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CLINICAL STUDY

Genetic investigation of four meiotic genes in women with premature ovarian failure

Béatrice Mandon-Pépin, Philippe Touraine[1], Frédérique Kuttenn[1], Céline Derbois[2], Agnes Rouxel[1], Fumihiko Matsuda[2], Alain Nicolas[3], Corinne Cotinot and Marc Fellous[4]

INRA, UMR 1198, ENVA, CNRS, FRE 2857, Biologie du Développement et Reproduction, Jouy-en-Josas F-78350, France, [1]APHP, Department of Endocrinology and Reproductive Medicine, GH Pitié Salpêtrière, 47-83 Bd de l'Hôpital, 75651 Paris Cedex 13, France, [2]Centre National de Génotypage, 2 rue Gaston Crémieux, 91057 Evry, Cedex, France, [3]CNRS, UMR144, Génétique Moléculaire de la Recombinaison, Institut Curie, 26 rue d'Ulm, 75248 Paris Cedex 05, France and [4]INSERM, U709, Genomics and Epigenetics of Placentary Pathology, Hôpital Cochin, 123 Bld de Port Royal, 75014 Paris, France

(Correspondence should be addressed to B Mandon-Pépin; Email: beatrice.pepin@jouy.inra.fr)

Abstract

Objective: The goal of this study was to determine whether mutations of meiotic genes, such as disrupted meiotic cDNA (*DMC1*), MutS homolog (*MSH4*), *MSH5*, and *S. cerevisiae* homolog (*SPO11*), were associated with premature ovarian failure (POF).
Design: Case–control study.
Methods: Blood sampling, karyotype, hormonal dosage, ultrasound, and ovarian biopsy were carried out on most patients. However, the main outcome measure was the sequencing of genomic DNA from peripheral blood samples of 41 women with POF and 36 fertile women (controls).
Results: A single heterozygous missense mutation, substitution of a cytosine residue with thymidine in exon 2 of *MSH5*, was found in two Caucasian women in whom POF developed at 18 and 36 years of age. This mutation resulted in replacement of a non-polar amino acid (proline) with a polar amino acid (serine) at position 29 (P29S). Neither 36 control women nor 39 other patients with POF possessed this genetic perturbation. Another POF patient of African origin showed a homozygous nucleotide change in the tenth of *DMC1* gene that led to an alteration of the amino acid composition of the protein (M200V).
Conclusions: The symptoms of infertility observed in the *DMC1* homozygote mutation carrier and in both patients with a heterozygous substitution in exon 2 of the *MSH5* gene provide indirect evidence of the role of genes involved in meiotic recombination in the regulation of ovarian function. *MSH5* and *DMC1* mutations may be one explanation for POF, albeit uncommon.

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Introduction

Premature ovarian failure (POF; OMIM no. 311360) is a cause of female infertility due to the loss of normal ovarian function in women before the age of 40 years (1). The condition is defined by the absence or cessation of normal menses for at least 6 months (primary or secondary amenorrhea), menopausal level of follicle-stimulating hormone (FSH) >40 mIU/ml, hypoestrogenism and infertility (2, 3). POF affects 1 and 0.1% of women by 40 and 30 years of age respectively. POF is not uncommon considering the incidence rate of 1–2% of women during their reproductive life.

Several mechanisms may be involved in POF pathogenesis such as viral or autoimmune inflammatory disease, environmental toxics, and radiation or chemotherapy, but the genetic contribution is a significant etiological component. However, the disorder can occur on a familial basis, and there is evidence for a genetic mechanism in at least some cases. Deletions and translocations involving three regions of the X chromosome (Xq13-22, Xq26-28, and Xp11.2-22.1) have been associated with POF (4–9). Several genes located on this chromosome (i.e., bone morphogenetic protein-15 *BMP15*, kit ligand *KITLG*) have been sequenced in cohorts of POF patients, and heterozygous variants were detected but their frequency remained rare and did not appear to be a common cause of POF (10–13).

Candidate gene approaches have revealed few mutations in the gonadotropins and their receptors (14, 15) except noteworthy missense variant Ala189 Val of the *FSH* receptor gene which was strongly associated with POF in the Finnish population but rare in other world populations (16–19). POF can also be associated in familial syndromes such as type 1 blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES; OMIM no. 110100) (20). Several *FOXL2* gene mutations have been reported in the type 1 BPES and nonsyndromic POF cases but are uncommon in diverse

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populations (21, 22). Recently, attention has been focused on members of the transforming growth factor-β (TGF-β) superfamily synthesized by the oocyte, growth differentiation factor-9, and BMP15. These studies identified several heterozygous variants that are significantly more prevalent among women with POF but they are not a major cause of ovarian insufficiency (13, 23–25). Mutations in autosomal genes (galactose-1-phosphate uridylyltransferase, *GALT1*; transforming growth factor beta receptor, *TGFBR3*; inhibin alpha, *INHa*; forkhead box E1, *FOXE1*; and *β-glycan*) have also been related to POF (23, 24, 26–30). Nevertheless, in most cases, the etiopathology of the disease remains unknown.

