerase chain reaction	34 non-pregnant and 31 pregnant women; cross-sectional	Fetal molecules shorter than maternal; fetal measurement restricted to male pregnancies through *SRY* targeting, and cohort small	Chan et al. (2004)
Aneuploidy detection by size	Proportion of short fragments per chromosome	Sequencing cohorts of 36 trisomy 21, 27 trisomy 18, 21 trisomy 13, 10 monosomy X with controls	Aneuploidy detectable without counting; denominators for trisomy 13 and monosomy X too small for precise performance estimation	Yu et al. (2014)

Application area	Fragmentomic feature	Population and design	Principal finding and constraint	Supporting references
Fetal fraction enrichment	Physical and *in vitro* size selection	Ethylenediaminetetraacetic acid gel tube samples; 435 women sampled from seven weeks zero days	Enrichment enables screening from eight weeks; 5.9% of male pregnancies still below 4% fetal fraction, and 4.4% spontaneous abortion before the later sampling point	Daryabari et al. (2022, 2026)
Computational size selection and origin attribution	In silico filtering of longer fragments	204 pregnancies; retrospective single-centre	All 37 low fetal fraction samples rendered analysable; 56 of 131 initial positives reclassified as false positives of predominantly maternal origin; not independently replicated	Yang et al. (2026)
Preferred ends and end motifs	Preferred end coordinates; four-nucleotide end motifs	Deep sequencing to 270-fold coverage; tissue comparison across liver, placenta and haematopoietic cells	Ends carry fetal or maternal selectivity and cluster by tissue; sequencing depth far exceeds routine diagnostic practice	Chan et al. (2016); Jiang et al. (2020); Sun et al. (2018)
Obstetric risk prediction	Transcription start site coverage, transcription start site score, Gini coefficient	1,058 pregnant women; two independent test sets	Area under the curve 0.903 early-onset and 0.850 late-onset preeclampsia; requires 600 million whole-genome sequencing reads per sample for metric stability	Xu et al. (2026)
Obstetric risk prediction	Promoter and transcription start site coverage	788 singleton pregnancies, 282 with fetal growth restriction, four centres	Area under the curve 0.70 and 0.69 in validation; discrimination substantially lower than in the preeclampsia cohort, with depth requirement not equivalently established	J. Wang et al. (2026)
Monogenic disorder diagnosis	Fragment size used only for fetal fraction estimation	152 pregnancies in routine service; 422 pregnancies in panel testing; 81 families for spinal muscular atrophy	Diagnostic determination derives from allelic or haplotypic dosage rather than from fragmentation; fragmentomics functions as a supporting parameter	H. Li et al. (2026); Young et al. (2020); Zhang et al. (2019)

Note. Performance figures are those reported by the cited authors. Entries in the constraint column reflect limitations identified in the source publications or evident from the reported design, and are not the product of a formal risk-of-bias assessment

5. Extrapolation to Rare Congenital Disorders: What the Evidence Does and Does Not Support

5.1 Structural and Single-Gene Congenital Conditions

Non-invasive prenatal diagnosis for specific congenital conditions is now delivered clinically, and its performance illustrates where the practical constraints actually lie. A retrospective cohort of 275 tests performed between 2017 and 2023 for suspected achondroplasia, of which 176 were requested following sonographic abnormality, confirmed 34.1% of sonographically indicated referrals; abnormality of the proximal femur, frontal bossing, and a pronounced nasal saddle were the most specific markers, and the presence of all three yielded a diagnostic yield of 87.5% (Verebi et al., 2025). The determinant of yield in this service was the quality of phenotypic selection rather than any property of the plasma assay. Improving referral specificity raised yield from approximately one third to seven eighths without any change in the molecular method.

This finding carries directly into any proposal to deploy fragmentomics for congenital disorders. For a condition with a birth prevalence in the range typical of individual rare diseases, a test with excellent analytical characteristics will still generate predominantly false positive results when applied without phenotypic enrichment, because positive predictive value is governed by prevalence. Fragmentomic features, which are continuous, population-variable, and sensitive to clearance and to circadian and demographic factors as described in Section 3, are less suited to unenriched application than allele-specific sequence detection, which is discrete and comparatively robust to those influences.

Where a targeted molecular question exists, targeted sequencing addresses it. Where no such question exists, the diagnostic problem is one of hypothesis generation, and it is here that fragmentomics has been proposed to contribute. The available evidence does not yet speak to that proposition in congenital disease. No accessible study has evaluated whether fragmentomic profiling of maternal or paediatric plasma identifies a category of congenital disorder in an undirected manner. The proposition remains open rather than refuted, but it should not be described as supported.

5.2 The De Novo Variant Problem

A substantial proportion of severe congenital disorders arise from de novo variants, which are invisible to carrier screening and to family-based haplotype approaches. Chan et al. (2016) detected fetal de novo mutations from maternal plasma at 85% sensitivity

and 74% positive predictive value, describing this as a 169-fold improvement in positive predictive value over previous attempts, and interrogated maternal inheritance at 618,271 of 656,676 heterozygous maternal single-nucleotide polymorphisms, a 90-fold enhancement in resolution. The result was obtained at 270-fold coverage in a single pregnancy and represents a technical demonstration rather than a clinical assay.

