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## Short Communication



## Revisiting *GDF9* variants in primary ovarian insufficiency: A shift from dominant to recessive pathogenicity?

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## ABSTRACT

**Background:** Primary ovarian insufficiency (POI) affects around 2–4% of women before the age of 40. Genetic factors play an important role in POI. The *GDF9* gene has been identified as a significant genetic contributor of POI. However, the pathogenicity and penetrance of *GDF9* variants remain uncertain.

**Methods:** A next-generation sequencing approach was employed to investigate the entire coding region of the *GDF9* gene in a cohort of 1281 patients with POI or diminished ovarian reserve (DOR). The frequency of each identified *GDF9* variant was then compared with that of the general population, taking into account the ethnicity of each individual.

**Results:** By screening the entire coding region of the *GDF9* gene, we identified 19 different variants, including 1 pathogenic frameshift variant. In total, 36 patients with POI/DOR (2.8%) carried at least one *GDF9* variant. With regard to missense variants, no significant overrepresentation of the most common variants was observed in our POI/DOR cohort in comparison to the general or specific ethnic subgroups. Only one homozygous subject had a frameshift loss of function variant.

**Conclusion:** This epidemiological study suggests that the vast majority of heterozygous missense variants could be considered as variants of uncertain significance and the homozygous loss-of-function variant could be considered as a pathogenic variant. The identification of a novel case of a homozygous POI patient with a heterozygous mother carrying the same variant with normal ovarian function strongly suggests that *GDF9* syndrome is an autosomal recessive disorder.

**Abbreviations:** ACMG, American College of Medical Genetics and Genomics; DNA, Deoxyribonucleic acid; DOR, Diminished ovarian reserve; GATK, Genome analysis toolkit; *GDF9*, Growth Differentiation Factor 9; gnomAD, Genome Aggregation Database; FMR1, Fragile X Messenger Ribonucleoprotein 1; FSH, Follicle stimulating hormone; PCR, Polymerase chain reaction; POI, Primary ovarian insufficiency; VUS, Variants of unknown significance.

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## 1. Introduction

Premature ovarian insufficiency (POI) occurs in 2–4 % of women. Various factors can lead to the ovarian follicular depletion or dysfunction, including iatrogenic factors, environmental factors, autoimmune diseases, and genetic defects (abnormal karyotype, X-fragile and *FMR1* premutation). Considering that 10–30 % of POI cases have familial inheritance and/or genetic causes (Qin et al., 2015), more than one hundred of genes have been implicated in the aetiology of POI. These genes are involved in various key biological processes in the ovary, such as meiosis, and DNA damage repair, homologous recombination, follicle development, granulosa cell differentiation and proliferation, and ovulation. Although there is no specific consensus or international recommendations, next-generation sequencing using panel-specific genes, whole exome sequencing or whole genome sequencing is revolutionising the field of POI and identifying a high number of genetic variants. These approaches can be very highly beneficial in elucidating the aetiology of POI. However, a significant proportion of variants are often rare and their role in the pathogenesis is uncertain, and are therefore classified as variants of unknown significance (VUS). Furthermore, incomplete penetrance and variable clinical expressivity limit their clinical interest and utility in genetic counselling.

Among the genes involved in ovarian development and folliculogenesis, Growth Differentiation Factor 9 (*GDF9*) was one of the first to be described in human POI (Takebayashi et al., 2000). Between 2005 and 2007, five independent studies identified heterozygous *GDF9* variants in different populations of women with POI (Dixit et al., 2005; Laissue et al., 2006; Chand et al., 2006; Kovanci et al., 2007; Zhao et al., 2007). The pathogenicity of these variants was suggested by their frequency in the population database, familial segregation (or autosomal dominant inheritance from the father), and effect according to in silico analysis. Moreover, a few functional studies have been performed showing that transfection of several *GDF9* missense mutants reduces or abolishes the production of mature proteins and reduces the biological activities of conditioned media from human embryonic kidney 293F cells compared to the corresponding wild-type *GDF9* (Inagaki and Shimasaki, 2010; Wang et al., 2013; Simpson et al., 2014).

