ARTICLE

Meiotic genes in premature ovarian insufficiency: variants in *HROB* and *REC8* as likely genetic causes

Elena J. Tucker[1,2]✉, Katrina M. Bell[1], Gorjana Robevska[1], Jocelyn van den Bergen[1], Katie L. Ayers[1,2], Nurin Listyasari[1,3], Sultana MH Faradz[3], Jérôme Dulon[4], Shabnam Bakhshalizadeh[1,2], Rajini Sreenivasan[1,2], Benedicte Nouyou[5], Wilfrid Carre[6], Linda Akloul[7], Solène Duros[8], Mathilde Domin-Bernhard[8], Marc-Antoine Belaud-Rotureau[5,9], Philippe Touraine[4,10], Sylvie Jaillard[5,9,10]✉ and Andrew H. Sinclair[1,2,10]

Premature ovarian insufficiency (POI), affecting 1 in 100 women, is characterised by loss of ovarian function associated with elevated gonadotropin, before the age of 40. In addition to infertility, patients face increased risk of comorbidities such as heart disease, osteoporosis, cancer and/or early mortality. We used whole exome sequencing to identify the genetic cause of POI in seven women. Each had biallelic candidate variants in genes with a primary role in DNA damage repair and/or meiosis. This includes two genes, *REC8* and *HROB*, not previously associated with autosomal recessive POI. *REC8* encodes a component of the cohesin complex and *HROB* encodes a factor that recruits MCM8/9 for DNA damage repair. In silico analyses, combined with concordant mouse model phenotypes support these as new genetic causes of POI. We also identified novel variants in *MCM8*, *NUP107, STAG3* and *HFM1* and a known variant in *POF1B*. Our study highlights the pivotal role of meiosis in ovarian function. We identify novel variants, consolidate the pathogenicity of variants previously considered of unknown significance, and propose *HROB* and *REC8* variants as new genetic causes while exploring their link to pathogenesis.

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INTRODUCTION

Reproductive success of human females relies on a myriad of genes involved in numerous cellular processes. Early in embryogenesis, the oocyte pool needs to be established, needs to be shielded from DNA damage, and needs to successfully commence and arrest in meiosis before cyclical hormonal induction and maturation occurs after menarche. If any of these processes is disrupted, premature ovarian insufficiency (POI) can result. POI is a common form of female infertility characterised by menstrual disturbance (amenorrhea or oligomenorrhea) with raised gonadotropins and low estradiol before the age of 40. It is known to have a strong genetic basis, however, the majority of patients have no established cause [1]. The only genetic analyses routinely offered to POI patients include *FMR1* premutation analysis, accounting for 3–13% of cases [2], and microarray/karyotyping detecting chromosomal rearrangements, responsible for 10–13% of cases [3].

Although further genetic studies are not routinely offered or clinically advised according to the European Society of Human Reproduction and Emrbyology (ESHRE) guidelines [4], they are increasingly being performed in a research context. Understanding genetic cause of POI allows for prompt and appropriate clinical care by the relevant medical professionals [5]. For example, certain genetic causes are associated with co-morbidities such as cancer predisposition or neurological problems. Specifically, when a diagnosis is made in a gene involved in DNA damage repair, the possibility of cancer predisposition arises. Chromosomal stability studies can be performed to determine patient sensitivity to DNA damage. With an elevated risk of DNA damage, patients should be vigilant to respond to any early signs of cancer. Variants in *MCM9*, for example, have been associated with a predisposition to colorectal cancer in addition to POI [6]. If such a genetic cause of POI is identified, affected individuals can engage with gastroenterologists and have regular colonoscopies for disease surveillance, leading to early detection and better prognosis and outcomes.

Many of the genes associated with POI pathogenesis are involved in meiosis (Fig. 1). For successful meiosis, several steps need to occur in a tightly regulated manner. Sister chromatids must be bound together by the ring-like cohesin complex. Sister chromatid cohesion allows the assembly of the synaptonemal complex (Fig. 1), which brings together homologous chromosomes. The synaptonemal complex consists of an axial/lateral

[1]Murdoch Children's Research Institute, Melbourne, VIC, Australia. [2]Department of Paediatrics, University of Melbourne, Melbourne, VIC, Australia. [3]Division of Human Genetics, Center for Biomedical Research (CEBIOR), Faculty of Medicine, Diponegoro University/Diponegoro National Hospital, Semarang, Indonesia. [4]Department of Endocrinology and Reproductive Medicine, AP-HP, Sorbonne University Medicine, Centre de Référence des Maladies Endocriniennes Rares de la Croissance et du Développement, Centre des Pathologies Gynécologiques Rares, Paris, France. [5]CHU Rennes, Service de Cytogénétique et Biologie Cellulaire, F-35033 Rennes, France. [6]CHU Rennes, UF Bioinformatique et Génétique Computationnelle, Service de Génétique Moléculaire et Génomique, F-35033 Rennes, France. [7]CHU Rennes, Service de Génétique Clinique, CLAD Ouest, F-35033 Rennes, France. [8]CHU Rennes, Département de Gynécologie Obstétrique et Reproduction Humaine, F-35033 Rennes, France. [9]Univ Rennes, CHU Rennes, INSERM, EHESP, IRSET (Institut de recherche en santé, environnement et travail) – UMR_S 1085, F-35000 Rennes, France. [10]These authors jointly supervised this work: Sylvie Jaillard, Andrew H. Sinclair. ✉email: Elena.tucker@mcri.edu.au; Sylvie.JAILLARD@chu-rennes.fr

