

Report of fertility in a woman with a predominantly 46,XY karyotype in a family with multiple disorders of sexual development

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All authors have nothing to declare.

Abstract

Context: We report herein a remarkable family in which the mother of a woman with 46,XY complete gonadal dysgenesis was found to have a 46,XY karyotype in peripheral lymphocytes, mosaicism in cultured skin fibroblasts (80% 46,XY and 20% 45,X) and a predominantly 46,XY karyotype in the ovary (93% 46,XY and 6% 45,X).

Patients: A 46, XY mother who developed as a normal woman, underwent spontaneous puberty, reached menarche, menstruated regularly, experienced two unassisted pregnancies, and gave birth to a 46,XY daughter with complete gonadal dysgenesis.

Results: Evaluation of the Y chromosome in the daughter and both parents revealed that the daughter inherited her Y chromosome from her father. Molecular analysis of the genes *SOX9*, *SF1*, *DMRT1*, *DMRT3*, *TSPYL*, *BPESC1*, *DHH*, *WNT4*, *SRY* and *DAX1* revealed normal coding sequences in both the mother and daughter. An extensive family pedigree across four generations revealed multiple other family members with ambiguous genitalia and infertility in both phenotypic males and females and the mode of inheritance of the phenotype was strongly suggestive of X-linkage.

Conclusions: The range of phenotypes observed in this unique family suggests that there may be transmission of a mutation in a novel sex determining gene or in a gene that predisposes to chromosomal mosaicism.

Introduction

Normal sexual differentiation in 46,XY individuals relies on a complex cascade of numerous genes, many of which have yet to be identified (1-11). Defects in these genes can cause disorders of sexual development of varying severity. The external genitalia and Müllerian structures are typically female in women with complete 46,XY gonadal dysgenesis in association with streak gonads bilaterally. As the gonads are dysgenetic and nonfunctional, spontaneous pubertal development seldom occurs in these women (12), and successful pregnancy is even more unusual; unassisted pregnancy is unheard of (1). There have been a few instances of fertility in 46,XX/46,XY true hermaphrodites (13), but no reports of fertility in a 46,XY woman. Pregnancy in Turner Syndrome is reported to be possible in about 2% of cases (13), though it is rare for unassisted pregnancy to occur in non-mosaic Turner patients possessing only a 45,X line (14).

Herein we report the extraordinary case of a fertile woman with normal ovaries and a predominantly 46,XY ovarian karyotype, who

gave birth to a 46,XY female with complete gonadal dysgenesis. The karyotype of this phenotypically normal mother was 46,XY in blood, 80% 46,XY and 20% 45,X in cultured skin fibroblasts, and 93% 46,XY; 6% 45,X; <1% 46,XX in the ovary. The family pedigree on the mother's side was notable for the presence of 7 individuals over four generations with either sexual ambiguity, infertility or failure to menstruate, including one individual with documented 45,X/45,XY mixed gonadal dysgenesis. Both the mother and the 46,XY daughter were screened for mutations in a number of genes known to be involved in mammalian testis-determination. In all genes screened (see below), the open reading frame (ORF) was found to be normal. This suggests that a mutation in a novel sex determination gene or a gene that predisposes to chromosomal mosaicism may be responsible for the phenotype in this family.

Materials and Methods

Methods

Informed written consent was obtained from the subjects.

LHRH stimulation test. Factrel® 100 µg was given intravenously with sequential blood drawn at baseline and at timed intervals for 2 hours.

ACTH stimulation test. Cortrosyn® 0.25 mg was given intravenously, and blood was drawn at baseline and one hour after injection.

hCG stimulation test. Human Chorionic Gonadotropin 5000 units was given intramuscularly daily for 3 days.

Hormone assays. Steroid hormone assays were performed by standard radioimmunoassay as described (15-17).

Karyotypic analyses. Fluorescence in situ hybridization (FISH) was performed on a paraffin section slide of the gonad using the CEP probe for Y and the chromosome X centromeric control probe (Vysis, Downer's Grove, IL).

Molecular analysis. Maternity testing was performed by using the STR kit AmpFISTR Profiler Plus (PE Applied Biosystems, Foster City, USA). Nine STRs were analyzed D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 using thermal cycling conditions and capillary electrophoresis were performed according to the manufacturer's

instructions, and products were analyzed using the ABI 3100 capillary electrophoresis instrument and GeneScan software (Applied Biosystems).

The Y chromosomes of the proband and her two parents were typed using the marker YAP (Y chromosome Alu polymorphism) as described (18). The entire open reading frame of the sex-determining region Y (*SRY*) gene was amplified and directly sequenced as described (2, 3).

