XX Ovarian Dysgenesis Is Caused by a *PSMC3IP/HOP2* Mutation that Abolishes Coactivation of Estrogen-Driven Transcription

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XX female gonadal dysgenesis (XX-GD) is a rare, genetically heterogeneous disorder characterized by lack of spontaneous pubertal development, primary amenorrhea, uterine hypoplasia, and hypergonadotropic hypogonadism as a result of streak gonads. Most cases are unexplained but thought to be autosomal recessive. We elucidated the genetic basis of XX-GD in a highly consanguineous Palestinian family by using homozygosity mapping and candidate-gene and whole-exome sequencing. Affected females were homozygous for a 3 bp deletion (NM_016556.2, c.600_602del) in the *PSMC3IP* gene, leading to deletion of a glutamic acid residue (p.Glu201del) in the highly conserved C-terminal acidic domain. Proteasome 26S subunit, ATPase, 3-Interacting Protein (PSMC3IP)/Tat Binding Protein Interacting Protein (TBPIP) is a nuclear, tissue-specific protein with multiple functions. It is critical for meiotic recombination as indicated by the known role of its yeast ortholog, Hop2. Through the C terminus (not present in yeast), *PSMC3IP* also coactivates ligand-driven transcription mediated by estrogen, androgen, glucocorticoid, progesterone, and thyroid nuclear receptors. In cell lines, the p.Glu201del mutation abolished PSMC3IP activation of estrogen-driven transcription. Impaired estrogenic signaling can lead to ovarian dysgenesis both by affecting the size of the follicular pool created during fetal development and by failing to counteract follicular atresia during puberty. *PSMC3IP* joins previous genes known to be mutated in XX-GD, the FSH receptor, and *BMP15*, highlighting the importance of hormonal signaling in ovarian development and maintenance and suggesting a common pathway perturbed in isolated XX-GD. By analogy to other XX-GD genes, *PSMC3IP* is also a candidate gene for premature ovarian failure, and its role in folliculogenesis should be further investigated.

Sex determination of the embryo is established by multiple molecular events that direct the development of germ cells, their migration to the urogenital ridge, and the formation of either a testis or an ovary from the indifferent or bipotential gonad.[1,2] Aberrations of this process can result in major structural genital anomalies, ambiguous genitalia, gonadal dysgenesis (GD), and infertility (reviewed in Biason-Lauber[3]). Ovarian development was originally considered to be a default pathway reflecting lack of *SRY*. Although a number of genes are now known to be involved in early ovarian development (e.g., *WNT4* [MIM 3603490], *NR0B1/DAX1* [MIM 300473], *FOXL2* [MIM 605597], and *RSPO1* [MIM 609595]), the details of female sex determination are not as well understood as male sex determination.[3] Elucidating the molecular defects underlying disorders of sex development (DSD) has provided critical insights into this process. Hypergonadotropic complete ovarian insufficiency (OI) with normal karyotype (46, XX), also known as XX female gonadal dysgenesis (XX-GD [MIM 233300]) is a rare, genetically heterogeneous disorder. It represents the severe end of the spectrum of ovarian insufficiency, which can range from lack of spontaneous pubertal development to premature menopause before age 40. XX-GD females have streak gonads, leading to low estrogen and progesterone levels and subsequently elevated gonadotropins. Clinical expression can be variable, but the common presentation is a lack of spontaneous pubertal development, primary amenorrhea, and uterine hypoplasia. Known causes of isolated XX-GD include recessive mutations in the follicle-stimulating hormone (FSH) receptor gene (*FSHR* [MIM 136435]), severe X-linked recessive mutations in the growth and differentiation factor *BMP15* (MIM 300247), and both recessive and dominant mutations in the *NR5A1/SF-1* (MIM 184757) transcriptional regulator.[4,5] Syndromes featuring XX-GD include XX-GD with immunodeficiency and pulmonary fibrosis (MIM 611926), XX-GD with short stature and recurrent metabolic acidosis (MIM 605756), and congenital muscular dystrophy with infantile cataracts and GD (MIM 254000). Ovarioleukodystrophy (MIM 603896), a leukoencephalopathy with vanishing white matter that is associated with XX-GD, is caused by mutations in the *EIF2B2* (MIM 606454), *EIF2B4* (MIM 606687) and *EIF2B5* (MIM 603945), genes, which encode subunits of the translation initiation factor EIF2B (reviewed in Fogli and Boespflug-Tanguy[6]). Perrault syndrome (MIM 233400), which also includes sensorineural deafness and, in some families, neurological manifestations, can be caused by mutations in *HSD17B4* (MIM 610860), which encodes a 17-beta-estradiol dehydrogenase involved in peroxisomal fatty acid

