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Original Article

Clinical Outcomes and Counseling Impact of Exome Sequencing in a Low-Risk Prenatal Cohort

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Abstract

Background: Prenatal exome sequencing (pES) is recommended mainly for fetuses with major or multiple structural anomalies when conventional karyotyping and chromosomal microarray are non-diagnostic. Outside these indications, its clinical value is uncertain.

Methods: We retrospectively reviewed 10 fetuses tested with pES at our center (2016–2025) despite lacking guideline-based indications. Indications included advanced maternal age (n=1), sex-chromosome anomalies (n=5), parental gonadal mosaicism risk (n=2), and isolated soft ultrasonographic marker (n=2). All cases had karyotype and/or chromosomal microarray (CMA) before pES.

Results: One fetus carried a pathogenic *SETD5* (NM_001080517.3) c.1541del, p.(Lys514Argfs*2) variant, along with 45,X/46,XY mosaicism and transient ascites/pleural effusion. The *SETD5* variant was considered incidental in the prenatal context, though it influenced management and led to medical termination. A second fetus had a maternally inherited heterozygous NSD1 (NM_022455.4) c.2410C>T, p.(Pro804Ser) variant of uncertain significance supporting pregnancy continuation, but the pregnancy was terminated in an external center. One other fetus was lost to follow-up; all other live-born infants appeared normal on postnatal examination.

Conclusion: In this small "out-of-indication" series, pES was management-changing in one pregnancy and created uncertainty in another, which complicated the family's decision-making process. The latter finding aligns with current recommendations discouraging pES in low-risk antenatal settings due to its limited yield. Nonetheless, in select situations involving unresolved diagnostic ambiguity, pES may still uncover variants with potential relevance to counseling and management.

Keywords: Exome sequencing, Chromosome Aberrations, Genetic Counseling, Genomics

INTRODUCTION

Prenatal genetic diagnosis has evolved from cytogenetic karyotyping to chromosomal microarray analysis (CMA) and, more recently, to genome-scale sequencing capable of detecting single-nucleotide and small indel variants. These technological advances have markedly improved diagnostic rates for fetuses with structural anomalies and have become integral to prenatal counseling and pregnancy management (1). Following a normal CMA, whole-exome sequencing (WES) provides an additional diagnostic yield of approximately 10–30% in fetuses with structural anomalies, with the highest rates observed in fetuses with skeletal dysplasia findings, while the yield falls below 2% in those with isolated increased nuchal translucency (2-4). Exome sequencing, despite its diagnostic power in anomaly-selected fetal cases, provides minimal (0.6-2.7%) incremental yield in fetuses without malformations (5-7). This contrast underpins current recommendations for selective use with major guidelines uniformly emphasizing that prenatal exome sequencing (pES) should be reserved for fetuses with one or more major structural anomalies that remain unexplained after standard genetic analyses (8-10). Moreover, pathogenic variants identified with antenatal

exome analysis are not always clinically actionable in the prenatal period, as many associated phenotypes display variable expressivity, reduced penetrance or age-related penetrance (11).

Despite this, clinicians encounter requests for prenatal exome sequencing (pES) outside anomaly-based selection. A common scenario is pES driven by parental anxiety after invasive testing performed for reasons other than fetal structural anomalies, such as advanced maternal age, increased risk in aneuploidy screening, or isolated soft markers. Another occurs when sex-chromosome abnormalities or CNVs of uncertain significance are detected, prompting pES to exclude additional monogenic findings that could influence counseling and decision-making. In these settings, decisions must balance the modest potential for meaningful findings against the risks of uncertainty, anxiety, and unnecessary follow-up. Thorough pre- and post-test counseling and clear reporting criteria help mitigate these challenges.

This study describes a single-center retrospective series of 10 fetuses that underwent pES without anomaly-based indications. We report indications, molecular results and fetal/postnatal outcomes, and outline how individual results influenced counseling and management. The goal is to provide pragmatic information for clinicians who face similar requests in low-risk settings and to illustrate when pES added value, when it did not, and how uncertainty was handled. Although our data come from the prenatal setting, the clinical questions it raises about the balance of diagnostic yield, psychological impact, and downstream decision-making resonate across many clinical application domains of genomic testing ranging from internal medicine and oncology to reproductive genetics. This is a systemic challenge that the wider medical community is beginning to face: how to integrate powerful genomic tools into routine practice without overextending their use, and how to support patients when the answers provided are uncertain or incidenta.

