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MCM9 compound heterozygosity in an adolescent with premature ovarian insufficiency

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Summary

Delayed puberty in girls is often related to late maturation but is occasionally the first sign of premature ovarian insufficiency (POI). POI is a condition that affects ovarian function and fertility, and its etiology is unknown in most cases. Genetic factors have recently been identified in 20–25% of women with POI, involving genes that regulate various aspects of ovarian development and maintenance. We report a case of delayed puberty due to POI in an adolescent from a non-consanguineous family who carried two variants in the *MCM9* gene. MCM9 is essential for DNA replication and repair, and its dysfunction can lead to chromosomal instability and ovarian failure. Our case highlights the importance of targeted gene panel analysis, particularly in POI patients with negative autoimmunity screening, and evidence of ovarian or uterine dysgenesis on pelvic imaging.

Learning points

- Delayed puberty in girls is often self-limiting, but it can also indicate underlying conditions with lifelong implications, such as premature ovarian insufficiency (POI).
- Patients with POI, negative autoimmune screening, a normal karyotype, and no FMR premutation should undergo further genetic testing, preferably through targeted gene panels.
- Compound heterozygous variants in *MCM9* can cause POI, presenting with delayed puberty and primary amenorrhea in girls without a consanguineous family.

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Background

Delayed puberty in girls is defined as the absence of breast development by 13 years of age or menarche by 15 years of age. Although delayed puberty is often self-limiting and merely a sign of late sexual maturation, it can occasionally be the first sign of an underlying condition with lifelong implications, such as premature ovarian insufficiency (POI) (1). POI is defined by amenorrhea for more than 4 months before age 40, excluding pregnancy, and elevated levels of serum follicle-stimulating hormone >25 U/L, confirmed by at least two blood draws >4 weeks apart. POI encompasses a wide range of phenotypes, including ovarian dysgenesis, primary amenorrhea (PA), secondary amenorrhea (SA), premature ovarian failure, and premature menopause (2).

The rate of follicular atresia in POI can be influenced by various factors such as iatrogenic interventions, autoimmune disease, metabolic disease, infections, and genetic defects involved in folliculogenesis or steroid hormone synthesis and response. A genetic predisposition in idiopathic cases is suggested by the high incidence of familial aggregation, which may account for up to 20–25% of cases (2). Given the heterogeneous genetic basis of POI, involving pathogenic variants in multiple genes, molecular genetic analysis of gene panels is crucial in the diagnostic workup. Genome-wide association studies have identified gene loci associated with meiosis or DNA repair pathways, such as minichromosome maintenance 8 (*MCM8*) and minichromosome maintenance 9 (*MCM9*), which may play a role in ovarian aging (3). Several studies reported homozygous and compound heterozygous variants in *MCM8* and *MCM9* genes in patients with POI, displaying phenotypic variations that range from PA to SA, and exhibiting short to normal stature (4, 5). Homozygous variants are hitherto exclusively found in consanguineous families of Arab, Turkish, or Jewish origin (6, 7).

This case report describes a 16-year-old girl from a non-consanguineous Belgian, white family who presented with delayed puberty and PA due to POI caused by compound heterozygous variants in *MCM9*. This case underscores the significance of genetic testing in evaluating idiopathic POI, particularly in patients with negative autoimmune screening and findings of atrophic ovaries or a hypotrophic uterus on pelvic imaging.

Case presentation

A 16-year-old girl presented to our pediatric endocrine clinic with delayed puberty. She exhibited an absence of pubic hair, no breast development, and no vaginal discharge. There were no symptoms of galactorrhea, hirsutism, hot flashes, or mood swings. The patient denied recent weight changes or monthly abdominal pain. Her medical history was uneventful, and she was not taking any medications. Family history revealed that both parents had a constitutional delay of puberty (her mother experienced menarche at 14 years, and her father received intramuscular testosterone treatment). Additionally, her sister had only started breast development at the age of 13.5. The parents were of Belgian origin, white descent, unrelated, and had normal height (mid-parental height *Z* score of −1.25). The family history was negative for autoimmune or endocrine disorders, early menopause, or fragile X syndrome.

On physical examination, her weight was 46.5 kg (*Z* score: −1.5), height 157.9 cm (*Z* score: −1.3), and blood pressure 119/66 mm Hg. She was at Tanner stage I for both breast and pubic hair development. Palpation of the thyroid gland revealed no abnormalities. Skin pigmentation and nail appearance were normal, and we observed no dysmorphic features.