Using the polymerase chain reaction (PCR) primers (Table 1), the open-reading frames of seven genes known to be involved in sexual development were sequenced:

The *DMRT1* and *DMRT3* genes were sequenced using lymphocyte DNA isolated from the patient. The conditions of amplification were: for *DMRT1* exon 1, incubation at 95°C for 5 min followed by 40 cycles of 95°C 1 min, 68°C 1 min and 72°C 30 sec. For *DMRT1* exons 2 and 4, incubation at 95°C for 5 min followed by 40 cycles of 95°C 1 min, 57°C 30 sec and 72°C 1 min. For *DMRT1* exon 3, incubation at 95°C for 5 min followed by 40 cycles of 95°C 1 min, 62°C 1.30 min and 72°C 30 sec. For *DMRT1* exon 5, incubation at 95°C for 5 min followed by 40 cycles of 95°C 1 min, 50°C 30 sec and 72°C 30

sec. For *DMRT3* exon 1 the PCR conditions were incubation at 95°C for 5 min followed by 40 cycles of 95°C 1 min and 62°C 1.30 min with no extension time. For *DMRT3* exon 2, two amplicons were used to amplify the entire exon for direct sequencing. Both primer pairs of each amplicon were used at the conditions of incubation at 95°C for 5 min followed by 35 cycles of 95°C 1 min and 60°C 1 min with no extension time.

Amplification of the coding region of the *BPESCI* gene was performed utilizing PCR reactions for 35 cycles at 95°C for 30 sec, 56°C for 30 sec, and 72°C for 30 sec. PCR products were directly sequenced using the forward primer for each amplicon.

The reaction conditions for amplifying and sequencing the *WNT4* open-reading frame were identical to that described for the *BPESCI* gene above. PCR products were sequenced using the forward primer of each amplicon.

Exon 1 of the *DAX1* open reading frame was amplified using three amplicons, and exon 2 was amplified in one step. PCR amplification was performed as indicated for the *BPESCI* gene as described above with the exception of the primer

pairs *DAX1* exon1 F2 / *DAX1* exon 1 R2 where the annealing temperature was 58°C and the annealing time was 45 sec. Direct sequencing of all amplicons was performed using both the forward and reverse primers.

Amplification and sequencing of the *DHH* gene was performed as described by Umehara, et al (19).

The *TSPYL* gene was sequenced using the conditions of 95°C for 5 min, followed by 35 cycles of 95°C 1 min, 60°C 1 min and 72°C 1 min. A final extension of 72°C for 5 min was included.

The amplification conditions for all *SOX9* amplicons were 95°C for 5 min, followed by 35 cycles of 95°C 1 min, 61°C 1 min and 72°C 1 min. A final extension of 72°C for 10 min was also performed.

The open reading frame of the *SFI* gene was amplified and directly sequenced using conditions described (20).

Case Histories

Patient 1 (daughter). This 17 year old woman from Croatia was the product of a 39-week

gestation, delivered by Caesarean section due to a maternal hip fracture. Birth weight was 3.8 kilograms, and length was 52 cm. She was breastfed for one year. She sought medical attention at age 17 years because of lack of breast development and primary amenorrhea. Intelligence was normal determined by her standing as a top student in her class.

On exam she was an articulate, tall, thin woman. The height was 187 cm (>95th %) with a mid-parental target height of 181 cm. Her weight was 68 kilograms. She had a normal frontal hairline. There was mild facial acne but no facial hair. She had Tanner stage I breasts and Tanner stage IV pubic hair. External female genitalia were normal, without clitoromegaly or labial fusion. The vaginal introitus was normal. Pelvic examination revealed a hypoplastic uterus with no palpable gonads. Bone age was 14 years at a chronological age of 17 years. Karyotype on peripheral blood (performed twice) was 46,XY. Further investigation revealed that multiple family members on the mother's side had ambiguous genitalia, infertility, or problems with sexual identity (Figure 2). This led to the decision to karyotype the mother.

Patient 2 (mother). This 52-year old phenotypically normal woman underwent normal pubertal development and reached spontaneous menarche at age 11 years. She had a history of two pregnancies, the first of which resulted in a spontaneous miscarriage. Her second pregnancy was uneventful, except that her daughter was delivered by Caesarean section due to a recent hip fracture in the mother from a motor vehicle accident. She breastfed the daughter for one year. She continued to have regular menses until menopause at age 49, after which time she received hormone replacement therapy for two years.

Physical examination revealed a feminine-appearing woman with a normal body habitus (Figure 1). The height was 177 cm. There was no receding hairline or balding of the scalp and no acne or facial hair. Breasts and pubic hair were Tanner stage V, although pubic hair was sparse. The external genitalia were normal with no clitoromegaly or labial fusion. The vaginal introitus was normal. Pelvic examination revealed a uterus in retroverted

position with no adnexal masses. The karyotype in peripheral blood was 46,XY (20 cells).