METHODS

Study Design and Setting

We conducted a retrospective review of all prenatal exome sequencing (pES) cases performed between January 2016 and June 2025 at the Medical Genetics Department of our tertiary referral center. Written informed consent for testing and secondary analysis of anonymized data was obtained from all couples before invasive sampling. Clinical and follow-up data were retrieved from institutional medical records. This study was approved by the Institutional Review Board of Koç University (IRB No: 2025.462.IRB2.215).

Inclusion and Exclusion Criteria

The inclusion criterion was performance of pES outside guideline-based indications. Cases tested under standard indications, including fetal structural or multisystem malformations, amniotic fluid or placental abnormalities, and growth or fluid regulation anomalies were excluded. Cases with a fetal chromosomal anomaly that explained the observed fetal findings were also included.

Cohort Description

The final cohort comprised ten fetuses: one with advanced maternal age, five with numerical sex-chromosome abnormalities, two with parental gonadal mosaicism risk due to a previously affected child, and two with isolated soft ultrasound markers (nasal bone hypoplasia). The decision to perform pES was made upon the family's request for comprehensive testing once fetal material was already available and after detailed pre-test counseling regarding the expected yield and interpretive limitations.

Sample Collection and Laboratory Methods

Samples were obtained by amniocentesis or chorionic villus sampling (CVS) using standard procedures. All cases underwent karyotype and/or chromosomal microarray (CMA) prior to sequencing. Exome sequencing was performed in accredited diagnostic laboratories using validated capture platforms and next-generation sequencing pipelines. All exome analyses achieved a mean coverage of at least >94% at ≥20x depth and >97% at ≥10x depth. Reads were aligned to the human reference genome (GRCh37 or GRCh38), and variants were annotated using population databases (gnomAD, 1000 Genomes) and disease databases (ClinVar, OMIM, HGMD). Variants were classified according to ACMG/AMP 2015 guidelines as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, or benign (12). Parental confirmation was performed with Sanger sequencing in peripheral blood DNA to determine inheritance and phase.

Follow-up and Outcomes

One family was lost to follow-up in the prenatal period due to transfer of care. In the rest, pregnancy outcomes were obtained from obstetric and pediatric records. For live births, postnatal evaluations were made or reviewed for growth, structural anomalies, or developmental concerns. For one terminated pregnancy, clinical and radiological postmortem examinations were performed.

STATISTICAL ANALYSIS

Given the descriptive and retrospective nature of the study, no formal statistical analyses were performed. Data were compiled and analyzed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) for tabulation and summarization. Because of the small cohort size (n=10) and the absence of a control group, no inferential or quantitative statistics were applied.

RESULTS

Molecular Findings

Of the ten fetuses, one carried a pathogenic *SETD5* (NM_001080517.3): c.1541del, p.(Lys514Argfs*2) variant and one had a variant of uncertain significance in *NSD1* (NM_022455.4): c.2410C>T, p.(Pro804Ser). The remaining eight showed no reportable sequence variants (**Table 1**).

Cytogenetic Findings

Among the ten cases, five fetuses had sex-chromosome aneuploidies: one with 47,XXX; one with 47,XXY (Klinefelter syndrome); one with 47,XYY; one with 45,X/46,XX mosaic Turner syndrome; and one with 45,X/46,XY mosaicism consistent with mixed gonadal dysgenesis. In four of these cases, the indication for invasive testing was an increased risk for sex-chromosome aneuploidy detected on noninvasive prenatal testing (NIPT). Only in the fetus with mixed gonadal dysgenesis, the invasive procedure was performed due to the presence of ambiguous genitalia with ascites and pericardial effusion, that were detected to be transient on follow-up.

Chromosomal Microarray (CMA) Results

All chromosomal microarray (CMA) results were consistent with the karyotype findings, except in Case 8, where CMA revealed two small deletions of uncertain significance: one on chromosome 2q13, encompassing the *NPHP1* gene, and another on 16p12.2, partially overlapping the *OTOA* gene. Both were considered carrier states for the respective autosomal recessive conditions; the *NPHP1*-related ciliopathies (MIM# 609583, #256100, #266900) and *OTOA*-related deafness (MIM#607039). In this case, pES was additionally performed to rule out possible inherited or de novo pathogenic variants that could result in disease expression when in trans.