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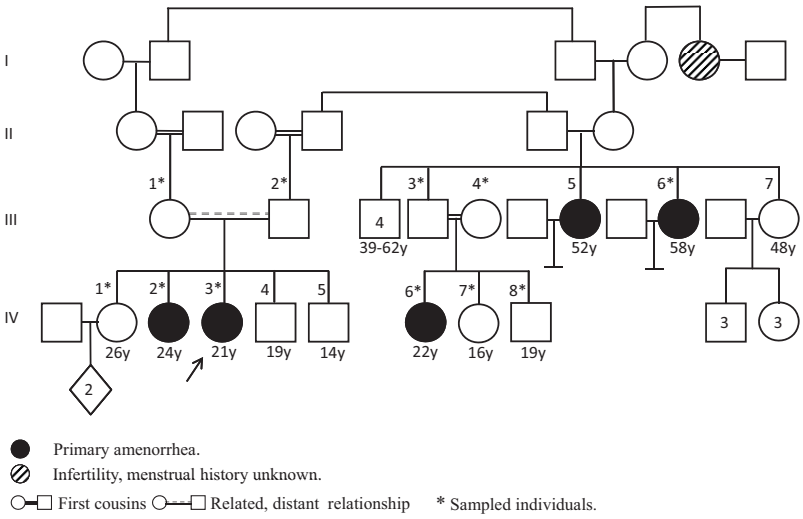
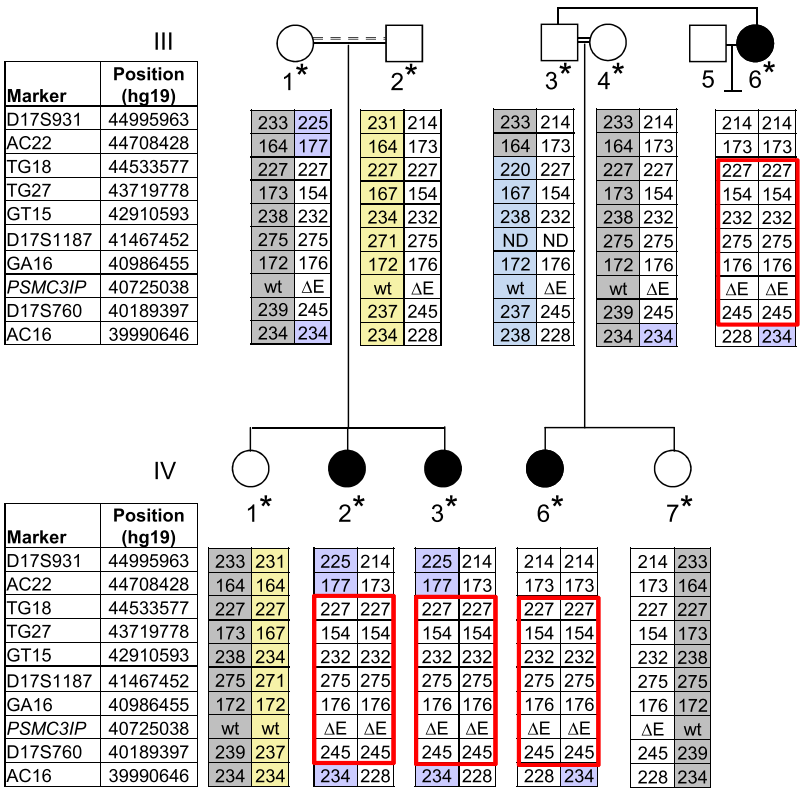


Figure 1. XX Ovarian Dysgenesis in a Consanguineous Family
To simplify pedigree structure, not all family members are indicated. Individual's pedigree numbers are indicated above the symbols and their ages are indicated under the symbols. The proband is indicated by an arrow.

beta-oxidation[7] and in *HARS2* (MIM 600783), which encodes the mitochondrial histidyl-tRNA synthetase.[8] However, the majority of isolated XX-GD cases remain unexplained and are thought to be caused by other autosomal-recessive mutations.

