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# Whole exome sequencing in a cohort of familial premature ovarian insufficiency cases reveals a broad array of pathogenic or likely pathogenic variants in 50% of families

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**Objective:** To study the diagnostic yield, including variants in genes yet to be incriminated, of whole exome sequencing (WES) in familial cases of premature ovarian insufficiency (POI).

**Design:** Cross-sectional study.

**Setting:** Endocrinology and reproductive medicine teaching hospital departments.

**Patients:** Familial POI cases were recruited as part of a nationwide multicentric cohort. A total of 36 index cases in 36 different families were studied. Fifty-two relatives were available, including 25 with POI and 27 affected who were nonaffected. Karyotype analysis, *FMR1* screening, single nucleotide polymorphism array analysis, and WES were performed in all subjects.

**Interventions:** None.

**Main Outcome Measures:** The primary outcome was a molecular etiology, as diagnosed by karyotype, *FMR1* screening, single nucleotide polymorphism array, and WES.

**Results:** A likely molecular etiology (pathogenic or likely pathogenic variant) was identified in 18 of 36 index cases (50% diagnostic yield). In 12 families, we found a pathogenic or likely pathogenic variant in a gene previously incriminated in POI, and in 6 families, we found a pathogenic or likely pathogenic variant in new candidate genes. Most of the variants identified were located in genes involved in cell division and meiosis ( $n = 11$ ) or DNA repair ( $n = 4$ ).

**Conclusions:** The genetic etiologic diagnosis in POI allows for genetic familial counseling, anticipated pregnancy planning, and ovarian tissue preservation or oocyte preservation. Identifying new genes may lead to future development of therapeutics in reproduction based on disrupted molecular pathways.

**Clinical Trial Registration Number:** NCT 01177891. (Fertil Steril® 2021; ■:■-■. ©2021 by American Society for Reproductive Medicine.)

**Key Words:** Genes in POI, hypergonadotropic hypogonadism, premature ovarian insufficiency, primary amenorrhea, whole exome sequencing



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**P**remature ovarian insufficiency or primary ovarian insufficiency (POI) affects 1%–4% of women before the age of 40. The diagnosis is based on the presence of menstrual disturbances, such as oligomenorrhea or amenorrhea, for at least 4 months and a follicle-stimulating hormone (FSH) level greater than 25 IU/L on 2 occasions more than 4 weeks apart (1). In most cases, POI is related to follicular and oocyte depletion (2). The causes of POI include a past history of chemotherapy and/or radiotherapy, autoimmune diseases, and genetic diseases. The most common genetic causes are chromosomal abnormalities, such as Turner syndrome, with a total or partial loss of an X chromosome, terminal deletions of the long arm of the X chromosome, and/or X, autosomal translocations. Premutations of *FMR1* genes are present in 2%–3% of sporadic cases of POI (3). Familial cases are reported in 10%–20% of cases (4). Few studies have reported whole exome sequencing (WES) in cohorts of patients with POI to identify new genes (5–8). More than 70 different candidate genes have been described in POI (5, 9). However around 70% of POI cases remain idiopathic. We therefore conducted a study in a large cohort of familial cases of POI patients, using WES.

## MATERIALS AND METHODS

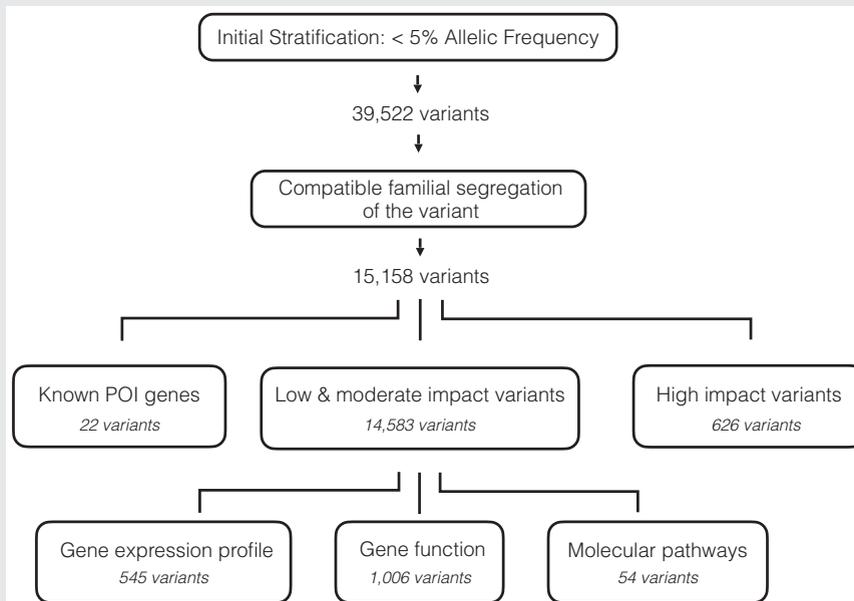
Cases of nonsyndromic familial POI, with at least one other POI case in a first-degree relative, were included in the course of 3 years in a multicentric nationwide cohort called Familial Cases of Premature Ovarian Failure (IRB number NCT 01177891). Sixteen French centers recruited familial cases of POI.

