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BRIEF REPORT

Prenatal identification of a pathogenic maternal *FGFR1* variant in two consecutive pregnancies with fetal forebrain malformations

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ABSTRACT

Objective: Holoprosencephaly (HPE) is the most common aberration of forebrain development, and it leads to a wide spectrum of developmental and craniofacial anomalies. HPE etiology is highly heterogeneous and includes both chromosomal abnormalities and single-gene defects.

Methods: Here, we report an *FGFR1* heterozygous variant detected by prenatal exome sequencing and inherited from the asymptomatic mother, in association with recurrent neurological abnormalities in the HPE spectrum in two consecutive pregnancies.

Results: Individuals with germline pathogenic variants in *FGFR1* (MIM: 136350) show extensive phenotypic variability, which ranges from asymptomatic carriers to hypogonadotropic hypogonadism, arhinencephaly, Kallmann's syndrome with associated features such as cleft lip and palate, skeletal anomalies, isolated HPE, and Hartsfield syndrome.

Conclusion: The presented case supports the role of exome sequencing in prenatal diagnosis when fetal midline structural anomalies are suggestive of a genetic etiology, as early as the first trimester of gestation. The profound heterogeneity of *FGFR1* allelic disorders needs to be considered when planning prenatal screening even in asymptomatic carriers.

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Introduction

Holoprosencephaly (HPE) is the most frequent aberration of forebrain development and it results from impaired midline cleavage of the embryonic prosencephalon [1]. Distinct anatomical subtypes are classically outlined in decreasing order of severity: alobar, semilobar, lobar, and middle interhemispheric fusion variants [2,3]. HPE clinical spectrum ranges from severe developmental and physical impairments to clinically unaffected carriers and usually reflects the severity of the radiological phenotype [4]. Milder midline brain defects, such as agenesis of the corpus callosum, arhinencephaly, and absent septum pellucidum, are variably related but not specific to HPE [5]. Craniofacial anomalies are present in most cases and include synophthalmia, proboscis, or cleft lip-palate, as well as traits such as hypotelorism, and single maxillary central incisor [6].

HPE is determined by both genetic and environmental (e.g. teratogenic) factors. Most HPE cases present a chromosomal abnormality; trisomy 13 is the most common cause of HPE [7]. Approximately, 25% of HPE cases are ascribable to single-gene defects, and distinct syndromic and nonsyndromic forms with both autosomal dominant (AD) and autosomal recessive (AR) transmission have been described. In some individuals, the genetic cause remains unknown [2].

HPE can be screened during pregnancy by fetal ultrasound (US) as early as the first trimester of gestation [8]. Fetal magnetic resonance imaging (MRI) is commonly performed to confirm the US findings and

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explore possible additional abnormalities [9]. However, prenatal counseling is often challenging due to the extreme phenotypic variability associated with HPE, particularly in nonsyndromic inherited forms that can differ greatly even among members of the same family [10]. The presented case demonstrates the recurrence of forebrain malformations in the HPE spectrum, in association with a maternally inherited heterozygous pathogenic variant in *FGFR1* (fibroblast growth factor (FGF) receptor 1, MIM: 136350) detected by prenatal exome sequencing.

Case presentation

A 34-year-old woman (gravida 3 para 1) with no personal or family medical history presented a recurrence of fetal midline brain defects in two consecutive pregnancies.

The first gestation resulted in the full-term birth of a healthy child after an uneventful pregnancy. The second gestation (Table 1a, case I) was terminated at 21 gestational weeks (GWs) due to the US appearance of unilateral cleft lip and palate, absent cavum septum pellucidum, and intrauterine growth restriction (IUGR). After unremarkable cytogenetic analysis, exome sequencing was performed on genomic DNA extracted from cultured amniocytes and parental peripheral blood samples using the NovaSeq6000 platform. The analysis detected a heterozygous *FGFR1* variant (NM_023110.3:c.296A>G p.(Tyr99Cys)) of maternal origin, which was classified as pathogenic according to the American College of Medical Genetics and Genomics standards and guidelines (Table S1) [11]. Upon physical examination, the proposita presented no clinical signs of HPE and her sex-hormonal profile was in range; brain MRI was not performed. The firstborn of the proposita and her healthy brother tested negative on subsequent Sanger-sequencing-based segregation analysis; the parents of the proposita were not available for genetic testing.

