A Novel Phenotype Combining Primary Ovarian Insufficiency Growth Retardation and Pilomatricomas With MCM8 Mutation

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Context: Primary Ovarian insufficiency (POI) affects 1% of women aged <40 years and leads most often to definitive infertility with adverse health outcomes. Very recently, genes involved in deoxyribonucleic acid (DNA) repair have been shown to cause POI.

Objective: To identify the cause of a familial POI in a consanguineous Turkish family.

Design: Exome sequencing was performed in the proposita and her mother. Chromosomal breaks were studied in lymphoblastoid cell lines treated with mitomycin (MMC).

Setting and patients: The proposita presented intrauterine and postnatal growth retardation, multiple pilomatricomas in childhood, and primary amenorrhea. She was treated with growth hormone (GH) from age 14 to 18 years.

Results: We identified a novel nonsense variant in exon 9 of the minichromosome maintenance complex component 8 gene (*MCM8)* NM_001281522.1: c0.925C > T/p.R309* yielding either a truncated protein or nonsense-mediated messenger ribonucleic acid decay.

The variant was homozygous in the daughter and heterozygous in the mother. MMC induced DNA breaks and aberrant metaphases in the patient's lymphoblastoid cells. The mother's cells had intermediate but significantly higher chromosomal breaks compared with a control.

Conclusion: We describe a novel phenotype of syndromic POI related to a novel truncating *MCM8* variant. We show for the first time that spontaneous tumors (pilomatricomas) are associated with an MCM8 genetic defect, making the screening of this gene necessary before starting GH therapy in patients with POI with short stature, especially in a familial or consanguineous context. Appropriate familial monitoring in the long term is necessary, and fertility preservation should be considered in heterozygous siblings to avoid rapid follicular atresia. **(*J Clin Endocrinol Metab* 105: 1–10, 2020)**

Key Words: Primary ovarian insufficiency, MCM8, chromosomal breakage syndrome, fertility preservation, growth hormone therapy, pilomatricoma

Abbreviations: BAM, binary alignment map; CADD, combined annotation dependent depletion; CGH array, comparative genomic hybridization array; DNA, deoxyribonucleic acid; EBV, Epstein-Barr virus; FSH, follicle-stimulating hormone; GH, growth hormone; IGV, integrative genomics viewer; IUGR, intrauterine growth retardation; LH, luteinizing hormone; M-CAP, mendelian clinically applicable pathogenicity score; MAF, minor allele frequency; MCM, minichromosome maintenance; MCM8, MCM complex component 8 gene; MMC, mitomycin; MMR, mismatch repair; mRNA, messenger ribonucleic acid; NMD, nonsense-mediated mRNA decay; POI, primary ovarian insufficiency; SD, standard deviation; SGA, small for gestational age; SIFT, sorting intolerant from tolerant; T4, thyroxine; TPO, thyroid peroxidase; TSH, thyroid stimulating hormone; WES, whole exome sequencing.

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Primary ovarian insufficiency (POI) is characterized by amenorrhea or oligomenorrhea >4 months with follicle-stimulating hormone (FSH) ≥ 25 IU/L in women aged <40 years. Although many iatrogenic (chemotherapy, surgery, or irradiation), autoimmune, or viral factors can cause POI, more than 70% of cases remain idiopathic. It is estimated that one-third of unexplained POI could be of genetic origin. There is a high genetic heterogeneity with more than 60 genes involved to date (1). They are implicated in the establishment of the ovarian reserve, in follicular growth, or in follicular atresia. Recent advances and especially whole exome sequencing (WES) have recently highlighted a role for meiosis and deoxyribonucleic acid (DNA) repair genes in POI, previously suspected from animal models. Indeed 15 of 21 POI genes identified by WES in the last 5 years are meiosis or DNA repair genes (2-16). However, these genes have been identified most often in single or rare families (2-16). Obviously, more patients need to be studied to describe the phenotypic spectrum of POI associated with specific gene defects.

Extrareproductive features of syndromic POI are important to evaluate because they need an adapted medical care such as with sensorineural deafness in Perrault syndrome, neonatal jaundice, failure to thrive in galactosemia, cardiopathy, and short stature in the classical Turner syndrome (1). For the latter condition, the use of growth hormone (GH) therapy is indicated in the presence of intrauterine growth retardation (IUGR) and short stature. However, care should be taken to ensure that the use of this therapy in patients with POI and short stature does not produce adverse effects when specific causes are involved such as in chromosomal breakage syndromes (17).

In this study, we describe in a consanguineous Turkish family with a novel phenotype of POI with primary amenorrhea and impuberism, including intrauterine and postnatal growth retardation and multiple pilomatricomas in childhood, associated with a nonsense variant of a DNA repair gene, *MCM8*. This is the first case of tumors observed in humans with *MCM8* variants. This observation makes the screening of this gene necessary before GH therapy, especially in familial or consanguineous POI. A long-term follow-up is needed. Fertility preservation should be proposed in heterozygous siblings to avoid rapid follicular atresia.