A systematic literature review of the detection of de novo or paternally inherited pathogenic single-nucleotide variants in maternal plasma identified 12 studies through a search conducted in February 2024 and four further studies through subsequent citation analysis, concluding that next-generation sequencing of gene panels or the whole exome can detect pathogenic single-nucleotide variants in fetuses with high positive predictive value (Valovičová et al., 2025). Detection in that literature is achieved through variant calling at sufficient depth to distinguish a low-fraction fetal allele from sequencing error, and the fetal fraction that determines feasibility can be estimated from fragment size. Fragmentomics again supports the determination without providing it.

A structural argument explains why this division of labour is likely to persist. Detecting a de novo variant requires locating a specific low-frequency allele against a background of maternal sequence, which is a problem of sequence discrimination and depth. Fragmentation features, by contrast, are aggregate properties of the molecular population and carry no allele-specific information. A fragmentomic signal could in principle arise from a de novo variant only where the variant produced a systemic change in chromatin structure, nuclease activity, or tissue turnover of sufficient magnitude to alter the plasma fragmentome measurably during gestation. Whether such conditions exist in a form detectable against normal biological variance is unknown, and no study has attempted to establish it.

5.3 Obstetric Phenotypes as Proof of Principle with Limited Transferability

The preeclampsia and fetal growth restriction studies discussed in Section 4.3 constitute the strongest demonstrations that fragmentomic features can discriminate a clinical phenotype in pregnancy. Their transferability to rare congenital disease is nonetheless limited by three features of their design. Both conditions are common relative to individual rare diseases, permitting the assembly of cohorts numbering in the hundreds. Both involve the placenta, which is the dominant non-haematopoietic contributor to maternal plasma cfDNA, so the affected tissue is well represented in the analyte. Both are functional disorders of placental physiology, in which altered tissue turnover and inflammatory activation provide a plausible route from pathology to fragmentome.

None of these conditions holds for most rare congenital disorders. Affected tissues such as brain, skeletal muscle, or bone contribute little to plasma cfDNA in the fetal or neonatal period as currently characterised. Many congenital disorders do not involve accelerated tissue turnover, so no additional cell death signal is generated. Cohort sizes are orders of magnitude smaller. An inference from preeclampsia to, for example, a skeletal dysplasia or a urea cycle disorder therefore requires assumptions about analyte representation and about the relationship between pathology and cell turnover that are not merely untested but implausible on present understanding for a substantial subset of conditions.

The appropriate conclusion is neither dismissal nor endorsement. The obstetric studies demonstrate that the measurement framework functions and can capture disease-associated variation under favourable conditions. They do not establish that the framework will capture disease-associated variation under the unfavourable conditions that characterise most rare congenital disease, and the burden of demonstration rests with those advancing the extension.

6. Postnatal Fragmentomics in Paediatric Rare and Metabolic Disease

6.1 Inherited Metabolic Disorders and Graft Injury Monitoring

The most direct application of fragment size analysis in paediatric metabolic disease concerns graft monitoring rather than primary diagnosis. Eleven patients with a range of inborn errors of metabolism requiring living-related liver transplantation were studied through serial plasma sampling at defined postoperative intervals, with graft-derived cfDNA quantified by Y chromosome capture in seven sex-mismatched recipients; graft-derived molecules were shorter than recipient-derived molecules, and a ratio of short to long fragments, calculated from windows of 105 to 145 and 160 to 170 base pairs, indicated an earlier trend of graft injury than alanine aminotransferase or aspartate aminotransferase (Ng et al., 2019).
The study is important for two reasons and limited for several. It establishes that the size differential exploited in the prenatal setting extends to a donor-recipient relationship in children with metabolic disease, and it does so without requiring donor genotype, which removes a practical barrier to implementation. The cohort of eleven patients with seven informative for the sex-mismatched quantification is very small, no independent validation cohort was reported, and the comparison against transaminases was made without a formal accuracy analysis. The application is prognostic monitoring of an established graft, not diagnosis of the underlying metabolic disorder.

Donor-derived cfDNA in paediatric transplantation has since been evaluated more rigorously, though using quantitative rather than fragmentomic measurement. In paediatric liver transplantation, evaluation across 98 patients under surveillance and 40 undergoing for-cause biopsy, with 60 biopsy-proven acute rejection events of which 46.7% were subclinical, found that donor-derived cfDNA alone distinguished subclinical rejection from rejection-free cases, achieving a sensitivity of 96.7% with a 95% confidence interval of 88.6 to 99.4 and a specificity of 34.6% with a 95% confidence interval of 25.0 to 45.7 at a threshold of 1.9% (Trezeguet Renatti et al., 2026). The performance profile is that of a rule-out test, and the low specificity is the operative constraint. Whether fragmentomic features would improve specificity in this setting has not been tested, and represents a tractable question.

For metabolic disease more broadly, cfDNA has been reviewed as a candidate biomarker principally in relation to common adult conditions including obesity, type 2 diabetes, and metabolic-associated fatty liver disease, with attention to cfDNA load and composition as reflections of tissue pathology and to a proposed role in adipose tissue inflammation (Pollastri et al., 2025). The paediatric inherited metabolic disorders are pathophysiologically distinct from these conditions, and the reviewed evidence does not extend to them. No accessible study has evaluated whether plasma fragmentomic profiling distinguishes children with an inborn error of metabolism from unaffected children, whether at diagnosis or during metabolic decompensation.