As to the third pregnancy (Table 1a, case II), the first trimester fetal US was unremarkable, and the fetal karyotype was normal. Targeted testing for the familial variant in *FGFR1* through Sanger sequencing on cultured amniocytes showed that the fetus carried the maternal variant (Table 1b, case II). US follow-up at 20w5d GA documented absent cavum septum pellucidum and hypoplastic corpus callosum (Figure 1(a,b)).

Table 1a. Clinical data of the second (I) and third (II) pregnancies of the proposita, both of which were terminated due to adverse fetal outcomes.

Case	Parental details			Gestation at diagnosis	Phenotypes (HPO terms)	Obstetric history	Family history	Outcome
I	Maternal	Age	33	Pregnancy previously terminated at 21w0d	Unilateral cleft lip-palate, absent septum pellucidum, intrauterine growth restriction.	Gravida 2, para 1.	Unremarkable	Pregnancy termination
		Medical history	Unremarkable					
	Paternal	Age	37					
		Medical history	Unremarkable					
II	Maternal	Age	34	19w0d	Hypoplasia of the corpus callosum, absent cavum septum pellucidum, lobar holoprosencephaly.	Gravida 3, para 1.	Unremarkable	Pregnancy termination
		Medical history	Unremarkable					
	Paternal	Age	38					
		Medical history	Unremarkable					

Table 1b. Genetic findings on amniotic fluid sampling in both pregnancies of the proposita.

Case	Procedure (gest. age)	Sample	Performed test	Result	Variant	ACMG classification	Inheritance and zygosity	Interpretation
I	Amniocentesis (20w0d)	Cultured amniocytes	Karyotype	46,XX	–	–	–	–
	Amniocentesis (as above)	Cultured amniocytes	CMA	arr(X-22)x2	–	–	–	–
	Amniocentesis (as above)	Cultured amniocytes and parentals' lymphocytes	Trio exome sequencing	*FGFR1* (ENST00000447712.7)	NM_023110.3: c.296A>G p.(Tyr99Cys)	Pathogenic	Maternal, heterozygous	Causative of fetal phenotype
II	Amniocentesis (16w0d)	Cultured amniocytes	Karyotype	46,XX	–	–	–	–
	Amniocentesis (as above)	Cultured amniocytes	*FGFR1* exon 3 Sanger sequencing	*FGFR1* (ENST00000447712.7)	NM_023110.3: c.296A>G p.(Tyr99Cys)	Pathogenic	Maternal, heterozygous	Causative of fetal phenotype

Abbreviations: CMA, chromosomal microarray; HPO, Ontology of Human Phenotype; ACMG, American College of Medical Genetics [11].