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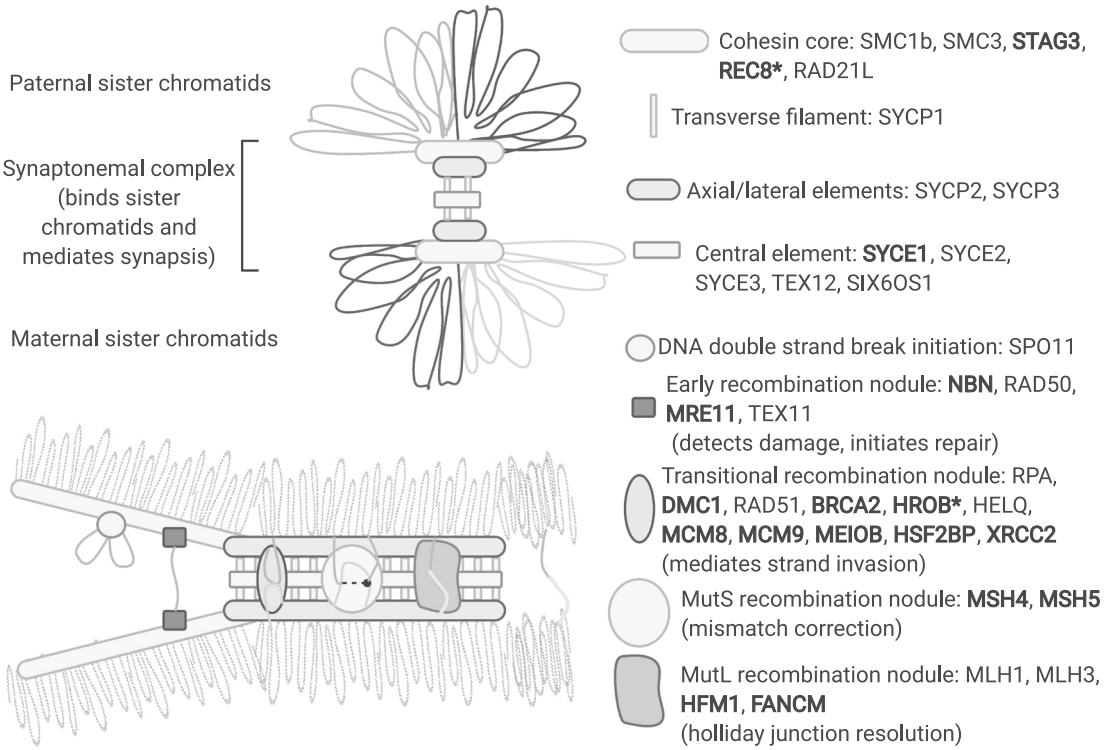

Fig. 1 Diagram depicting proteins involved in meiosis. Those in bold have been implicated in human POI. Those with asterisks were identified for the first time in this study.

element (SYCP2, SYCP3), a transverse filament (SYCP1) and a central element (SYCE1, SYCE2, SYCE3, SIX6OS1, TEX12) [7]. Paired chromosomes then need to be positioned appropriately, which requires genes involved in chromosomal tethering to the nuclear envelope and spindle migration, such as *NUP107* and *POF1B* [8, 9].

Meiotic cell division relies on effective DNA damage and repair to mediate homologous recombination and cross-over events (Fig. 1). This begins with the initiation of DNA double strand breaks by SPO11, and is regulated by accessory proteins such as MEI1, MEI4 and REC114. DNA double strand breaks are detected by the early recombination machinery, including the MRN complex (composed of MRE11A, RAD50 and NBN) (Fig. 1). EXO1 excises DNA at double strand breaks to generate single-stranded DNA, which can then be loaded by the meiosis-specific recombinases, DMC1 and RAD51, promoting strand invasion. Helicases have an important role in strand invasion via their ability to unwind the D loop to make homologous DNA accessible to the invading strand. There are two parallel helicase pathways, one involving the helicase, HELQ, and another involving the helicases MCM8 and MCM9, recruited by HROB [10, 11]. After strand invasion DNA is resolved by the late recombination machinery (Fig. 1), involving mismatch repair proteins, MSH4, MSH5, and endonucleases MLH1 and MLH3 [12, 13]. A number of factors tightly regulate the relative number of cross over events, such as HFM1 and FANCM [14, 15].

Variants in many of these "meiosis" genes cause POI, as summarised in Supplementary File 1. The dual role of many genes in meiosis as well as somatic DNA damage repair, means variants can manifest as POI-related syndromes. This makes it particularly important to understand the consequence of their disruption, to delineate which patients will be at risk of associated co-morbidities, such as cancer. Indeed, women with certain cancers, such as BRCA1-related breast cancer, have a greater likelihood of diminished ovarian reserve [16]. Women with POI do not have a general increased risk of cancer [17], however, individuals with POI resulting from certain genetic aberrations can have high cancer risk. Prompt recognition of genetic cause therefore enables surveillance for cancer development in relevant individuals, potentially improving prognosis.

Our aim was to identify new causes of POI in affected women via whole exome sequencing (WES). We found novel likely causative variants in six genes, all with a relevant role in meiosis, and including two genes never before associated with autosomal recessive human POI, *REC8* and *HROB*.

METHODS

Participants

Patients were recruited after clinical consultation as part of our ongoing research program investigating the genetics of POI ($N = 80$ patients, to date). Available family and medical history is included in Table 1. All patients had POI, defined by menstrual disturbance and elevated FSH (>20 mIU/mL) measured twice at least one month apart as per the ESHR guidelines [18], had no history of ovarian surgery, infection or gonadotoxic therapy, had normal 46,XX chromosomal complement, were negative for *FMR1* premutation and negative for ovarian auto-antibodies. Patients with North African ancestry resided in France.

General molecular techniques

Genomic DNA was extracted from EDTA-blood manually with the NucleoSpin® Blood XL kit (Macherey-Nagel) or with an automated system, Hamilton Microlab STAR and Nucleospin® Blood L kit (Macherey-Nagel), and were assessed by NanoDrop™ 1000 spectrophotometer and Qubit dsDNA BR Assay (Thermo Fisher Scientific). Selected variants were validated by Sanger sequencing using BigDye v3.1 Terminators (Applied Biosystems) and ABI 3130X. Primer sequences are available on request.