In the ovary, primordial germ cells enter into meiosis from week 9 post-conception, oocytes pass through leptotene, zygotene, and pachytene stages before arresting in the last stage of meiotic prophase I, the diplotene, or dictyate stage at about the time of birth. It is widely accepted, although recently debated, that in mammals a female is born with a fixed number of oocytes within the ovaries (31, 32). The fertile lifespan of a female depends on the size of the oocyte pool at birth and the rapidity of the oocyte pool depletion. The phenotype of ovaries in null mutant mice for several meiotic genes could be strikingly similar to clinical observations found in human infertility and POF. In female mice lacking the *Dmc1* gene, normal oogenesis was aborted in embryos, and germ cells disappeared in the adult ovary (33, 34). The ovaries of *Msh5*−/− female mice are normal in size at birth, but degenerate progressively to become rudimentary, concomitant with the decline in oocyte numbers from day 3 pp until adulthood (35). The aim of this study was to screen a cohort of 41 clinically well-characterized patients who present unexplained infertility (normal XX karyotype, women with POF) for mutations in four meiotic genes. For this purpose, the exons of these four genes (*DMC1*, *SPO11*, *MSH4*, and *MSH5*) were sequenced and compared with the human corresponding gene to evaluate the impact of meiotic prophase arrest in 46 XX females with ovarian disorders.

Materials and methods

Patients and control population

Patients ($n=41$) were mainly ($n=35$) recruited from the reproductive endocrine unit of Pitie-Salpetriere Hospital, Paris, France. The diagnostic criteria for POF include at least 6 months of amenorrhea before the age of 40 years, with high serum FSH levels (>40 IU/l). In two cases, however, patients were included without fulfilling these criteria. The first one had an FSH level of 38 mUI/l but with a familial history of POF. The second patient had clinical symptoms suggesting Turner's syndrome (short size, bradymetacarpia, and multiple nevi) but with a normal karyotype. However, hypoestrogenism was associated with mild increase of FSH level (18 mUI/l). Since a mutation of one of the studied meiotic genes has been identified in this patient, we considered it necessary to still maintain the patient in our cohort. Karyotyping with a high-resolution GTG banding was carried out for all the patients. This study was approved by the institutional review board of the hospitals, and all participants gave their written informed consent. The control population provided by the Centre National of Genotypage (CNG) included 36 Caucasian women having at least one child and no history of infertility. A second group of control population originating from Senegal ($n=32$) was also tested for the tenth exon of *DMC1* gene.

DNA extraction and PCR

Genomic DNA was isolated from peripheral blood samples using the standard phenol–chloroform procedure. The *DMC1*, *MSH4*, *MSH5*, and *SPO11* genes are composed of 14, 20, 25, and 13 exons respectively. The sequencing project was performed at the CNG (Evry). All primers were designed using the software Primer 3 (36) (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The first PCR amplification, using intronic oligonucleotide primers flanking the exons, was performed in a 15 μl volume containing 25 ng genomic DNA, 2.5 pM of each primer, and 0.75 U Taq polymerase (ExTaq, Takara, Cambrex, MD, USA). After an initial denaturation step at 94 °C for 5 min, 34 cycles of amplification were performed consisting of 5 s at 98 °C, 30 s at 60 °C, a 30 s elongation step at 72 °C, and one 10 min terminal elongation step. Primer sequences of *DMC1* and *MSH5* genes are given in Tables 1 and 2.

DNA sequencing and in silico analysis

All the PCR products containing the exons and flanking regions of each gene were purified using BioGel P-100 (Bio-Rad laboratories). To 2 μl sense or antisense sequencing primer (1.5 μM) and 3 μl Bigdye terminator mix (Applied Biosystems, Foster City, CA, USA), 1 μl purified PCR products was added. The amplification consisted of an initial 5 min denaturation step at 96 °C, 25 cycles of 10 s of denaturation at 96 °C, and a 4 min annealing/extension step at 60 °C. The purified reaction products (G50 Sephadex spin column, Boehringer Mannheim) were sequenced on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems).