All probands were evaluated clinically by endocrinologists and/or gynecologists. Patients with clinical signs supporting Turner syndrome, blepharophimosis, past surgery of the ovaries, or previous chemotherapy or pelvic radiotherapy were excluded from the study. Patients were also excluded if they had a past history of autoimmune diseases, such as thyroid disease, type 1 diabetes, celiac disease, or systemic lupus erythematosus. The clinical data collected were age at menarche, history of menses, previous pregnancies, and age at diagnosis of POI. Biologic data included FSH and estradiol levels.

The study was approved by the ethics committee. All patients and their relatives provided informed consent before enrollment. Authorization was obtained by the National Commission for Data Protection and Liberties (Commission Nationale de l'Informatique et des Libertés or CNIL) to collect genealogic trees from all families (AR 111839).

Peripheral blood samples were collected from probands and their relatives for karyotype analysis, *FMR1* screening (mutations and mutations/premutations), single nucleotide polymorphism (SNP) array analysis (Illumina, San Diego, CA, USA), and WES. Karyotypes were performed in the Medical Genetics Department at Trousseau Hospital according to conventional techniques with G and R banding. Genomic DNA extraction from venous blood was performed by proteinase K digestion and phenol-chloroform-based methods according to the manufacturer's instructions (Illumina). Assessment for *FMR1* premutation and SNP was also performed in the Medical Genetics Department at Trousseau Hospital.

WES was performed in all probands as well as their affected and nonaffected relatives. Library preparation, exome capture, sequencing, and data analysis were conducted by IntegraGen SA (Evry, France). Genomic DNA was captured by the Agilent in-solution enrichment method (SureSelect XT Clinical Research Exome; Agilent, Santa Clara, CA, USA) with their biotinylated oligonucleotides probes library (SureSelect XT Clinical Research Exome - 54 Mb), followed by paired-end 75 bases massively parallel sequencing on Illumina HiSeq4000. For detailed explanations of the process, see also Gnirke et al. (1). Sequence capture, enrichment, and elution were performed according to the manufacturer's instructions and protocols (SureSelect) without any modification except for library preparation performed with NEBNext Ultra kit (New England Biolabs, Ipswich, MA, USA). For library preparation, 600 ng of each genomic DNA was fragmented by sonication and purified to yield fragments of 150–200 bp. Paired-end adaptor oligonucleotides from the NEBNext Ultra kit were ligated and repaired, and then purified and enriched by 8 polymerase chain reaction (PCR) cycles. Twelve hundred nanograms of these purified libraries were then hybridized to the SureSelect oligo probe capture library

**FIGURE 1**

Variant filtering and annotation. Variants were first filtered based on their allelic frequency (<5%) and their familial segregation, leading to 15,158 variants. Variants in known premature ovarian insufficiency genes (22 variants) and high-impact variants (626 variants) were manually curated. Low- and moderate-impact variants were further filtered, based on the genes' expression profiles (545 variants), physiologic functions (1,006 variants), and molecular pathways shared with already incriminated premature ovarian insufficiency genes (54 variants). POI = premature ovarian insufficiency.

Rouen. Exome sequencing in familial POI cases. *Fertil Steril* 2021.

for 72 hours. After hybridization, washing, and elution, the eluted fraction was PCR-amplified with 9 cycles, purified, and quantified by quantitative PCR to obtain sufficient template DNA for downstream applications. Each eluted-enriched DNA sample was then sequenced on an Illumina HiSeq4000 as paired-end 75-b reads. Image analysis and base calling were performed with Illumina Real-Time Analysis (2.7.6) with default parameters (10).

Base calling was performed with the use of the Real-Time Analysis software sequence pipeline (2.7.7) with default parameters. Sequence reads were mapped to the human genome build (hg38/GRCh38.p7) by the Burrows-Wheeler Aligner tool. The duplicated reads (e.g., paired-end reads in which the insert DNA molecule showed identical start and end locations in the human genome) were removed using Sambamba tools (11). Variant calling, allowing the identification of genetic alterations as well as single nucleotide variations and small insertions or deletions (up to 20 bp), was performed with the Broad Institute GATK Haplotype Caller GVCF tool (3.7) for constitutional DNA.

Identification of loss of heterozygosity regions and evaluation of consanguinity were performed on data from SNP arrays as well as WES. To investigate B-allele frequency, we used the Bioconductor DNACopy package (DNACopy 1.32.0) with mutated allele frequencies of SNVs known in the 1000 Genomes Project. This implements the circular binary segmentation algorithm to segment B-allele frequency data.

For variant calling (Fig. 1), we retained variants with an allelic frequency <5% in ExAC and gnomAD. We excluded variants with incompatible familial segregation from this pool and then excluded variants that were synonymous or noncoding. High-impact variants (frameshift and truncating, splice variants,  $n = 626$ ) were manually annotated, as well as variants in genes previously incriminated in POI in humans. Moderate variants (missense, protein altering, inframe insertions and deletions) as well as low-impact variants (splice region variants, incomplete terminal codon, stop retained variants) were further filtered ( $n = 14,583$ ). From this moderate- and low-impact group, we selected variants ( $n = 545$ ) in genes that had an expression pattern compatible with the reproductive phenotype (ovary or pituitary gland). Second, we selected variants ( $n = 1,006$ ) in genes that had a function, based on Online Mendelian Inheritance in Man searches, that might suggest an involvement in POI (keywords: "fertility," "gametogenesis," "oogenesis," "meiosis," "DNA repair," "synaptonemal complex," "reproduction," and "crossover"). Finally, we selected variants ( $n = 54$ ) in genes involved in the same molecular pathways as genes already involved in POI. Two operators manually annotated the selected variants. Variants were assessed at the cohort level, and their pathogenicity was evaluated based on the type of variant and its effects on the protein, its conservation through evolution, its prevalence in existing databases (ExAC, gnomAD, 1000 Genomes), and its score on functional prediction tools (SIFT,