6.2 Mitochondrial Disorders and Cell-Free Mitochondrial DNA

Primary mitochondrial diseases present a diagnostic problem for which a plasma-based approach would be valuable, since definitive investigation has historically required tissue biopsy and since less invasive sources such as leucocytes, urinary sediment, and buccal cells may not reflect the mitochondrial DNA content or heteroplasmy of affected tissue. Plasma cell-free mitochondrial DNA has been shown to be recoverable and sequenceable, permitting haplogroup assignment in a comparison of seven patients with biopsy-confirmed mitochondrial disease and seven controls, with the circular topology of the mitochondrial genome proposed as a factor limiting its degradation (Newell et al., 2018).

Haplogroup assignment is not diagnosis. The clinically decisive measurements in mitochondrial disease are the identification of a pathogenic variant and the quantification of heteroplasmy in the relevant tissue, and neither was established by that study. Current assessments of the non-invasive biomarker landscape in primary mitochondrial disease identify circulating protein markers including fibroblast growth factor 21, growth differentiation factor 15, and neurofilament light chain, together with nicotinamide adenine dinucleotide-related signatures, functional and digital endpoints, and neuroimaging, and note that multi-omic approaches are moving towards integrated molecular phenotyping (Arena et al., 2026). Complementary analysis of the diagnostic gap reaches similar conclusions regarding the state of biomarker development (Makwikwi et al., 2026). Cell-free DNA fragmentation does not appear in these assessments as an established or emerging modality.

A specific mechanistic obstacle deserves statement. Mitochondrial DNA is not organised into nucleosomes, so the nucleosomal periodicity that underlies most fragmentomic inference does not apply to it. Fragmentation of cell-free mitochondrial DNA is governed by different processes, and the analytical tools developed for nuclear fragmentomics are not directly transferable. Any claim that fragmentomics offers a route to mitochondrial disease diagnosis must therefore specify whether it refers to nuclear cfDNA reporting on tissue injury or to mitochondrial cfDNA analysed by methods yet to be established. The accessible literature does not currently support either formulation as a diagnostic proposition.

6.3 Monogenic Nuclease Deficiencies as a Reverse-Inference Paradigm

A distinct and more defensible application arises where the genetic defect directly affects the machinery of DNA fragmentation. Deficiency of deoxyribonuclease 1-like 3 causes monogenic paediatric-onset systemic lupus erythematosus and has been described in an overlap syndrome with antineutrophil cytoplasmic antibody-associated vasculitis (Triaille et al., 2025). The fragmentomic consequences of enzyme loss are established in the mouse, comprising an excess of molecules shorter than 120 base pairs, an excess of long multinucleosomal molecules, and reduced frequency of the commonest end motifs (Serpas et al., 2019). In humans, reduced plasma protein levels of the enzyme have been observed in individuals with the highest cfDNA concentrations, accompanied by the corresponding shifts in fragment size and end-motif composition (Malki et al., 2025).

This constitutes the strongest available case that a fragmentomic measurement could contribute to the diagnosis of a paediatric monogenic disorder, because the causal chain from genotype to measured phenotype is short and mechanistically specified rather than inferred from correlation. The limitations are equally clear. The disorder is very rare, the human evidence consists of case reports and of observational association in healthy cohorts rather than of a diagnostic accuracy study, and functional assays of enzyme activity together with sequencing of the gene already provide a diagnostic route. Fragmentomic profiling would need to demonstrate an advantage in sensitivity, timeliness, or cost against those comparators, and no such comparison has been performed.

The broader point is that the genetic architecture of fragmentomic traits identifies where reverse inference is likely to succeed. The loci associated with end-motif frequencies at genome-wide significance include *DFFB* and *DNASE1L3* together with *PANX1* and *DNASE1L1* (Zhu et al., 2026). Monogenic disorders affecting these genes and their functional partners constitute the plausible target set for a fragmentomics-first diagnostic strategy. Conditions outside this set would need to act on the fragmentome indirectly through altered tissue turnover, and the magnitude of such indirect effects relative to normal variance has not been characterised.

6.4 Tissue-of-Origin Deconvolution in Paediatric Disease

Where fragmentomics is frequently invoked, methylation-based deconvolution is often what has actually been demonstrated, and the distinction is analytically consequential. Reference atlases of cell-type-specific methylation permit inference of the tissue origins of plasma cfDNA (Loyfer et al., 2023; Moss et al., 2018), and this approach has been applied successfully in paediatric populations. Methylation markers specific to immune and gastrointestinal epithelial cells in children with appendicitis found appendix epithelial cfDNA undetectable, neutrophil and regulatory T-cell cfDNA elevated in appendicitis, and eosinophil cfDNA most markedly elevated in perforated compared with non-perforated disease, with cross-validation by a machine-learning approach (Fox-Fisher et al., 2025). Cell-free DNA methylomic analysis has similarly been used to identify tissue injury patterns in children with acute respiratory distress syndrome (Yehya et al., 2025).