Patients and Methods

Clinical data and medical history

The pedigree of this Turkish consanguineous family is illustrated in Figure 1. The parents (III.1 and III.2) are first cousins connected by at least 2 consanguineous weddings (Fig. 1A). The proband IV.2 was investigated in Belgium at the age of 14 years for small stature, lack of pubertal development, and primary amenorrhea. She was born at 40 weeks of gestation after IUGR. At birth, she weighed 2450 g (–2.6 standard deviation [SD]) with a height of 45 cm (–2.9 SD). The first years of life were unremarkable except for hypotrophy and recurrent otitis that necessitated adenoidectomy and ear tube insertion at 7 years of age. She had no psychomotor developmental delay. At the age of 13 years, the dermatologist diagnosed multiple pilomatricomas (Fig. 2). She was also referred to an endocrinologist and a clinical geneticist to investigate the persistence of small-proportionated stature and late puberty with questions about the etiology, GH therapy, and pubertal induction. The auxological parameters were height of 133 cm (–4.3 SD), head circumference of 50 cm (–3.2 SD), weight of 24.4 kg (–5.7 SD), body mass index of 13.9 kg/m^2 (–2.9 SD), and growth velocity of 2.8 cm/year (based on growth Turkish data) (18). The Tanner score was A1P1M1 with primary amenorrhea. She had a 3-year bone age delay. Hormonal assays revealed high FSH (124 IU/L) and luteinizing hormone (LH) (34 IU/L) levels and low estradiol (<5 pg/mL) plasma levels. POI was diagnosed. Prolactin, cortisol, and insulin-like growth factor 1 (IGF-1) plasma levels were normal. Pelvic ultrasonography and magnetic resonance imaging revealed a small prepubertal uterus and streak ovaries (right 14 mm and left 10 mm) without follicles. Anti–thyroid peroxidase (TPO) antibodies were slightly elevated at this stage (37 IU/L at age 14 years, normal range < 34 IU/L) with normal thyrotropin (TSH) and thyroxine (T_4) plasma concentrations. A second blood assay at the age of 19 years confirmed high FSH and LH levels (73 IU/L and 34 IU/L, respectively) and a very low antimüllerian hormone (0.03 µg/L) level. TSH and T_4 levels were normal (see Table 1). Ovarian and adrenal antibodies were negative. IGF-1 plasma levels were within normal ranges: 278 [213-654] ng/mL. The karyotype was 46,XX, and *FMR1* premutation screening was negative. Array CGH (180K) was normal. At age 14 years, she started a GH therapy for persistent small stature after IUGR. GH therapy was initiated at a dose of 0.03 mg/kg/day and continued for 4 years. Pubertal induction was started at age 15 years, with good response. Her final height after treatment is 153 cm (–2.3 SD; target height 156.1 cm), identical to her mother. Because of the potential link between multiple pilomatricomas and Gardner syndrome, a condition characterized by adenomatous colonic polyposis, we performed a sigmoidocolonoscopy at the age of 22 years, which precluded diagnosis in the absence of a polyp.

The older sister of the proband is healthy. She was also born small for gestational age (SGA) (weight 2500 gr [–2.5 SD] and height 47 cm [–2.5 SD]). She had menarche at the age of 11 years, with normal puberty. She is currently 21 years old with a height of 150 cm (–2.4 SD) in the context of familial short stature and has no health problem. Blood and hormonal assays at the age of 22 years in another center revealed no abnormalities especially for FSH, LH, TSH, and T_4 plasma assays and anti-TPO, anti–21-hydroxylase, and ovarian antibodies were negative.

The patient's mother (III.1) has a height of 153 cm (–2.2 SD) (body mass index [BMI] 25.7 kg/m^2) and head circumference of 56 cm (0 SD). She did not originate from a consanguineous marriage. She had 2 pregnancies at ages 24 and 25 years in Turkey. Cessation of menses occurred 3 years after the last pregnancy at the age of 28 years. She