PolyPhen2, MutationTaster). The variants were scored according to the 2015 American College of Medical Genetics criteria (12). In addition to “pathogenic” and “likely pathogenic,” we identified 106 variants of unknown significance that were annotated on the basis of the same criteria.

## RESULTS

A total of 36 index patients (and related individuals) were studied. All had a familial history of POI, with at least one first-degree relative affected. In these families, apart from the proband, 25 relatives with POI and 27 nonaffected relatives were available to be sequenced. In 10 families, the father of the proband was included to evaluate the mode of transmission of potential pathogenic variants.

All the index patients were of Caucasian ethnicity. Among them, 10 of 36 had primary amenorrhea. Among the patients with secondary amenorrhea (26 of 36), the mean age at menarche was  $12.9 \pm 1.5$  SD years. Their mean

age at diagnosis of POI was  $25.3 \pm 8.8$  years. At diagnosis of POI, their mean FSH level was  $85.4 \pm 53$  IU/L, their mean luteinizing hormone level was  $32.4 \pm 19$  IU/L, and their mean serum estradiol level was  $55.4 \pm 48.8$  pmol/L (Table 1).

Karyotype and *FMR1* analysis gave normal results in all patients. Analysis of SNP arrays was normal except in one patient (family F1) in whom we found a 1.6-Mb microdeletion in the long arm of chromosome 15 involving the *CPEB1* gene.

WES of our familial POI cohort identified a likely molecular diagnosis in 18 of 36 index patients (50% diagnostic yield). Figure 2 shows the pedigree for families F1 to F18 in which a pathogenic or likely pathogenic variant was identified, with the exception of EPB41L2, in which we identified a variant of unknown significance. The variants are shown in Table 2. The first group contains variants in genes previously reported in human cases of POI (n = 12) (Table 2 and Fig. 2A): *CPEB1*, *DIPAH2*, *EIF2B5*, *FSHR*, *MSH5*, *NR5A1*, *NUP107*, *PGBD3*, *POF1B*, *SPIDR*, *STAG3*, and *SYCE1*. The second group contains variants in heretofore unreported