However, the pathogenesis of several *GDF9* variants and the context of autosomal dominant inheritance are questionable for at least four independent reasons: 1- some variants do not appear to be very rare, with a prevalence of 1 %-4% in certain ethnic groups; 2- *GDF9* missense and frameshift variants have been identified in twin mothers (Palmer et al., 2006) and in polycystic ovarian syndrome in the absence of POI (Takebayashi et al., 2000); 3- a homozygous loss-of-function variant (c.783del; p.Ser262HisfsTer2) was identified in a 19-year-old woman with POI (França et al., 2018), while her sister and her mother carrying the variant were unaffected, suggesting an autosomal recessive inheritance; and 4- mice carrying a null *Gdf9* mutation had POI, while the heterozygous female did not show an abnormal phenotype (Carabatsos et al., 1998).

All these recent data raised questions about the pathogenicity of *GDF9* variants in the context of autosomal dominant inheritance. To analyse the pathogenicity of the different variants according to specific ethnic origins, we compared the prevalence of *GDF9* variants identified in our large cohort of 1281 unrelated patients with POI/DOR from different ethnic-geographical origins with the frequency reported in the general population in the gnomAD database.

## 2. Materials & methods

### 2.1. Patients

We recruited 1281 unrelated women under 40 years of age over a 3-year period (January 2020 to July 2023), 704 with idiopathic, sporadic or familial POI, primary or secondary amenorrhoea, elevated FSH (>25 UI/L) on two measurements, and 577 with DOR (diminished ovarian

reserve) defined by a slightly elevated FSH level and a reduced number of ovarian follicles. We excluded from the statistical analysis 65 POI patients with a known aetiology of POI (such as medical treatments (radiotherapy, chemotherapy, pelvic surgery), autoimmune diseases (autoimmune thyroiditis, systemic lupus erythematosus), genetic pathogenic variants, primary adrenal insufficiency, and Perrault syndrome). The women were divided into four groups according to their ethnic origin: European (n = 739), North African (n = 160), Sub-Saharan African (n = 306) and Asian (n = 11). To compare the frequencies of the variants, we used the public gnomADv3 and gnomADv4 databases (<https://gnomad.broadinstitute.org/>)(v2.1.2). Our work complies with the principles laid down in the Declaration of Helsinki. All POI/DOR patients gave informed written consent to participate and signed an informed consent form approved by the local ethics committee.

