

## Brief Report

### HYPOGONADOTROPIC HYPOGONADISM IN A FEMALE CAUSED BY AN X-LINKED RECESSIVE MUTATION IN THE *DAX1* GENE

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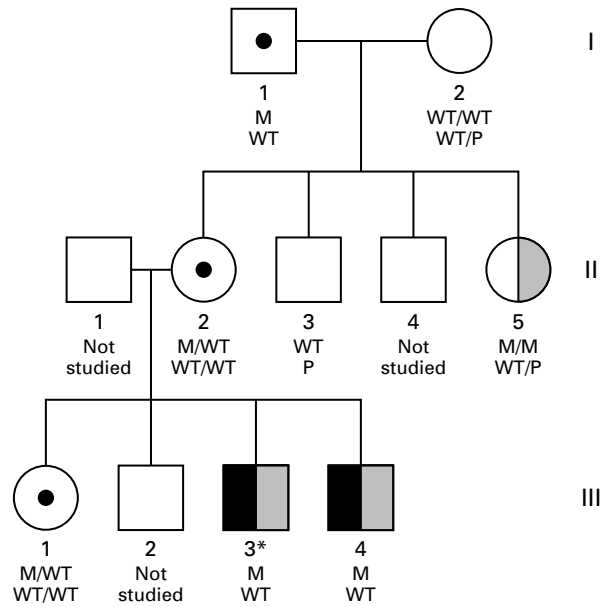
**A**DRENAL hypoplasia congenita is a rare X-linked disorder characterized by primary adrenal insufficiency and hypogonadotropic hypogonadism.<sup>1</sup> All patients described to date have been male, and female carriers have had no clinical symptoms.<sup>1-10</sup> Although most patients present with adrenal crisis in the neonatal period, the onset of adrenal insufficiency varies, even within a family, from the neonatal period to 10 years of age.<sup>1,3,4,9</sup>

The gene responsible for this disorder, *DAX1*, is on the short arm of the X chromosome<sup>11</sup> and encodes a 470-amino-acid member of the nuclear hormone receptor superfamily. The DNA-binding domain consists of amino acid repeats rather than the zinc fingers characteristic of other nuclear hormone receptors.<sup>11</sup> It has no known ligand.<sup>11,12</sup> *DAX1* is expressed in the adrenal cortex, gonads, hypothalamus, and pituitary.<sup>13</sup> Little is known about the function of *DAX1* or the genes that it regulates. The majority of reported *DAX1* mutations are nonsense or frame-shift mutations, suggesting that severe alterations are necessary to cause the clinical symptoms. The few reported missense mutations of the *DAX1* gene are in the presumptive ligand-binding domain.<sup>1,7,9,10</sup> No relation between the onset of adrenal insufficiency and the site of the mutation has been demonstrated.

We conducted molecular genetic and clinical studies in a kindred with X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. The two affected family members were brothers who were hemizygous for a *DAX1* mutation; their unaffected mother was heterozygous for the mutation. The mutation, a nonsense mutation in the DNA-

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**Figure 1.** Pedigree of a Family with Adrenal Hypoplasia Congenita and Hypogonadotropic Hypogonadism.

Squares denote male family members, circles female family members, open symbols unaffected family members, symbols with a dot unaffected carriers, symbols with stippling family members with hypogonadotropic hypogonadism, and half-solid symbols family members with adrenal insufficiency. The genotype is displayed below each symbol: the upper row indicates the presence of the mutation (M) or the wild-type (WT) sequence, and the lower row the presence of the upstream polymorphism (P) or the wild-type sequence. The asterisk indicates the proband.

binding domain that should prevent synthesis of a functional protein, was also found in their maternal grandfather, who was unaffected. Their maternal aunt, who had isolated hypogonadotropic hypogonadism, was homozygous for the mutation. This homozygosity apparently resulted from gene conversion, the nonreciprocal transfer of DNA from one parental allele to the other. This family illustrates an extraordinary range of phenotypes associated with the same *DAX1* mutation. Moreover, the apparently spontaneous gene conversion in the aunt illustrates a novel molecular cause of isolated hypogonadotropic hypogonadism and suggests a new molecular mechanism by which X-linked recessive disease may occur in a female.