Both strands from all patients and controls were sequenced for the entire coding region and the exon/intron boundaries. Alignment and single nucleotide polymorphism (SNP) analysis were performed with Genalys software developed by the CNG (37).

The sequence of each variant was confirmed by a new round of PCR amplification and sequencing. The potential deleterious effect of the amino acid

Table 1 Sequences of PCR and sequencing primers for the human *DMC1* gene.

DMC1 gene	PCR primers	Sequencing primers
Exon 1	5′-TCCCAGGTTCAAGCGAT-3′	5′-TCAGGCATCTGTGTGCATGT-3′
	5′-GCCATACCAGCTGTTAAG-3′	5′-GTAGCTAACAGGGAAGGAAC-3′
Exon 2	5′-TGAAATGAAATCAGAGGCCC-3′	5′-CAGCCCTTTCAATGTTGGTG-3′
	5′-GAAAAGCCTGTTGGTGGAAA-3′	5′-TCAAAGGCTGGATTTCTGCC-3′
Exon 3	5′-TTTCCACCAACAGGCTTTTC-3′	5′-CATTCTTGGGAAATCAGGGC-3′
	5′-ACCTGGAAGTTACTGCCCCT-3′	5′-CCAGGTCTCTTAATCCCTAC-3′
Exon 4	5′-CACTGTTGCATGTTTGACCC-3′	5′-TTGAACCTAGAAAGGGCAGC-3′
	5′-CTTGCTCCTCCAAGCAGTCT-3′	5′-AATCTTGCTCCTCCAAGCAG-3′
Exon 5	5′-CAGCCAAGAATTGCTGTTCA-3′	5′-GGCATGCTATTTGTTCAGCC-3′
	5′-GTGAAACCCCGTCTCCACTA-3′	5′-GCGAGACTCCGTCTCAAAAA-3′
Exon 6	5′-TGTAATCCCAGCTACTCAGG-3′	No forward sequencing primer
	5′-TGTAATCCCAGCTACTCAGG-3′	5′-TCAGGCACATAGTAGATGTTTG-3′
Exon 7	5′-GCAACAGCAGATTCCATGTG-3′	5′-CAACTATGCTGGCAGAATAC-3′
	5′-TTACCCAAACAGGTTCTGCC-3′	5′-CCATATGAAGAAGTGAAACC-3′
Exon 8	5′-TGCAGGTGCACTTAGTTTGC-3′	5′-TGGTTGCTAGCATCCTCTAG-3′
	5′-CTTGAAGCCAGGAGTTGGAG-3′	5′-TCTGCCTTAGCATGTATACC-3′
Exons 9+10	5′-GTAGCATTTGGTATACATGC-3′	5′-TATTTTGCCTGGCTCCCAAG-3′
	5′-AAGAGTTGTAAAGCCGGG-3′	5′-CGCTGCCTCCTGACATTATA-3′
Exon 11	5′-ACTTTGCAGAGAAGCTTGG-3′	5′-AGCCCGGCTTTACAACTCTT-3′
	5′-GCGCCCAGTAATAAAGTG-3′	5′-CGGAGTAGCTGAGATTACAG-3′
Exon 12	5′-GAGGTTGCAGTGAGTGAGAT-3′	5′-ACACAGCTAGACTCCATCTC-3′
	5′-GTTAGGGAAAGGTTCCCTGA-3′	No reverse sequencing primer
Exon 13	5′-CCTGTTTCCAAGTTTGGAGT-3′	5′-GGCACATAATGCCTGTGACA-3′
	5′-GCCCAGCCCTGGAATTTT-3′	5′-CCAGCCCTGGAATTTTCATG-3′
Exon 14	5′-CCTGTTTCCAAGTTTGGAGT-3′	5′-GTTGTTGGGAAAGGAGTACG-3′
	5′-GCCCAGCCCTGGAATTTT-3′	5′-AAGCACATGCCACTGCACTT-3′

changes was determined using PolyPhen software (http://tux.embl-heidelberg.de/ramensky/index.shtml). The multiple protein sequence alignment was realized with BioEdit and ClustalW (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

Results

Sequence analysis

The analysis of the coding sequence of *DMC1* revealed a homozygous substitution in the tenth exon of one case (patient A), g.33551A>G (with respect to the sequence AY520538 in Genbank; Fig. 1). This leads to the change in amino acid M200V. The patient A was of African origin (from Senegal, Sarakholé ethnic group). In order to determine the frequency of this genetic perturbation in a control population originating from Senegal, 32 additional DNA samples provided by Pasteur Institute (Dakar) were tested for exon 10. All individuals originated from the same geographic region and ethnic group (Sarakholé) as the patient's family.