Whole-exome sequencing (WES)

DNA underwent WES with SureSelect Human All Exon V6 (Agilent) capture and sequencing on the NovaSeq 6000 (Illumina) or with SureSelect Human All Exon V7 (Agilent) and sequencing on the NextSeq 500/550 (Illumina). WES data were processed using Cpipe [19] or C-GeVarA pipeline (Constitutional Genetc Variant Analysis).

Table 1. Summary of patient clinical details.

Patient	Gene	Age at diagnosis	Karyotype	Amenorrhea	Secondary sex characteristic	US	FSH IU/l	LH IU/l	Estradiol pg/ml	AMH (ng/mL)	Other
1	MCM8	28	46,XX	Primary	Delayed	Small ovaries: Right 0.45 cm^2 Left 0.43 cm^2	82.1	24.9	6	0.15	No known consanguinity, Senegalese
2	STAG3	16	46,XX	Primary	Normal	Atrophic ovaries	75.7	43.6	12	0.01	Consanguineous, Algerian, affected cousin
3	POF1B	17	46,XX	Primary	Delayed	Ovaries of normal size Right 4.08 cm^2 Left 1.73 cm^2 Devoid of follicles	56.1	ND	6	0.07	No known consanguinity, Malian, two affected sisters
4	NUP107	20	46,XX	Primary	Tanner III	One detectable ovary Confirmed by MRI 2.4 cm^2	62	32	<5	<0.1	Consanguineous, Moroccan
5	HFM1	33	46,XX	Secondary	Normal	Ovaries of normal size Right 2.46 cm^2 Left 1.36 cm^2 Devoid of follicles	96	46	29	ND	Consanguineous, affected mother and maternal grandmother, Algerian
6	REC8	19	46,XX	Secondary	Normal	ND	114	59	11	<0.1	Non-consanguineous, French
7	HROB	33	46,XX	Primary	Delayed	No detectable ovaries	34	ND	5	ND	No known consanguinity, Guinean

US ultrasound, *AMH* anti-Mullerian hormone, *FSH* follicle stimulating hormone, *LH* luteinising hormone, *ND* not determined.

We performed two phases of analysis (Table 2) as previously described [1] – the first was gene-centric and used two gene lists (POI genes and meiosis genes, Supplementary File 2) and the second was an unbiased variant-centric approach. Variant-centric analysis focused on high-priority variants (those considered likely to lead to loss of function: frameshift, nonsense and splice site variants) in any gene and with any inheritance, or potentially bi-allelic moderate-high priority variants (missense, in-frame indels, frameshift, nonsense or splice spite). Only variants with MAF < 0.005 in 1000 genomes and gnomAD (https://gnomad.broadinstitute.org/) and with high quality scores (Q > 50 and allele balance >25) were considered. Heterozygous "high" priority variants were subsequently discounted if they affected genes tolerant of LoF according to gnomAD data, affected genes not expressed in gonadal tissue according to GTEx data, fell within minimally-expressed transcripts that had not been validated and/or affected genes with no discernable link to ovarian biology. Missense variant pathogenicity was predicted in silico using Mutation Taster (http://www.mutationtaster.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT/Provean (http://provean.jcvi.org/), and CADD (Combined Annotation-Dependent Depletion) (https://cadd.gs.washington.edu/snv). The conservation of affected residues was assessed by Multiz Alignments of 100 vertebrates (UCSC Genome Browser https://genome.ucsc.edu/). Described variants were submitted to ClinVar (SCV001810162 - SCV001810169).

RESULTS

Variants in known POI genes

A homozygous MCM8 variant: c.1905 + 1G > C. Patient 1, born to consanguineous parents of Senegalese descent, experienced primary amenorrhea and was diagnosed with POI at age 28, with an FSH level of 82.1 IU/l. Ultrasound revealed small but detectable ovaries (0.45 cm^2 right, 0.43 cm^2 left). WES identified a homozygous *MCM8*:NM_182802.3 c.1905 + 1 G > C variant (Supplementary Fig. 1, Table 3). This variant is present in the gnomAD database, however, at a low frequency of 0.000016 (0.000062 in African population), and never homozygous. The variant is predicted to disrupt a canonical splice donor site (https://www.genomnis.com/access-hsf).

A homozygous STAG3 variant: c.2627G > A p.(Gly876Glu). Patient 2, born to consanguineous parents of Algerian descent, was diagnosed with POI at the age of 16 following primary amenorrhea and an FSH level of 75.7 IU/L. Pelvic ultrasound revealed atrophic ovaries. She has a similarly affected female cousin, however, DNA was not available for analysis. WES identified a homozygous *STAG3*: NM_012447.4 c.2627 G > A p.(Gly876Glu) variant (Supplementary Fig. 2, Table 3). Although not falling within a known functional domain, glycine at this site is moderately conserved (Supplementary Fig. 2). The only alternative amino acid in mammals is also small and non-polar (alanine). A large negatively charged residue, like glutamate, is never observed. The variant is absent from public databases, such as gnomAD. Most, but not all, online algorithms predict this to be a likely deleterious variant (Table 3).

A homozygous POF1B variant: c.986G > A p.(Arg329Gln). Patient 3, of Mali descent (no known consanguinity), was diagnosed with POI at age 17 following delayed puberty and primary amenorrhea. Her FSH was elevated at 56.1 IU/L and she had low AMH at 0.07 ng/ml. She had two similarly affected sisters, however, their DNA was not available for analysis. Pelvic ultrasound detected two ovaries with substantial surface area (1.73 cm^2 on the left and 4.08 cm^2 on the right), however no detectable ovarian follicles. WES identified a homozygous *POF1B*: NM_024921.3 c.986 G > A p.(Arg329Gln) variant (Supplementary Fig. 3, Table 3). This variant has conflicting annotation in Clinvar. Predictions of pathogenicity using online algorithms are also conflicting (Table 3). The overall frequency of this variant is 0.002642 (African population, 0.004854), and it is never detected in homozygous state in gnomAD.