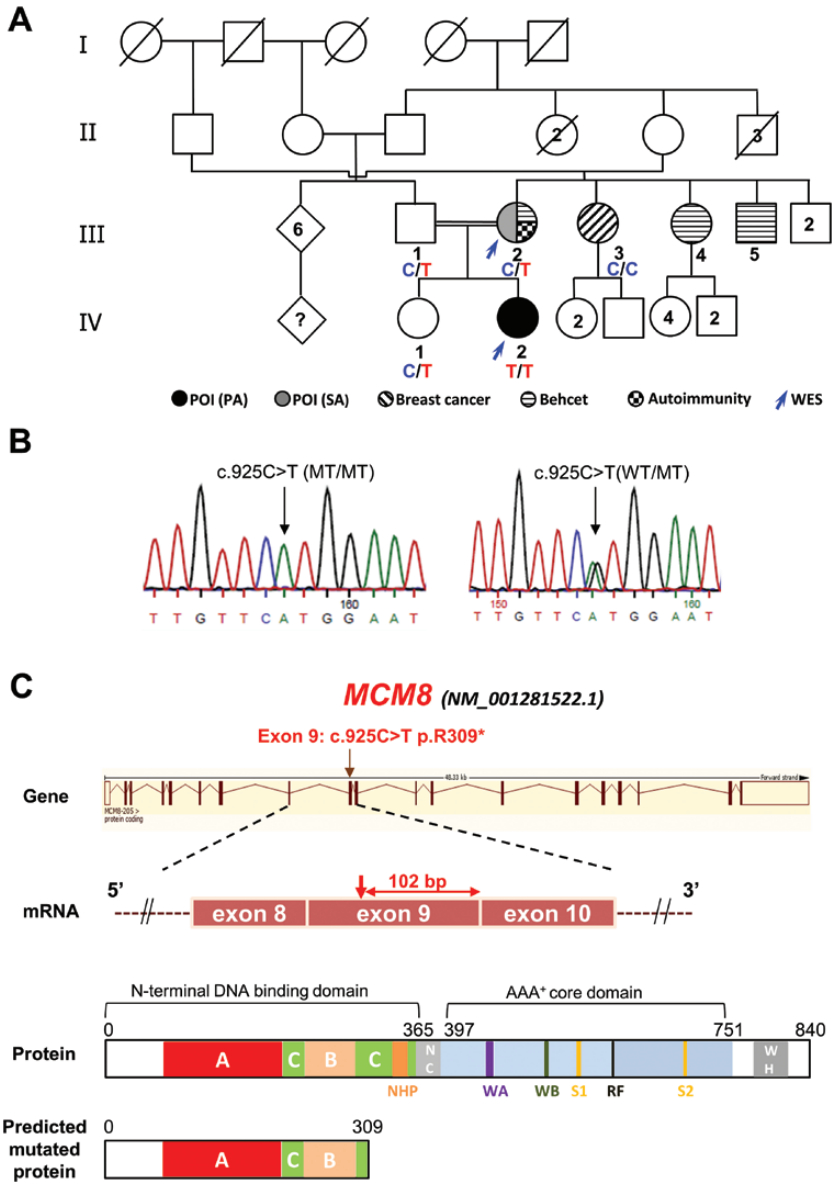

Figure 1. Pedigree of the family with *MCM8* pathogenic variant and molecular analysis. A) Pedigree of the family. Double lines indicate consanguineous unions. The proband and the mother (blue arrows) were analyzed by whole exome sequencing (WES). The genotypes of *MCM8* are indicated: normal wild type (WT) allele C in blue, mutated allele T in red. MCM8, minichromosome maintenance complex component 8; POI, primary ovarian insufficiency. B) Sanger sequencing electropherograms showing the heterozygous (on the left) and homozygous (on the right) MCM8 variant. MT, mutated; WT, wild type. C) Structure of the MCM8 gene, mRNA, and protein and position of the variant. The structure of the region of the transcript surrounding the variant and of the normal protein are shown below the genomic structure, the coding exons represented as colored rectangles (references NM: 001281522.1). According to the "50-bp rule" (26), the pathogenic variant can cause degradation of the corresponding mRNA if it is more than 50 bp upstream from the 3'exon-exon junction. Alternatively, this variant may yield a truncated protein devoid of the 531 C-terminal residues (predicted mutated protein). The different domains are indicated: NHP, N-terminal hairpin; WA, walker A; WB, walker B motifs for ATP hydrolysis; S1, sensor 1; S2, sensor 2; RF, arginine finger; WH, winged helix; N-C N-terminal to C-terminal linker, A, B, and C structural domains. mRNA, messenger ribonucleic acid.

was investigated in Belgium at age 39 years. Hormonal assays confirmed the diagnosis of POI with high FSH (36.3 IU/L) plasma concentration. LH (23 IU/L) plasma level was high and anti-müllerian hormone was low (0.03µg/L). She reported being diagnosed with hypothyroidism at age 12 years in Turkey. A treatment was started, but medication adherence was poor, and she stopped medication, showing no symptoms since the age of 22 years. All TSH and T_4 levels available from age 34 to 46 years were within normal ranges. There were persistent high levels of anti-TPO antibodies (145 IU/mL; normal < 30 IU/mL) (see Table 1). At the age of 46 years, a total thyroidectomy was performed due to a multinodular goiter, and histology confirmed benign lymphocyte thyroiditis. The mother also suffered from Behcet disease and was treated for 1 year with colchicine at age 34 years. One brother (III.5) and 2 sisters (III.3 and III.4) also developed Behcet disease. In addition, her sister III.3 was treated at the age of 46 years for breast cancer with a good clinical outcome.