These studies demonstrate that tissue-of-origin inference from paediatric plasma is feasible and can yield clinically discriminating information, but they are methylation studies rather than fragmentomic studies. The finding that appendix epithelial cfDNA was

undetectable in a condition centred on the appendix is instructive about the limits of the analyte: an affected tissue contributing insufficient cfDNA to be measured cannot report on itself, and the diagnostic signal derived instead from the immune response. For paediatric rare disease, where affected tissues are frequently those with low turnover, this constraint is likely to be common rather than exceptional.

Combined fragmentomic and methylomic characterisation is emerging and represents the more promising direction, since the two modalities report on partially independent properties. Longitudinal profiling in health has characterised both together and identified circadian and demographic structure in each (Aerden et al., 2026), and age- and sex-associated methylation signatures have been mapped across the plasma methylome in adults aged 22 to 77, including the development of an epigenetic clock comprising 125 cytosine-guanine dinucleotides and the identification of age-related and sex-related variation in monocyte, granulocyte, and hepatocyte contributions (Chen et al., 2025). The absence of equivalent paediatric normative characterisation is a specific and remediable deficiency.

The postnatal paediatric evidence is limited in volume and concentrated in a small number of application areas that do not include primary genetic diagnosis. Table 3 sets out the accessible studies, the analyte and feature examined, the population, and the principal finding together with its limitation. Assembling the evidence in this form makes clear that no study in the reviewed literature evaluates fragmentomic profiling as a means of diagnosing a rare congenital or inherited metabolic disorder in a child, and that the paediatric applications demonstrated to date concern injury monitoring and inflammatory characterisation.

Table 3. Accessible postnatal paediatric studies of cell-free DNA fragmentation and tissue-of-origin inference

Clinical context	Analyte and feature	Population and design	Principal finding and limitation	Supporting references
Liver transplantation for inborn errors of metabolism	Nuclear cell-free DNA; fragment size distribution and short-to-long fragment ratio	11 recipients, 7 informative through sex mismatch; serial sampling to day 60	Graft-derived molecules shorter than recipient molecules; ratio indicated graft injury earlier than transaminases; very small cohort, no validation set, no formal accuracy analysis	Ng et al. (2019)
Paediatric liver transplantation	Donor-derived cell-free DNA; quantitative fraction rather than fragmentomic features	98 surveillance and 40 for-cause patients; 60 biopsy-proven rejection events	Sensitivity 96.7% (95% CI 88.6-99.4) and specificity 34.6% (95% CI 25.0-45.7) at a 1.9% threshold; rule-out profile with specificity as the limiting property	Trezeguet Renatti et al. (2026)
Primary mitochondrial disease	Cell-free mitochondrial DNA; sequence recovery	7 patients with biopsy-confirmed disease and 7 controls	Haplogroup assignment achievable from plasma; haplogroup is not diagnostic, and heteroplasmy quantification in affected tissue was not addressed	Newell et al. (2018)
Monogenic deoxyribonuclease 1-like 3 deficiency	Nuclear cell-free DNA; fragment size and end motifs	Murine gene deletion; human case reports; observational association in 862 healthy adults	Causal chain from genotype to fragmentomic phenotype is short and specified; human evidence is case-level and observational, with no diagnostic accuracy study	Malki et al. (2025); Serpas et al. (2019); Triaille et al. (2025)
Paediatric appendicitis	Cell-free DNA methylation markers for tissue of origin	Children with appendicitis and control groups	Eosinophil cell-free DNA elevated in perforated disease; appendix epithelial cell-free DNA undetectable, illustrating that low-turnover affected tissue may not be represented in the analyte	Fox-Fisher et al. (2025)
Paediatric acute respiratory distress syndrome	Cell-free DNA methylome; tissue injury patterns	Children with severe lung injury	Tissue injury patterns identifiable from plasma; methylation-based rather than fragmentomic, and directed at injury characterisation rather than genetic diagnosis	Yehya et al. (2025)

Note. CI = confidence interval. No study identified in this review evaluated fragmentomic profiling for the primary diagnosis of a rare congenital or inherited metabolic disorder in a paediatric population. Entries describing methylation-based analyses are included because these are frequently conflated with fragmentomics in discussion of paediatric liquid biopsy.

7. Methodological Constraints on Translation

7.1 Pre-Analytical Variability and the Absence of Paediatric Standards

Fragmentomic measurements are unusually vulnerable to specimen handling because the quantity being measured is the length and terminal structure of a degradable molecule, and because leucocyte lysis introduces genomic DNA whose size profile differs from that of circulating material. Systematic assessment of pre-analytical variables affecting cfDNA from blood and urine has documented the influence of collection tube chemistry, processing interval, centrifugation protocol, and storage on yield and

integrity (Peng et al., 2024). Extraction contributes further variance, with evaluation across plasma and urine demonstrating substantial variability in recovery efficiency and supporting the use of spike-in normalisation to render measurements comparable (Sandberg et al., 2025).

The consequences for a size-based measurement are direct rather than incidental. Contamination with high-molecular-weight genomic DNA shifts the fragment distribution towards longer molecules, which mimics the profile associated with reduced nuclease activity or elevated cfDNA concentration described in Section 3.3. A study reporting a longer fragment profile in an affected group relative to controls cannot distinguish disease biology from differential specimen handling unless collection and processing were identical and documented. Several of the small paediatric studies reviewed here do not report sufficient pre-analytical detail for that judgement to be made.