Table 2. Filtering summary.

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
	Median coverage	124	124	138	151	103	126	123
	Percentage of bases >x10	97.9	97.55	97.69	99.32	97.4	99	97.58
	Mod-high (all)	**541**	**469**	**484**	**1184***	**483**	**731**	**547**
Gene centric	**Mod-high (POI)**	**12 variants (12 genes)**	**19 variants (18 genes)**	**15 variants (15 genes)**	**43 variants (39 genes)**	**12 variants (12 genes)**	**21 variants (19 genes)**	**22 variants (20 genes)**
	Mod-high (meiosis)	**1 variants (1 gene)**	**4 variants (4 genes)**	**3 variants (3 genes)**	**2 variants (2 genes)**	**4 variants (4 genes)**	**4 variants (3 genes)**	**3 variants (3 genes)**
Variant centric	**Potentially bi-allelic (all)**	**68 variants (39 genes)**	**106 variants (73 genes)**	**80 variants (48 genes)**	**416 variants (172 genes)**	**46 variants (28 genes)**	**87 variants (55 genes)**	**80 variants (43 genes)**
	High (all)	**19 variants (16 genes)**	43 variants (41 genes)	32 variants (31 genes)	51 variants (49 genes)	**23 variants (23 genes)**	**47 variants (46 genes)**	**25 variants (25 genes)**
	Pathogenic/likely pathogenic	**MCM8**	**STAG3**	**POF1B**	**NUP107**	**HFM1**	**REC8**	**HROB**

The number of variants detected at each filtering stage is indicated. Bold indicates the categories in which the likely pathogenic variants fell. Brackets indicate the number of different genes in which the variants were found as sometimes there were multiple variants detected in a gene.
POI: gene list containing 572 genes known or predicted to be involved in premature ovarian insufficiency, adapted from (Tucker et al. 2016). Meiosis gene list containing 60 genes as per Supplementary File 2. These are (1) known to be associated with human POI or POI-related syndromes, (2) associated with a POI-like phenotype in deficient animal models or (3) have a strong functional link to known POI genes (Supplementary File 1). High: variants of high priority such as frameshift, splice site or nonsense variants. Mod-high: variants of high priority as well as variants of moderate priority, including missense variants and in-frame deletions/insertions.
*This patient had an excess of variants called. Upon manual inspection of WES data, this was accounted for by an excess of false positives. All variants discussed were Sanger-verified.

A homozygous NUP107 variant: c.1064G > A p.(Arg355His). Patient 4, born to consanguineous parents of Moroccan descent, received a POI diagnosis at age 21 after experiencing primary amenorrhea associated with elevated FSH of 62 IU/L. Estradiol and AMH were low (5 pg/ml and 0.1 ng/ml) and her pubertal development was Tanner III. Pelvic MRI identified uterine hypoplasia and only one ovary of 2.4 cm^2 without follicles. The apparent uterine "hypoplasia" could be due to estrogen deficiency; however, she was not evaluated for uterine recovery post-hormone replacement therapy. WES identified a homozygous *NUP107*: NM_020401.4 c.1064 G > A p.(Arg355His) variant that is invariably predicted pathogenic by online algorithms (Supplementary Fig. 4, Table 3).

A homozygous HFM1 variant: c.2410G > T p.(Glu804Ter). Patient 5 is from a consanguineous Algerian pedigree and presented with secondary amenorrhea, not receiving a POI diagnosis until the age of 33 when she had an FSH level of 96 IU/L. She had an affected mother who was post-menopausal at 40 and an affected maternal grandmother who was post-menopausal at 28, as well as an infertile brother with azoospermia (Supplementary Fig. 5). She had a sister with regular menses but intellectual disability. Unfortunately, familial DNA was not available for analysis. Pelvic ultrasound revealed ovaries of normal size, however, they were devoid of follicles. Puberty and secondary sexual characteristics were normal. WES in this patient identified a homozygous *HFM1*: NM_001017975.4 c.2410 G > T p.(Glu804Ter) (Supplementary Fig. 5, Table 3). This is present in gnomAD, at a frequency of 0.000004 and only in heterozygous state. This nonsense variant falls within the 21st coding exon of this 39-exon gene and therefore is predicted to induce nonsense mediated decay [20], although this was not further investigated by RNA studies.

New genetic causes of POI

Biallelic loss-of-function REC8 variants. Patient 6 is from a French non-consanguineous pedigree and experienced menarche at 14 years of age with regular menstruation for 1.5 years followed by infrequent menstruation (~3 monthly) and amenorrhea from age 16. She commenced hormone treatment and was re-evaluated at age 19, when elevated FSH was elevated (114.1 IU/L) and POI was diagnosed (Table 1).

WES identified two heterozygous variants in *REC8*: NM_001048205.1:c.1035_1036dup p.(Glu346Glyfs*72) and c.624 + 1 G > A p.? (Fig. 2). The c.1035_1036dup introduces a frameshift into the 13th coding exon of this 19 exon gene, and is predicted to cause nonsense mediated decay [20]. The c.624 + 1 G > A affects a canonical donor splice site, and is predicted to disrupt gene splicing. Sequencing parental DNA revealed that these variants were compound heterozygous (Fig. 2C). Both variants are absent from gnomAD and predicted disease-causing (Table 3). REC8 is likely under selection against loss-of-function (LoF) variants having an observed:expected ratio of 0.42, and with no homozygous LoF variants being detected in gnomAD.