Two DNA controls presented a heterozygous substitution at the same position. This variant frequency (3%) was comparable with those previously described in Genbank database. This variant is predicted to be probably damaging by the Polyphen program prediction (PSIC score difference=2.053). The familial analysis revealed that both parents and one sister are carriers of the same mutation with heterozygous status (Fig. 2A).

We have also detected, in the second exon of *MSH5*, a heterozygous transition g.2547C>T (with respect to the sequence AY943816 in Genbank) that altered codon 29 of the protein resulting in a proline-to-serine change (P29S). This mutation leads to the change from a medium size hydrophobic amino acid (P) to a small polar amino acid (S), and this variant is predicted to be possibly damaging by the Polyphen program prediction (PSIC score difference=1.800).

This variant was present in two POF patients (patients B and C). It was not found in any control ($n=36$). The sequencing of one patient's family (patient B) revealed the presence of the variant in the DNA of the father and the young sister (Fig. 2B).

The sequencing of *MSH4* and *SPO11* genes revealed no intragenic mutation. We detected only several SNPs present in similar frequency in patients and controls (data not shown).

Patient's phenotype

The mean patient age was 26.5 (15–39) years. The patients presented with the following clinical patterns: primary amenorrhea with absence of or interrupted puberty ($n=6$) and secondary amenorrhea with normal puberty ($n=23$). Eleven patients had a familial history of POF. Mean FSH level was 73.2 mUI/l (18–141).

DMC1-M200V

Patient A was a 28-year-old African woman. Puberty occurred normally when she was 15, with regular

Table 2 Sequences of PCR and sequencing primers for the human MutS homolog 5 (*MSH5*) gene.

MSH5 gene	PCR primers	Sequencing primers
Exon 1	Sense: 5′-ATGTCCCAGTAGGGGTGT-3′	5′-AATCAGCGTCCAGACTCTTC-3′
	Antisense: 5′-TGTGGACACAGGAGGTGA-3′	5′-AGATTGTGGGAAACTCCACG-3′
Exon 2	Sense: 5′-ATGAGGGTGGGGCGC-3′	5′-CCTCTGTGAATCGTTGCTTC-3′
	Antisense: 5′-TAGGCATCATCACCCCCA-3′	5′-GGCTCCCAACCCTCTTTTAT-3′
Exon 3	Sense: 5′-AGATTGCTCCACTGCACTTC-3′	5′-CTAAATGGGGGTGATGATGC-3′
	Antisense: 5′-GGTTGAGTCAGGAGAATTGC-3′	5′-GAGGAATTCATGGTTCCATC-3′
Exons 4+5	Sense: 5′-GAATCTGCCATCACGCCT-3′	5′-GAGGGCTATGGGTTTTCTCT-3′
	Antisense: 5′-CTGAGGCAGTGCCTTTTG-3′	5′-GGAACAGGGAGTTAGGCTAA-3′
Exons 6–8	Sense: 5′-ACTGCCTCAGTGACCCTT-3′	5′-TACAAGACCGTTCCCTTTGC-3′
		5′-AGCCCCCAGGAGATTTAAGA-3′
	Antisense: 5′-CCCCTTCCCTTTCCTTCA-3′	5′-CCACAACTCCACTTCCTTTG-3′
		5′-AGCATGCCTCCACCTCTTTA-3′
Exon 9	Sense: 5′-GTAATCCCAGCCACTCAGGA-3′	5′-AAAGACGTGATCTCAGGAGG-3′
	Antisense: 5′-ACAAGGTCTCCCAAAGTCCC-3′	5′-GGAGCCAATTGCTTTTCTGG-3′
Exon 10	Sense: 5′-CCTGTGAGTGTCCATCCCTT-3′	5′-AGCTTCCTCAACAACCAGCA-3′
	Antisense: 5′-AATCCAAGGTTCATGGCTTG-3′	5′-GAAATGCAGTTAGCCAGTGC-3′
Exons 11+12	Sense: 5′-CCTCAGAGTGAGCTGCAGTG-3′	5′-GTAACTTGTAGTACCCCCAC-3′
	Antisense: 5′-GTGTTGAAACTGCATGGTGG-3′	5′-GGCCTTTACCTGGACTTTTG-3′
Exons 13+14	Sense: 5′-TCTGTCTTCCTTCCTAGACTG-3′	5′-CTGTGATCTTCCCTACTGGT-3′
	Antisense: 5′-GACCACCTGCCAAGGATG-3′	5′-TGCCAAGGATGGTACTCCAT-3′
Exons 15–18	Sense: 5′-CGCAGTGATGGAGTACCAT-3′	5′-AGGGCAGGAGACTCACTTTT-3′
		5′-AAGTCCACAGCTTTGAACCC-3′
	Antisense: 5′-TTGGGCCCCTCATGTCTA-3′	5′-CATCACTCACCTTACAGAGG-3′
		5′-CTCATGTCTATTCCTCCACC-3′
Exons 19–21	Sense: 5′-TAGACATGAGGGGCCCAA-3′	5′-CTGGGGGTTCATCTATCTTG-3′
		5′-TCCTGTTTCACCCTGTCCAT-3′
		5′-TGCGTTACGGGCTTCCAATA-3′
	Antisense: 5′-CATATGCCCCTCTGCACT-3′	5′-CTCACTGTCATGCTCCTTCA-3′
Exons 22–25	Sense: 5′-GCTGTGTGGGCAGAAAAGAA-3′	5′-AATGCTAACCTCTGCCCTCT-3′
		5′-CTCCCACCTTCTTGCTTGTT-3′
	Antisense: 5′-TACTGAGGCAGGGCAGGT-3′	5′-GGTGGTTGCACATTTGGATC-3′
		5′-CCTGCTCTGTGTTTTGGATC-3′