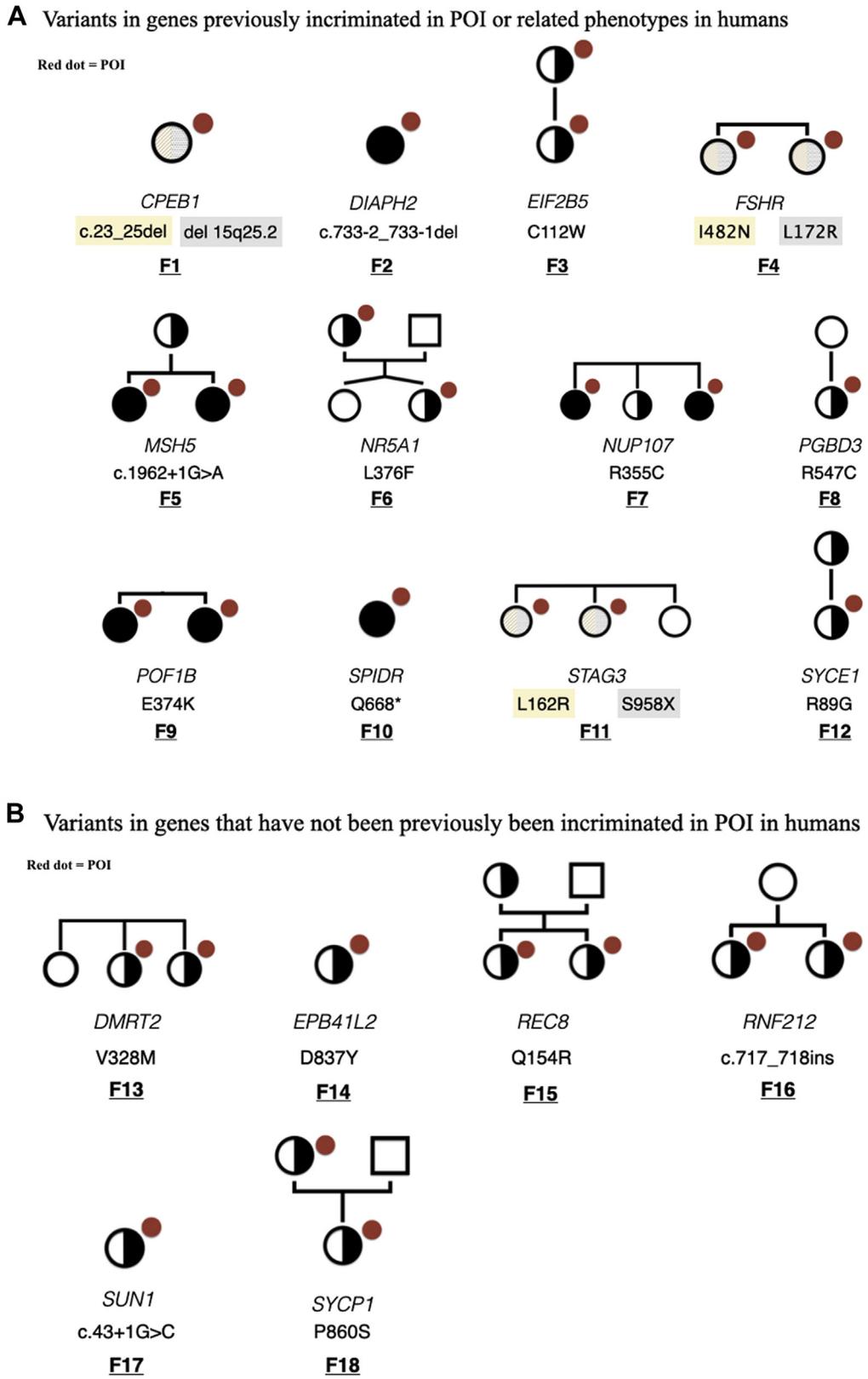
**TABLE 1**

**Clinical and hormonal characteristics of index patients.**

Index case (family no.)	Age at menarche (y)	Age at diagnosis of POI (y)	FSH (IU/L) N: 2–10	LH (IU/L) N: 2–8	Estradiol (pmol/L) N: 120–1,500	Pregnancies
F1	PA	20.0	255.0	45.0	106.4	2 P after OD
F2	12	37.0	38.0	28.0	NA	2 SP
F3	14	18.0	51.0	26.0	NA	0
F4	PA	16.0	84.0	29.0	7.3	0
F5	14	36.0	43.0	58.0	183	0
F6	14	17.0	37.0	11.0	55.1	0
F7	PA	15.0	41.0	17.0	3.7	0
F8	PA	14.0	112.0	33.0	7.3	0
F9	13	17.0	170.0	42.0	77.4	0
F10	12	30.0	30.0	10.0	18.4	1 SP
F11	PA	13.0	86.0	27.0	NA	0
F12	PA	16.0	160.0	49.0	33.0	0
F13	15	26.0	106.0	83.0	36.7	2 SP
F14	11	37.0	160.0	NA	110.0	2 SP and 1 M
F15	PA	19.0	40.0	16.0	7.3	0
F16	12	38.0	36.0	14.0	80.7	0
F17	12	29.0	94.0	40.0	14.7	0
F18	12	40.0	47.0	13.0	77.1	1 M
F19	PA	16.0	128.0	30.0	36.7	0
F20	12	34.0	54.0	37.0	147.0	2 P
F21	14	17.0	30.0	25.0	77.4	TP after OD
F22	14	33.0	38.0	NA	77.4	1 P after ovarian stimulation
F23	14	27.0	40.0	30.0	77.1	NA
F24	14	37.0	30.0	11.0	77.1	1 SP and 1 M
F25	PA	16.0	56.0	24.0	47.7	0
F26	17	28.0	30.0	13.0	3.7	2 P after clomiphene citrate and 1 M
F27	15	35.0	71.0	59.0	NA	1 MTP
F28	12	31.0	90.0	6.0	NA	1 MTP
F29	10	21.0	80.0	38.0	44.0	0
F30	11	13.0	149.0	64.0	55.1	0
F31	13	34.0	154.0	NA	44.0	0
F32	13	31.0	108.0	69.0	154	1 P on HRT
F33	13	21.0	54.0	47.0	18.4	0
F34	12	35.0	61.0	17.0	51.4	0
F35	PA	13.0	119.0	23.0	NA	0
F36	11	24.0	101.0	27.0	7.3	0

Note: Clinical and hormonal characteristics of each index patient with premature ovarian insufficiency are shown, including age at menarche and age at diagnosis of premature ovarian insufficiency. FSH = follicle-stimulating hormone; HRT = hormonal replacement therapy; LH = luteinizing hormone; M = miscarriage; MTP = medical termination of pregnancy; NA = not available; OD = oocyte donation; P = full-term pregnancy; PA = primary amenorrhea; POI = premature ovarian insufficiency; SP = spontaneous pregnancy; TP = twin pregnancy.

**FIGURE 2**



Pedigrees for all the families and individuals with sequenced exomes. Red dots represent affected individuals. Filled shapes represent homozygosity, and half-filled shapes represent heterozygosity. The figure displays variants in gene that were previously associated with premature ovarian insufficiency (A) and variants in new genes in premature ovarian insufficiency (B). F = family; POI = premature ovarian insufficiency.