A homozygous loss-of-function HROB variant. Patient 7, of Guinean descent and not known to be consanguineous, experienced delayed puberty and primary amenorrhea. Her first hormonal assessment was not until the age of 33, when FSH was found to be elevated at 34 IU/L, and ovaries could not be visualised by ultrasound.

WES identified an apparently homozygous *HROB*: NM_024032 cDNA c.421delG p.(Glu141ArgfsTer62) (Fig. 3). Parental DNA was not available to confirm true homozygosity, but based on WES coverage there was no evidence of a deletion of one allele. This variant falls in the third coding exon of this ten exon gene, and introduces a frameshift, likely leading to nonsense-mediated decay [20]. The variant is detected twice in heterozygous state in gnomAD giving a minor allele frequency of 0.000008 (Table 3) and is not detected in the African population in gnomAD. No homozygous LoF variants in this gene are reported in gnomAD.

DISCUSSION

A novel causative splice site variant in MCM8 associated with isolated POI

Given *MCM8* is a known autosomal recessive POI gene, with previously reported LoF causative variants, we conclude that the

Table 3. Molecular detail of candidate variants in Patients 1–7.

Patient	Gene	RefSeq	State	gDNA variant	cDNA variant	Protein variant	gnomAD	Polyphen	Mutation Taster	CADD	SIFT	Provean	HSF	Other
1	MCM8	NM_182802.3	Hom	chr20:5965647 G > C	c.1905 + 1 G > C	p.?	0.000016 (4 het) 0.000062 (1het): African	NA	Disease-causing (score 1)	NA	NA	NA	Broken donor site	AR LoF variants known to cause POI [21]
2	STAG3	NM_012447.4	Hom	chr7:99800140 G > A	c.2627 G > A	p.(Gly876Glu)	0	Possibly damaging (0.656)	Disease-causing (0.974)	17.07	Tolerated (0.066)	Deleterious (−2.62)	NA	- AR missense variants are a known cause of POI [24]
3	POF1B	NM_024921.3	Hom	chrX:84563194 C > T	c.986 G > A	p.Arg329Gln	0.002642 (507 het) 0.0049 (88het/ hemi): African	Benign (0.392)	Polymorphism*	23.4	Damaging (0.050)	Neutral (−1.87)	NA	- Previously reported variant [8, 28]
4	NUP107	NM_020401.4	Hom	chr12:69109501 G > A	c.1064 G > A	p.(Arg355His)	0.0001076 (27het) 0:African	Probably damaging (score 1)	Disease-causing (score 1)	32	Damaging (0.001)	Deleterious (−4.75)	NA	- Unique genomic variant, previously reported pathogenic locus [29]
5	HFM1	NM_001017975.4	Hom	chr1:91790249 C > A	c.2410 G > T	p.(Glu804Ter)	0.000004 (1 het)	NA	Disease-causing (score 1)	NA	NA	NA	NA	- AR LoF variants known to cause POI [33]
6	REC8	NM_001048205.1	Cmpd Het	chr14:24647853_24647854dup	c.1035_1036dup	p.(Glu346GlyfsTer72)	0	NA	Disease-causing (score 1)	NA	NA	NA	NA	- Mouse model has POI and functional link to human POI genes [36, 37]
	REC8	NM_001048205.1	Cmpd Het	chr14:24646111 G > A	c.624 + 1 G > A	p.?	0	NA	Disease-causing (score 1)	NA	NA	NA	Broken donor site	
7	HROB	NM_024032.5	Hom	chr17:42225592delG	c.421delG	p.(Glu141ArgfsTer62)	0.000008 (2 het) 0:African	NA	Disease-causing (score 1)	NA	NA	NA	NA	- Mouse model has POI and known functional link to human POI genes [10]

AR autosomal recessive, *hom* homozygous, *het* heterozygous, *cmpd het* compound heterozygous, *LoF* loss of function.
*Erroneously, MutationTaster suggests this variant is detected in homozygous state 79x in ExAC. According to gnomAD, it is detected 507x but never in homozygous state. This may contribute to the benign pathogenicity prediction.