Case 8: SETD5 Variant and Phenotypic Correlation

Case 8 with ambiguous genitalia exhibited 45,X/46,XY mosaicism and transient ascites and pleural effusion that resolved by the late second trimester. While hydropic findings in 45,X/46,XY mosaicism are recognized but uncommon, exome sequencing was pursued to explore whether the transient hydrops phenotype could be better explained by a single-gene disorder (13). Exome analysis and familial studies revealed a de novo heterozygous frameshift *SETD5* variant, classified as likely pathogenic and compatible with a molecular diagnosis of autosomal dominant Intellectual Developmental Disorder 23 (MRD23, MIM# 615761) (14).

Antenatal phenotypes related to *SETD5* pathogenic variants are not well characterized; however, increased nuchal translucency has been reported in one fetus (15). While *SETD5* could have theoretically contributed to the fetal findings, the absence of a well-described prenatal phenotype made a causal link uncertain, and the variant was therefore considered incidental in this context. The *SETD5* finding greatly influenced counseling: as *SETD5* pathogenic variants are associated with intellectual disability and behavioral anomalies, the family opted for medical termination at 24 weeks following detailed counselling.

Case 3: NSD1 Variant

Case 3 with a 47,XYY karyotype underwent pES and was found to carry a missense *NSD1* variant initially classified as a VUS. Segregation analysis demonstrated maternal inheritance from a clinically unaffected mother, supporting reclassification to likely benign. Although the family was informed that the prenatal phenotype was not expected to be associated with neurodevelopmental impairment, they opted for termination of the pregnancy at an external center.

Summary of Remaining Cases and Outcomes

No pathogenic or likely pathogenic variants were identified in the remaining eight fetuses through pES. Among all pregnancies, one was lost to follow-up, while the remaining seven resulted in live births. All infants were clinically normal on neonatal and early postnatal examinations, with no structural malformations or growth abnormalities.

DISCUSSION

This retrospective series illustrates the complex realities of performing pES outside established indications, and both the potential value and pitfalls of such testing. The small size of this cohort reflects the rarity of out-of-indication prenatal exome sequencing in routine clinical practice. Over nearly a decade, fewer than ten such cases were encountered at our tertiary center, underscoring both the limited demand and the cautious clinical attitude toward testing beyond guideline recommendations. Even small, carefully documented series like this can contribute valuable real-world data for future reviews addressing the clinical utility and ethical implications of pES in low-risk settings.

Case 8 with the *SETD5* variant demonstrates that actionable, or at least management-influencing findings can occasionally arise in low-yield settings and help families make decisions. Conversely, Case 3 with 47,XYY and the *NSD1* variant differs in that it represents emotional challenges and confusion for the family and misguided parental decisions. The family with the *SETD5*-positive fetus initially planned to continue the pregnancy, as the detected 45,X/46,XY mosaicism and genital findings were not considered absolute indications for termination. Their decision changed after learning that *SETD5*-related disorders are associated with intellectual disability and behavioral impairment. Conversely, the family of the fetus with 47,XYY remained undecided about termination until the *NSD1*

Table 1. Clinical and laboratory findings of fetuses with low-risk indications undergoing pES.

	GA (weeks) / Consanguinity	Indication for invasive procedure	Cytogenetic / Array findings (GRCh37)	Fetal USG findings & WES results	Outcome
1	16 / No	Advanced maternal age (41)	46,XY .arr(1–22)x2,(XY)x1	Normal; No variant detected	Healthy newborn
2	16 / No	Increased risk for sex-chromosome anomaly on NIPT	47,XXX .arr(X)x3	Normal; No variant detected	Healthy newborn
3	19 / No	Increased risk for sex-chromosome anomaly on NIPT	47,XYY .arr(X)x1,(Y)x2	Normal; Maternal het VUS in *NSD1* (NM_022455.4): c.2410C>T p.(Pro804Ser)	ToP
4	19 / No	Increased risk for sex-chromosome anomaly on NIPT	47,XXY .arr(X)x1,(Y)x2	Normal; No variant detected	Lost to follow-up
5	17 / No	Increased risk for sex-chromosome anomaly on NIPT	mos 45,X/46,XX [21/39] .arr(X) x1[0.3]	Normal; No variant detected	Healthy newborn
6	23 / No	Ambiguous genitalia, ascites, and pericardial effusion	mos 45,X/46,XY [17/3] .arr(X) x1,(Y)x0[0.6]	Transient ascites and effusion; De novo het LP variant in *SETD5* (NM_001080517.3): c.1541del p.(Lys514Argfs*2)	ToP
7	23 / No	Soft ultrasound marker on anomaly screening	46,XY .arr(1–22)x2,(XY)x1	Normal; No variant detected	Healthy newborn
8	22 / No	Isolated small nasal bone	46,XX .arr 2q13(110874327_111388468)x1; 16p12.2(21405328_21737414)	Small NB; No variant detected	Healthy newborn
9	21 / –	1% gonadal mosaicism risk for *COMP* (NM_000095.2): c.1417_1419del, p.(Asp473del)	46,XY .arr(1–22)x2,(XY)x1	Normal; No variant detected	Healthy newborn
10	13 / No	1% gonadal mosaicism risk for *IQSEC2* (NM_001111125.2): c.2983C>T, p.(Arg995Trp)	46,XX .arr(1–22,X)x2	Normal; No variant detected	Healthy newborn