We report a highly consanguineous Arab Palestinian family in which at least five females are affected with complete XX-GD, all sharing the same clinical and laboratory features (Figure 1). The proband (Figure 1, IV-3) is the youngest daughter of distantly related parents (III-1 and III-2, Figure 1). Following normal pregnancy and delivery (birth weight 3000 g) and normal growth and development in childhood, she failed to develop spontaneous puberty and at 15 years of age, breast development and pubic hair were at Tanner stage 1 and 2, respectively. Hormonal testing of individual IV-3 revealed high basal gonadotropin levels (LH, 19.9 IU/L, FSH, 89.3 IU/L; normal values are: pubertal LH, 1.0–14.7 IU/L; menopausal LH, 11.0–40.0; pubertal FSH, 3.0–21.0 IU/L; menopausal FSH, 21.1–73.0 IU/L) that increased further during luteinizing hormone-releasing hormone (LHRH) stimulation. Estradiol and progesterone were undetectable (estradiol $<$ 73.4 pmol/l, progesterone $<$ 0.64 nmol/l; normal values for the follicular and luteal stage are 100–900 pmol/l and 1–63 nmol/l, respectively) and androgen levels were normal (the testosterone level was 2.2 nmol/l; the normal level is 1–3 nmol/l). Thyroid, adrenal, and growth hormone functions were normal. Karyotype was that of a normal female, 46,XX, and no SRY sequence was detected by PCR. Abdominal ultrasound and magnetic resonance imaging revealed uterine hypoplasia and undetectable ovaries. Hormone replacement therapy resulted in normal breast development and induction of regular menstruation, but uterus size remained relatively small. We subsequently examined the proband's sister (IV-2, Figure 1), who was then 18 years old and was receiving low-dose estradiol replacement therapy for primary amenorrhea of unknown cause. Pubertal development had commenced only following treatment and was only partial (Tanner stage 3) as a result of insufficient estrogen dosage and lack of progesterone replacement therapy. Her laboratory and imaging results were similar to those of the proband. In the extended family, the proband's father reported that two of his paternal cousins, both women currently in their 50s (III-5, III-6, Figure 1), were childless. These women also had primary amenorrhea and achieved puberty and menarche only following hormonal replacement. In both cases, the karyotype was 46,XX, and abdominal ultrasounds showed hypoplastic uterus and undetectable ovaries. In individual III-6 these findings were confirmed in an explorative laparotomy. In the following year, the proband's second cousin (IV-6) presented at age 17 years with primary amenorrhea and lack of pubertal development. Results of laboratory and imaging studies were similar to those of the proband. Individual IV-6 is the child of first-cousin parents (III-3 and III-4) who are members of the same extended family. Other adult sisters of affected females (IV-1, IV-7, and III-7; Figure 1) are healthy and have normal sexual development and fertility (IV-1 and III-7). Given the high degree of consanguinity, we hypothesized that XX-GD in this family was autosomal recessive, as result of homozygosity by descent. Following approval of the institutional review board and the National Helsinki Committee for Genetic Studies, informed consent was obtained and homozygosity mapping was performed on all sampled individuals indicated in Figure 1. We did not include male siblings of affected females in this analysis because, as in other XX-GDs, the phenotype might be sex-limited. DNA extracted from blood was genotyped with the Affymetrix GeneChip 250K Nsp SNP array. SNP data were examined for informative genomic regions longer than 2 Mb that were homozygous and shared among affected women but were not homozygous for the same allele in the unaffected sisters.

Data analysis was performed with KinSNP[9] and SNP Express.[10] A single 4.8 Mb region fulfilling homozygosity criteria was identified on chromosome 17 (40,174,841-44,987,261 hg19). Fine mapping of this region with 27 short tandem repeat (STR) markers defined a homozygous region of similar size (39,990,510-44,708,028 hg19) (Figure 2). Combining these results, the boundaries of the region were 40,174,841 to 44,708,028, a 4.5 Mb region harboring 157 genes (UCSC Genome Browser). An obvious candidate gene in this region was *BRCA1* (MIM 113705) because it is highly expressed in oocytes,[11] plays a critical