**TABLE 2**

**Variants in genes previously incriminated in POI (F1 to F12) and not previously incriminated in POI (F13 to F18).**

Family no.	Gene	Locus	dbSNP	NM ID	Nucleotide change	Amino acid change	State	Type	gnomAD allelic frequency	Grantham distance	PolyPhen-2 (0-1)	MutationTaster (0-1)	Evolutionary conservation	ACMG scoring	ACMG annotation
F1	<i>CPEB1</i>	15q25.2	rs542858157	NM_030594.4	c.23_25del	NA	Het.	Inframe deletion	0	NA	NA	NA	NA	PM2 PM4 PM3 PM2	Likely pathogenic
—	<i>CPEB1</i>	15q25.2	NA	NA	1.6-Mb deletion	83223426 - 84832932	Het.	Deletion	0	NA	NA	NA	NA	PM2 PM4 PM3 PM2	Likely pathogenic
F2	<i>DIAPH2</i>	Xq21.33	rs1254336920	NM_007309.3	c.733-2_733-1del	NA	Hem.	Splice acceptor variant	0.005549	NA	NA	NA	NA	PM4 PVS1 PS3 PM2	Pathogenic
F3	<i>EIF2B5</i>	3q27.1	rs139538250	NM_003907.2	c.336C>G	C112W	Het.	Missense	0.00002121	215	0.99	DC (1)	<i>Tetraodon nigroviridis</i>	PP1 PM2 PP3 PP5 PP4	Likely pathogenic
F4	<i>FSHR</i>	2p16.3	NA	NM_181446.2	c.515T>G	L172R	Comp. Het.	Missense	NA	102	0.99	DC (1)	<i>Tetraodon nigroviridis</i>	PP5 PM3 PM2 PVS1	Pathogenic
—	<i>FSHR</i>	2p16.3	NA	NM_181446.2	c.1445T>A	I482N	Comp. Het.	Missense	NA	149	0.94	DC (1)	<i>Bos taurus</i>	PP5 PM3 PM2 PVS1	Pathogenic
F5	<i>MSH5</i>	6p21.33	rs777668732	NM_172166.3	c.1962+1G>A	NA	Hom.	Splice donor variant	0.000008164	NA	NA	NA	NA	PVS1 PM4 PM2	Pathogenic
F6	<i>NR5A1</i>	9q33.3	rs772109827	NM_004959.4	c.1126C>T	L376F	Het.	Missense	0.000004246	22	0.94	DC (1)	<i>Danio rerio</i>	PP5 PM2 PVS1	Pathogenic
F7	<i>NUP107</i>	12q15	rs375755220	NM_020401.2	c.1063C>T	R355C	Hom.	Missense/Splice acceptor	0.00004386	180	0.98	DC (1)	<i>Drosophila melanogaster</i>	PVS1 PP3 PM2 PP5	Pathogenic
F8	<i>PGBD3</i>	10q11.23	rs571669209	NM_170753.3	c.1639C>T	R547C	Het.	Missense	0.00001415	180	0.99	P (1)	<i>Homo sapiens</i>	PP5 PM2 PP1 PP4 PP3 PVS1	Pathogenic
F9	<i>POF1B</i>	Xq21.1	rs146172066	NM_024921.3	c.1120G>A	E374K	Hem.	Missense	0.00005061	56	1	DC (0.99)	<i>Xenopus tropicalis</i>	PP4 PP5 PP3 PM2 PVS1	Pathogenic
F10	<i>SPDR</i>	8q11.21	rs778343717	NM_001080394.3	c.2002C>T	NA	Hom.	Stop gain	0.00001604	NA	NA	NA	NA	PM2 PP5 PP4 PVS1	Pathogenic
F11	<i>STAG3</i>	7q22.1	NA	NM_001282718.1	c.3052del	S958X	Comp. Het.	Frameshift	0.000003978	NA	NA	NA	NA	PM2 PP5 PP4 PM4 PVS1	Pathogenic
	<i>STAG3</i>	7q22.1	NA	NM_001282718.1	c.485T>G	L162R	Comp. Het.	Missense	NA	102	0.99	DC (1)	<i>Saccharomyces cerevisiae</i>	PM2 PP5 PP4 PVS1	Pathogenic
F12	<i>SYCE1</i>	10q26.3	rs141668584	NM_130784.2	c.265A>G	R89G	Het.	Missense	0.0021	125	0.99	DC (0.64)	<i>Ornithorhynchus anatinus</i>	PM2 PP5 PP4 PVS1	Pathogenic
F13	<i>DMRT2</i>	9p24.3	rs140004479	NM_181872.4	c.982G>A	V328M	Het.	Missense	0.002928	21	0.99	DC (1)	<i>Danio rerio</i>	PP3 PM1 PM2 PP5	Likely pathogenic
F14	<i>EPB41L2</i>	6q23.1	rs560502444	NM_001431.3	c.2509G>T	D837Y	Het.	Missense	0.0002439	160	0.99	DC (0.7)	<i>Caenorhabditis elegans</i>	PP3 PM2 PP5	Uncertain significance
F15	<i>REC8</i>	14q12	NA	NM_001048205.1	c.461A>G	Q154R	Het.	Missense	NA	43	0.84	NA	<i>Danio rerio</i>	PP3 PM1 PM2 PP5	Likely pathogenic
F16	<i>RNF212</i>	4p16.3	rs757048057	NM_001193318.2	c.717_718ins	NA	Het.	Frameshift	NA	NA	NA	NA	NA	PP3 PM2 PM2 PM4 PP5	Likely pathogenic
F17	<i>SUN1</i>	7p22.3	rs192103672	NM_001171945.1	c.43+1G>C	NA	Het.	Splice donor variant	0.0001206	NA	NA	NA	NA	PP3 PM2 PM2 PM4 PP5	Likely pathogenic
F18	<i>SYCP1</i>	1p13.2	rs150731809	NM_001282541.1	c.2578C>T	P860S	Het.	Missense	0.00001142	74	1	DC (1)	<i>Danio rerio</i>	PP3 PM1 PM2 PP5	Likely pathogenic