Investigation

Previous hormonal analysis indicated elevated levels of follicle-stimulating hormone (81.1 IU/L, reference range (RR): 3.5–12.5 IU/L) and luteinizing hormone (29.4 IU/L, RR: 2.6–12.6 IU/L), low estradiol levels (<25 ng/L, RR: 31–90 ng/L), and decreased levels of antimüllerian hormone (AMH; <0.03 µg/L, RR: 0.66–8.4 µg/L) and inhibin B (<10 ng/L, RR: <100 ng/L). Serum prolactin, thyroid stimulating hormone, and fT4 levels were within normal ranges. Repeat laboratory testing confirmed hypergonadotrophic hypogonadism. Screening for anti-ovarian, anti-21-hydroxylase, anti-thyroid peroxidase, and anti-parietal cell antibodies yielded negative results. Skeletal maturation was delayed, with a bone age of 12 years at a chronological age of 16 years. A pelvic MRI showed a hypotrophic uterus and atrophic ovaries. The patient's CGH microarray and *FMR1* triplet repeat analysis were both normal.

Genetic testing through a POI gene panel (see Methods section) identified two variants in *MCM9*: a heterozygous frameshift variant NM_017696.3:c.2237_2238dup, p.(Phe747Ilefs*78) and a heterozygous nonsense variant NM_017696.3:c.820C>T, p.(Gln274*). According to the latest American College of Medical Genetics and Genomics (ACMG) guidelines, both *MCM9* variants were classified as likely pathogenic (PVS1 + PM2) (8) (Table 1).

The c.820C>T variant introduces a premature stop codon at position 274, whereas the c.2237_2238dup variant results in an amino acid change at position 747, followed by a frameshift and a stop codon after 78 amino acids. As a result, the nuclear localization signals situated in the C-terminal domain of the protein will be disrupted and subsequently lead to a dysfunctional protein (9). Based on this, c.2237_2238dup can be considered a null variant, and hence the ACMG category PVS1 can be utilized.

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Table 1 Variant features of MCM9:c.820C>T and MCM9:c.2237_2238dup.

	MCM9:c.820C>T	MCM9:c.2237_2238dup
dbSNP	rs1297657246	Not referenced
gDNA	g.119238810G>A	g.119137181_119137182dup
pNomen	p.(Gln274*)	p.(Phe747Ilefs*78)
Coding effect	Nonsense	Frameshift
Allele frequency	0.000005576	0.000001290
ACMG criteria	PVS1, PM2	PVS1, PM2
ACMG pathogenicity class	Likely pathogenic (class 4)	Likely pathogenic (class 4)

Allele frequency: frequency of variant in all genetic ancestry groups combined in gnomAD v4.1.0 database; dbSNP, SNP ID number in NCBI dbSNP database; gDNA, genomic position of variant; pNomen, predicted protein change.

Parental segregation analysis showed that the c.2237_2238dup variant was inherited paternally and the c.820C>T maternally, which confirms the compound heterozygosity in the index case. Moreover, a heterozygous, known pathogenic maternal variant in *FSHR*, NM_000145.4:c.1222G>T, p.(Asp408Tyr), was detected (Fig. 1).

Methods

Exome library preparation and sequencing

DNA is enzymatically fragmented with the NEBNext® Ultra™ II FS DNA Library Prep Kit according to the manufacturer's instructions and modified to obtain an Illumina-compatible DNA library. Genes of interest are captured with the IDT xGenTM Exome Hyb Panel v2 and xGen™ Custom Hyb Panel-Accel v1. The captured fragments are then sequenced on an Illumina NovaSeq 6000 machine.

Exome data analysis

The sequencing reads are aligned to the human reference genome hg19 with BWA-mem. The aligned reads are sorted and quality-controlled with samtools. Duplicate reads are marked with Picard. The reads are further optimized by GATK and undergo a quality control with Picard. The coverage in the sequenced regions is determined using samtools and an in-house developed R script. Variant calling is performed with GATK. Variant annotation and filtering are done with the Highlander software v16.1 (https://sites.uclouvain.be/highlander/).

POI gene panel

The POI gene panel contains the coding regions and 14 base pair (bp) flanking intronic sequences of the following genes: *AFF2, AMH, AMHR2, BMP15, DACH2, DAZL, DIAPH2, DMC1, ESR1, FIGLA, FMR1, FOXL2, FOXO1, FOXO3, FSHR, GDF9, GPR3, HFM1, INHA, LHCGR, MCM8, MCM9, MSH5, NOBOX, NR5A1, PATL2, PGRMC1, POF1B, SOHLH1, SOHLH2, STAG3, TGFBR3, TUBB8,* and *XPNPEP2*.

Limitations of the POI gene panel analysis

Larger genomic deletions and duplications, structural variants, repeats, deep intronic variants, and variants in the promoter regions are not detected with this test.

Treatment

Pubertal induction was initiated with estradiol valerate 0.5 mg daily, incrementing progressively to 2 mg daily until Tanner's stage 4 breast development and a mature uterus on sonography were achieved.