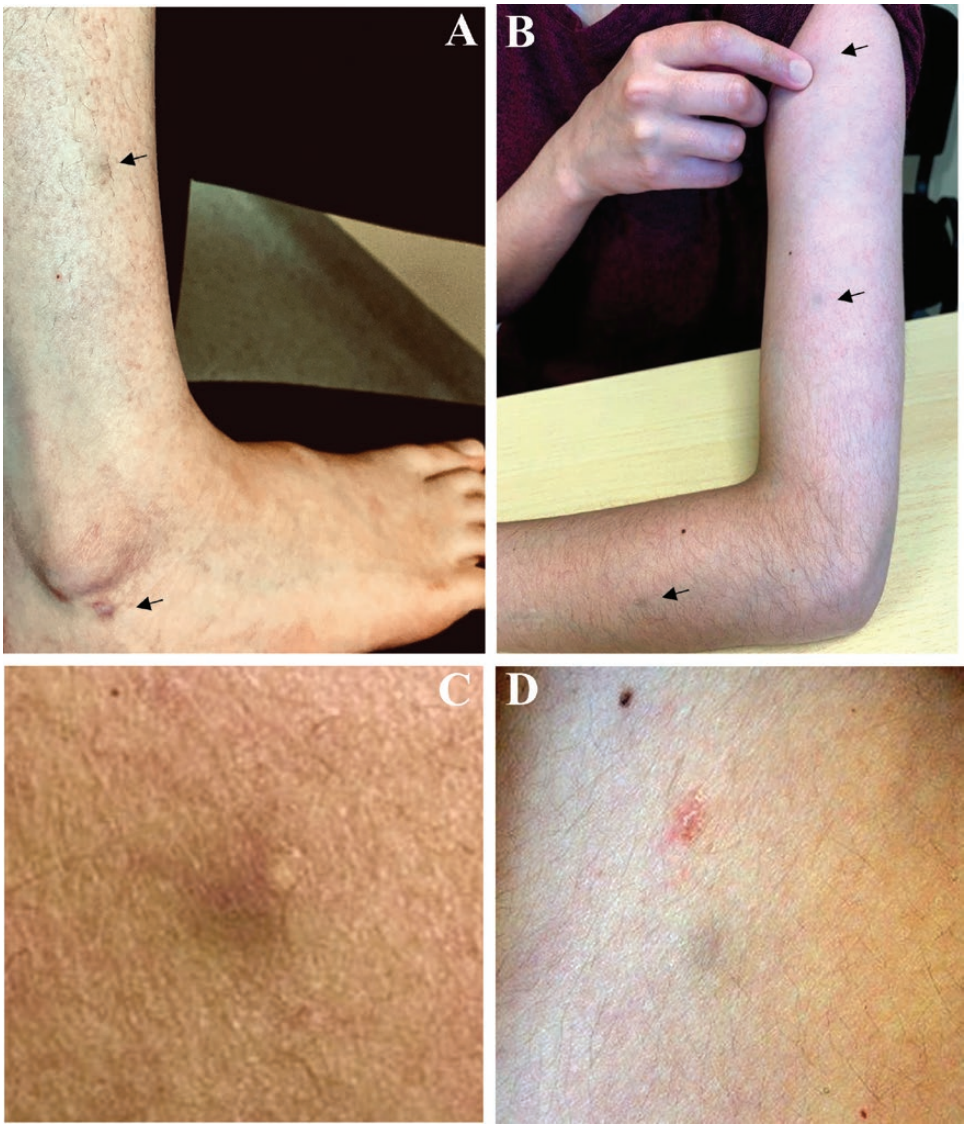

Figure 2. Pilomatricomas in the proposita. Pilomatricomas appear as papular, palpable bluish lesions. A and C: leg and foot. B and D: arm and forearm.

WES and homozygosity mapping

Exome analysis was performed on genomic DNA extracted from the peripheral blood of the proposita (IV.2) and her mother (III.2). Library preparation, exome capture, sequencing, and data processing were performed by IntegraGen SA (Evry, France) according to their in-house procedures (10). Data analysis was performed as described in heddar et al (19). Briefly, exon enrichment was performed on 600 ng of DNA, using the Agilent SureSelect Human All Exons kit version CRE (Agilent Technologies, Santa Clara, USA). Exon-enriched libraries were subjected to a 75 bp paired-end sequencing on a HiSeq2500, according to the manufacturer's protocol. Reads alignment to the human reference genome (GRCh38) and variant calling were performed using the Illumina pipeline (CASAVA 1.8.2). Variant annotation was performed using the Ensembl Variant Effect Predictor Tools. The variants were filtered using SIRIUS, an IntegraGen in-house pipeline platform. Homozygosity mapping of the proband and her mother using HomozygosityMapper was performed (20).

Sanger genomic sequencing

Relevant pathogenic variant detection was confirmed by direct genomic Sanger sequencing of *MCM8* using the followings pair of primers:

5'-TTGCAGAAACCATCCAAAGTAAGT-3'
5'-GAGGTTCAGGAGCATCTTAGAAA-3'

Chromosome breakage analysis

To confirm the functional impact of the *MCM8* nonsense variant, we performed chromosomal breakage studies. Epstein-Barr virus (EBV)–immortalized lymphoblastoid cell lines derived from the patient, her mother, and a healthy woman as control were established at the Institute of Pathology and Genetics, Gosselies, Belgium, following a standard in-house protocol. EBV-immortalized cells were cultured under standard conditions for karyotyping. DNA damage was induced using mitomycin C (MMC, Sigma) added for 72 hours according to the in-house protocol of the laboratory specialized in the diagnosis of Fanconi anemia in our University (Department of Medical Biology and Pathology, Gustave Roussy, Villejuif,