Paediatric practice introduces additional constraints that have not been addressed in the standardisation literature. Blood volumes obtainable from neonates and small children are limited, which reduces the cfDNA input available and thereby the sequencing depth achievable, precisely the parameter on which coverage-based features depend most heavily. Sampling in acute paediatric presentations occurs at clinically determined times, which interacts with the diurnal variation in concentration and mononucleosomal proportion documented in adults (Aerden et al., 2026). No paediatric consensus on collection, processing, and reporting for fragmentomic analysis was identified in this review, and the absence of such guidance is a barrier to the accumulation of comparable data across centres.

7.2 Statistical Learning Applied to Small Populations

Genome-wide fragmentation profiling in its established form generates a large number of features per sample and relies on classifier training to reduce them to a diagnostic output. In the oncological setting where the approach was developed, training cohorts comprise hundreds of cases and controls (Cristiano et al., 2019; Mouliere et al., 2018). The ratio of features to samples is already unfavourable in that setting, and it becomes untenable for a condition affecting a few children per hundred thousand births. A classifier trained on a cohort of twenty affected children and forty controls, using thousands of genomic windows, will separate the groups; the separation carries no assurance of generalisation.

The confounders documented in Sections 3.2 and 3.3 make this failure mode more likely rather than less. Germline variation at nuclease loci contributes to end-motif phenotypes (Zhu et al., 2026), cfDNA concentration co-varies systematically with fragment profile (Malki et al., 2025), and age, sex, and time of day structure both the methylome and the fragmentome (Aerden et al., 2026; Chen et al., 2025). In a small cohort, cases and controls will differ on several of these axes by chance, and a flexible classifier will exploit those differences. The resulting model may achieve high apparent accuracy while encoding ancestry, age, or collection time. Detecting this requires external validation in an independently collected cohort, which for a rare disorder may take years to assemble.

Analytical choices in the processing pipeline compound the problem. Correction for guanine-cytosine composition bias must be applied in a fragment-length-specific manner, since the bias interacts with fragment length rather than acting uniformly (Rahman et al., 2025). Metric stability in coverage-based analysis has been shown to require very deep sequencing (Xu et al., 2026). A pipeline that applies a global bias correction at moderate depth may generate features that appear informative but that reflect residual technical structure, and batch differences correlated with case status would be indistinguishable from biology in the resulting model.

The implication is not that statistical learning is inappropriate but that its application must be matched to the sample sizes available. Approaches with fewer parameters, features selected on mechanistic grounds rather than empirically, and pooling of biologically related disorders into functionally defined groups are more defensible in this setting than genome-wide classifier training. None of these approaches has been systematically evaluated in paediatric rare disease.

7.3 Technological Developments and Their Realistic Contribution

Long-read sequencing addresses several limitations of short-read fragmentomics by measuring fragment length directly rather than inferring it from paired-end alignment, and by resolving the structure of fragment termini. Long-read analysis has identified aberrant fragmentation patterns associated with elevated cfDNA concentrations in cancer (Berman et al., 2026), and dedicated nanopore-based analytical pipelines for fragmentomic feature extraction have been developed (Ficorilli et al., 2026). Nanopore sequencing has also been applied to plasma from critically ill patients to perform tissue-of-origin and pathogen detection simultaneously, which is relevant to the acute paediatric setting where infection and genetic disease frequently enter the differential diagnosis together (Willemart et al., 2025).

Methodological refinement of end characterisation is proceeding in parallel, with holistic treatment of fragment termini capturing end position, motif, and single-stranded structure as related aspects of a single cutting process rather than as independent summary statistics (Jiang et al., 2026). Such refinement improves the biological interpretability of measurements, which is of particular value where cohort sizes preclude empirical feature selection.

These developments improve measurement rather than establish clinical utility. The constraints identified above are not principally measurement constraints. Insufficient representation of affected tissue in the analyte is not resolved by measuring the analyte more accurately. Small cohort size is not resolved by richer features per sample, and may be worsened where additional features increase model complexity. Pre-analytical heterogeneity persists regardless of the sequencing platform employed. Technological advance is a

necessary condition for progress in this field but is not the binding constraint, and treating it as such has contributed to the gap between the claims made for fragmentomics in paediatric genetics and the evidence supporting them.

7.4 Positioning Within the Existing Diagnostic Pathway

Any assessment of clinical value must be made against the alternatives actually available. Genome sequencing in critically ill infants achieves a confirmed diagnostic yield of 32%, rising to a potential 39%, within a median of 13 days (Moreno et al., 2026), and rapid genome sequencing has been examined as an approach to metabolic conditions in the newborn period (Zheng & Yang, 2025). Prenatal and neonatal genetic testing increasingly form a continuum in which findings from one phase inform the other (Harris & Vora, 2025). A fragmentomic assay entering this pathway would need to identify conditions that sequencing misses, to deliver results substantially faster, or to operate at a cost permitting broader application. No published evidence addresses any of these propositions in paediatric rare disease.