Marker	Position (hg19)	III-1*	III-2*	III-3*	III-4*	III-5	III-6*
D17S931	44995963	233 225	231 214	233 214	233 214	214 214	214 233
AC22	44708428	164 177	164 173	164 173	164 173	173 173	173 164
TG18	44533577	227 227	227 227	220 227	227 227	227 227	227 227
TG27	43719778	173 154	167 154	167 154	173 154	154 154	154 173
GT15	42910593	238 232	234 232	238 232	238 232	232 232	232 238
D17S1187	41467452	275 275	271 275	ND ND	275 275	275 275	275 275
GA16	40986455	172 176	172 176	172 176	172 176	176 176	176 172
PSMC3IP	40725038	wt ΔE	wt ΔE	wt ΔE	wt ΔE	ΔE ΔE	ΔE wt
D17S760	40189397	239 245	237 245	237 245	239 245	245 245	245 239
AC16	39990646	234 234	234 228	238 228	234 234	228 234	228 234

Marker	Position (hg19)	IV-1*	IV-2*	IV-3*	IV-6*	IV-7*
D17S931	44995963	233 231	225 214	225 214	214 214	214 233
AC22	44708428	164 164	177 173	177 173	173 173	173 164
TG18	44533577	227 227	227 227	227 227	227 227	227 227
TG27	43719778	173 167	154 154	154 154	154 154	154 173
GT15	42910593	238 234	232 232	232 232	232 232	232 238
D17S1187	41467452	275 271	275 275	275 275	275 275	275 275
GA16	40986455	172 172	176 176	176 176	176 176	176 172
PSMC3IP	40725038	wt wt	ΔE ΔE	ΔE ΔE	ΔE ΔE	ΔE wt
D17S760	40189397	239 237	245 245	245 245	245 245	245 239
AC16	39990646	234 234	234 228	234 228	228 234	228 234

Figure 2. Haplotypes in the Chromosome 17q21 Region
Individuals are numbered as in Figure 1. Selected STR markers (of 27 genotyped) are listed telomeric (D17S931) to centromeric (AC16). For PSMC3IP the following abbreviations are used: ΔE, the p.Glu201del mutation; wt, wild-type sequence. The disease-associated haplotype is shown in white, and the homozygous region shared by all affected individuals is boxed in red. Primer sequences for all STRs and for *PSMC3IP* sequencing are detailed in Table S1.

role in meiosis,[12,13] and functions as a ubiquitin ligase of estrogen receptor (ER)α.[14] *BRCA1* sequencing of individuals IV-2 and IV-6 revealed no mutations (primer sequences are detailed in Table S1, available online). After ruling out *BRCA1*, other genes in the candidate interval were assessed for function and tissue expression relevant to XX-GD. We performed Sanger sequencing of *MEOX1* (MIM 600147), *NAGLU* (MIM 609701), *UBTF* (MIM 600673), and *PSMC3IP* (MIM 608665) (primer sequences are detailed in Table S1). This revealed a homozygous 3 bp deletion in the *PSMC3IP* gene (Figure 3A), NM_016556.2 (PSMC3IP_v001):c.600_602del (p.Glu201del). This 3 bp deletion is predicted to result in deletion of one of two adjacent, highly conserved glutamate residues at positions 200 and 201 and is designated as PSMC3IP p.Glu201del (Figure 3B). *PSMC3IP* c.600_602del was not found in genomic DNA of 254 chromosomes of healthy controls of Arab Palestinian origin.

From genomic sequence alone, it was not possible to determine whether the 3 bp deletion, designated c. 600_602del, altered the *PSMC3IP* intron7/exon 8 acceptor splice site. Five alternative 3 bp deletions at genomic location chr17:40,725,038-40,725,044 (hg19) could lead to the mutant intron 7/exon8 AG/GAA sequence (Figure 3A). These include deletions of the exon 8 acceptor splice site, although the canonical AG remains intact in all cases (Figure 3A). To determine whether the deletion affects splicing, we performed RT-PCR spanning exons 5–8 on leukocyte cDNA from affected and unaffected individuals. Alternative splicing was not observed, suggesting the mutation does not affect *PSMC3IP* splicing (data not shown). The *PSMC3IP* locus partly overlaps with the *MLX* gene (MIM 602976), which is transcribed in the opposite direction, and *PSMC3IP* c. 600_602del corresponds to a 3 bp deletion within the *MLX* 3′ UTR, 1417 bp downstream of the *MLX* stop codon and 150 bp upstream of the *MLX* polyA signal. Quantitative RT-PCR of *MLX* expression in leukocyte cDNA from homozygous affected and heterozygous and homozygous unaffected individuals revealed no differences in *MLX* expression. We conclude that the pathogenic effect of the 3 bp deletion is highly unlikely to be due to the altered *MLX* 3′ UTR sequence.