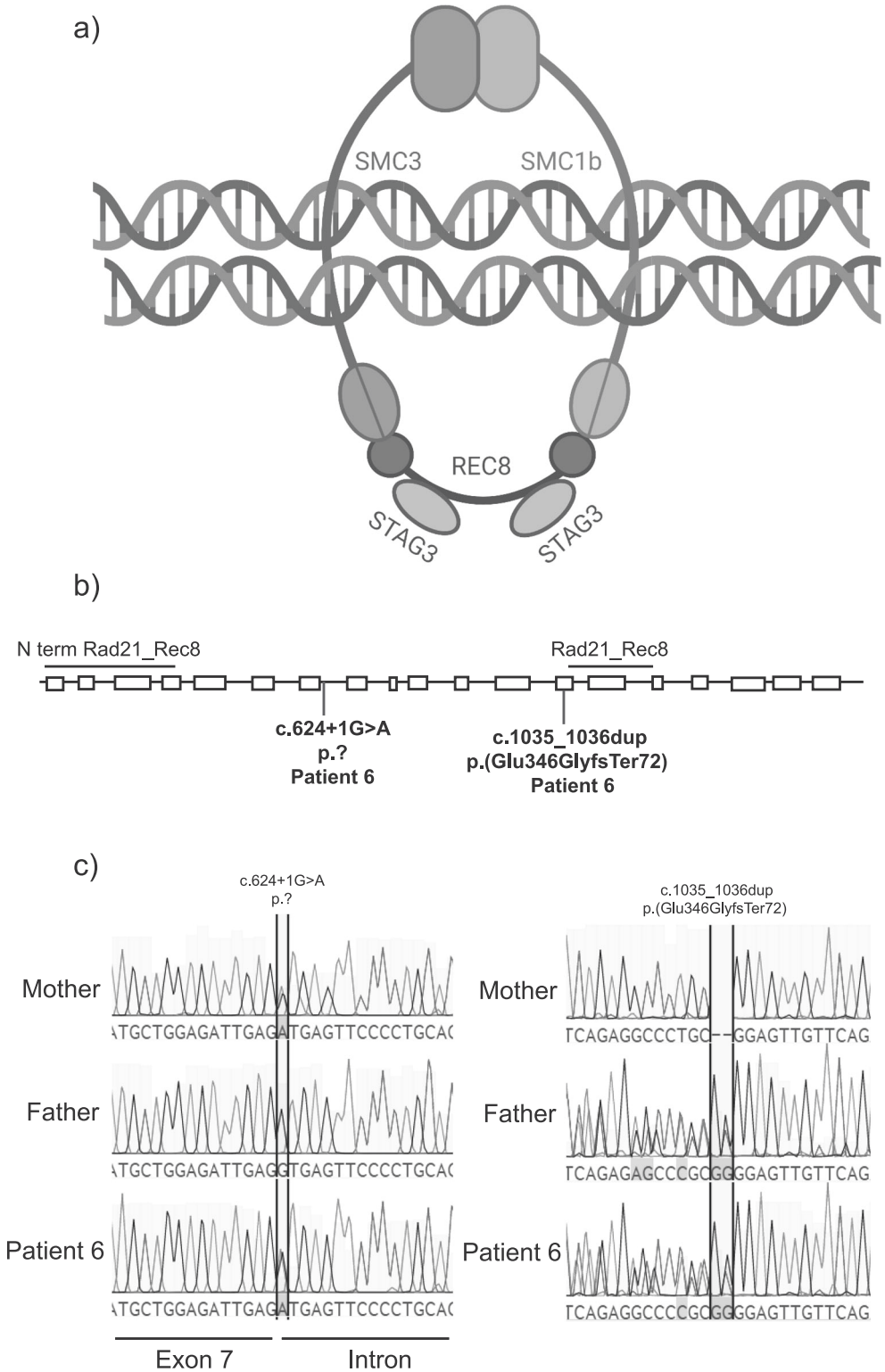

Fig. 2 WES identifies *REC8* as a novel meiosis gene underlying human POI. A Diagram depicting meiotic cohesion with REC8 closing the ring-like structure surrounding DNA. **B** Schematic diagram of the *REC8* gene indicating variant location and conserved domains. **C** Sanger sequencing demonstrates parental segregation consistent with compound heterozygosity. Left panel: the c.624 + 1 G > A splicing variant is paternally inherited. Right panel: the c.1035_1036dup p.(Glu346Glyfs*72) variant is maternally inherited.

novel splicing variant in Patient 1 is responsible for POI (Table 4). Six pathogenic variants in *MCM8* have been reported in the literature, all but one being predicted LoF alleles, like the c.1905 + 1 G > C variant in Patient 1 that abolishes a canonical splice site (Supplementary Fig. 1). Interestingly, *MCM8* has been associated with both isolated POI [21] and syndromic POI involving short stature and pilomatricomas [22]. MCM9, the binding partner of MCM8, has similarly been associated with both isolated and syndromic POI, including cancer predisposition [6, 23], highlighting the role of this complex in both somatic and germ cell

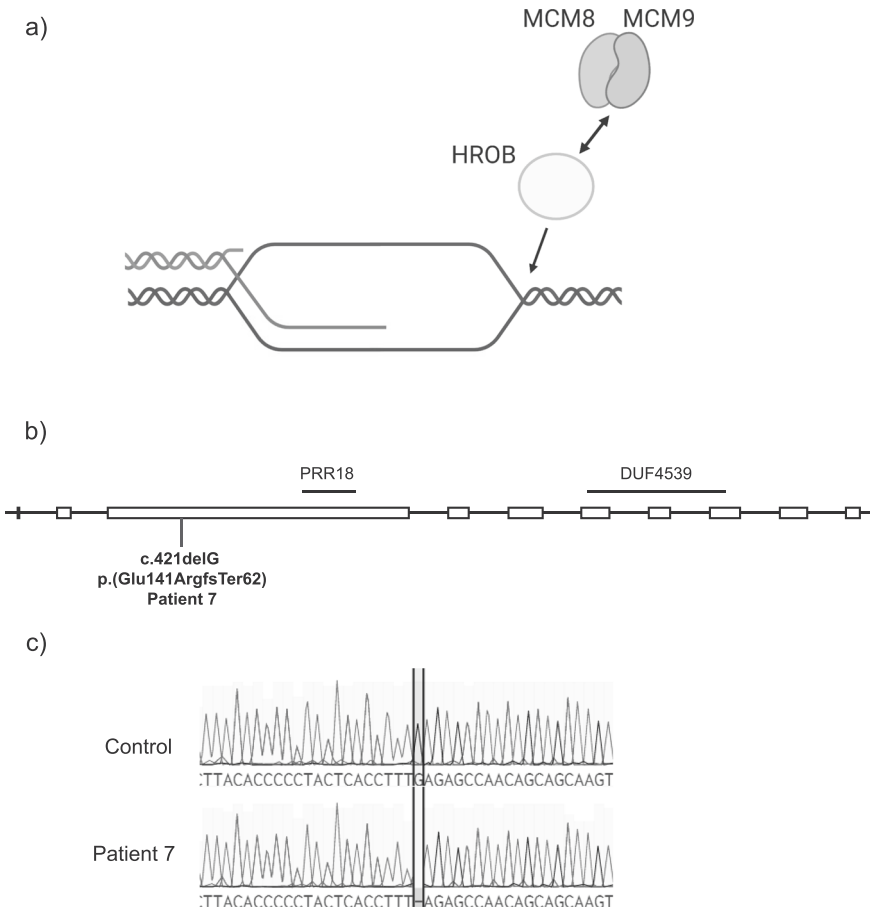

Fig. 3 WES identifies *HROB* as a novel meiosis gene underlying human POI. A Diagram depicting the role of HROB in recruiting the helicase MCM8/9 complex to DNA double strand breaks. **B** HROB gene diagram indicating variant location and domains above (PPR proline rich region, DUF domain of unknown function). **C** Sanger validation of the homozygous *HROB* c. 421delG variant.