Two positions within the pathway are nonetheless defensible on the current evidence. The first is as a quantitative adjunct that improves the performance of sequence-based prenatal assays, which is the role fragment size already occupies and where the evidence is strongest (Daryabari et al., 2026; Yang et al., 2026; Yu et al., 2014). The second is as a monitoring modality in conditions where a defined injury process is expected to alter tissue turnover, which is the role occupied in transplantation (Ng et al., 2019; Trezeguet Renatti et al., 2026). The position most frequently claimed, namely primary undirected diagnosis of rare genetic disease, is the position least supported.

Implementation considerations extend beyond analytical performance. Expansion of non-invasive prenatal testing into new indications raises questions about the scope of conditions for which screening is appropriate, which have been examined in relation to whether a threshold of seriousness should constrain that scope (Taylor-Sands et al., 2025). Systematic review of healthcare professionals' perspectives and experiences identifies recurring difficulties in conveying the probabilistic nature of screening results and in supporting informed decision-making (Warton & Vears, 2025). A fragmentomic risk score produces a continuous output whose interpretation depends on prior probability, and communicating such a result for a rare condition would compound the difficulties already documented for categorical screening outputs.

The constraints described in this section differ in their tractability, and distinguishing those that can be addressed by better study design from those that reflect the biology of the analyte is necessary for setting research priorities. Table 4 classifies the principal constraints accordingly, identifies the evidence establishing each, and indicates whether the constraint is remediable within current technology. The classification underlies the priorities developed in the following section.

Table 4. Principal constraints on the translation of fragmentomics into paediatric rare disease diagnosis

Constraint	Nature of the problem	Evidence establishing the constraint	Remediable within current technology	Supporting references
Absent paediatric normative data	Reference distributions for fragment length, end motifs and coverage indices in healthy children are not established, so deviation cannot be quantified	Normative characterisation exists for adults, including circadian, age and sex structure, with no paediatric equivalent identified	Yes; requires cohort assembly rather than methodological advance	Aerden et al. (2026); Chen et al. (2025)
Pre-analytical heterogeneity	Collection tube, processing interval and extraction efficiency alter the measured fragment profile in the same direction as several disease hypotheses	Documented effects of pre-analytical variables on yield and integrity, and of extraction variability requiring spike-in normalisation	Yes; requires consensus protocols and reporting standards for paediatric practice	Peng et al. (2024); Sandberg et al. (2025)
Confounding by clearance and concentration	Fragment profile co-varies with cfDNA concentration and hepatic clearance independently of the cellular source	41-fold cfDNA increase in sepsis attributable to impaired clearance rather than cell death; systematic profile shift across a healthy concentration range	Partially; requires concurrent measurement of concentration and nuclease status in study design	Cano-Gamez et al. (2025); Malki et al. (2025); Malki and Lo (2026)
Germline genetic structure of fragmentomic traits	Common variation at nuclease loci contributes to inter-individual variance in the features used for classification	15 study-wide significant loci for end-motif frequencies identified in 28,016 women, including *DFFB*, *DNASE1L3*, *PANX1* and *DNASE1L1*	Partially; requires genotype-aware analysis and ancestrally matched controls	Zhu et al. (2026)
Sample size relative to model complexity	Feature counts typical of genome-wide profiling exceed attainable cohort sizes in individual rare disorders by orders of magnitude	Established profiling methods developed in adult oncology cohorts numbering in the hundreds	Partially; requires mechanistically constrained feature sets and pooled disorder groupings	Cristiano et al. (2019); Mouliere et al. (2018)
Sequencing depth	Coverage-derived indices	Metric stability shown to	Partially; long-read	Rahman et

Constraint	Nature of the problem	Evidence establishing the constraint	Remediable within current technology	Supporting references
requirements	require read depths incompatible with limited paediatric sample volume and routine budgets	require 600 million whole-genome sequencing reads; guanine-cytosine bias correction is fragment-length dependent	platforms measure length directly but do not reduce depth requirements for coverage indices	al. (2025); Xu et al. (2026)
Representation of affected tissue in the analyte	Tissues affected in many rare disorders contribute little cfDNA, so no signal is available to detect	Appendix epithelial cfDNA undetectable in children with appendicitis, with the diagnostic signal deriving instead from the immune response	No; reflects the biology of cfDNA release rather than measurement capability	Fox-Fisher et al. (2025)
Comparator performance	Genome sequencing already achieves substantial yield within a clinically actionable interval	Confirmed diagnostic yield of 32%, potential yield 39%, median turnaround 13 days in critically ill infants	Not applicable; defines the threshold any new modality must exceed	Moreno et al. (2026)

Note. cfDNA = cell-free DNA. Assessment of remediability reflects the judgement of the present synthesis based on the cited evidence, and is intended to support prioritisation rather than to constitute a formal appraisal.

8. Future Directions

The priorities set out here follow from the constraints classified in Table 4, and are ordered by the extent to which addressing them is prerequisite to addressing the others. Each is stated with the design required rather than as a general aspiration.