To exhaustively analyze the 4.5 Mb candidate interval, we carried out whole-exome, massively parallel sequencing for individuals IV-3 and IV-6 (Figure 1). Libraries prepared from the genomic DNA of individuals IV-3 and IV-6 (Figure 1) were hybridized to biotinylated cRNA oligonucleotide baits from the SureSelect Human All Exon Kit (Agilent Technologies, Santa Clara, CA). Each exome-enriched library was sequenced with 50 bp single-end reads on two lanes of an ABI SOLiD 3 plus analyzer. The sequences were aligned to the hg19 human genome assembly (February 2009) with Burrows-Wheeler Aligner (BWA);[15] Samtools[16] was used to list high-quality DNA variants, and Annovar[17] was applied to this list in order to filter out those variants listed in dbSNP130 or predicted to be benign according to SIFT.[18] The remaining variants were then classified by Annovar as missense, nonsense, frameshift, or splice-site variants. To corroborate these results, we repeated the entire analysis with the CLC Bio Genomic Workbench (CLC Bio, Aarhus, Denmark). The proportion of the entire exome covered by more than five reads in individuals IV-3 and IV-6 was 65% and 70%, respectively, in the first analysis, and 63% and 68%, respectively, in the second analysis. In both analyses, the only functional variant shared between IV-3 and IV-6 within the homozygous candidate region in chromosome 17, was the *PSMC3IP* 3 bp deletion described above (Table 1, Table S2).

PSMC3IP (Proteasome 26S subunit, ATPase, 3-Interacting Protein) is also known as *TBPIP* (Tat Binding Protein

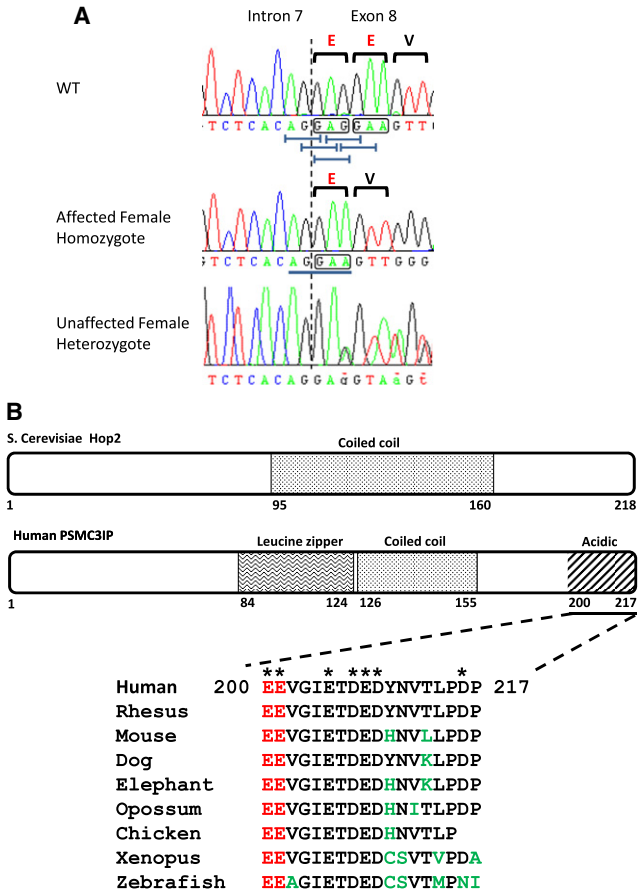


Figure 3. The *PSMC3IP* c.600_602del Mutation, Which Encodes the p.Glu201del Mutation

(A) Genomic sequence at the exon 8 acceptor splice junction. Affected females are homozygous for a 3 bp deletion, eliminating one of two adjacent glutamic acid residues (codons circled in black). Blue bars indicate five possible deleted triads that would lead to the mutant AG|GAA sequence resulting in p.Glu201del. The vertical dashed line indicates the intron 7-exon 8 boundary. The AG|GAA splice sequence is maintained in the homozygous mutant.