Table 1. Clinical and Biological Studies of the POI Family

Case	Cycles	Age at Evaluation (Years)	BMI (kg/m²) (Height cm/ weight kg)	HC cm (SD)	FSH IU/L	LH IU/L	E2 ng/L	AMH µg/L	InhB ng/L	T nmol/L	17OHP ng/mL	D4 (ng/dL)	DHEA (µg/dL)	PRL ng/mL	TSH mU/L	T4 pmol/L	Anti-TPO IU/mL	Ovarian surface Size R/L (mm)
Proposita	PA	14	14.1 (133/25)	50 (-3)	110.5	23	-	<0.1	<10	0.17	0.18	-	85	-	0.889	16.2	37	not visualized
		15			106	31.6	<5	<0.1	<9.5	0.10		30	104	7.7	1.54	15.8	45	10/14
		21	-	-	59.5	26.6	120	<0.1	-	-	0.40	-	-	-	1.85	15.1	142	-
Sister	Regular menses	22	20.5 149.3/45.7	55 (0)	4.7	5.8	142	1.1	163	0.55	0.66	-	199	19.5	1.51	14.8	<1	-
Mother	SA (29 years)	39	25.7 153.5/60.3	56 (0)	36.3	23.3	74	<0.03	<0.1	<0.69	<30	<30	47	6.92	0.63	16.7	145	18/27

Normal plasma values: AMH: 2.2-6.8 ng/mL; testosterone: 0.4-2 nmol/L; 17OHP: 10-100 ng/dL; D4: 40-410 ng/dL; DHEA: 35-430 ng/dL; PRL: 0.7-25 ng/mL; TSH: 0.3-4.2 mU/L; inhibine B: 10-320 ng/L; T_4: 11.5-22.7 pmol/L; anti-TPO antibodies: N < 35 IU/mL. Respectively, follicular, ovulatory, luteal phases and menopause: FSH (IU/L): (2.9-12), (6.3-24), (1.5-7), (17-95); LH: (IU/L) (1.5-8), (9.6-80), (0.2-6.5), (8-33); E2 (ng/L): (19.5-144.2), (63.9-356.7), (55.8-214.2), (≤ 32.2).

Abbreviations: 17OHP, 17 hydroxyprogesterone; AMH, anti-müllerian hormone; BMI, body mass index; D4, delta4 androstenedione; DHEA, dehydroepiandrosterone; E2, estradiol; FSH, follicle-stimulating hormone; HC, head circumference; InhB, inhibine B; LH, luteinizing hormone; P, progesterone; PA, primary amenorrhea; PRL, prolactin; SA, secondary amenorrhea; T, testosterone; T_4, thyroxine; TPO, thyroid peroxidase; TSH, thyrotropin; US, ultrasonography.

France) (10). For each sample, 3 conditions were tested: without MMC (to analyze spontaneous damage), with 10^{-7} M MMC, and with 10^{-6} M MMC. Chromosomal breakages and rearrangements were scored by an experiment cytogeneticist on at least 20 metaphases.

Results

Exome sequencing and Sanger sequencing results

Exome sequencing was performed on peripheral blood DNA from patient IV.2 and III.2, her mother (Fig. 1A). All exome metrics are summarized in Table 2. Briefly, across the 2 exomes, we obtained an average of 6 Gb of sequences with more than 99% of mappable reads and a mean depth of 75x. More than 90% of bases were covered to a minimum depth of 25x, and the Q score was above 30 for most reads (>95%). First, variants with coverage less than 5x and/or Q score below 20 were filtered out. Then, variations were filtered in the last updates of the following human variation database using a minor allele frequency (MAF) of 1%: 1000 Genomes; ExAC, EVS and GnomAD (exome and Genomes). In this consanguineous family, a recessive genetic transmission was expected with pathogenic variants homozygous in the patient and heterozygous in the mother. Among a total of a 45212 variants sequenced with a coverage of at least 5X, 111 have an MAF < 1%, are located in coding sequences or in splicing regions, fit the criteria of pathologic variant using in silico prediction tools with Varsome website, and fulfill an autosomal recessive transmission (Table 3 and in heddar et al (21) among the 111 variants, 36 variants can be excluded because of their presence in our control in-house database of fertile women. Of the 75 remaining variants, 31 are synonymous variants without impact on splicing, and 28 correspond to false positives by careful examination of the BAM. Five out of the 16 variants are ruled out with M-CAP and CADD scores. Therefore, the 11

Table 2. Whole Exome Sequencing Metrics in the Family With *MCM8* Mutation

WES Metrics	Mother (III0.2)	POI (IV0.2)
Gbases	6.4	5.6
Number of reads (millions)	9.6	8.4
% Alignment	99.2	99.3
% Mismatch rate R1	0.16	0.16
% Mismatch rate R2	0.37	0.35
% ≥Q30 bases	95.5	95.6
Mean quality score	39	39
Mean depth (X)	77.7	72.1
% of bases covered at 25X	92.6	90.8

Abbreviations: POI, primary ovarian insufficiency; WES, whole exome sequencing.