menstrual cycles up to 21 years. A secondary amenorrhea occurred definitely since then. She was referred to our department when she was 28. POF was confirmed with a high FSH level (91 mUI/l); estradiol and inhibin B levels were low (<10 pg/ml and 15 ng/ml respectively). No anti-ovarian antibodies were found positive. Pelvic ultrasonography showed a small uterus (50 mm in its maximal length) and small-sized ovaries; no follicle was observed. An ovarian biopsy was performed confirming the follicular depletion. Familial study identified one sister with a long-standing history of infertility with eight spontaneous abortions.

MSH5-P29S

The first heterozygous patient (patient B) was a Caucasian woman who was 18 years old when she was referred to our department. She first had menstruations when she was 14 with a normal puberty. However, an oligomenorrhea and a secondary amenorrhea appeared progressively. Clinically, she was short (1.46 m) and associated with an obesity (BMI: 32); had rough face, bradymetacarpia, and a mild intellectual deficiency. Hormonal evaluation identified a mild increase of FSH level (18 mUI/l); inhibin B was low (12 pg/ml). Ten days of progestin treatment induced vaginal bleeding. Ultrasonography identified two ovaries that are small in size with multiple follicles depicted. Turner's syndrome was suggested but high-resolution karyotype was found normal and repeated twice. Since the syndrome appeared uncommon, an ovarian biopsy was performed, identifying multiple primary follicles; a secondary follicle was also observed.

The second heterozygous patient (patient C) was also a Caucasian woman who was 36 years old when she was referred to our department. She had her first menstruations, associated with a normal pubertal development, when she was 13. She had oligomenorrhea between 13 and 18 years of age and then used oral contraceptive pills until she was 30. She became pregnant 6 months later and gave birth to a normal boy. She had menstruations following this but a secondary amenorrhea occurred when she was 32. Hormonal results confirmed the existence of POF with a high FSH level (71 mUI/l). Pelvic ultrasonography identified two ovaries small in size without follicles. An ovarian biopsy was performed, depicting small streak gonads with a complete follicular depletion. Table 3 showed major characteristics of these three patients with mutation in meiotic gene.