DNA repair. The identification of a novel *MCM8* variant associated with isolated POI supports the critical role of this gene primarily for meiosis.

A novel homozygous missense variant in STAG3 is a variant of uncertain significance

Missense variants in *STAG3* are a recently identified cause of non-syndromic POI and azoospermia [24, 25]. Patient 2, who harbours the homozygous c.2627 G > A p.(Gly876Glu) missense variant in *STAG3*, presents with primary amenorrhea and atrophic ovaries, a phenotype consistent with previously reported patients, who usually have streak gonads. Without functional analysis, it remains possible that this is a benign variant and that POI is due to another genetic cause. If familial DNA could be obtained including from the similarly affected cousin, segregation studies could provide more definitive genetic diagnosis. Importantly, one of the reported patients with biallelic *STAG3* variants suffered a germ cell tumour [26], suggesting cancer surveillance may be warranted, although the small case number prevents prediction of a statistically significant increase in risk.

Recurrence of a POF1B missense variant consolidates its status as pathogenic

The homozygous *POF1B* c.986 G > A p.(Arg329Gln) variant is previously reported to cause POI, but is classed as a variant of uncertain significance (VUS) in ClinVar. It was initially described as pathogenic because it fell within a 21 Mb linkage region determined by homozygosity-by-descent mapping, with an identical haplotype shared by four affected sisters but not an unaffected sister in a large consanguineous pedigree. Functional studies demonstrate this variant disrupts POF1B binding to non-muscle actin filaments [8] and results in disorganised F-actin at tight junctions [27]. Furthermore, this variant has been detected in a POI patient with a reciprocal translocation of the other X chromosome that causes non-random X-inactivation and therefore, functional homozygosity of the p.Arg329Gln variant [28]. We present a third independent family harbouring this variant in association with POI. Given the established functional evidence suggesting this variant is deleterious, and its recurrence in POI pedigrees, this variant is likely causative in Patient 3 (Table 4). Our study adds weight to the *POF1B* variant being pathogenic and suggests a need to update public resources, such as ClinVar.

A novel NUP107 causative variant consolidates a genotype: phenotype correlation

Interestingly, the c.1064 G > A p.(Arg355His) *NUP107* variant detected in patient 4 is a novel genomic variant that alters the same amino acid as a previously reported variant, c.1066 C > T, p.Arg355Cys [29]. Although segregation studies and in silico analyses supported the previously reported variant being pathogenic, a knock-in mouse model had grossly normal ovarian pathology, and the authors concluded that using the ACMG guidelines, the variant was a VUS until additional human studies could confirm its pathogenicity [29]. Our discovery of a variant altering the same residue, and similarly abolishing its positive charge, provides this data, and establishes these variants as causative (Table 4).

NUP107 is ubiquitously expressed and encodes a component of the nuclear pore complex. Variants in *NUP107* are an established

Table 4. Variant based criteria.

Patient	Gene	Evidence	Strength criteria	Curation
1	MCM8 c.1905 + 1 G > C	Rare in population databases (<0.0005 for recessive disease) Predicted null variant in a gene where LOF is known pathogenic Predicted protein length changing variant Patient's phenotype is specific for changes in the gene	Moderate (PM) Very strong (PVS) Moderate (PM) Supporting (PP)	1PVS + 2PM + 1PP Pathogenic
2	STAG3 c.2627 G > A	Absent in population databases Missense in gene with known pathogenic missense variation Patient's phenotype is specific for changes in the gene	Moderate (PM) Supporting (PP) Supporting (PP)	1PM + 2PP VUS
3	POF1B c.986 G > A	Rare in population databases Missense in gene with known pathogenic missense variation Co-segregation with disease in multiple affecteds (literature) Detected in trans with a likely pathogenic variant (literature) Previously reported pathogenic variant Patient's phenotype is specific for changes in the gene	Supporting (PP) Supporting (PP) Moderate (PM) Supporting (PP) Supporting (PP) Supporting (PP)	1PM + 5PP Likely pathogenic
4	NUP107 c.1064 G > A	Rare in population databases (<0.0005 for recessive disease) Novel missense at previously reported pathogenic site Missense in gene with known pathogenic missense variation Patient's phenotype is specific for changes in the gene	Moderate (PM) Moderate (PM) Supporting (PP) Supporting (PP)	2PM + 2PP Likely pathogenic
5	HFM1 c.2410 G > T	Rare in population databases (<0.0005 for recessive disease) Predicted null variant in a gene where LOF is known pathogenic Patient's phenotype is specific for changes in the gene	Moderate (PM) Very strong (PVS) Supporting (PP)	1PVS + 1PM + 1PP Pathogenic
6	REC8 c.1035_1036dup	Absent in population databases Multiple lines of evidence support deleterious impact Detected in trans with a likely pathogenic variant Patient's phenotype is specific for changes in the gene Predicted protein length changing variant	Moderate (PM) Supporting (PP) Moderate (PM) Supporting (PP) Moderate (PM)	3PM + 2PP Likely pathogenic
	REC8 c.624 + 1 G > A	Absent in population databases Multiple lines of evidence support deleterious impact Detected in trans with a likely pathogenic variant Patient's phenotype is specific for changes in the gene Predicted protein length changing variant	Moderate (PM) Supporting (PP) Moderate (PM) Supporting (PP) Moderate (PM)	3PM + 2PP Likely pathogenic
7	HROB c.421delG	Rare in population databases (<0.0005 for recessive disease) Multiple lines of evidence support deleterious impact Functional studies in human cells show a deleterious effect of LoF Predicted protein length changing variant Patient's phenotype is specific for changes in the gene	Moderate (PM) Supporting (PP) Strong (PS) Moderate (PM) Supporting (PP)	1PS + 2PM + 2PP Pathogenic