#### CASE REPORT

The proband (Subject III-3 in Fig. 1) was seen at the age of 16 days because of vomiting, lethargy, dehydration, hyponatremia, and hyperkalemia. His genitalia were normal, but the scrotum was hyperpigmented. No 17-ketosteroids or pregnanetriol was detected in a 24-hour urine collection. He was treated with cortisone acetate and desoxycorticosterone acetate. He presented to our clinic at the age of 16 years with delayed puberty. Physical

**TABLE 1.** ENDOCRINOLOGIC CHARACTERISTICS OF THE PROBAND AND HIS MATERNAL AUNT.\*

| CHARACTERISTIC   | PROBAND | AUNT                           | NORMAL RANGE                               |
|--|---------|--------------------------------|--|
| Serum cortisol ( $\mu\text{g}/\text{dl}$ )                       |         | 10                             | 7–25                                       |
| Serum aldosterone ( $\text{ng}/\text{dl}$ )                      |         | 5                              | 1–21                                       |
| Serum corticotropin ( $\text{pg}/\text{ml}$ )                    |         | 29                             | 0–60                                       |
| Plasma renin activity ( $\text{ng}/\text{ml}/\text{hr}$ )        |         | <0.6 (supine)<br>2.7 (upright) | <0.6–3.0                                   |
| Serum dehydroepiandrosterone sulfate ( $\mu\text{g}/\text{dl}$ ) | <1.0    | 82                             | Men, 100–400<br>Women, 60–230              |
| Serum luteinizing hormone (U/liter)                              | 5.1     | 5.2                            | Men, 6–26<br>Women, follicular phase, 6–27 |
| Serum follicle-stimulating hormone (U/liter)                     | 6.2     | 4.6                            | Men, 5–25<br>Women, follicular phase, 6–27 |
| Serum estradiol ( $\text{pg}/\text{ml}$ )                        | <6.9    | <5.5                           | Women, follicular phase, 10–100            |
| Serum testosterone ( $\text{ng}/\text{dl}$ )                     | 14.6    | <8.4                           | Men, 200–1000<br>Women, 20–80              |

\*Measurements were obtained in the proband at the time of the diagnosis of hypogonadotropic hypogonadism at the age of 16 years, while he was receiving a glucocorticoid and a mineralocorticoid. Measurements were made in his aunt at the age of 50 years, five weeks after the discontinuation of estrogen therapy. Unstimulated values (i.e., before challenge) are given. To convert values for serum cortisol to nanomoles per liter, multiply by 27.6; to convert values for serum aldosterone to picomoles per liter, multiply by 27.7; to convert values for corticotropin to picomoles per liter, multiply by 0.22; to convert values for plasma renin activity to nanograms per liter per second, multiply by 0.28; to convert values for serum dehydroepiandrosterone sulfate to nanomoles per liter, multiply by 0.027; to convert values for serum estradiol to picomoles per liter, multiply by 3.67; and to convert values for serum testosterone to picomoles per liter, multiply by 34.7.

examination revealed small testes (volume, 4 to 6 ml each) and Tanner stage 3 pubic hair. Serum gonadotropin and testosterone concentrations were in the prepubertal range (Table 1). The boy was treated with testosterone.

His younger brother (Subject III-4) began to vomit and to have trouble gaining weight at the age of two weeks, and he was also treated with a glucocorticoid and a mineralocorticoid. He also had hypogonadotropic hypogonadism and was subsequently treated with testosterone.

The boys' maternal aunt (Subject II-5) was evaluated at the age of 16 years for delayed puberty and primary amenorrhea. She was given a diagnosis of hypogonadotropic hypogonadism and treated with estrogen and progesterin. She never had symptoms or signs of adrenal insufficiency. At the age of 50 years, she was evaluated at our clinic. She was tall (174 cm), with eunuchoidal proportions (an arm span of 182 cm and a ratio of the upper to the lower segment of 0.68). There was no hyperpigmentation. Five weeks after the discontinuation of estrogen therapy, serum gonadotropin concentrations were low (Table 1) and did not increase in response to gonadotropin-releasing hormone. Adrenal function was normal both basally (Table 1) and in response to corticotropin (peak serum cortisol concentration, 27  $\mu\text{g}$  per deciliter [745 nmol per liter]). Computed tomography of the abdomen revealed normal adrenal glands, and magnetic resonance imaging of the head revealed a small pituitary gland (4 mm in the largest dimension). Transvaginal ultrasonography revealed a small uterus and small ovaries (right, 1.1 by 1.2 by 1.2 cm; left, 1.0 by 0.9 by 1.0 cm).

Blood was collected from five other family members (the boys' sister, mother, maternal uncle, and maternal grandparents), none of whom had a history of adrenal insufficiency or delayed puberty. Adrenal function was normal in the grandfather (morning serum cortisol concentration, 18  $\mu\text{g}$  per deciliter [497 nmol per liter]).

The protocol was approved by the institutional review board, and informed consent was obtained from each family member studied.