Note: The table lists variants in genes with gene symbol, family identification number, database identification number, nucleotide and amino acid change, effect type, allelic frequency, suspected effect (Grantham distance, pathogenicity scores), evolutionary conservation, variant novelty, age of onset, and annotation based on the ACMG criteria. ACMG = American College of Medical Genetics; Comp. Het. = composite heterozygous; Hem. = hemizygous; Hom. = homozygous; NA= not available; POI = premature ovarian insufficiency.

genes in POI and/or disorders or differences of sexual development ( $n = 6$ ) (Table 2, B and Fig. 2, B): *DMRT2*, *EPB41L2*, *REC8*, *RNF212*, *SUN1*, and *SYPC1*.

Of the 18 variants identified, 5 were considered to be of autosomal recessive transmission, 11 of autosomal dominant transmission, and 2 of X-linked recessive transmission. Except for one gene (*STAG3*) (13), all the variants identified were novel. We did not find any family or any individual with more than one potential causative mutated gene.

## DISCUSSION

We present a large cohort of patients with familial POI and their relatives who had a precise phenotypic evaluation as well as an extensive genetic evaluation including karyotype, screening for *FMR1* premutation, and SNP-array analysis. We used high-density SNP arrays that allowed us to detect both copy number variants and genomic areas of loss of heterozygosity suggesting consanguinity. Because all those investigations were normal except for one patient (family F1, 1.6-Mb deletion in *CPEB1*), we performed WES.

In our cohort, a likely molecular diagnosis of POI was found in 50% of the families. In two thirds of these cases, we were able to find variants in genes already known to be implicated in POI. In the remaining third, we identified variants in genes that are candidate genes, based on their ovarian expression, their function, and their role in molecular pathways known to be involved in reproduction and sex determination. The genes identified have a broad array of physiologic and molecular functions, highlighting the complexity of ovarian and reproductive function. For each gene, we discuss animal models, its physiologic function, previous descriptions in human cases of POI when available, as well as each variant's pathogenicity.

### Genes Involved in Reproductive Physiology

**FSHR.** Pathogenic variants in *FSHR* have already been reported with an autosomal recessive transmission in 46,XX patients with POI. FSH is of paramount importance in reproduction in both males and females. The transcription of *FSHR* begins early in follicular growth, which explains the cases of POI associated with *FSHR* pathogenic variants. We identified compound heterozygosity in the *FSHR* gene in 2 affected sisters in family F4. The 2 predicted pathogenic missense variants (c.515T>G; L172R and c.1445T>A; I482N) are located in 2 conserved protein domains (part of the LRR superfamily and the 7tm-GPCRs superfamily, respectively).

**NR5A1.** 46,XY gonadal dysgenesis and 46,XX primary ovarian insufficiency have been reported in cases with heterozygous variants in *NR5A1* (14), as well as in cases of female-to-male sex reversal (15–18). *NR5A1* is a transcription factor that regulates several genes involved in sexual differentiation and steroidogenesis (19). In family F6, we identified a heterozygous predicted pathogenic missense variant (c.1126C>T; L376F) in the affected proband, as well as in her affected mother. This variant, located in a ligand-binding site, has been reported with an allelic frequency of

0.00042% in the general population. Neither her nonaffected sister nor her father had the variant. This suggests an autosomal dominant mode of inheritance.

### Genes Involved in Cell Division and Meiosis

**CPEB1.** In the mouse model generated by Tay and Richter (2001) (20), female mice had vestigial ovaries and absent oocytes, which were shown to be arrested at pachytene and to have fragmented chromatin, as well as absent synaptonemal complexes. Groisman et al. (21) confirmed the defects in germ cell development in *Cpeb1*-null mice. In family F1, we found an apparently homozygous in frame deletion in *CPEB1* (c.23\_25del), with a reported allele frequency of 0.04%. This case had already been described by our team when we identified a large 1.6-Mb deletion by SNP-array (22). It appears that this patient consequently presents with a hemizygous 3 bp *CPEB1* deletion, in trans of the large deletion.

**DIAPH2.** Several studies (23–26) have reported patients with POI associated with a balanced t(X;12)(q21;p13) translocation with one of the breakpoints located in *DIAPH2*. An important event in mitosis and meiosis is microtubule attachment to kinetochores. *Dia3*, a mouse homologue of *DIAPH2*, has been found to play an essential role in metaphase chromosome alignment related to microtubule binding (27, 28). We found an affected woman in family F2 with a hemizygous splice acceptor variant (c.733-2\_733-1del) in *DIAPH2*. This variant is predicted to affect a conserved protein domain and was reported with an allele frequency of 0.5% in gnomAD.

**NUP107.** Homozygosity for a pathogenic variant in *NUP107* was reported in 4 women with ovarian dysgenesis in a Palestinian family (29). *NUP107* is part of the nuclear pore complex, which mediates transport between the nucleus and the cytoplasm. It associates with *NUP160* during interphase, and the complex interacts with kinetochores during prophase. Depletion of the *NUP107* subunit induces failure of the kinetochore to establish microtubule attachment. We found a homozygous predicted pathogenic missense variant (c.1063C>T; R355C) in *NUP107* in the 2 affected sisters in family F7. Her nonaffected sister was heterozygous for this variant, suggesting an autosomal recessive transmission.