### 2.2. Next generation Sequencing, mutation validation and in silico analysis

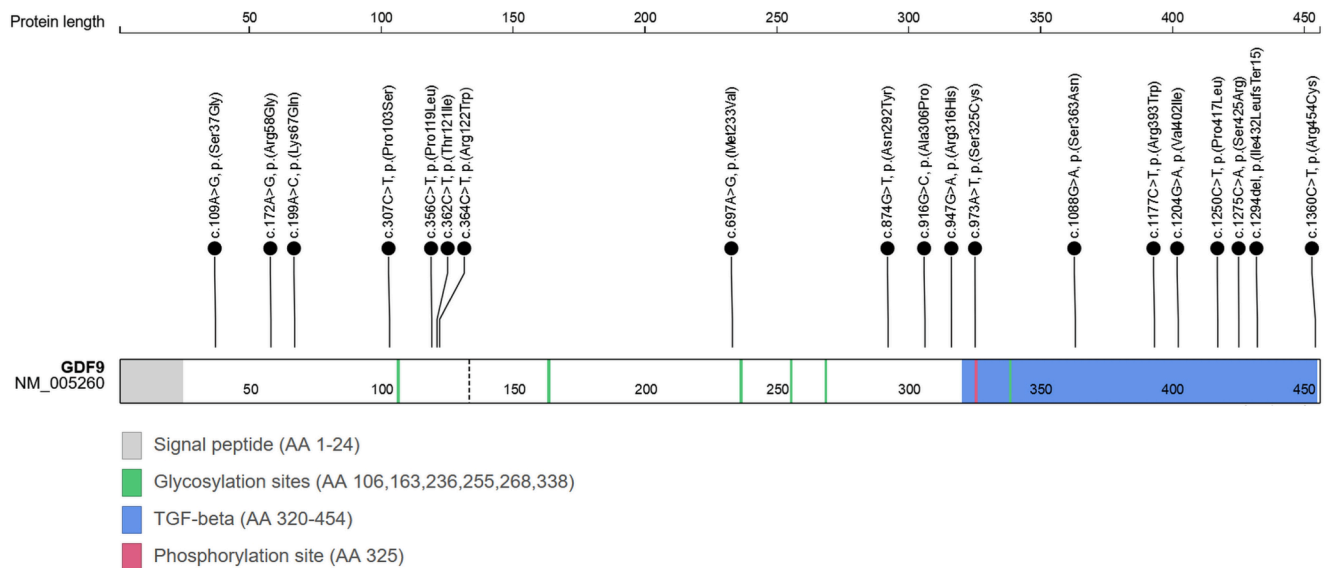
Genomic DNA was extracted from peripheral blood samples of women with POI/DOR and purified using the Chemagic 360 Nucleic Acid Extractor (Perkin Elmer) and the Wizard Genomic DNA Promega Kit (Promega Corporation). A custom target capture array, previously described (Jordan et al., 2024), was designed by Roche NimbleGen to cover seven selected known causative genes, including *GDF9* (NM\_005260.7) (Qin et al., 2015; Luo et al., 2023). Briefly, 1 µg of genomic DNA was fragmented using an enzymatic approach. The sample library was prepared using a KAPA Library Preparation Kit (KAPA, KR0935). Target regions were captured using the SeqCap EZ library Kit (Roche NimbleGen), followed by multiplex sequencing on the Illumina MiSeq System at an average coverage of 200x according to the manufacturer's recommendations. Following the filtration of substandard reads, the human genome reference (NCBI build37/hg19 version) was utilized in mapping the remaining reads through the Burrows-Wheeler Aligner (BWA). The genome analysis toolkit (GATK) and Polyweb (<http://www.polyweb.fr/>) were applied for variant identification and filtration as previously described (<https://www.broadinstitute.org/gatk/guide/topic?name=bestpractices>) (Jordan et al., 2024). All calls with a read coverage ≤ 5×, a Phred scaled SNP quality of ≤ 20 and a MAF > 3 % were filtered out. Variants were annotated using an in-house Paris Descartes bioinformatics platform pipeline based on the Ensembl database (release 67). Variant classification followed the latest ACMG guidelines. All rare genetic variants were verified by Sanger sequencing on an ABI3500 xL DX DNA analyser according to the manufacturer's instructions (Applied Biosystems, Life Technologies). Primer sequences and positions, PCR conditions and product sizes are available on request. The pathogenic effects of each variant were assessed using various bioinformatics tools (CADD (combined annotation dependent depletion); Alamut v.2.4 (Interactive Biosoftware) (<https://www.interactive-biosoftware.com/alamut>)).

### 2.3. Statistical analysis

Statistical analyses were performed using SAS software (SAS institute, Inc.n Cary, NC) and the R environment (<https://www.R-project.org/>). Fisher's exact test was used to assess the differences between the cohort and the general population and to examine the estimated odds ratio. Data are presented as a percentage or mean and SD for quantitative variables. P < 0.05 was considered as statistically significant.

## 3. Result

Screening of the entire coding region of the *GDF9* gene allowed us to identify 19 different variants, 1 frameshift variant, and 18 different missense variants (Fig. 1; Table 1). Eleven variants were located in the propeptide region (amino acids 25–319) and 8 in the mature chain (aa



**Fig. 1.** Location of the different *GDF9* variants identified in the cohort of women with POI/DOR (n = 1281).

320–454). One variant p.(Ser325Cys) was located in the phosphorylation site of the protein.

Only one of these variants was not previously reported (gnomADv4; Table 1). In total, 36 patients with POI/DOR (2.8 %) carried out at least one *GDF9* variant. Thirty-five patients carried out a heterozygous variant and one individual was homozygous for a frameshift variant. None of these patients had another pathogenic variant in the other genes tested.