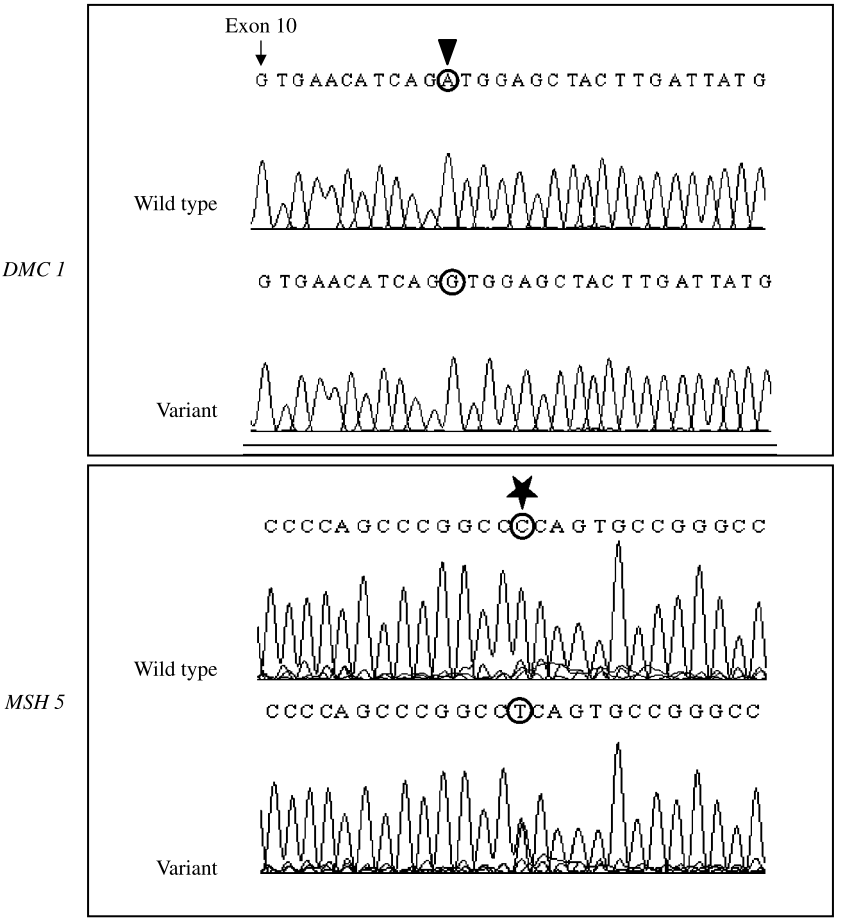

Figure 1 Analysis of *DMC1* and *MSH5* coding sequences. Electropherogram showing (above) the sequence of exon 10 *DMC1* variant in comparison with the sequence of wild type and (below) the sequence of a part of the exon 2 *MSH5* variant in comparison with the sequence of wild type. The arrowhead indicates nucleotide 12 of exon 10 with the A>G homozygous substitution. The star indicates nucleotide 97 of exon 2 with the C>T heterozygous substitution.

Discussion

The POF syndrome is a very heterogeneous clinical disorder probably due to the complex genetic networks controlling human oogenesis and folliculogenesis. It is often associated with small pedigrees that make it difficult to perform genetic linkage analysis to identify responsible genes. An alternative approach is to test candidate genes on the basis of existing knowledge of ovarian physiology.

Several meiotic genes known in yeast have been isolated in mammals, including *Dmc1*, *Msh4*, *Msh5*, and *Spo11* genes (38–41).

Dmc1 is important for meiotic recombination in many organisms; for example, mice with targeted mutations of the *Dmc1* gene are sterile and show hallmarks of poorly repaired DNA double-strand breaks. At birth, the mutant ovaries formed in *Dmc1*−/− mice contained a high proportion of oocytes whose nuclear features were characteristic of leptonema or zygonema, in contrast to the littermates, in which the majority of oocytes had progressed to the pachytene stage. Histological analysis showed that the adult ovaries from *Dmc1*−/− deficient mice contained no follicle at any developmental stage (33, 34). These results indicate that while germ cells are indeed formed in *Dmc1*−/− ovaries, the meiotic progression is blocked leading to progressive death of oocytes and subsequent complete depletion in the ovary by adulthood. The description of our clinical case is perfectly compatible with the animal model. Patient A had a normal gonadal function during a few years, which disappeared when she was 21. Since then, ovarian description, either by ultrasonography or histology, showed a complete absence of follicular reserve and/or maturation.

Msh5 is a member of a family of proteins known to be involved in DNA mismatch repair (42). *Msh5*−/− mice are viable but sterile (35). Meiosis in these mice is affected due to the disruption of chromosome pairing in prophase I. The ovaries of *Msh5*−/− females are normal in size at birth, but degenerate progressively to become rudimentary (35). The phenotype of *Msh5*−/− females differs from *Dmc1*−/− mice, in that the few oocytes remaining at 4–5 day pp in *Msh5*−/− ovaries are normal in appearance and have formed follicles (43). In contrast, ovaries from *Dmc1* knockout females were very small and contained no follicle at any developmental stages. A less severe