GA, gestational age (weeks); NIPT, noninvasive prenatal testing; USG, ultrasonography; arr, chromosomal microarray result; mos, mosaic; het, heterozygous; LP, likely pathogenic; VUS, variant of uncertain significance; ToP, termination of pregnancy; NB, nasal bone

variant of uncertain significance was reported. Although segregation analysis confirmed maternal inheritance and the variant was interpreted as likely benign, and the family was counseled in detail that the prenatal phenotype was not expected to be associated with severe outcomes, the additional information increased parental anxiety and contributed to the decision to terminate the pregnancy (Table 1).

These examples show that pES outside standard indications can alter management even when variants are incidental or uncertain, sometimes by shifting parental perception of risk. At the same time, they highlight the potential for harm through anxiety, overinterpretation, and decisional pressure, which are amplified when testing is not phenotype-driven.

Our diagnostic yield (1/10) is higher than expected for such unselected cases, but this reflects the small sample size rather than a trend. Larger meta-analyses report <2% incremental yield when pES is applied in low-risk or structurally normal fetuses. Thus, the key message is not the yield itself but the interpretive and counseling demands that follow. Every pathogenic variant or VUS requires thoughtful multidisciplinary discussion and careful communication with the family. The medical implications for the family must be addressed during pre-test counseling, particularly when residual uncertainty is likely to persist even after testing.

Lastly, although the *SETD5*-positive fetus also had mixed gonadal dysgenesis, the detailed postmortem examination provided valuable clinical information that may contribute to understanding the prenatal phenotype associated with *SETD5*-related disorders. Findings such as clinodactyly and distal phalangeal hypoplasia of the second and fifth fingers, in addition to mild craniofacial and genital anomalies, enrich the limited phenotypic data available for *SETD5* in the antenatal period. These observations, though confounded by the coexisting karyotypic abnormality, highlight the importance of thorough postmortem assessment in expanding knowledge of prenatal genotype-phenotype correlations. Future multicenter studies with standardized inclusion criteria, systematic follow up, and consideration of cost-effectivity issues are needed to better define the clinical value of pES in low-risk pregnancies or fetal cases where chromosomal anomalies already explain the phenotype. These studies may ultimately guide the balanced integration of "out-of-indication" pES into clinical practice.

This study is limited by its retrospective design and relatively small sample size, which may restrict the generalizability of the findings. Additionally, deep phenotyping information was incomplete except for one case, and long-term postnatal outcomes were not systematically assessed.

CONCLUSION

In this small "out-of-indication" cohort, pES provided additional information in only one pregnancy but created diagnostic uncertainty in another, ultimately influencing

both families' decisions. In the first family, sequencing clarified prognosis and guided decision-making, whereas in the second, it complicated the process. This shows that pES remains a powerful tool capable of generating management-influencing data, but its use outside guideline-based indications should remain selective and purpose-driven. Careful counseling before and after testing, transparent communication of uncertainty, and multidisciplinary interpretation are essential to balance potential benefits against emotional and ethical costs. Lastly, our findings contribute valuable postmortem data to the limited body of knowledge on prenatal SETD5-related phenotypes, revealing features such as clinodactyly and distal phalangeal hypoplasia of the second and fifth fingers. These observations may help refine future understanding of early *SETD5*-associated manifestations. .

DECLARATIONS

Ethics Committee Approval: This study was approved by the Institutional Review Board of Koç University (IRB No: 2025.462.IRB2.215). All procedures were conducted in accordance with the principles of the Declaration of Helsinki.

Data Availability: The datasets generated and analyzed during the current study are available from the corresponding author, upon reasonable request.

Competing Interests: The authors declare that they have no competing interests.

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