Although several variants (e.g. p.(Lys67Gln), p.(Pro103Ser), p.(Ser425Arg), and p.(Arg454Cys)), identified in our study, have shown different degrees of in vitro functional impairment in vitro, including defects in transcriptional activity and biological activities (Inagaki and Shimasaki, 2010; Wang et al., 2013), the pathogenicity of these variants remains unclear. Using data from our POI cohort, we compared the frequency of the *GDF9* variants in our population of POI/DOR patients (n = 1216) and in the general population. When we compared the frequency of the variants in our POI/DOR population with the frequency in the general population considering only women (Table 1), no significant differences were observed for 11 rare variants (Fig. 2; for 14 variants using gnomADv3, Fig.S1). Overall, only eight variants were found to be significantly overrepresented in POI/DOR (Fig. 2). The odds ratio (OR) of the variant carriers ranged from 14.85 to infinity (Fig. 2). The clinical characteristics of POI patients carrying these variants are shown in Table 2.

It is worth noting that several *GDF9* variants have been identified globally, though with a somewhat uneven geographical and ethnic distribution. It would appear that the variants p.(Arg58Gly), p.(Pro119Leu), p.(Met233Val), p.(Val402Ile), and p.(Pro417Leu) were most frequently reported in African/African Americans, whereas the variant p.(Lys67Gln) was predominantly reported in subjects of South Asian origin (Table 1). It would also appear that p.(Ser425Arg) is more commonly found in subjects of East Asian origin, while p.(Pro103Ser), p.(Thr121Ile), p.(Asn292Tyr), p.(Ser363Asn), p.(Arg393Trp), and c.1294del are more frequently observed in subjects of European origin (Table 1). Given these differences in distribution, we decided to perform an analysis of *GDF9* variants with ethnic subgroups. When looking at the ethnic subgroups, no significant difference was observed for ten variants, which are mainly observed in certain ethnic populations (Fig.S2). However, six variants, including p.(Ala306Pro), p.(Ser325Cys), p.(Arg393Trp) and c.1294del remain overrepresented in POI/DOR when considering the general ethnic population (Fig.S1). In addition, bioinformatic prediction by both SIFT, Polyphen-2, CADD and REVEL

suggested no pathogenic effect for four variants, but a pathogenic effect for four variants (Table 1). Overall, two of these 18 missense variants (p.(Thr121Ile) and p.(Arg454Cys)) were considered likely to be benign, three were considered likely to be pathogenic (p.(Ala306Pro), p.(Ser325Cys) and p.(Arg393Trp)) and the others were considered variants of uncertain significance (Table 1).

In contrast to the most previously described heterozygous missense variants, we report a homozygous 1-bp deletion (c.1294del) in the *GDF9* gene in a French patient with primary-secondary amenorrhoea. At first presentation, a 23-year-old woman was characterised by elevated FSH (51 UI/L) and LH (luteinising hormone) (18 UI/L). The patient's height was 155 cm. Breast development and pubic hair were Tanner 5 and 5 (S5P5). The karyotype was 46,XX, determined by the analysis of at least 50 metaphases. IVF by oocyte donation was performed and she was pregnant but miscarried. She has one brother and 3 asymptomatic sisters. One of the 3 sisters has an asymptomatic child. The variant c.1294del followed the guideline of ACMG guideline to be considered pathogenic: it was very rare in all international databases; it was a null variant in a gene where loss-of-function is a known mechanism of disease. Both parents were probably heterozygous and the patient's mother went through the menopause at the age of 53.

#### 4. Discussion

*GDF9* variants are often described as one of the top 20 common genetic explanations for POI (Jiao et al., 2021; Luo et al., 2023). However, frameshift, nonsense and splice site variants are very rare in the general population and in POI cohorts. To date, only one frameshift variant and one large duplication variant have been identified in women with POI (Norling et al., 2014; França et al., 2018). In this study, we identified 1 rare loss-of-function variant (Table 1). However, the vast majority of *GDF9* variants were missense variants for which pathogenicity remains elusive, especially as few missense variants are commonly observed in the general population. To help classify these variants, we analysed the OR values according to their molecular consequences and to their ethnic frequency.

In our large cohort of POI/DOR, we identified 18 different missense variants, and found no significant overrepresentation of 11 variants in our 1216 POI/DOR patients compared to the general population using data from the gnomADv4 database. Overall, we observed a significant overrepresentation of only 7 missense variants in our POI/DOR cohort. The OR of these variant carriers varied between 14.85 and infinity

**Table 1**  
*GDF9* variants identified in the cohort of 1281 individuals. Results of pathogenicity prediction using different software (CADD, SIFT, Polyphen-2, Revel) are given for each variant. The results of the frequency in the gnomADv3 and V4 databases are given. Classification of variants according to the ACMG rules is shown. The position of each variant in the subunits of the preproprotein is indicated: the peptide signal (amino acids (aa) 1–24); the prodomain (aa 25–319); the mature region (aa 320–454). Nd: not identified; \*, phosphorylation site.