Results

Patient 1 (daughter).

Repeat karyotype revealed:

Blood: 46,XY (100%) (20 nuclei)

Skin: 46,XY (100%) (50 nuclei)

Gonad: 46,XY (99.25%),45, X (0.75%)
(400 nuclei)

Hypothalamic-pituitary-gonadal axis (Table 1):

LHRH stimulation test was consistent with gonadal failureabsent gonads.

Gonadal function (Table 2): hCG stimulation test revealed high baseline testosterone with little response to hCG.

Adrenal function (Table 3): No evidence of a steroidogenic defect was demonstrated.

Pelvic Ultrasound: Normal kidneys and a left extra-renal pelvis were noted. The ultrasound was hypoplastic. A small left gonad was present. No gonad was noted on the right.

Surgical Pathology: The patient underwent laparoscopic gonadectomy. No gonadal tissue was identified on the right, although there was a normal fallopian tube on that side. *Histology:* On

the left she had a small fragment of fibrous tissue with a small focus of ovarian stroma. Inhibin staining was positive.

Serum testosterone: Repeat testosterone after gonadectomy was 51.8 ng/dL (nl range 23.0-69.1)

Patient 2 (mother)

Repeat karyotype:

Blood: 46,XY (100%) (20 nuclei)

Skin: 46,XY (80%) / 45,X (20%) (50 nuclei)

Gonad: 46,XY (92.9%), 45,X (5.9%), 46,XX (0.6%), 47,XXY (0.6%) (1000 nuclei)

Hypothalamic-pituitary-gonadal axis (Table 2):

LHRH stimulation test was consistent with menopause or absent gonads.

Gonadal function (Table 3): hCG stimulation test revealed no evidence of testicular function.

Adrenal function (Table 4): No evidence of a steroidogenic defect was demonstrated.

Pelvic ultrasound: The uterus measured 8.8 cm x 4.5 cm x 5.5 cm and was normal in echotexture.

The endometrial stripe measured 4 mm. The right ovary measured 3.3 cm x 2.5 cm x 2.7 cm with normal venous and arterial flow. The left ovary

measured 3.0 cm x 1.6 cm x 2.5 cm with normal flow. There was no free fluid.

MRI of pelvis: The uterus appeared mildly atrophic and had a mildly thickened endometrial stripe. There were probable small myomas in the lower uterine segment. Both ovaries were atrophic with no follicles or masses noted.

Surgical Pathology: The mother agreed to undergo gonadectomy because of the increased risk of gonadoblastoma in gonads containing a Y chromosome. The internal structures were those of a normal woman (see Figure 1). No Wolffian remnants were seen. The uterus and fallopian tubes were left intact, and the ovaries were removed.

Histology: Pathology revealed a histologically unremarkable right ovary with several corpora albicans, suggestive of prior ovulation. The left ovary contained fibromuscular tissue with ovarian hilar cells, and an immunohistochemical stain for inhibin showed a focus of positive cells confirming the presence of ovarian stromal elements.

Family History and Genetic Studies

Family History

There is a remarkable family history of ambiguous genitalia and infertility affecting both phenotypic men and women across 4 generations (Figure 2). The daughter inherited her Y chromosome from the father (see below), thereby excluding involvement of the Y chromosome in the development of sex reversal in this family. The pedigree is strongly suggestive of X-linked inheritance of the phenotype, although autosomal dominant sex-limited transmission cannot be excluded.

Genetic evaluation

Maternity was established at a probability exceeding 99.15%. The analysis of the Y chromosome polymorphism YAP revealed that this insertion was present in the Y chromosome of the mother (defining her Y chromosome haplogroup as D/E), and the insertion was not present in the Y chromosome of the daughter or her father (Figure 3). These data indicate that the daughter inherited the Y chromosome from her father and the sequence was identical to that of a normal male. The molecular analysis of the coding sequences of nine genes known to be involved in sexual development (*SOX9*, *SFI*,

DMRT1, *DMRT3*, *TSPYL*, *BPESC1*, *DHH*, *WNT4*, *SRY* and *DAX1*) revealed coding sequences in both the mother and daughter that are identical to the normal reference sequences. Polymorphisms were not identified in any of the analyzed genes.