Table 3. Whole Exome Sequencing Variant Filtering in the Family With *MCM8* Mutation

Variant Filters	Number of Variants
Filter "PASS"	45515
Minimum depth at variant ≥ 5	45212
Homozygous in patient and heterozygous in mother	5381
In protein coding gene	2236
In coding sequence or splice	2005
MAF < 1% (ExAC)	111
Not homozygous in fertile controls	75
Not synonymous	44
Careful examination with IGV	16
CADD > 20, MCAP > 0.025	**11**

Abbreviations: CADD, combined annotation-dependent depletion; ExAC, variant database; IGV, integrative genomics viewer; MAF, minor allele frequency; MCAP, Mendelian clinically applicable pathogenicity; PASS, variants with satisfactory Next Generation Sequency quality criteria (Q score > 20).

remaing variants are detailed in heddar et al (21). The only relevant gene with a deleterious impact variant and a known role in ovarian function according to existing animal model and pathophysiology in humans is the *MCM8* variant. There is no other homozygous pathogenic variant in another gene in tandem that could explain the patient's extraovarian phenotype (intrauterine growth retardation, small stature, and pilomatricomas). The *MCM8* variant was a nonsense variant in exon 9 of *MCM8* (NM_001281522.1:c0.925C > T), yielding either a truncated protein p.R309* deleted of the last 531 residues of *MCM8* or a degradation of the corresponding transcript by a nonsense-mediated mRNA decay (NMD) (Fig. 1b and 1c). This nonsense variant was recorded in dbSNP as rs201115244. It was absent in all population variant databases (1000 Genomes Project, ESP6500, ExAC) but was reported in one male and one female at the heterozygous state in the GnomAD data base. The MAF was of 8.13e-5 (2/246146 alleles).

Sanger genomic sequencing in the family confirmed the presence of the nonsense variant (c0.925C > T; p. Arg309*), homozygous in the patient and heterozygous in both parents. The healthy older sister was heterozygous for the allelic variant. The maternal aunt (III.3) who developed breast cancer inherited both wild-type alleles (Fig. 1A).

Autozygosity mapping has been performed in the patient and her mother using HomozygosityMapper (20). As shown in heddar et al (21), various homozygous regions can be highlighted in several chromosomes in the patient, with MCM8 being located on chromosome 20. By contrast, no homozygous regions are found in the mother except in chromosome 4, with a low score and not shared in her daughter (21). Furthermore, as shown in heddar et al (21), no relevant homozygous pathogenic variant could be detected in the mother. This confirms that the mother does not originate form a consanguineous marriage and excludes a pseudodominance mode of inheritance.

Chromosomal instability studies

EBV-immortalized cell lines derived from the patient, her mother, and a healthy control were exposed to MMC to study chromosomal instability. No spontaneous chromosomal breakage was observed in the 3 samples in the absence of MMC. At 10^{-7} M MMC, few breakages were observed in the patient's cells but not in the mother's cells and the control's cells. At 10^{-6} M, the patient's cells showed 2 times more breaks per cell when compared with her mother's cells. The mother's cells exhibited 5 times more breaks per metaphase when compared with the control's cells. Furthermore, more aberrant metaphases were observed in the patient's cells compared with her mother's cells and with the control's cells. (Fig. 3A).

Discussion

Recent advances in the genetics of POI with the use of WES have highlighted a growing role for meiosis and DNA repair genes, including *MCM8/9* (6, 7). However, these genes have been identified most often in single or rare families. Minichromosome maintenance (MCM) proteins are a group of 10 highly conserved proteins involved in DNA replication (22). MCM8 and MCM9 form a heterodimer that has an important role in DNA replication, homologous recombination, DNA double-strand break repair, and also has recently been implicated in mismatch repair (MMR) (22–25).

In the present study, we identified by WES a novel nonsense (c0.925C > T; p.R309*) variant in exon 9 of the *MCM8* gene in a Turkish consanguineous family with POI. This novel variant may yield a truncated protein deleted of the 531 C-terminal residues or a degradation of the corresponding mRNA by NMD. According to the "50-bp rule" established in 1998 (26) and confirmed by large transcriptomic analysis in humans (27), the aberrant mRNA should undergo NMD if the stop codon is located more than 50 to 55 nucleotides upstream of the 3'exon-exon junction. As shown in Figure 1, the nonsense variant of *MCM8* detected in our patient is located 102 bp upstream of the exon 9/exon10 junction. Therefore, it is very likely that the aberrant transcript would be degraded by NMD.

In our study, we show that EBV-derived lymphoblastoid cells from the proband exhibited higher MMC-induced breaks per cell with complex rearrangements when compared with a control. These findings

A

	Aberrant Metaphase			Breaks/ Metaphase			Breaks/ Aberrant Metaphase		
	Untreated	10^{-7}M	10^{-6}M	Untreated	10^{-7}M	10^{-6}M	Untreated	10^{-7}M	10^{-6}M
POI Patient	0/50	4/50 (4%)	**15/19 (79%)**	0/50	4/4 (1)	**40/19 (2.11)**	0/50	4/4(1)	**40/5(2.66)**
Mother	0/50	2/50 (2%)	**13/19 (68%)**	0/50	2/2 (1)	**20/20 (1)**	0/50	2/2(1)	**20/13(1.54)**
Control	0/50	1/50 (1%)	**3/19 (16%)**	0/50	1/1(1)	**4/19 (0.211)**	0/50	1/1(1)	**4/3(1.33)**