The most immediate priority is the establishment of paediatric normative fragmentomic reference data. Deviation from normal cannot be quantified where normal is undefined, and every diagnostic proposition in this field depends on that quantification. The required study is a multi-centre cross-sectional cohort of healthy children stratified by age from the neonatal period through adolescence, with sampling times recorded, using a single standardised pre-analytical protocol and a defined sequencing depth. Outcome measures are the distribution and within-individual stability of fragment length metrics, end-motif frequencies, and coverage-derived indices, together with their covariance with age, sex, ancestry, cfDNA concentration, and time of sampling. Adult work has established that such structure exists and is measurable (Aerden et al., 2026; Chen et al., 2025), so the methodology is available and the obstacle is cohort assembly rather than technique. Without these data, effect sizes in paediatric case-control studies cannot be interpreted.

A parallel and equally tractable priority is pre-analytical standardisation for paediatric fragmentomics. The variables requiring specification are collection tube chemistry, maximum interval to processing, centrifugation protocol, extraction method, and minimum reported metadata including sampling time and blood volume. The relevant evidence base already exists in general form (Peng et al., 2024; Sandberg et al., 2025) and requires adaptation to the volume constraints of paediatric practice rather than original derivation. The appropriate output is a consensus protocol adopted across participating centres, since the value of standardisation is realised only through comparability of data generated independently.

The third priority is the systematic evaluation of reverse inference in disorders of the fragmentation machinery. This is the application with the shortest causal chain from genotype to measurable phenotype and therefore the most likely to succeed. The design required is an international cross-sectional study of children with confirmed pathogenic variants in *DNASE1L3*, *DFFB*, and the further loci implicated in end-motif determination (Zhu et al., 2026), with age-matched controls, comparing fragmentomic profiles against enzyme activity assays and sequencing as reference standards. Outcome measures are sensitivity and specificity for detection of the enzymatic defect, and the correspondence between fragmentomic severity and clinical phenotype. Given the rarity of these conditions, international registry-based recruitment is necessary, and the timescale is measured in years. A positive result would establish the first genuinely diagnostic application of fragmentomics in paediatric genetics; a negative result would appropriately constrain claims for the wider field, since success is less likely where the causal chain is longer.

The fourth priority concerns the systematic characterisation of analyte representation. The proposition that fragmentomics can report on a disorder requires that the affected tissue contributes measurable cfDNA, and the observation that appendix epithelial cfDNA was undetectable in appendicitis indicates that this cannot be assumed (Fox-Fisher et al., 2025). The required work is a mapping of tissue contributions to plasma cfDNA across paediatric age ranges using combined methylation and fragmentomic deconvolution, generating an empirical basis for determining which disorders are candidates for plasma-based detection and which are not. This would prevent the expenditure of scarce rare-disease cohorts on hypotheses that are excluded on analyte grounds before any assay is designed, and would constitute a screening step preceding the design of individual disease studies.

A fifth priority, contingent on the preceding four, is methodological work on inference in small samples. The requirement is the development and evaluation of approaches that use mechanistically selected feature sets, incorporate concentration and genotype as covariates rather than ignoring them, and pool functionally related disorders where the pathophysiological rationale supports pooling. Evaluation should be conducted against simulated and real cohorts of realistic size, with external validation as a required rather than optional component. The existing literature applies methods developed for large cohorts to small ones without addressing the mismatch, and the resulting performance estimates are not reliable.

Two areas warrant longer-term attention. The first is the integration of fragmentomic with methylation-based analysis, which is proceeding in adults (Aerden et al., 2026) and which is likely to be more informative than either modality alone, since fragmentation reports on nuclease exposure and chromatin accessibility while methylation reports on cell identity. The second is the evaluation of long-read platforms in paediatric application, where direct length measurement and simultaneous pathogen detection are of specific value in the acutely unwell child (Ficorilli et al., 2026; Willemart et al., 2025). Neither should be prioritised ahead of the normative and standardisation work, since richer measurement of an uncharacterised baseline does not yield interpretable results.

Finally, where a diagnostic application does reach evaluation, implementation research should proceed alongside rather than after analytical validation. The interpretive difficulties documented for existing prenatal screening (Taylor-Sands et al., 2025; Warton & Vears, 2025) will be greater for a continuous risk score in a rare condition, and designing the communication of such results after the assay has been validated has repeatedly proved inadequate in related fields.

9. Conclusions

The biological foundations of cell-free DNA fragmentomics are secure. Fragmentation is the product of a defined enzymatic system operating on chromatin, in which deoxyribonuclease 1-like 3, deoxyribonuclease 1, and DNA fragmentation factor subunit beta act on nucleosomally organised DNA, and in which chromatin accessibility differences between tissues generate corresponding differences in fragment length, terminal position, and end-motif composition. This account rests on genetic manipulation, on genome-wide association evidence in a large cohort, and on convergent observation across multiple clinical contexts, and it is among the better-established mechanistic accounts underlying any liquid biopsy modality. The conclusion that fragmentomic measurements carry interpretable biological information is well supported.

The clinical evidence is far more restricted than that mechanistic security might suggest, and its distribution is uneven in ways that bear directly on the review question. The strongest clinical demonstrations lie in obstetrics, where fragment size functions reliably as a quantitative parameter for estimating and enriching the fetal contribution to maternal plasma, and where coverage-based features have discriminated placental phenotypes in cohorts numbering in the hundreds. In every prenatal application that yields a molecular diagnosis of a single-gene disorder, the diagnostic determination is made by sequence analysis and fragmentomic information enters only in support. Describing these achievements as fragmentomic diagnosis conflates a supporting parameter with the basis of a diagnostic conclusion.