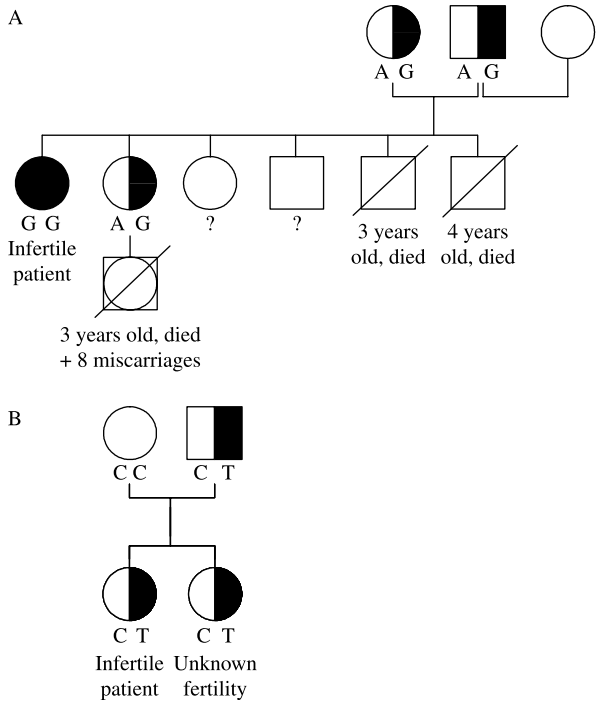

Figure 2 (A) Pedigrees of the infertile patient's family (patient A) with *DMC1* homozygous mutation. Circles, females; squares, males; and slashes through symbols, deceased. (B) Pedigrees of the infertile patient's family (patient B) with *MSH5* variant. Circles, females and squares, males.

phenotype for *Msh5* versus *Dmc1* mutation has also been observed in yeast and worms (44, 45).

This type of phenotype is in accordance with those observed in POF patients with a progressive loss of activity of the ovary leading to gonads reduced in size without germ cells; the oocytes having failed to progress to the dictyate stage *in utero* and subsequently degraded. Nevertheless, the MSH5 protein of the POF patients is probably not completely defective and phenotypes could be less severe than those observed in null mice. However, in our patients, only heterozygous *MSH5* mutation was described. The interspecific genotype difference (heterozygous in humans and homozygous in mice) could be explained by a more dosage-sensitive system in humans.

In both cases with *MSH5* alteration, gonadal function appeared normal with a progressive involution. The most surprising data concern the youngest woman who presented with syndromic features. However, similarities in the ovarian phenotype in female *Msh5*−/− mice and Turner's syndrome patients have been reported (35). It is also probable that this young woman will present with a complete POF in the near future. Indeed, the variability observed in clinical phenotypes (complete or partial infertility) could result from the age that the patients consult with clinicians. The sequencing of *MSH5* gene in the family of this patient revealed that her 20-year-old young sister was also a carrier of the same variant. Until now, she had normal menses but she could be considered as a carrier of a genetic predisposition to develop POF in the future.

The resulting P29S alteration within MSH5 protein is located within the N-terminal region and it is conceivable that this amino acid change could directly impact the integrity of the protein interaction between MSH5 and MSH4. Amino acid sequence analysis revealed that the MSH5 N-terminal region contains a contiguous (Px)5 dipeptide repeat flanked by two PxxP motifs (46). This dipeptide repeat is disrupted in the *MSH5* P29S variant. Moreover, this same mutation has previously been described in genomic DNA of patients with ovarian carcinoma (47). To address the effect of P29S alteration on the interaction between MSH4 and MSH5, a quantitative two-hybrid analysis has been performed. This alteration causes moderate but significant reduction between both proteins and could affect the formation of MSH4–MSH5 heterocomplex (47). The functionality of both proteins in meiotic homologous recombination process probably needs a precise interaction between them and any deviation from this precise coordination will be expected to affect the accuracy of DNA recombination. It is noteworthy that this alteration was found in two patient populations with ovarian pathology; the previous with ovarian cancer and the present with POF. These two pathologies could affect the capacity of DNA repair leading to either a progressive loss of germ cells or cancer formation. For this reason, it will be crucial to follow the degeneration of the ovary from our two patients on a long-term period to prevent an eventual ovarian carcinoma.

In summary, one homozygous missense mutation in *DMC1* gene and one heterozygous in *MSH5* were described here, in 3 of 41 POF patients. The *DMC1* M200V mutation seems to generate a deleterious effect only in homozygous states since the mother and the

Table 3 Major characteristics of the patients with mutation in meiotic genes.