Sequence variation	Amino acid variation	ACMG criteria v3	ACMG classification v3	ACMG criteria v4	ACMG classification v4	Number of cases	gnomAD v3 (women)	Asian	European	African	Latino/Admixed American	gnomAD v4 (women)	Asian	European	African	Latino/Admixed American	CADD phred	SIFT	Polyphen-2	Revel
c.109A > G	p.(Ser37Gly)	PM2, BP4	Variant of Unknown Significance	PM2, BP4	Variant of Unknown Significance	1	5/77828	2/3662	1/39354	nd	2/6788	47/812320	5/23186	31/611572	nd	11/32462	4.25	Tolerated	Benign	Benign
c.172A > G	p.(Arg58Gly)	PM2, BP4	Variant of Unknown Significance	PS4, PM2, BP4	Variant of Unknown Significance	2	10/77854	nd	nd	6/22146	4/6792	29/812480	nd	nd	20/41488	5/32486	8.30	Tolerated	Benign	Benign
c.199A > C	p.(Lys67Gln)	PM2	Variant of Unknown Significance	BS2, PM2	Variant of Unknown Significance	1	0/77820	nd	nd	nd	nd	39/812456	36/23192	nd	nd	nd	23.00	Damaging	Possibly damaging	Uncertain
c.307C > T	p.(Pro103Ser)	PM1, PP3, BS1	Variant of Unknown Significance	PM1, PP3, BS1, BS2	Variant of Unknown Significance	8	170/77842	nd	122/39356	6/22152	5/8492	2612/812450	2/23194	2115/611614	17/41494	22/32478	25.60	Tolerated	Probably damaging	Uncertain
c.356C > T	p.(Pro119Leu)	PM1, PM2, BS1	Variant of Unknown Significance	PS4, PM1, PM2 BS1	Variant of Unknown Significance	2	28/77860	nd	nd	28/22148	nd	45/812504	nd	1/611658	42/41474	nd	26.60	Damaging	Probably damaging	Uncertain
c.362C > T	p.(Thr121Ile)	BS1, BP4	Likely benign	BS1, BS2, BP4	Likely benign	1	39/77850	nd	20/39356	3/19304	nd	284/812478	nd	258/611632	7/41490	4/32486	15.71	Tolerated	Benign	Benign
c.364C > T	p.(Arg122Trp)	PM2, BP4	Variant of Unknown Significance	PS4, PM2, BP4	Variant of Unknown Significance	1	5/77826	nd	1/39352	4/22138	nd	14/812462	nd	7/611638	6/41464	nd	23.90	Damaging	Possibly damaging	Benign
c.697A > G	p.(Met233Val)	PM2, BP4	Variant of Unknown Significance	PM2, BP4	Variant of Unknown Significance	1	16/77864	nd	nd	16/22158	nd	31/812122	nd	nd	27/41498	2/32488	8.21	Tolerated	Benign	Uncertain
c.874G > T	p.(Asn292Tyr)	PM2, BP4	Variant of Unknown Significance	PS4, PM2, BP4	Variant of Unknown Significance	1	1/77844	nd	1/39364	nd	nd	11/812426	nd	11/611606	nd	nd	16.47	Damaging	Possibly damaging	Uncertain