(B) PSMC3IP domains and conservation of the acidic C-terminal region. Yeast Hop2 and its mammalian ortholog, PSMC3IP, are shown with known domains.[19,20,42] Numbers below the bars indicate the residue number. Within the conserved acidic domain, the adjacent Glu residues are indicated in red and nonconserved residues in green, an asterisk (*) denotes acidic residues. Acidic domains are important for interaction with transcriptional coactivators.[43]

Interacting Protein) and is the ortholog of *S. cerevisiae Hop2*. The gene encodes two protein isoforms of 217 and 205 residues and has adjacent leucine zipper and coiled-coil domains in the middle region of the protein[19] and a C-terminal acidic domain starting at Glu200 (Figure 3B).[20] This acidic domain is not present in the yeast ortholog but is highly conserved from zebrafish to human, and both Glu200 and Glu201 are invariant in all species (Figure 3B). Human *PSMC3IP* was originally described as a transcript in the chromosome 17 *BRCA1* region[21] and was later identified in a yeast two-hybrid screen for proteins interacting with the glucocorticoid receptor DNA-binding domain.[22] *PSMC3IP* encodes a nuclear protein found at high levels in the testis of fetal and adult mouse, rat, and human and is also present in the fetal and adult ovary, spleen, and thymus[22–24] (Figures S1 and S2). In addition to the glucocorticoid receptor, PSMC3IP binds the DNA-binding domains of other hormone receptors, including the estrogen receptors (ER) α and β, the thyroid hormone receptor β1, the androgen receptor, and the progesterone receptor. PSMC3IP acts as a coactivator of ligand (hormone)-dependent transcription mediated by all of these nuclear receptors.[22] Deletion analysis has shown that although the PSMC3IP leucine zipper is critical for nuclear receptor binding, ligand-dependent transcriptional coactivation cannot be localized to a single domain.[22] However, ligand-dependent transcriptional coactivation is particularly dependent on the PSMC3IP C-terminal region (aa 181-217), which includes Glu201 and the acidic domain[22] (Figure 3B).

We therefore examined the effect of the *PSMC3IP* p.Glu201del mutation on hormonal signal transduction and specifically on the coactivation of estrogen-dependent transcription by using the assay described by Ko et al.[22] in an ovarian carcinoma cell line IGROV1 (Figure 4)[25] and a breast cancer cell line, MCF-7. Wild-type and mutant *PSMC3IP*-expression constructs were cotransfected with a reporter construct containing a firefly luciferase gene downstream of an estrogen-response element (ERE, Addgene, Cambridge, MA), in addition to an ERα-expression construct (Addgene, Cambridge, MA) and a *Renilla* luciferase construct to control for transfection efficiency. Relative luciferase activity was measured with the Dual-Luciferase Reporter Assay System (Promega), with and without β-estradiol treatment. Coactivation of estrogen-dependent transcription was compared for the mutant *PSMC3IP* p.Glu201del construct, the wild-type *PSMC3IP* construct, and a C terminus-deleted *PSMC3IP* construct previously reported to have significantly reduced estrogen-induced activity (*PSMC3IP* c.541_651del, corresponding to PSMC3IP p.Thr181_Pro217del)[22] (Figure 4). Whereas in IGROV1 cells wild-type PSMC3IP-induced estrogen-dependent activity by 48% ($p < 0.02$), PSMC3IP p.Glu201del had no significant coactivating activity, similar to PSMC3IP p.Thr181_Pro217del (Figure 4). Compared to wild-type PSMC3IP, under estradiol treatment the p.Glu201del mutant had 68% less activity ($p < 0.02$) in IGROV1 cells (Figure 4) and 18% less activity in MCF-7 cells ($p < 0.01$).

These results demonstrate that the *PSMC3IP* p.Glu201del mutation significantly impairs PSMC3IP function as an ER coactivator, consistent with the uniformly severe phenotype observed in all affected females in the family described. Combined with the genetic evidence, we conclude that a *PSMC3IP* mutation is the genetic basis for XX hypergonadotropic ovarian dysgenesis in this family.