Outcome and follow-up

Our patient was also referred to a fertility specialist to explore potential options for future reproduction. After counseling, it was decided that oocyte cryopreservation would not be offered due to her low AMH levels.

Discussion

We present a rare case of an adolescent girl presenting with delayed puberty due to POI caused by heterozygous compound mutations in *MCM9*. Recently, studies in POI have identified causal mutations in genes involved in human meiotic recombination, such as Helicase For Meiosis 1 (*HFM1*), MutS Homolog 4 (*MSH4*), MutS Homolog 5 (*MSH5*), *MCM8*, and *MCM9* (3). *MCM8* and *MCM9* are members of the minichromosome maintenance family of genes (*MCM2-9*). Both genes are considered to ensure oocyte quantity and quality by homologous recombination-mediated repair during meiosis. Other processes that require their involvement are fork progression during replication and mismatch repair post-replication. To fulfill their function, MCM8 and MCM9 must operate in the MCM8/9 heterohexameric complex. What sets MCM9 apart from MCM8 and other MCM proteins is its distinctive and long C-terminal domain (CTD), encompassing approximately 530 amino acid residues. This CTD likely acts as a regulatory domain, facilitating protein–protein interactions (such as recruiting RAD51)

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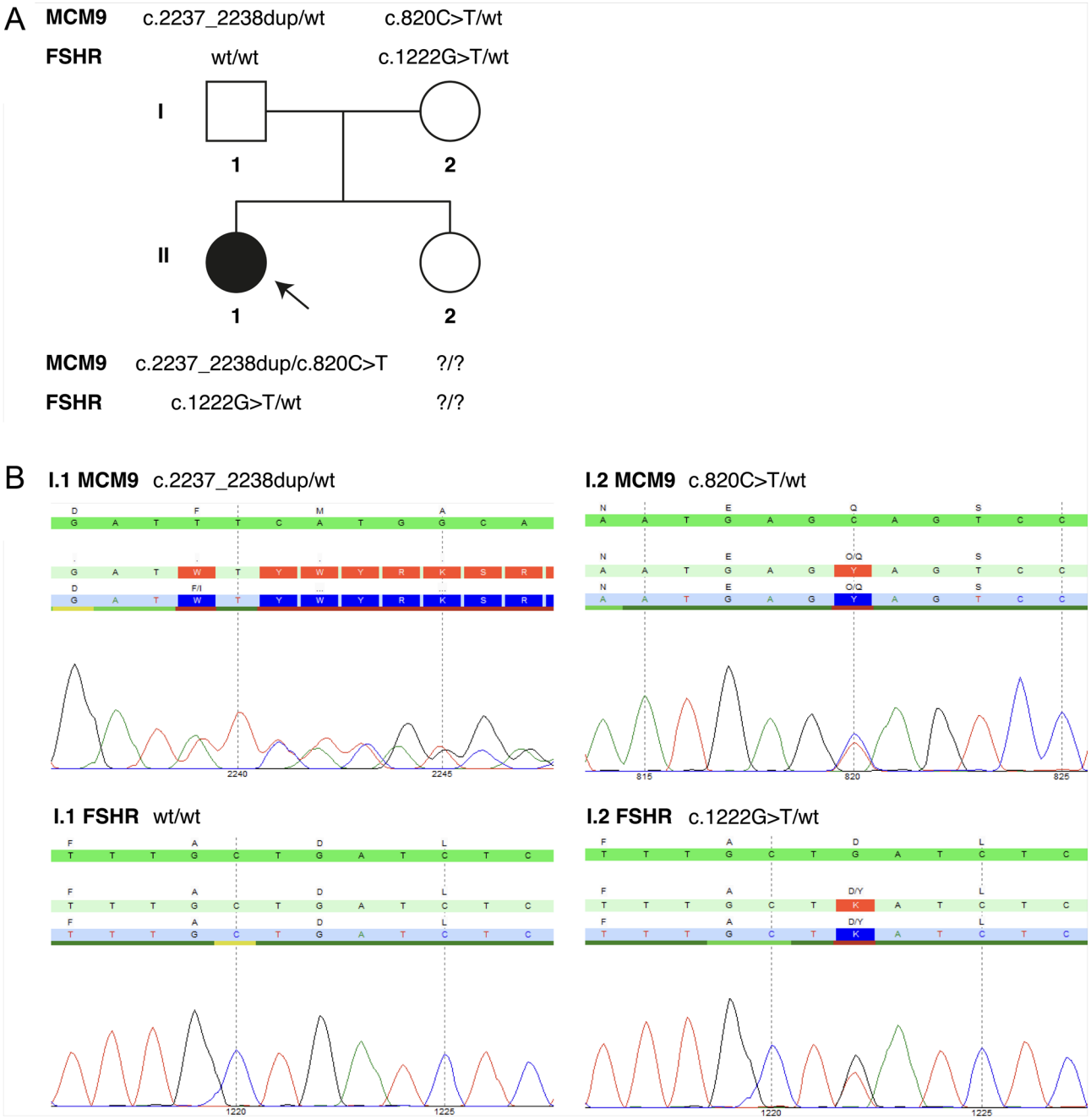