c.916G > C	p.(Ala306Pro)	PM1, PM2, BP4	Variant of Unknown Significance	PS4, PM1, PM2, BP4	Likely pathogenic	1	1/77850	nd	nd	nd	1/6792	9/812336	nd	3/611508	nd	1/32482	3.17	Tolerated	Benign	Benign
c.947G > A	p.(Arg316His)	PM2, PP3	Variant of Unknown Significance	PM2, PP3	Variant of Unknown Significance	1	1/77848	nd	1/39364	nd	nd	36/812286	14/23204	18/611468	nd	nd	23.40	Damaging	Probably damaging	Uncertain
c.973A > T	p.(Ser325Cys)	PM1, PM2, PP3	Variant of Unknown Significance	PS4, PM1, PM2, PP3	Likely pathogenic	1	0/77842	nd	nd	nd	nd	0/809738	nd	nd	nd	nd	13.91	Damaging	Probably damaging	Uncertain
c.1088G > A	p.(Ser363Asn)	PM1, PP3	Variant of Unknown Significance	PM1, PP3, BS2	Variant of Unknown Significance	1	23/77864	nd	19/39364	nd	1/6790	388/812320	14/23194	338/611482	1/41492	6/32486	22.50	Damaging	Probably damaging	Damaging
c.1177C > T	p.(Arg393Trp)	PM1, PM2, PP3	Variant of Unknown Significance	PS4, PM1, PM2, PP3	Likely pathogenic	1	0/77830	nd	nd	nd	nd	7/810254	nd	6/609652	nd	1/32468	26.10	Damaging	Probably damaging	Damaging
c.1204G > A	p.(Val402Ile)	PM1, PP3, BS1	Variant of Unknown Significance	PM1, PP3, BS1	Variant of Unknown Significance	1	103/77832	nd	1/39352	98/22138	2/6792	218/811394	nd	1/610656	200/41460	8/32486	24.30	Damaging	Probably damaging	Uncertain
c.1250C > T	p.(Pro417Leu)	PM1, PP3, BS1	Variant of Unknown Significance	PM1, PP3, BS1, BS2	Variant of Unknown Significance	1	164/77840	nd	1/39354	157/22150	4/6788	382/811626	4/23172	30/610874	325/41466	8/32482	27.90	Damaging	Probably damaging	Damaging
c.1275C > A	p.(Ser425Arg)	PM1, PM2, BS1	Variant of Unknown Significance	PM1, PM2, BS2	Variant of Unknown Significance	1	11/77842	7/2274	nd	4/22156	nd	66/809738	46/22960	1/609152	11/41418	nd	23.60	Tolerated	Possibly damaging	Damaging
c.1294del	p.(Ile432LeufsTer15)	PVS1, PS4, PM2	Likely pathogenic	PVS1, PS4, PM2	Likely pathogenic	1	0/77850	nd	nd	nd	nd	7/810576	nd	6/609896	nd	1/32480	28.00			
c.1360C > T	p.(Arg454Cys)	PP3, BS1, BP6	Likely benign	PP3, BS1, BS2, BP6	Likely benign	9	242/77824	3/1166	166/39352	14/22142	46/6784	2974/812008	65/23136	2533/611258	30/41478	126/32480	32.00	Damaging	Probably damaging	Damaging