Hop2, the yeast ortholog of *PSMC3IP*, is required for homologous pairing and recombination in meiosis[26] as part of the Hop2-Mnd1 heterodimer essential for activity

Table 1. Whole-Exome Sequencing in Two Affected Individuals

Individual (Figure 1)	Number of DNA variants					
	Total[a]	Not in dbSNP130.	SIFT Score [$p < 0.05$]	Nonsynonymous or Splice Junction Variants[b]	Within the Chromosome 17 Homozygous Region	Shared by Both Affected Individuals
IV-3	32,899	5,643	5,333	2,080	10	1
IV-6	42,328	7,060	6,678	2,252	5	1

Results shown are for analysis with BWA,[15] Samtools,[16] and Annovar.[17]
[a] Variants with a minimum quality score of 20, covered by at least four reads.
[b] Splice mutations are defined as those within 10 nt of an intron/exon junction.

of Dmc1, the meiosis-specific recombinase.[27] Murine *Hop2*/*Psmc3ip* knockouts[28] have defects in gametogenesis but are otherwise normal, suggesting that *PSMC3IP* is not developmentally essential. Female knockout mice show severe reduction in ovarian size, tubulo-stromal hyperplasia, and a lack of follicles. Male knockout mice have testicular hypoplasia, hyperplasia of interstitial cells, a lack of spermatogenesis, and a block in meiosis I. Consistent with *Hop2*'s role in meiosis, homologous chromosomes in spermatocytes of *Psmc3ip* knockout mice fail to synapse, and double-strand breaks at recombination sites are not repaired.[28] The possibility of a meiotic defect in the human patients with GD cannot be directly examined because the affected females were diagnosed years after the onset of meiosis. However, the p.Glu201del mutation alters the PSMC3IP acidic domain, which is not present in the yeast ortholog, and in the mouse, deletion of *Psmc3ip*'s 36 most C-terminal residues, including the entire 18 residue acidic domain, does not impair Mnd1 complex formation or DNA binding.[19] Thus, current evidence does not indicate a direct meiotic role for the PSMC3IP acidic domain but does not preclude the possibility that p.Glu201del could have a meiotic effect by affecting the overall conformation of the protein. Our direct evidence (Figure 4) supports the hypothesis that the ovarian dysgenesis phenotype observed with the *PSMC3IP* p.Glu201del mutation is related to impaired estrogen-dependent transcription.

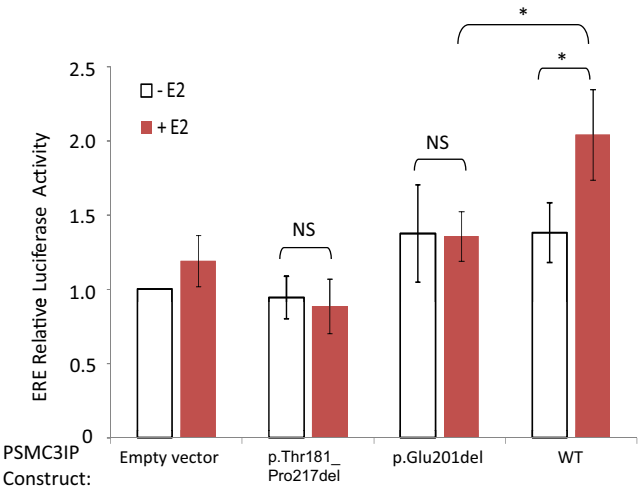


Figure 4. Reduced Estrogen-Induced Transcriptional Activity of PSMC3IP p.Glu201del
Ovarian carcinoma IGROV1 cells, which do not express the estrogen receptor (ER),[41] were cotransfected with a reporter including luciferase downstream of an ERE (100ng), ERα (10ng), and either an empty pcDNA3.1+ vector (Invitrogen), or one of three forms of *PSMC3IP* cloned into pcDNA3.1(+): wild-type (WT), C-terminal deletion, p.Thr181_Pro217del, and the p.Glu201del mutation we identified.
We used the Promega Dual-Luciferase Reporter Assay System (Promega), in which reporter activity is normalized against a *Renilla* luciferase reporter controlled by the HSV-thymidine kinase promoter (pRL-TK). Total DNA amounts were balanced with pcDNA3.1 vector.
Relative luciferase activity of the ERE-luciferase reporter construct is shown for each *PSMC3IP* construct, with (+E2) or without (−E2) treatment with 30nM β-estradiol in RPMI medium.
The error bars indicate mean values of triplicate transfections ± SEM, normalized to the empty vector control. For indicated comparisons, *$p < 0.02$. NS is an abbreviation for not significant. The PSMC3IP WT and p.Glu201del constructs were produced by RT-PCR of RNA extracted from lymphocytes of a healthy individual and the proband, respectively. Following PCR, products were cut by restriction enzymes and ligated into the pcDNA3.1+ (Invitrogen) multicloning-site. The p.Thr181_Pro217del construct was produced in a similar manner, and primers were designed specifically to remove residues 181–217 of the protein (primer sequences are indicated in Table S1).