Patient	**Age** (years)	**Origin**	**FSH levels** (mUI/l)	**Follicle**	**Mutated gene**
A	28	African	91	None	*DMC1:* g.33551A>G Homozygous mutation
B	18	Caucasian	18	Multiple primary, 1 secondary	*MHS5:* g.2547C>T Heterozygous mutation
C	36	Caucasian	71	None	*MHS5:* g.2547C>T Heterozygous mutation

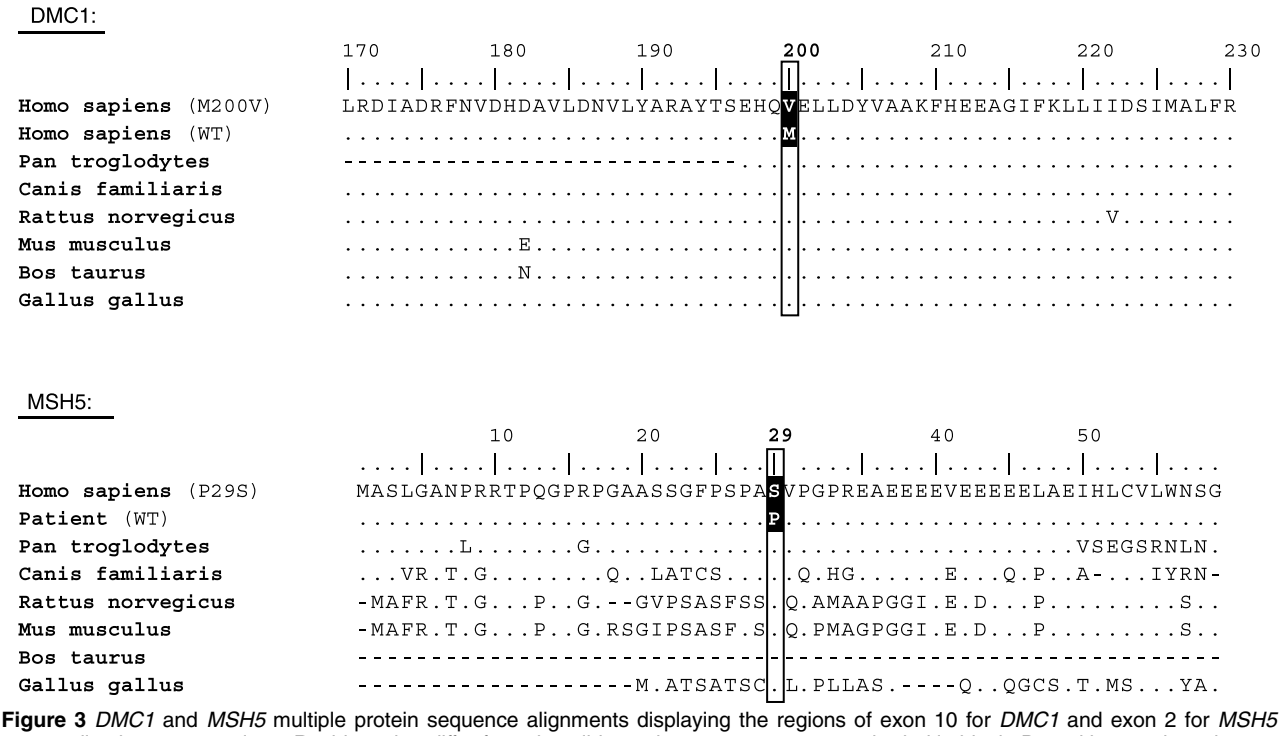

Figure 3 *DMC1* and *MSH5* multiple protein sequence alignments displaying the regions of exon 10 for *DMC1* and exon 2 for *MSH5* surrounding human mutations. Residues that differ from the wild-type human sequence are shaded in black. Dotted boxes show the positions of each mutation (amino acid numbers referred to human sequence).

sister of this patient, carriers of the same mutation with heterozygous status are fertile. A multiple sequence alignment showed that the amino acid M200V was highly conserved across vertebrates, from chicken to primates (Fig. 3), suggesting a functional or structural role. Accordingly, Polyphen program prediction suggested a potential damaging effect of this mutation. The encoded substitution of a methionine for a valine at this residue is non-conservative in nature and affects a Dmc1 protein region which has been shown to facilitate binding. In addition, preliminary results on yeast have shown that the DMC1 M200V mutation of the conserved residue when introduced into *Schizosaccharomyces pombe* causes a significant decrease in meiotic recombination frequencies (work in progress).

However, further investigations will be needed to confirm the pathological role of these mutations such as screening of increased numbers of patients, and controls and generation of *in vivo* models using knock-in alleles in which missense mutations are introduced that mimic the kinds of mutations found in POF patients.

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