Figure 1

Pedigree and segregation analysis. (A) Pedigree of the family. White shapes, unaffected; black shapes, affected; squares, males; circles, females. The arrow indicates the index case. The genotypes of the MCM9 and FSHR variants are shown. (B) Electropherograms of the identified variants in the parents of the index case. WT, wild-type.

crucial to MCM9's function. Nonsense mutations that result in MCM9 truncation or complete deletion have primarily been associated with infertility. Apart from the CTD, most missense mutations are found within the ATPase domain of *MCM8* and the DNA-binding domain of *MCM9*, which negatively impact their respective activities during replication or repair processes (10). In our patient, the identified pathogenic variants were in exon 4, near the DNA binding domain, and in exon 12 within the CTD, and were not reported before. These novel variants in *MCM9* result in the generation of a premature stop codon. Nonetheless, it remains uncertain whether these variants lead to a truncated, dysfunctional MCM9 protein or if the MCM9 mRNA will undergo degradation through nonsense-mediated mRNA decay.

Several mouse studies have demonstrated arrested follicular development in *Mcm8* knockout mice and germ cell depletion in *Mcm9* knockout mice. Besides their role in gonadal development, mouse models have confirmed that *Mcm9* knockout mice have an increased risk of hepatocellular carcinoma in males and ovarian tumors in females. Individuals affected with variants in these double-strand break repair genes have chromosomal instability and an increased risk for tumor development in somatic cells, namely in fibroblasts and T lymphocytes exposed to DNA cross-linking agents (11).

In human studies, mutations in *MCM9* have been implicated in causing hypergonadotrophic hypogonadism. In general, variants in *MCM9* have an autosomal recessive inheritance pattern, as most causative *MCM9* variants are reported in either a homozygous or compound heterozygous state (4, 5). For monoallelic variants, Guo *et al.* hypothesized that there could be a dosage-dependent effect with heterozygous variations causing SA owing to residual functional MCM9 (5). However, their study did not include exome or gene panel sequencing to rule out potentially causative variants in other genes associated with POI, and the scope of their functional analysis was too restricted to definitively ascertain the role of MCM9 haploinsufficiency in POI.

Most of the patients described with biallelic variations were siblings from consanguineous, Middle Eastern, or Turkish families presenting with PA (6, 7). In our patient, the POI gene panel revealed three mutations. First, we found compound heterozygous variants in *MCM9*, a c.820C>T nonsense variant and a c.2237_2238dup frameshift variant in *MCM9*. Both variants are classified as likely pathogenic (ACMG class 4) since they result in a premature stop codon (PVS1) and have an extremely low frequency in the gnomAD population database (gnomAD v4.1.0) (PM2) (Table 1) (8). Secondly, a heterozygous c.1222G>T variant was present in *FSHR*. This known variant has been classified as pathogenic (ACMG class 5) because of supportive *in vitro* and *in vivo* evidence of its damaging effect, its location in a mutational hot spot, and its absence from controls (6). Inheritance of *FSHR*-related POI is nevertheless recessive, indicating that the heterozygous pathogenic c.1222G>T variant is most likely not implicated in the patient's phenotype. Apart from early menopause due to decreased ovarian follicles, *MCM9* variants have been linked to infantile uteri and/or invisible or small ovaries, delayed puberty and bone age, osteoporosis, and occasional short stature (6, 7). Except for normal stature, our patient resembled other cases with delayed puberty, atrophic uterus and ovaries on pelvic MRI, and a delayed bone age. Measurement of bone mineral density to screen for osteoporosis was not performed.

Moreover, the relationship in humans between *MCM9* germline mutations and predisposition to tumors caused by microsatellite instability has still been elusive. Several studies and case reports describe homozygous *MCM9* pathogenic variant carriers with either colonic polyposis or colorectal carcinoma (7). More genetic studies are required to determine the correlation between (compound) heterozygous variants in *MCM9* and penetrance of tumor risk. As our patient is still an adolescent, we plan to start surveillance for colorectal and cervical cancer from the age of 18 years.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Patient consent
Written informed consent for publication of their clinical details was obtained from the patient.

Author contribution statement
EN wrote the original draft. MDV, AG, BJHD, FH, AVK, SV, JV, IG, JDS, and WS supervised the writing. SV and WS treated the patient. All authors approved the final manuscript. The authors want to thank Dr. Maartje Nielsen (Leiden University Medical Center LUMC, The Netherlands) for expert advice on cancer surveillance.

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