Although estrogen is not required for the initial steps in ovarian differentiation in mammals, it plays a critical, species-specific role in two stages of ovarian follicular development: (1) in the formation of primordial ovarian follicles and development of healthy oocytes within follicles during fetal development and the early postnatal period, and (2) in the postquiescence development of follicles destined for ovulation during the reproductive span (puberty to menopause).[29] Whereas in mice estrogens inhibit primordial follicle development,[30] in primates, development of a healthy follicle pool available for adult ovarian function is an intrauterine estrogen-dependent event[31,32] (reviewed in Albrecht and Pepe[29]). Recent data from normally progressing, second-trimester human fetuses show that the fetal ovary expresses the machinery required to produce and respond to estrogen, progesterone, and androgen signaling, including aromatase (CYP19A1), a key enzyme in estrogen synthesis, and both ER α and β.[33] The limited existing literature on human fetal

estradiol levels suggests that circulating estradiol levels are high,[34,35] and gene-expression data show that *PSMC3IP* is expressed in the second-trimester fetal ovary (Figure S2).[23] It is therefore plausible that *PSMC3IP* mutations would result in reduced folliculogenesis even during fetal development.

The second stage of follicular development commences with puberty, which is marked by increased follicle stimulating hormone (FSH) secretion. Binding of FSH receptors that are present exclusively in developing follicles activates estrogen production in granulosa cells, and leads to normal ovulation. Defects in this process lead to follicular atresia and ovarian dysgenesis and a lack of sexual development as exemplified by individuals with *FSHR* mutations (MIM 233300).[36] Our results raise the possibility that *PSMC3IP* mutations can compromise the estrogenic pathway downstream of the FSHR and suggest that ovarian dysgenesis associated with *PSMC3IP* mutations might be caused by the combination of a reduced fetal follicular pool and increased postnatal follicular atresia. This hypothesis could be tested by appropriate mouse models with the caveat of species-specific differences in the effect of estrogen signaling on fetal ovarian development.

Homozygous *FSHR* mutations cause variable defects in spermatogenesis but not azoospermia or complete infertility.[37] At this time, we cannot determine whether PSMC3IP p.Glu201del homozygosity affects males. Fathers III-2 and III-3 (Figure 1) of the affected individuals are heterozygous for this mutation (Figure 2), as is a postpubertal male sibling of affected individuals (IV-8, Figure 1). Other postpubertal males (e.g., the four brothers of III-3, Figure 1) have not been available for testing. Notably, there are no reports of hypogonadism or infertility among any males in the extended family. However, this does not preclude the possibility that PSMC3IP p.Glu201del or other mutations in *PSMC3IP*, especially those primarily affecting its meiotic function, would have such effects.

As a gene mutated in complete XX-GD, *PSMC3IP* is also a candidate gene for related phenotypes, including the milder phenotype of premature ovarian failure (POF, i.e., secondary amenorrhea before age 40) or the opposite clinical phenotype of ovarian hyperstimulation syndrome (OHSS [MIM 608115]).[38] By analogy, whereas inactivating *FSHR* mutations cause ovarian dysgenesis, FSHR mutations that increase ligand promiscuity result in OHSS.[39] Also, by analogy, severe dominant negative mutations of *BMP15* cause ovarian dysgenesis, whereas milder *BMP15* mutations that hamper posttranslational processing and lead to reduced amounts of mature BMP15 protein might be associated with POF (reviewed in Persani et al.[38] and Otsuka et al.[40]). Identification of a *PSMC3IP* mutation as a cause of ovarian dysgenesis warrants examination of the role of this gene in folliculogenesis. Genetic analysis of *PSMC3IP* in individuals with XX-DSD, POF, and OHSS could reveal additional mutations and uncover genotype-phenotype correlations.

Supplemental Data

Supplemental Data include two figures and two tables and can be found with this article online at http://www.cell.com/AJHG/.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/SNP/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
UCSC Genome Browser, http://genome.ucsc.edu/

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