**POF1B.** Lacombe et al. (30) in 2006 reported 5 sisters with primary amenorrhea from a consanguineous Lebanese family carrying a hemizygous pathogenic variant in *POF1B*. *POF1B* has been suggested to play a critical role in early ovarian development leading to the final number of germ cells (31), as well as a role in germ cell division through the binding of the *POF1B* protein to nonmuscle actin filaments. In family F9, we found a hemizygous predicted pathogenic missense variant (c.1120G>A, E374K) in the *POF1B* gene in the 2 sisters with POI. This variant was located in a conserved protein domain and was found with an allele frequency of 0.0051% in gnomAD.

**STAG3.** Homozygous pathogenic variants in *STAG3* have been described in Lebanese, Palestinian, and Chinese familial

cases of POI (32, 33). A homozygous missense variant has been recently reported in a patient with POI and her brother with nonobstructive azoospermia (34). STAG3 is a subunit of the cohesin ring, a protein complex involved in chromosome pairing and segregation during meiosis (35). Specifically, cohesin plays a role in maintenance of sister chromatid cohesion. In family F11, we found compound heterozygosity for 2 predicted pathogenic variants in *STAG3* in 2 affected sisters. The first variant (c.3052del; S958X) is a truncating frameshift variant, reported with an allelic frequency of 0.0004% in gnomAD. The second variant (c.485T>G; L162R) is a missense variant evaluated as damaging by SIFT, MutationTaster, and PolyPhen.

**SYCE1.** Both male and female mice knocked out for this gene are sterile, with the female exhibiting reduced ovaries, meiosis arrested at prophase I, and absent synaptonemal complexes (36). SYCE1 is part of the synaptonemal complex (37). One report of 2 consanguineous sisters with POI with a homozygous variant in that gene suggested an autosomal recessive transmission (38). In family F12, we found affected a heterozygous predicted pathogenic missense variant in *SYCE1* (c.265A>G; R89G) in both the affected proband and her non-affected mother. The variant is located in a conserved protein domain, and it is not reported in common genetic variants databases. An additional genetic factor could be involved in our proband, explaining her POI.

**EPB41L2.** Knockout of the mouse homologue 4.1G is associated with male infertility, reduced testis weight with immature spermatogenic cells, and absence of cell contacts between Sertoli and germ cells (39). Even though there is no description of female *EPB41L2*-null mice, the protein is expressed predominantly in the ovaries. EPB41L2 is a member of a protein family of adaptors between transmembrane proteins and the cytoskeleton. In family F14, a heterozygous predicted pathogenic missense variant in the *EPB41L2* gene (c.2509G>T; D837Y) was described in the proband. The allele frequency is 0.024%, and the variant was considered pathogenic based on several prediction tools, such as PolyPhen-2 (0.994, probably damaging), and MutationTaster (0.698, disease causing). Furthermore, a pLI of 0.99 for this gene suggests a low tolerance for heterozygous loss of function (and therefore a dominant transmission). Therefore, even though we lack familial segregation data, we hypothesize that *EPB41L2* could play a role in human female fertility.

**REC8.** Xu et al. (40) in 2005 showed that Rec8-null mice of both sexes were infertile. Male infertility is related to a failure of meiotic cells to complete prophase I, with the formation of synaptonemal complexes between sister chromatids rather than between homologous chromosomes. REC8 is a phosphoprotein (41) and a key regulator of meiosis, contributing to cohesin removal and establishing the meiotic chromosome segregation pattern (42). *REC8* has not previously been involved in human phenotypes. In family F15, we found a heterozygous predicted pathogenic missense variant (c.461A>G; Q154R) in *REC8* in the 2 affected daughters. This variant has an allele frequency of 0.0004% in gnomAD and is “possibly damaging” according to PolyPhen-2 (score: 0.841). The nonaffected mother carried this variant,

suggesting the role of additional factors explaining the phenotypic differences between the daughters and the mother.

**RNF212.** Both male and female *Rnf212* *-/-* mice are sterile (43). Males have absent crossover complexes in spermatocytes. Heterozygous *Rnf212* *+/-* male mice are fertile. The mechanisms involved in the infertility of female *Rnf212* *-/-* mice have not been described. *RNF212* was shown to be associated with increased recombination rates in humans in a genome-wide association study (44). In family F16, we found 2a predicted pathogenic heterozygous frameshift variant (c.717\_718ins) in the 2 affected sisters. The variant was not found in their nonaffected mother. Our results suggest that this variant has a dominant effect in humans.

**SUN1.** Both male and female *Sun1*-null mice are sterile (45). In male mice, spermatids are absent and apoptosis of spermatozoa is increased. No data are available regarding female *Sun1*-null mice. Heterozygous mice are fertile. SUN1 is an inner nuclear membrane protein that has a role in nuclear-cytoplasmic transport across the nuclear envelope. Specifically, it fosters contact between the nuclear lamina and the cytoskeleton, contributing to nuclear positioning. In family F17, we found a heterozygous splice donor variant in the *SUN1* gene (c.43+1G>C). Our result suggests a dominant effect in humans.