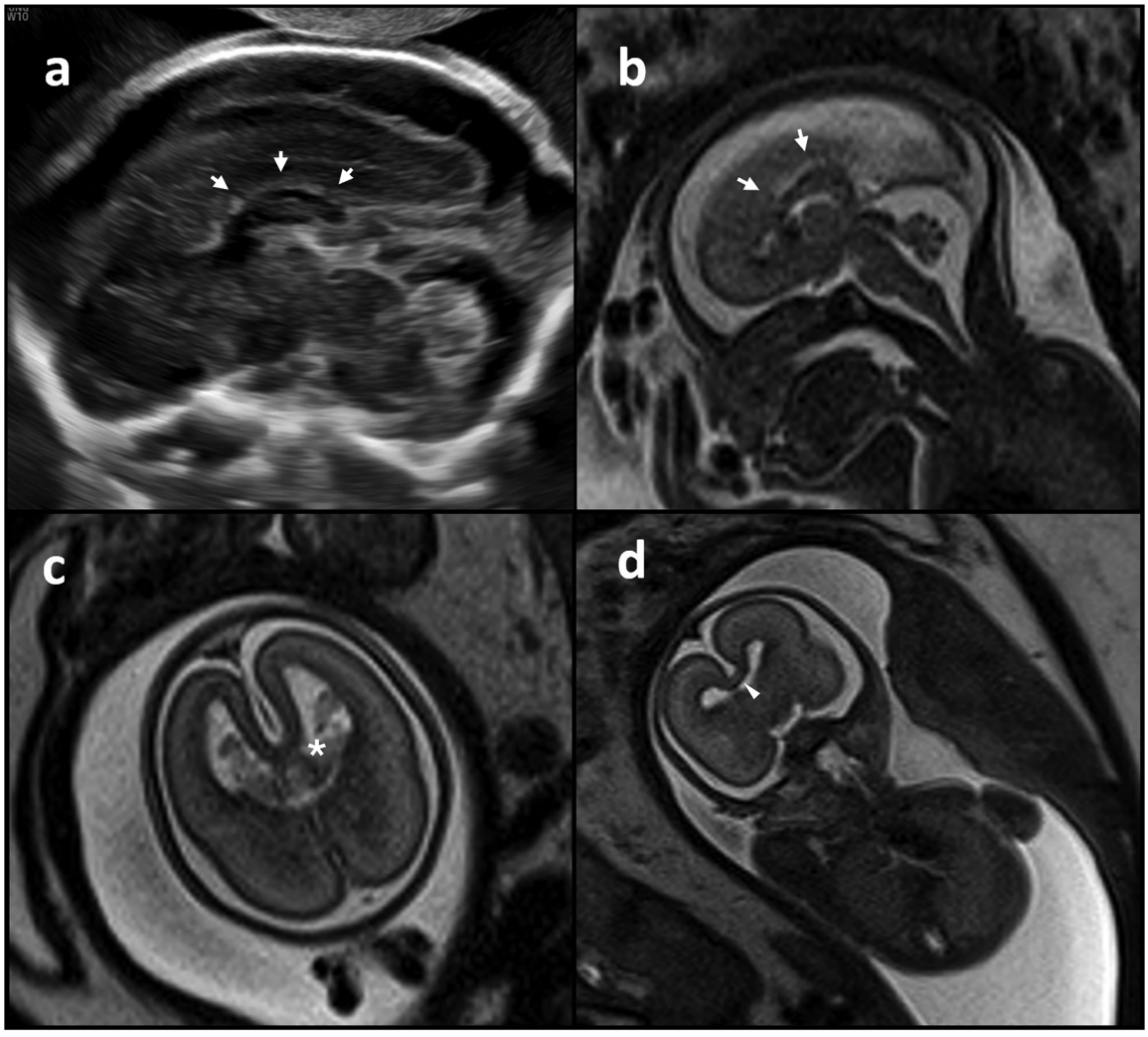

Figure 1. Fetal neuroimaging findings. Ultrasound (a) and magnetic resonance imaging (b) of the sagittal view showing the hypoplasia of the corpus callosum (white arrows). Magnetic resonance imaging of the transverse (c) and coronal (d) view showing partial separation of the frontal lobe (white asterisk), incomplete development of the corpus callosum and the presence of the sagittal scissure (white arrowhead), suggestive of lobar holoprosencephaly.

A subsequent fetal MRI confirmed the previous US findings and identified lobar HPE; craniofacial anomalies were absent and intrauterine growth was within range (Figure 1(c,d)). Following these results and a counseling session, the couple opted to terminate the pregnancy at 21 GWs. Products of conception in both pregnancies were not available for examination.

Discussion

HPE is a highly heterogeneous disorder, both phenotypically and etiologically. Several distinct genetic entities in which HPE is an occasional or an isolated finding have been described; most of these disorders are rare [12]. Among them, *FGFR1* is emerging as a major genetic driver in the development of HPE [13]. *FGFR1* encodes a receptor tyrosine kinase for FGFs with a pivotal role in the axial organization and mesoderm patterning during embryogenesis.