Discussion

Although there have been reports of fertility in 46,XX/46,XY true hermaphrodites with ovotestes (13) and in patients with mosaic and non-mosaic Turner Syndrome (21), we believe this to be the first report of fertility in a woman with a predominantly 46,XY karyotype in the ovary. The fact that this mother had normal functioning ovaries, menstruated regularly, and achieved unassisted pregnancy twice is remarkable. Additionally, her hormonal findings are compatible with a normal menopausal woman. Of course, it should be noted that the incidence of normal fertile females who have a 46,XY karyotype is not known because it is not routine to check the karyotype in fertile women. Although the demonstration of 5.9% 45,X cells in the ovary is difficult to interpret, most cytogeneticists agree that 5% does not indicate mosaicism. The finding of 20% 45,X cells in fibroblasts cultured from

skin indicates that she is a 46,XY/45,X mosaic, at least in the skin. Individuals with a karyotype of 46,XY/45,X usually have ambiguous genitalia or a male phenotype, though occasionally they can have a Turner female phenotype (21). Our case is unique, however, because the presence of bilateral ovaries or unassisted pregnancy has not previously been reported in this form of mosaicism. Moreover, ovarian cells were predominately 46,XY; the small percentage of X (5.9%) out of 1000 cells counted in the gonad might be due to artifact or technical error. Pregnancy is believed to occur in about 2% of women with Turner syndrome (14). Although fertility did occur in a woman with mosaicism of an isodicentric Y-chromosome (22), we believe that our case of fertility in a female with a predominant 46,XY karyotype in the ovary is unprecedented. Of note XY female wood lemmings (*Myopus shisticolor*), carrying an Xp mutation, are fertile and produce X-containing oocytes (23). There have also been reports of potential fertility in XY sex-reversed female mice. In the B6.YDOM sex-reversed female mouse, almost all of the XY female mice, while they lack estrous cyclicity, are able to mate and ovulate

after treatment with gonadotropins (24).

Likewise, fertility has been described in XY female horses (25).

The fact that this mother gave birth to a 46,XY female is even more remarkable. However, the daughter's clinical picture, unlike that of her mother, is more typical of 46,XY complete gonadal dysgenesis, in which spontaneous puberty is rare and fertility is unreported. The significant family history of ambiguous genitalia and sex reversal across several generations presents a unique opportunity to explore the genetics of sexual differentiation and perhaps identify a novel gene involved in gonadal determination.

The mother and daughter were both screened for mutations in a number of genes that are either known to be necessary or are excellent candidate genes for males in human testicular development. Some of the genes known to be involved in gonadal differentiation are outlined in Figure 4 (4-7, 26). Both the mother and daughter had normal *SRY* (sex-determining region of the Y chromosome) sequences, and the daughter inherited the Y chromosome from her father, thereby excluding involvement of the non-

recombining male-specific portion of the Y chromosome, including the *SRY* gene, in the development of sex reversal in this family.

Included in the screen were the *DMRT1* and *DMRT3* genes that, when deleted, are associated with male-to-female sex-reversal. The analysis of another gene that is associated with gonadal dysgenesis (*TSPYL*) also revealed a normal sequence. Mutations in this gene are also associated with sudden infant death and testicular dysgenesis in an Amish family (27).

The *BPESCI* gene, which exhibits testis-specific expression and is located within the homologous region in the human at 3q23 (28), was normal in both mother and daughter. The *DAX1* gene is located on Xp22 and appears to be necessary for correct testis determination and, in the mouse at least, necessary for the upregulation of *Sox9* expression (29). The gene *WNT4* is critical for normal ovarian and female sexual development. A mutation in *WNT4* leads to Mullerian duct regression and virilization in a 46,XX female (8), whereas duplication of the locus containing *WNT4* leads to 46,XY sex reversal (30). In the XY mouse the absence of *Wnt4* is associated with a lack of Sertoli cell

differentiation, suggesting that the gene is involved in mammalian testis determination (31). Sequence analysis of both *DAX1* and *WNT4* genes revealed normal wild type male coding sequences in both the mother and daughter.

The Desert hedgehog gene (*Dhh*) is a member of a family of signaling genes that play an important role in regulating morphogenesis. Mutations in the human *DHH* gene have been reported in a patient with 46,XY partial gonadal dysgenesis accompanied by minifascicular neuropathy (19) and in women with complete gonadal dysgenesis and the absence of somatic anomalies (32).

Other possible genes to explore in this remarkable family are the *follistatin* (*Fst*) and the *bone morphogenetic protein 2* (*Bmp2*) genes, which are both expressed in the mouse embryonic ovary and appear to be important for ovary organogenesis. *Fst* acts downstream of *Wnt4* to inhibit the formation of the XY-specific coelomic vessel and to maintain germ cell survival in the cortical domain of the ovary. *Bmp2* appears to also act downstream of *Wnt4* but independently of *Fst* (9, 33). Mutations in *Gata4* or *Fog2* can also

cause sex reversal in mice (10, 34, 35) and are potential candidate genes to be explored.