4

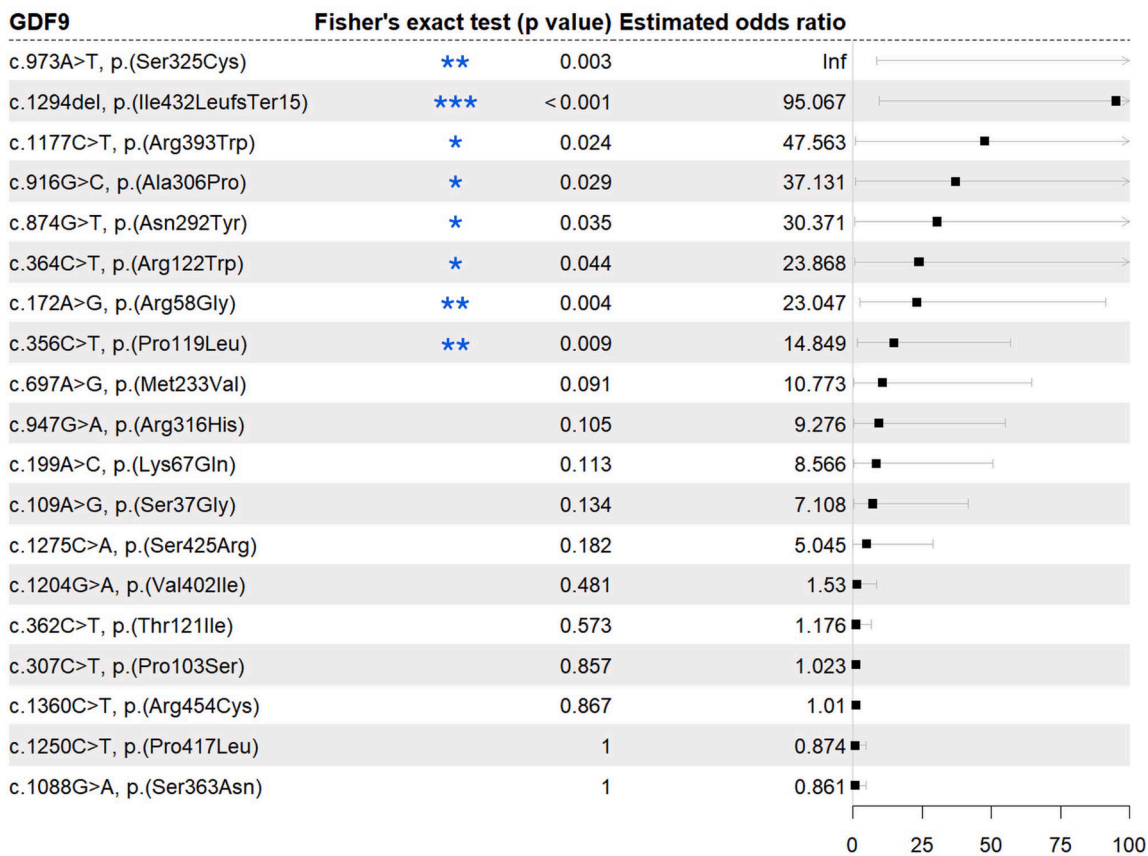


Fig. 2. Forest plot showing the odds ratios for the risk of POI/DOR in women with POI/DOR associated with *GDF9* variants (POI/DOR cohort versus controls from the gnomADv4 general population controls). Note: Confidence interval (min = minimum, max = maximum) is shown.

Table 2

Clinical characteristics of POI/DOR patients with potentially pathogenic *GDF9* variants. Nd = not determined. G/P (Gravida Para Scoring, G: pregnancy; P: live birth).

Patient ID	18ME00603	2121ME000053	2120ME000141	2120ME000224
Variant	c.973A > T	c.1294del	c.1177C > T	c.916G > C
Aminoacid substitution	p.(Ser325Cys)	p.(Ile432Leufs15)	p.(Arg393Trp)	p.(Ala306Pro)
POI/DOR	POI	POI	POI	POI
Amenorrhea (P/S)	S	Primary-secondary	S	S
Ethnic origin	South-American (Peru)	European (Portugal)	African (Ivory Coast)	European
Current age (year)	37	41	34	45
Age of first menstruation (years)	15	12	14	13
Age of amenorrhea (years)	34	12	32	30
No of pregnancies (G/P)	G0P0	G1P0 (egg donation)	G3P2	G3P3
FSH (IU/L)	70	51	137	25
LH (IU/L)	35	18	75.5	9
Estradiol (pg/ml)	57	nd	41	51
AMH (ng/ml)	0.02	nd	0.014	nd
BMI (kg/m <sup>2</sup> )	24.5	nd	30	nd
Height (cm)	155	nd	158	nd
Syndromic features	none	none	none	none
Associate phenotype	none	none	none	none

(Fig. 2). Furthermore, when we considered the ethnic subgroups, we found no significant difference for two of these variants (p.(Arg58Gly); p.(Pro119Leu)), suggesting that there are ethnic-specific variants (Fig. S2). In addition, bioinformatic predictions suggested that only one overrepresented missense variant (p.Arg393Trp) was likely to be deleterious. Overall, our epidemiological study using the ACMG classification showed that of the 18 rare *GDF9* missense variants identified, two are likely to be benign, three are likely to be pathogenic, and the others were considered variants of uncertain significance.

Until recently, the vast majority of human *GDF9* variants described so far in different POI cohorts were heterozygous missense variants. A

mutational screening of *GDF9* in 127 patients with POI revealed two rare heterozygous missense variants in six patients with secondary amenorrhea of the cohort (Dixit et al., 2005). In 2006, Laissue and colleagues identified the heterozygous p.(Ser186Tyr) variant in a patient with secondary amenorrhoea from a European cohort of 203 women with POI (Laissue et al., 2006). The *GDF9* variant was also found as a rare explanation for POI in the US cohort. In 61 subjects, most of them Caucasian, only one patient carried a heterozygous missense variant (p.(Pro103Ser)) (Kovanci et al., 2007). All these previous data suggest that POI due to *GDF9* variant is a dominant autosomal disorder.