For postnatal paediatric rare and inherited metabolic disease, the position must be stated plainly. No study identified in this review evaluated fragmentomic profiling for the primary diagnosis of such a condition in a child. The accessible paediatric evidence concerns graft injury monitoring after transplantation, in cohorts of eleven and of a few hundred, and tissue injury characterisation through methylation rather than fragmentation. The proposition that fragmentomics constitutes a diagnostic frontier in paediatric genetics is therefore best characterised as unevaluated rather than as either established or refuted, and the distinction between an untested hypothesis and a supported one has not been consistently observed in this literature.

Three obstacles are decisive rather than incidental. Paediatric normative reference data are absent, so deviation cannot be quantified against a defined baseline. The features that would be most diagnostically useful are simultaneously the most sensitive to confounding by cfDNA concentration, hepatic clearance, germline variation at nuclease loci, and circadian and demographic factors, each of which produces effects of a magnitude comparable to the differences a diagnostic classifier would seek to detect. Most decisively, tissues affected in many rare congenital disorders contribute little cell-free DNA to plasma, and no improvement in measurement can recover a signal that is not present in the analyte.

Two applications are nonetheless defensible on current evidence and merit development. Reverse inference in monogenic disorders of the fragmentation machinery itself has the shortest causal chain from genotype to measurable phenotype and is supported by convergent evidence from murine ablation, human case reports, and observational association in healthy cohorts. Fragmentomic refinement of graft injury monitoring addresses a documented performance limitation, namely the low specificity of quantitative donor-derived cell-free DNA measurement, in a setting where serial sampling is routine and cohorts are attainable. Both are narrower than the ambitions frequently expressed for the field, and both are correspondingly more likely to yield results that survive independent evaluation.

The broader significance of this synthesis lies in the distinction it draws between a measurement framework and a diagnostic technology. Fragmentomics is a well-founded way of extracting information from plasma DNA, and its foundations will support clinical application where the biology permits. Whether the biology permits application in paediatric rare disease is an empirical question that the field has largely asserted rather than investigated. Establishing normative data, standardising pre-analytical practice, and determining which disorders are candidates on analyte grounds are unglamorous prerequisites, but no diagnostic claim in this domain can be evaluated until they are met.

10. Limitations

This review is a narrative synthesis and carries the interpretive limitations intrinsic to that form. Studies were selected purposively rather than through a systematic protocol with predefined screening and extraction procedures, and the emphasis placed on particular studies reflects the judgement of the synthesis rather than a reproducible algorithm. Another reviewer applying different selection judgements could reasonably assemble a somewhat different evidence base and reach different emphases, particularly regarding the weight to be placed on mechanistic as against clinical evidence. The critical conclusions drawn should be understood as arguments open to challenge rather than as determinations.

Access to the literature was incomplete. Subscription-restricted indexes including Web of Science Core Collection, Scopus, and Embase were unavailable, and searching relied on Europe PMC together with Crossref Metadata Search and targeted retrieval. Publications indexed only in the unavailable resources, and those in journals not covered by the accessible indexes, may have been missed. Restriction to English-language publications is a further source of incompleteness that is likely to be consequential for this topic, since substantial prenatal cfDNA research is conducted in settings where publication occurs in other languages. Where full text was inaccessible, appraisal relied on the abstract and on publisher metadata, which permits verification of principal findings but not detailed evaluation of methods.

The evidence base itself is limited in ways that constrain the conclusions. Study designs are heterogeneous in population, comparator, outcome definition, sequencing platform, and analytical pipeline, so quantitative synthesis was not possible and comparisons between studies are qualitative. Several key paediatric studies involve very small cohorts without independent validation, and the resulting performance estimates are imprecise to a degree that individual figures should not be relied upon. Much of the relevant clinical evidence is observational and retrospective, so associations reported cannot be interpreted causally. Geographical concentration is pronounced, with the mechanistic and prenatal fragmentomic literature dominated by a small number of research groups working in a limited range of settings, which constrains generalisability to populations differing in ancestry and in clinical practice.

Publication bias is likely to operate in a specific direction here. Studies reporting successful discrimination by fragmentomic features are more likely to be published than those finding no discrimination, and the resulting literature will overstate the consistency with which such features distinguish clinical groups. Given the small cohorts characteristic of paediatric rare disease research, the effect of this bias is amplified rather than diminished. The absence of published negative findings in paediatric fragmentomics should not be read as an absence of unsuccessful attempts.

Comparability among studies is further limited by inconsistent reporting of pre-analytical handling and sequencing depth, both of which materially affect fragmentomic measurements and neither of which is uniformly specified. Long-term evidence is essentially absent, since no reviewed study reported longitudinal fragmentomic follow-up of children with a rare congenital or metabolic disorder over a period sufficient to assess stability or clinical correlation. The field is developing rapidly, and publications appearing after the final search date may alter aspects of the synthesis, particularly regarding long-read applications and combined fragmentomic and methylomic analysis, which were areas of active development at the time of searching.

Consent and 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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