Alternatively, because at least one other member of this extended family has 46,XY/45,X mixed gonadal dysgenesis (V-28), disorders of sexual development in this family may be due to a mutant gene that predisposes to chromosomal mosaicism and mixed gonadal dysgenesis. In either scenario, the transmission of the phenotype is strongly suggestive of X-linked inheritance, although an autosomal dominant sex-limited mutation cannot be formally excluded. Various studies of familial 46,XY gonadal dysgenesis have suggested the existence of an X chromosome locus that is necessary for testis determination (36-39), perhaps on proximal Xp (40). The latter finding is consistent with observations of deletions of Xp associated with 46,XX SRY-negative true hermaphroditism (11).

The serendipitous discovery of a predominantly 46,XY karyotype in this fertile mother of a 46,XY daughter suggests that perhaps all mothers of 46,XY (SRY+) females with complete gonadal dysgenesis should be carefully examined for an XY karyotype as well. This extraordinary family affords an exceptional

opportunity to investigate potential factors that can induce ovarian differentiation and function without the Xq critical region. Meanwhile, DNA has been obtained on 19 family members (3 affected, 16 unaffected) across 3 generations in the hopes of identifying the etiology of sex reversal in this interesting family. Linkage analysis is a potential next step as efforts are being made to obtain DNA from more affected members. A genome-wide search for deletions or

duplications in this mother and her daughter may serve to uncover novel gene mutations responsible for sex reversal or may even reveal a genetic cause for chromosomal mosaicism and mixed gonadal dysgenesis.

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Figure 1. Patient 2 (mother) and Laparoscopic photograph of right ovary of patient 2

Figure 2. Family Members

II-3. Woman with masculine appearance; no breasts; infertile; moved away from hometown because of unacceptable appearance and died during World War II at age of 62.

II-4. Woman who died at age 76 years

II-6. Woman who died at age 68 years

II-7. Man who died at age 64 years

III-5,6. Woman with hirsutism. Died around age 60 years.

III-9. Ambiguous genitalia with hirsutism (beard); raised as female; family was ashamed of her and hid her from public; died at age 55 yrs

III-10. Man with confused gender identity; infertile; committed suicide at age 24 yrs

III-13. Normal man who died at age 70

III-14. Normal woman

*IV-3. 46,XY fertile man

*IV-4. Fertile woman with a predominantly 46,XY ovary (Patient 2, mother)

*IV-5. 46,XX fertile woman

IV-7. Woman with absent uterus and ovaries (established outside of Zagreb, and history was obtained from Patient 1 (daughter)); on hormone replacement; died at age 42 yrs from multiple sclerosis

*IV-8. Normal fertile woman

*IV-9. Normal fertile man

*IV-10. 46,XX fertile woman

*IV-11. Normal fertile man

*IV-12. 46,XY normal fertile man

*IV-27. 46,XX fertile woman

*V-3. 46,XY complete gonadal dysgenesis.

*V-5. 46,XX fertile woman

*V-6. 46,XY male with ambiguous genitalia, bifid scrotum, and hypospadias;

hypoplastic testes in scrotum; stretched penile length 5 cm; high gonadotropins (LH 20.9

IU/L, FSH 59.1 IU/L); infertile; testosterone 15.4 nmol/L; estradiol 0.04 nmol/L;

prolactin 4.3 ng/mL

*V-7. Normal woman

*V-8. Normal man

*V-9. Normal woman

*V-10. Normal man

*V-11. 46,XX normal woman with normal hormones

*V-12. Normal woman

*V-13. Normal man

*V-16. Normal woman

*V-20. Normal man

V-21. Infertile woman. Tried *in vitro* fertilization without success.

V-23. Born with ambiguous genitalia and raised as a male. No facial hair.

*V-28. 46,XY/45,X Mixed gonadal dysgenesis; gonadoblastoma

*VI-1. Normal woman

*VI-2. Normal woman

*VI-3. Normal woman

*VI-4. Normal woman

*VI-5. Normal man

* Personal examination by M. Dunic

Figure 3. PCR amplification of the YAP insertion polymorphism on the Y chromosome

Lane 1, 100 bp molecular weight marker; 2, water negative control; 3, father of patient 2; 4, patient 2 (daughter); 5, patient 1 (mother). The Y chromosome of the mother has the YAP insertion, which is absent from the daughter and father.