B

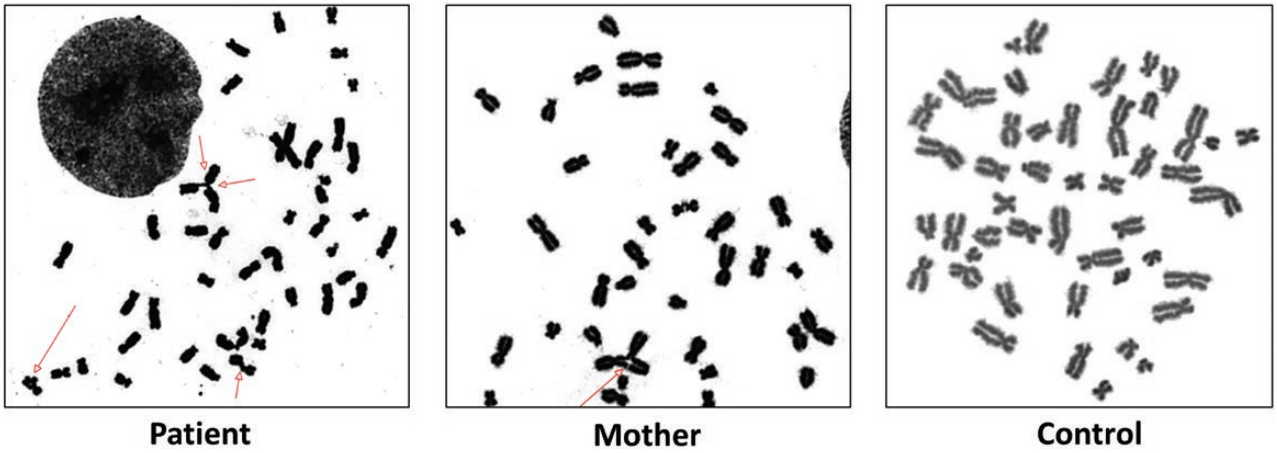

Figure 3. Chromosomal instability studies. (A) Spontaneous and mitomycin C-induced chromosomal breakage in lymphoblastoid cell lines from the patient, her mother, and a healthy control. A minimum of 20 metaphases were scored for each sample. (B) Examples of MMC-treated metaphases from the patient, her mother, and a control with 10^{-6} M MMC. Arrows indicate breaks and chromosomal rearrangements. MMC, mitomycin; POI, primary ovarian insufficiency.

support a causative role for the *MCM8* variant in the POI of the proband.

Until now, 4 *MCM8* biallelic homozygous pathogenic variants impairing MCM8 function have been described in 3 consanguineous families (7, 28, 29). In all cases, the phenotype described was primary amenorrhea with lack of pubertal development. When available (2/3 cases), ultrasonography revealed an infantile uterus with streak gonads. MMC-induced chromosomal instability was found in cells derived from the patients (7, 28, 29). The reproductive phenotype in our patient is in line with the previously reported patient with primary amenorrhea lack of pubertal development, and streak ovaries. In one family, hypothyroidism was noted (7). However, the main difference with previous observations is that our patient had a syndromic POI, including small stature and multiple pilomatricomas. Interestingly, in families harboring MCM9 pathogenic variants (6, 30, 31) syndromic POI with short stature was also observed (6). Since MCM8 and MCM9 form a stable complex and the absence or low concentration of 1 of the 2 partners destabilizes the heterodimer (32), similar functional consequences are expected. The birth weight of the older sister is 2500 g according to the mother, and therefore she could be considered born small for gestational age. However, we have no information on the exact term of pregnancy in Turkey and no medical information could be obtained. Furthermore, the postnatal growth of the sister was normal as her final height is similar to her mother's (153 cm). On the contrary, the patient has a growth curve lower than that of her sister, and she catches up to the familial height only after GH therapy and pubertal induction. It is known that short stature is associated with other DNA repair disorders and especially with POI with MCM9 defects (6). Furthermore, our patient developed multiple pilomatricomas in childhood. Those are benign tumors derived from hair follicles, either isolated or in multiple forms (33). Multiple pilomatricomas are often associated with other syndromes such as Gardner or Turner syndromes (33-35). We ruled out both affection with normal karyotype and rectosigmoidoscopy, in addition to a careful examination of all *APC* variants detected in the patient (Table 4). Interestingly, constitutional mismatch repair deficiency, a rare disease related to biallelic germline mutations in MMR genes (*MLH1, MSH2,MSH6 or PMS2)*, was described in 4 patients

Table 4. Variants in *APC* Detected by Whole Exome Sequencing in the Patient and Her Mother (NM_000038.5)