**SYCP1.** Male and female *Sycp1*-null mouse are sterile, with small gonads (46). In males, spermatocytes are arrested in pachytene, with rare crossovers in metaphase I. Similarly to SYCE1, SYCP1 is a component of the synaptonemal complex, a protein structure that allows synapsis and recombination between homologous chromosomes during meiosis I (47). In family F18, we found a heterozygous missense variant in the *SYCP1* gene (c.2578C>T; P860S). The allele frequency in gnomAD was 0.4%. The variant was found in the affected proband as well as in her affected mother and not in her father. Considering the high pLI score, our result suggests that this variant is dominant and the cause of POI in this family.

## Genes Involved in DNA Repair

There is a growing body of evidence for relationships between DNA break repair and ovarian aging. It has been shown that the expression of *BRCA* decreases with age and that women with pathogenic variants in *BRCA* appear to have a reduced number of primordial follicles (48). Although we did not find variants in genes belonging to the DNA double-strand break repair pathways in our cohort, we did find variants in genes for other DNA repair pathways.

**EIF2B5.** Although *EIF2B5* is not involved in DNA repair per se, it has a role in the initiation of translation (49). A single patient has been reported so far with ovarioleukodystrophy and a compound heterozygosity for *EIF2B5* (50). In family F3, we identified a heterozygous variant in the *EIF2B5* gene (c.336C>G; C112W) in a female patient and her affected mother. GnomAD gives an allele frequency of 0.0021% for this variant. Our patient had no neurologic or cognitive abnormalities. She did, however, experience recurrent

headaches. We hypothesize that heterozygosity in EIF2B5 could cause a milder ovarian phenotype without neurologic signs.

**MSH5.** *Msh5*  $-/-$  female mice are infertile with small ovaries. Histology showed no developing follicles and no germ cells. The same team reported 2 Chinese sisters homozygous for a pathogenic transversion variant in *MSH5* and POI (51). *MSH5* is involved in the DNA mismatch repair pathway as well as in meiotic recombination processes. It acts in meiotic double-stranded break repair (52, 53). We found a homozygous splice donor variant (c.1962+1G>A, MaxEntScan score = 4.30) in the 2 affected sisters in F5. This variant was present but heterozygous in their nonaffected mother.

**PGBD3.** *PGBD3* is part of the nucleotide excision repair pathway, which has been shown to protect DNA from various insults, such as ultraviolet or DNA cross-links (54). Four cases of POI in a Chinese family over 2 generations with a heterozygous variant in *PGBD3* have been reported, suggesting an autosomal dominant transmission (Qin et al., 2015). In family F8, we found a heterozygous missense variant (c.1639C>T; R547C) in *PGBD3*. According to PolyPhen-2, this variant is “probably damaging,” with a score of 0.996. It was not found in her nonaffected mother.

**SPIDR.** *SPIDR* is one of the nuclear scaffolding proteins. It is involved in limiting sister chromatid exchange during meiosis, as well as chromosome aberrations and cell sensitivity to DNA damage (55). A biallelic mutation in the *SPIDR* gene has been reported in a patient with POI (56). On the basis of, a functional assay DNA double-strand repair activity confirmed the role of this variant in the phenotype. In family F10, we identified a homozygous stop gain variant (c.2002C>T; Q668\*, ie, stop codon). It is present with an allele frequency of 0.0016% in gnomAD. It is predicted to be pathogenic.

### Genes Involved in Sex Determination

**DMRT2.** Several cases of ambiguous genitalia with hypergonadotropic hypogonadism have been described, associated with distal 9p monosomy and loss of *DMRT1* and *DMRT2* (57). Sequencing of the remaining copies of *DMRT1* and *DMRT2*, as well as *SRY*, was normal. Therefore, a role of *DMRT1* and *DMRT2* in sex determination has been suggested. The 2 genes share 80% amino acid identity. It has been suggested that *DMRT1* is essential for the maintenance of testicular differentiation, and that its low expression would foster ovarian differentiation (58). Neither of the 2 genes has been previously implicated in POI.

In family F13, we found 2a heterozygous missense variant in *DMRT2* (c.982G>A; V328M) in the 2 affected sisters. This variant was absent in the nonaffected sister.

Considering our patients' fertility, the number of pregnancies among the 18 patients in whom we identified variants is rather low. Eleven pregnancies have been reported: 7 full-term spontaneous pregnancies, 2 miscarriages, and 2 pregnancies after oocyte donation. Spontaneous pregnancies occurred before the diagnosis of POI in women carrying variants of *DMRT2*, *SPIDR*, and *EPB41L2*. These findings are

interesting, as they may improve genetic counseling in patients with POI.

In conclusion, our study suggests that a large proportion of familial cases of POI are genetic, and that WES is able to find a likely molecular etiology in 50% of cases. Most variants identified are involved in cell division, meiosis, or DNA repair. WES in POI provides a high yield of diagnosis. This allows for genetic counseling and improvement of patient care, as pregnancy planning, oocyte cryopreservation, and ovarian tissue preservation may be discussed. Future studies should aim at investigating the existence of variants in the candidate genes we identified in familial as well as nonfamilial cases of POI and performing whole genome sequencing on similar cohorts.

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