Figure 4. Legend

WT-1	Wilms' tumor 1
SF-1	Steroidogenic factor 1
LHX1	LIM homeobox 1
LHX9	LIM homeobox 9
DMRT1 & 3	Doublesex and Mab3-related transcription factors 1 and 3 (located on ch 9p24)
SRX	Sex-determining region of Y chromosome
SOX9	SRX-box-related 9
DAX1	Dosage-sensitive sex reversal locus-adrenal hypoplasia congenita-critical region on the X, gene 1
WNT-4	Wingless-type MMTV integration site family family, member 4. Member of Wnt family of locally secreted growth factors
RSPO	R-spondins
FST	Follistatin
BMP2	Bone morphogenetic protein 2
GATA-4	GATA-binding protein 4. Codes for a zinc finger transcription factor
AR	Androgen receptor
AMH-R	Anti-mullerian hormone receptor

Table 1. Primer pairs used for the amplification of genes involved in mammalian sex-determination.

DMRT1 exon 1 F	GGCAGACCTCGCCACTCCAG
DMRT1 exon 1 R	AAGGCTGAACCCGGGCTCCC
DMRT1 exon 1 F	TCTGTGTTTTGGCAAAGCTG
DMRT1 exon 2 R	CTGCTTCTGTGGCTGCAA
DMRT1 exon 3 F	GCAGGTCTTGGGTAGGAAGG
DMRT1 exon 3 R	CATGTGGCTTTCACACAACC
DMRT1 exon 4 F	CAAGGTGTCGGGAACATAGG
DMRT1 exon 4 R	CTCTCTCAACCCCAAATCCA
DMRT1 exon 5 F	GGAGAGCGTCACTTTCTTTGTT
DMRT1 exon 5 R	CCATGCAGATGGTAGTCACG
DMRT3 exon 1 F	CGGAGCACACACGACCAC
DMRT3 exon 1 R	GTCCTCCCAAGTGGAGCTG
DMRT3 exon 2 F1	TGCATTTGCTCTTCCAAA
DMRT3 exon 2 R1	AGAGTCGGCAGAAAACCTCA
DMRT3 exon 2 F2	AACTTCCGCAGAACCTGAGA
DMRT3 exon 2 R2	AGATGTGGCCTCTCCTCAGA
BPESC1 F	AAGGTGACTTAAGGGCAGAGC
BPESC1 R	GCCTGTCTCCAGACAAGAGTG
WNT4 exon 1 F	CCCAGGTAACCCCATCCT
WNT4 exon 1 R	GGTGTGCAGAGGGACGTT
WNT4 exon 2 F	ACAGCATTTCCACTCCCTTG
WNT4 exon 2 R	TCCTTTATGCCCTCACTTGG
WNT4 exons 3/4 F	GGGTGCCTAGCACATGATTT
WNT4 exons 3/4 R	TGAGAGCCTGCACAAATGTT
WNT4 exon 5 F	CACAACGGCAAATCTGACTG
WNT4 exon 5 R	TGAGGACCCAAAAACCAAAC
DAX1 exon 1 F1	ACAGCATCCAGGACATAGTGG
DAX1 exon 1 R1	TGCCTCCTGGGACCTATTTAT
DAX1 exon 1 F2	CGTGCGCGCTAGGTATAAAT
DAX1 exon 1 R2	AAGCAGCAGCGGTACAGAAG
DAX1 exon 1 F3	ACTAGCTCAAAGCAAACGCAC
DAX1 exon 1 R3	TCCTCTTGGCTGAGTTTCTGA
DAX1 exon 2 F1	AGCAAAGGACTCTGTGGTGAG
DAX1 exon 2 R1	GCAGGTTCCATGAAATTGCTA
TSPYLF	GCCGCTGAAATGTTAGTGAGA
TSPYLR	GGAAACAGGGTFCAGAAAAG
SOX9exon1F	GCGCCTTCCTAAGTGCTC
SOX9exon1R	GCAAATCAGCCCTGACCA
SOX9exon2F	TGACCCCTCTCCCTCTTTTT
SOX9exon2Ra	TGCCTCTTAGGCTCTGGGTA
SOX9exon3F1	GCACAGCCCTTGTTGATTTT
SOX9exon3R1	CTCAGCTGCTCCGTCTTGAT
SOX9exon3F2	ATCAAGACGGAGCAGCTGAG
SOX9exon3R2	AGCGAACGCACATCAAGAC

Table 2a. LHRH stimulation test of daughter

Time (min)	LH (mIU/mL)	FSH (mIU/mL)	T (ng/dL)	E ₂ (ng/dL)	Δ ⁴ A (ng/dL)	DHEA (ng/dL)
0	135.7	194.6	91	1.1	78	574
15	162.5	118.6				
30	232.7	133.6				
45	241.5	156.1				
60	229.7	143.7				
90	193.9	147.6				
120	156.1	147.3	72	0.5	91	718