Genomic Position (hg38)	Ref	Var	SNP Reference	Type	cDNA	Protein	Mother	Patient	SIFT	PolyPhen	1000G MAF	ExAC MAF
chr5:112839543	G	C	rs1801166	Missense	c0.3949G > C	E1317Q	GG	GC	Tolerated (0.06)	Benign (0.009)	0.0030	0.004126
chr5:112841059	T	A	rs459552	Missense	c0.5465T > A	V1822D	AA	AA	Tolerated low Confidence (0.5)	Benign (0)	0.1346	0.798
chr5:112827157	T	C	rs2229992	Synonymous	c0.1458T > C	Y486Y	TC	TC	.	.	0.4900	0.577
chr5:112828864	G	A	rs351771	Synonymous	c0.1635G > A	A545A	GA	AA	.	.	0.3339	0.648
chr5:112840073	G	A	rs41115	Synonymous	c0.4479G > A	T1493T	GA	AA	.	.	0.3345	0.648
chr5:112840628	G	A	rs42427	Synonymous	c0.5034G > A	G1678G	GA	AA	.	.	0.3333	0.650
chr5:112840859	G	A	rs34506289	Synonymous	c0.5265G > A	A1755A	GG	GA	.	.	0.0042	0.004472
chr5:112840862	T	G	rs866006	Synonymous	c0.5268T > G	S1756S	TG	GG	.	.	0.3331	0.650
chr5:112841474	G	A	rs465899	Synonymous	c0.5880G > A	P1960P	GA	AA	.	.	0.3335	0.648
chr5:112783896	A	G	rs111933129	Intronic	c0.645 + 2993A > G	.	AG	AG	.	.	0.0246	.
chr5:112822734	G	A	rs2545162	Intronic	c0.1408 + 743G > A	.	GA	AA	.	.	0.3209	.

Frequency of each variant in 1000 Genomes (1000G) and ExAC databases is reported.
Abbreviations: cDNA, complementary deoxyribonucleic acid; MAF, minor allele frequency; Ref, reference base; SIFT, sorting intolerant from tolerant; SNP, single nucleotide polymorphism; Var, variant base.

displaying multiple pilomatricomas (33, 36, 37). As MCM8 is also involved in the MMR pathway, in the same way, biallelic alterations of *MCM8* are also expected to be associated with pilomatricomas. This observation therefore corresponds to a novel phenotype associated with *MCM8* variant and illustrates the phenotypic variety of patients carrying mutations of this gene.

SGA or IUGR is a frequent manifestation found in some chromosomal breakage syndromes like Bloom syndrome or Fanconi anemia, as in our patient. Our observation, combined with previous reports, confirms that DNA damage is a driver for growth delay and may explain IUGR in chromosome instability syndromes (38). GH therapy is used in short children born SGA without catch-up growth. They correspond to a heterogeneous group. Currently available data do not indicate any increase in cancer risk during or after GH therapy (39). However, in some disorders such as in chromosomal breakage syndromes and DNA repair disorders, GH therapy is contraindicated because patients have an increased risk to develop cancer at a younger age. In these cases, GH may stimulate malignant cell growth (the SAGhE European cohort study) (39). Our patient, SGA, was treated during adolescence with GH without knowing that she had chromosomal instability. This may not be uncommon. Similar to recommendations for other chromosomal breakage syndromes such as Bloom syndrome (17, 38), our observation highlights the necessity to perform genetic analysis of *MCM8* before starting GH therapy in adolescents presenting with POI with small stature and normal karyotype in a familial or consanguineous context (17, 38).

The heterozygous mother developed secondary amenorrhea at the age of 29 years. In the mother, MMC induced an intermediate but significant increase of chromosomal breaks when compared with a control, and complex chromosomal rearrangements were observed (Fig. 3). In previously reported families with pathogenic *MCM8* or *MCM9* pathogenic variants, altered DNA repair ability was also observed in the heterozygous mothers, but they did not develop POI (6, 7, 28, 29). In this observation, the mother presented apparently transient hypothyroidism related to autoimmune thyroiditis and Behcet disease treated by colchicine. The presence of these environmental factors may explain the difference with the reported heterozygous carriers of *MCM8* pathogenic variants. Indeed, these factors, especially autoimmunity, might have played an additive role to alter ovarian function. Such heterozygous carriers of *MCM8* pathogenic variants should have fertility preservation to avoid precocious depletion of the follicular pool, particularly in the presence of other environmental factors.

Mice deficient in *Mcm8* or *Mcm9* developed ovarian tumors or hepatocellular carcinoma (32, 40). This is expected as both proteins are involved in DNA repair and MMR. However, the link between *MCM8-9* variants and tumor/cancer is unclear in humans. The screening of a large Australian cohort of more than 100 patients with Lynch-like syndrome failed to identify clearly pathogenic variants of *MCM9* (41). Furthermore, a homozygous nonsense *MCM9* variant was found in 2 sisters with POI and hereditary colorectal cancer (31). However, the 2 sisters also harbored 2 compound heterozygous variants of *MUTYH*, a known gene causing colorectal cancer (31). Our patient had pilomatricomas. This is the first report of tumors associated with *MCM8/9* pathogenic variants in humans. Long-term monitoring of such patients should be discussed in a multidisciplinary team.

In conclusion, we report a novel phenotype associated with a homozygous mutation of *MCM8* combining POI, IUGR, postnatal short stature, and multiple pilomatricomas. This observation has wide implication in the management of patients with POI and SGA and/or small stature. Mutations of *MCM8* should be searched before starting GH therapy in such cases, in a familial or consanguineous context, and similarly to other chromosomal breakage syndromes. On the other hand, heterozygous carriers of pathogenic *MCM8* variant may develop POI with SA in the presence of additional exogenous factors. An appropriate management and eventual fertility preservation have to be discussed. A long follow-up of such patients may contribute to better estimate cancer risk in this population and to draw clinical guidelines.

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