The presented case demonstrates the recurrence of fetal forebrain malformations in the HPE spectrum in association with an *FGFR1* defect inherited from an asymptomatic parent, thus supporting the highly variable involvement of the FGF signaling pathway in HPE etiopathogenesis. The p.(Tyr99Cys) variant has been previously reported in unrelated individuals diagnosed with Kallmann's syndrome (KS) or hypogonadotropic hypogonadism (HH) [14,15].

Available data do not allow for any genotype–phenotype correlations in *FGFR1* allelic disorders, whose clinical picture can range from asymptomatic carriers to HH, arhinencephaly, KS or craniosynostosis [16]. Additionally, loss-of-function *FGFR1* variants have been described in patients diagnosed with isolated HPE as

well as with Hartsfield syndrome (HS; MIM: 300571), which is characterized by the association of HPE and ectrodactyly with recurring additional features [13,17].

Chromosomal analysis is the first-line genetic testing in all individuals with HPE. In clinical suspicion of aneuploidy, karyotype analysis should be performed, otherwise, a chromosomal microarray (CMA) should be considered beforehand [4]. Exome sequencing has reported a diagnostic yield of about 20% in patients with both syndromic and nonsyndromic HPE who have previously undergone inconclusive genetic investigations, and it should be additionally contemplated [18].

If HPE is radiologically diagnosed prenatally, genetic testing may be performed during pregnancy to identify associated chromosomal abnormalities or genomic disorders. Recently, a prenatal diagnosis of HS based on the US findings of semilobar HPE, bilateral upper and lower extremity ectrodactyly, and bilateral cleft lip and palate has been reported. Exome sequencing on the fetal sample identified a de novo *FGFR1* pathogenic variant [19]. Our report provides another striking example of the role of exome sequencing strategies for prenatal causal diagnosis in fetuses with otherwise unexplained isolated or complex anomalies in the spectrum of HPE [20]. In the case of nondiagnostic karyotype or microarray, exome sequencing may indeed prove to be a cost-effective tool, with benefits in terms of costs, diagnostic efficiency and pregnancy outcomes [21].

In conclusion, to the best of our knowledge, this is the first described case of recurrent forebrain anomalies in the HPE spectrum due to an *FGFR1* defect, prenatally diagnosed by exome sequencing. Determining a specific genetic cause of fetal HPE can help discuss prenatal and perinatal management, and parental decision making, likely resulting in fewer stillbirths, neonatal deaths, and affected infants. Nevertheless, the profound inter- and intra-family heterogeneity of *FGFR1* allelic disorders should be considered while providing preconception and prenatal genetic counseling, even in asymptomatic carriers. Systematic studies on larger cohorts are needed to establish a possible genotype–phenotype correlation, and thus define tailored diagnostic approaches.

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Author contributions

Conceptualization, L.G. and S.N.; methodology, L.G.; software, L.M.; validation, A.N. and G.N.; formal analysis, E.P.; investigation, S.N. and L.B.S.; resources, G.N.; data curation, A.M.N.; writing – original draft preparation, L.G. and M.L.C.; writing – review and editing, I.M. and M.R.D.; visualization, A.M.N.; supervision, A.N. and I.M.; project administration, G.N.

Ethics statement

Ethical approval was not required for the studies involving humans because the submitted report is derived from a hospital case of a patient with evidence of fetal anomalies during her pregnancy, which was addressed to our institution by the attending physician. Therefore, Ethical Committee approval was unnecessary, since no supplementary analysis was performed on the patient, except for the diagnostic genetic test for developmental defects. The internal Ethical Committee approves entire research projects and not reports based on single cases. Ethical approval was not required for this study by local/national guidelines.

Consent form

The study was conducted according to the guidelines of the Declaration of Helsinki. We obtained written consent from the patient beforehand, as required by our regulations. The human samples used in this study were acquired from a by-product of routine care or industry.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The datasets supporting the conclusions of this article are included within the article (and its additional files).

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