cause of autosomal recessive nephrotic syndrome and Galloway-Mowat Syndrome-7 [30, 31]. Recently, the role of this gene in human ovarian function has been uncovered by the study of a large Palestinian pedigree with four individuals affected by isolated POI [32]. A homozygous missense variant segregated with POI in the family, and transgenic rescue experiments in fly validated that the variant impaired protein function and resulted in female infertility. The affected residue, p.Asp447, forms a salt bridge with the p.Arg355 residue affected by the variant identified in our study, and the other previously reported variant [32]. Taking into account the literature, and our newly described pathogenic variant, we speculate that variants affecting residues of this salt bridge, may disrupt NUP107 structure and/or function in a way that renders it incapable of its role in meiosis, but still able to perform its role in nephrotic function. Functional studies are required to investigate this theory.

HFM1 is an autosomal recessive POI gene that may also be involved in male infertility

Biallelic variants in *HFM1* are a known, albeit rare, cause of POI, with one publication describing three affected individuals from two independent families. All three affected individuals presented with secondary amenorrhea in young adulthood, like Patient 5 of this study [33]. This is the first report of a pathogenic nonsense variant (Supplementary Fig. 5, Table 4). It is intriguing that this woman had an azoospermic brother, which raises the possibility that defects in *HFM1* may account for infertility of both sexes in humans. This is in keeping with both male and female infertility of the *HFM1* knockout mouse model [14]. To investigate further, familial DNA is required, but was not available.

Variants in REC8 are a new cause of autosomal recessive POI

The compound heterozygous LoF variants in *REC8*, identified in Patient 6, represent a new genetic cause of POI. *REC8* encodes a meiosis-specific component of the cohesin complex that binds sister chromatids (Fig. 2A). The cohesin complex is a ring-like structure wrapped around chromosomes [34]. In humans, the cohesin complex responsible for binding sister chromatids during meiosis is composed of SMC1β and SMC3 which heterodimerise to form a V-like structure. This V-shape is closed to form a tripartite ring by a kleisin, either REC8 or RAD21L (Fig. 2A). STAG3 binds REC8 to stabilise the cohesin complex [35]. Consistent with an essential role in meiosis, male and female Rec8-deficient mice are sterile and have meiotic arrest with synapsed sister chromatids and the absence of crossovers [36, 37].

Heterozygous *REC8* variants have been detected in POI patients, however these have had no functional validation or functional studies have revealed no impact on protein stability [38, 39]. One *REC8* variant was confirmed to be inherited from an unaffected mother [39]. This suggests these variants are not causative in isolation, and potential oligogenic inheritance was proposed [39]. Furthermore, the mother of Patient 6 of this study, harbours one

LoF allele and is currently 49 years of age and has not reached menopause, suggesting haploinsufficiency of *REC8* is tolerated.

Given the role of REC8 in mammalian meiosis, the intimate relationship between *REC8* and the known POI gene, *STAG3*, the POI-like phenotype of the *Rec8*-deficient mouse model and the severe nature of the biallelic variants identified in Patient 6, we consider these *REC8* variants likely causative (Table 4).

Variants in HROB are a new cause of autosomal recessive POI

This study describes the first likely causative variant in *HROB* in a patient with isolated POI. HROB, also known as C17ORF53 or MCM8IP, is a recently identified factor required for homologous recombination [10]. It interacts with the MCM8/MCM9 helicase required for unwinding DNA at sites of damage to allow strand invasion and DNA synthesis-mediated repair [10] (Fig. 3A). Mice deficient in *Hrob* have meiotic arrest, leading to a POI-like phenotype [10].

Given variants in *MCM8* and *MCM9*, encoding the helicases with which HROB interacts, cause POI [21, 23], it is likely that deleterious *HROB* variants are similarly causative. The POI-like phenotype in mice further indicates that *Hrob* is essential for mammalian meiosis, and is supportive of the homozygous frameshift *HROB* variant being causative (Table 4).

Expansion of the genotypic spectrum of POI patients with meiotic defects

Six patients of this study had likely causative biallelic variants in genes with a role in meiosis. We describe the first nonsense variant in *HFM1*, identify a novel *NUP107* variant affecting a salt bridge previously implicated in POI, add new evidence to support the pathogenicity of a previously reported *POF1B* variant, and identify novel variants in *MCM8* and *STAG3* in association with POI. Importantly, we describe likely causative biallelic LoF variants in *REC8* and *HROB*, genes that have not previously been shown as a cause of autosomal recessive human POI. The role of these genes in POI pathogenesis will be consolidated by finding additional affected families, but concordance with mouse model phenotypes, the relevance of the affected genes and the severe nature of the biallelic variants support these being *bone fide* new genetic causes. This study highlights the important role of meiosis in ovarian function and female reproduction.

DATA AVAILABILITY

The full WES datasets generated during the current study are not publicly available due to their potential to impinge patient anonymity, but are available from the corresponding author on reasonable request. The variants described within the text are available in the ClinVar repository (https://www.ncbi.nlm.nih.gov/clinvar/).

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AUTHOR CONTRIBUTIONS

EJT, SJ, PT and AHS contributed to conception and design of the study. All authors contributed to the acquisition and/or analysis of data. EJT and SJ wrote the manuscript. All authors revised it critically and approved the final version.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Written informed consent was obtained from all participants. All procedures were approved by the Ethics Committee of Rennes University Hospital and the French law (CCTIRS Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé) or the Human Research Ethics Committee of the Royal Children's Hospital, Melbourne (HREC/22073).

ADDITIONAL INFORMATION

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Correspondence and requests for materials should be addressed to Elena J. Tucker or Sylvie Jaillard.