T, testosterone; E₂, estradiol; Δ⁴A, androstenedione; DHEA, dehydroepiandrosterone; LH, luteinizing hormone; FSH, follicle stimulating hormone

Table 2b. LHRH stimulation test of mother

Time (min)	LH (mIU/mL)	FSH (mIU/mL)	T (ng/dL)	E ₂ (ng/dL)	Δ ⁴ A (ng/dL)	DHEA (ng/dL)
0	22.9	48.9	22	0.6	42	96
15	101.7	70.7				
30	114.5	81.8				
45	115.5	86.7				
60	109.2	84.0				
90	96.8	84.5				
120	75.1	77.5	20	0.3	37	102

Normal values (mean ± SD):

	T (ng/dL)	E ₂ (ng/dL)	Δ ⁴ A (ng/dL)	DHEA (ng/dL)
XY	329 ± 166	2.5 ± 2.7	49 ± 20	226 ± 110
XX	31 ± 17	9.5 ± 5.8	87 ± 26	296 ± 218

Table 3a. hCG stimulation test of daughter

Time (hours)	T (ng/dL)	E ₂ (ng/dL)	Δ ⁴ A (ng/dL)	DHEA (ng/dL)
Baseline	90	0.5	29	255
24 hrs	110	0.3	76	424
48 hrs	85	0.3	53	207

hCG, human chorionic gonadotropin

24 hrs, drawn 24 hours after last dose of hCG

48 hrs, drawn 48 hours after last dose of hCG

Table 3b. hCG stimulation test of mother

Time (hours)	T (ng/dL)	E ₂ (ng/dL)	Δ ⁴ A (ng/dL)	DHEA (ng/dL)
Baseline	25	2.7	44	157
24 hrs post	46	0.5	33	157

48 hrs post	30	0.3	38	98
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Table 4a. ACTH stimulation test of daughter

Time (min)	17OHP ng/dL	17 Δ^5 P ng/dL	Δ^4 A ng/dL	DHEA ng/dL	T ng/dL	E ₂ ng/dL	DOC ng/dL	B μ g/dL	F μ g/dL	Aldo ng/dL	DHT ng/dL
0	36	321	78	574	91	1.1	25	0.48	12.5	5	7
60	73	886	98	821	94	1.5	56	3.36	24.7	9	10

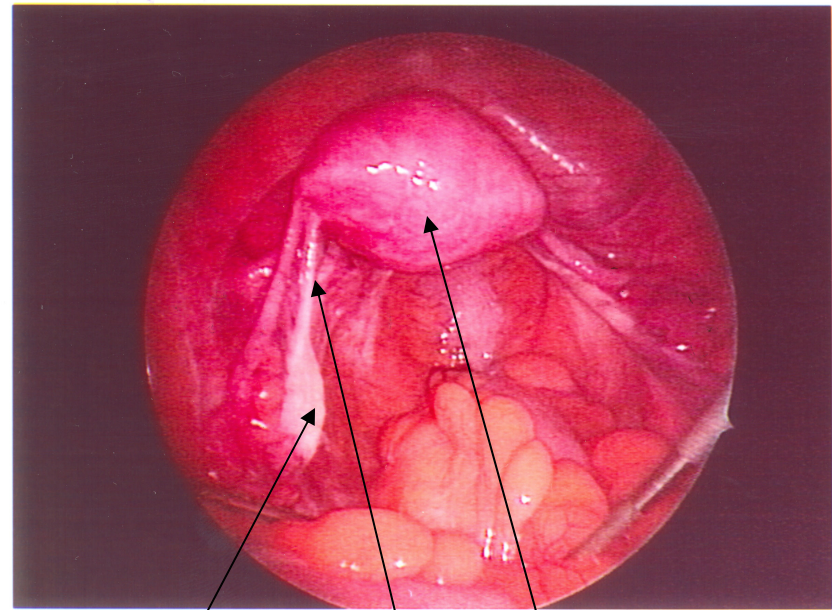
Min, minutes; 17OHP, 17-hydroxyprogesterone; 17 Δ^5 P, 17-hydroxypregnenolone; DOC, deoxycorticosterone; B, corticosterone; F, cortisol; Aldo, aldosterone; DHT, dihydrotestosterone

Table 4b. ACTH stimulation test of mother

Time (min)	17OHP ng/dL	17 Δ^5 P ng/dL	Δ^4 A ng/dL	DHEA ng/dL	T ng/dL	E ₂ ng/dL	DOC ng/dL	B μ g/dL	F μ g/dL	Aldo ng/dL	DHT ng/dL
0	19	78	44	157	25	2.7	19	0.24	9.0	10	5
60	159	797	118	415	34	3.2	69	2.92	31.1	11	7