In contrast to most previously described heterozygous missense

mutations, we report a homozygous 1-bp deletion (c.1294del) in the *GDF9* gene in a French patient with primary-secondary amenorrhoea. This was a null variant in a gene where loss-of-function is a known disease mechanism. According to the Prosite\_SIB protein domain database, the TGF- $\beta$  domain region in *GDF9* is located between 316 and 454 amino acids. In addition, variants in the mature region could affect the normal growth and maturation of ovarian cells. Our variant (p.Ile432-LeufsTer15) could result in a truncated protein lacking the end of the TGF- $\beta$  domain (aa 432–454), leading to a loss-of-function of the *GDF9* protein. Although maternal DNA was not available, our next-generation sequencing analysis excluded the presence of a deletion affecting one or more exons of the *GDF9* gene, suggesting that the variant identified in the POI patient is a homozygous deletion of A at position c.1294. As the patient's mother carrying the variant was unaffected (menopause at the age of 53), our report supports the recent conclusion by Franca and colleagues that *GDF9* is involved in an autosomal recessive disorder (França et al., 2018). These conclusions are supported by *Gdf9*-null mouse models. *Gdf9*-null female mice are infertile due to blockage at the primary follicle stage, whereas heterozygous *Gdf9*<sup>+/-</sup> females are fertile (Carabatsos et al., 1998).

However, it should be noted that our study has several limitations. Firstly, there is significant phenotypic heterogeneity within the group of women with fertility disorders (POI/DOR). It is possible that some of these women may have had at least one child before POI or after POI, with or without the use of assisted reproduction. Secondly, the gnomADv4 database brings together data from over 807,162 individuals through a worldwide collaborative data sharing effort. The majority of the sequence data were generated for case-control studies of common adult diseases and may include women with known or unknown POI (~2% in the 35–40-year age group), who are considered to be healthy. Finally, it is possible that another pathogenic variant exists in different genes, which would suggest that POI has a polygenic or oligogenic origin (Shekari et al., 2023). Whole genome sequencing should be considered in the future management of these patients, especially in familial cases of POI or in the presence of a heterozygous pathogenic *GDF9* variant.

Despite these limitations and unresolved issues, our analysis identified one rare loss-of-function variant, three likely pathogenic missense variants (including one novel one), two likely benign missense variants and 13 missense variants of uncertain significance. Our report strongly supports the notion that *GDF9* syndrome is an autosomal recessive disorder. However, we cannot exclude that biallelic variants show more severe phenotype than those with heterozygous variants, and that the heterozygous variants are causally associated with pathogenic variants in other genes. Although *GDF9* variants explain less than 0.1 % of POI/DOR, these data support routine genetic screening of patients in clinical practice.

#### CRedit authorship contribution statement

**Pénélope Jordan:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Camille Verebi:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Data curation. **Bérénice Hervé:** . **Sandrine Perol:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation. **Valérie Bernard:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation. **Daphné Karila:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation. **Eva Jali:** Resources, Methodology, Investigation, Formal analysis, Data curation. **Aude Brac de la Perrière:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation. **Virginie Grouthier:** Resources, Methodology, Formal analysis, Data curation. **Sophie Jonard-Catteau:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Data curation, Conceptualization. **Philippe Touraine:** Writing – review & editing, Resources, Investigation, Formal analysis. **Corinne Fouveau:** Methodology,

Formal analysis, Data curation. **Geneviève Plu-Bureau:** Resources, Methodology, Formal analysis, Data curation. **Jean Michel Dupont:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anne Bachelot:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation. **Sophie Christin-Maitre:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Data curation, Conceptualization. **Thierry Bienvenu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bienvenu reports was provided by Public Assistance Hospitals Paris. Bienvenu reports a relationship with Public Assistance Hospitals Paris that includes: employment and non-financial support. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

The data that support the findings of the study are available from the corresponding author and the first author upon reasonable request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gene.2024.148734>.

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