Normal baseline values (mean \pm SD):

	17OHP ng/dL	17 Δ^5 P ng/dL	DOC ng/dL	B μ g/dL	F μ g/dL	Aldo ng/dL	DHT ng/dL
46,XY	210 \pm 110	180 \pm 180	10 \pm 6	0.65 \pm 0.34	15.6 \pm 6.5	15 \pm 9	38 \pm 15
46,XX	150 \pm 120	196 \pm 187	18 \pm 11	0.35 \pm 0.39	12.0 \pm 6.7	11 \pm 7	13 \pm 8



Right ovary

Uterus

Right fallopian tube

Figure 1. Patient 2 (mother) and Laparoscopic photograph of right ovary

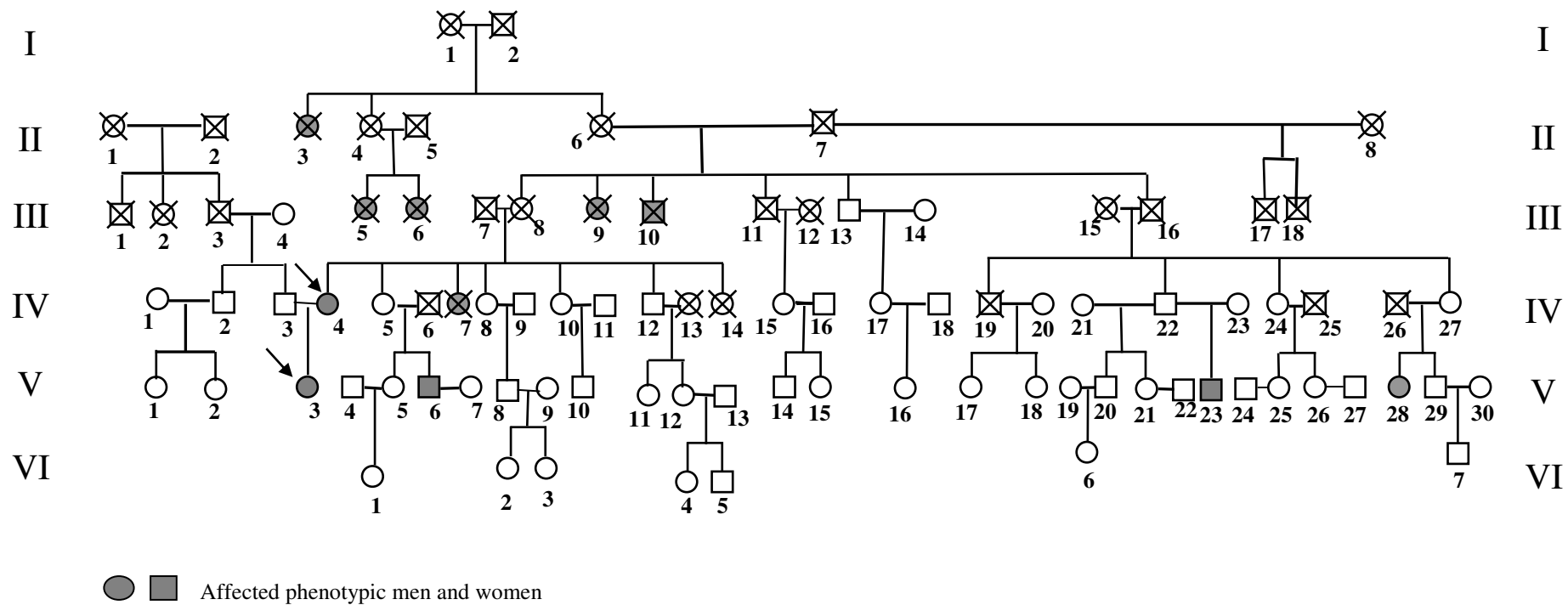


Figure 2. Pedigree. The arrows depicts daughter and mother.

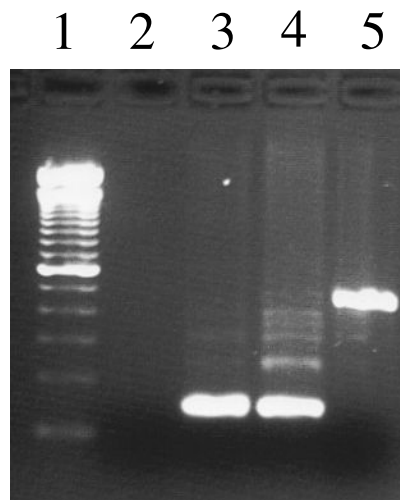


Figure 3. PCR amplification of the YAP polymorphism on the Y chromosome

Figure 4. Genes involved in sexual differentiation