## METHODS

### DNA Extraction, Amplification, and Sequencing of the *DAX1* Gene

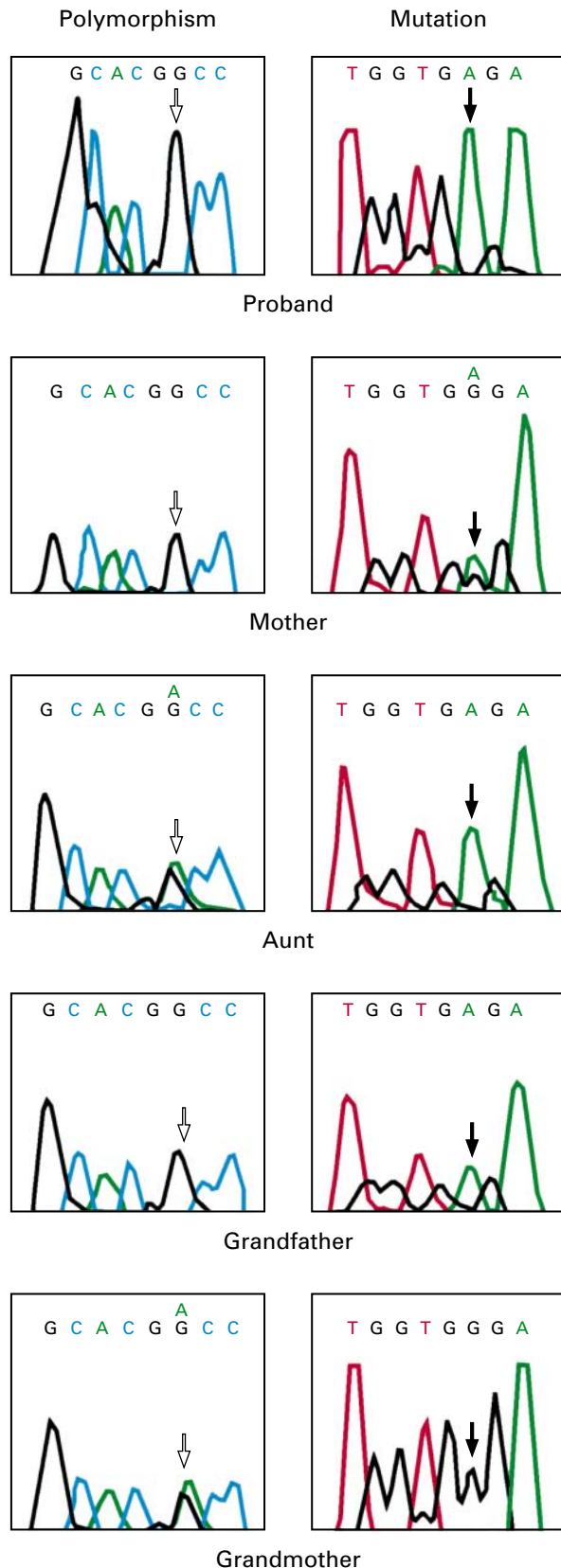
Genomic DNA was prepared from peripheral-blood leukocytes, skin, and urinary sediment according to standard procedures.<sup>14</sup> The *DAX1* gene was amplified by the polymerase chain reaction (PCR) with the use of previously described primer pairs.<sup>4</sup> The PCR products were sequenced from both strands by the dideoxy method with an automated fluorescence sequencing system (model 373A, Applied Biosystems, Foster City, Calif.).<sup>15</sup>

### Subcloning

Primers 3 and 4<sup>4</sup> were used to amplify DNA from peripheral-blood leukocytes from the proband's mother and maternal aunt. The PCR products were subcloned into pCR2.1 vector (Invitrogen, San Diego, Calif.). The resulting construct was used to transform *Escherichia coli* strain JM109 (Promega, Madison, Wis.). Both strands of positive clones were sequenced with the use of primers 3 and 4 according to the dideoxy method.

### Sequence-Specific Oligonucleotide Hybridization

Genomic DNA was amplified by PCR with use of primer 4 in conjunction with primer 5'GCAGCATCCTCTACAGCTT3'<sup>9</sup>. The concentration of sodium dodecyl sulfate in the hybridization solution was 0.2 percent. Filters were hybridized at 52°C for 20 hours. The wild-type and mutant oligonucleotides were 5'GGC-GCGTGTTGGACCGCTCCT3' and 5'GGCGCGTGGTGAGACCGCTCCT3', respectively. After hybridization, each filter was washed three times at room temperature for 10 minutes and once at 72°C for 7 minutes in a solution containing 6× saline sodium citrate (1× saline sodium citrate is 0.15 M sodium chloride and 0.015 M sodium citrate) and 0.2 percent sodium dodecyl sulfate.



**Hormonal Measurements**

Plasma luteinizing hormone, follicle-stimulating hormone, estradiol, testosterone, and dehydroepiandrosterone sulfate were measured by radioimmunoassay by Covance Laboratories (Vienna, Va.). Plasma cortisol was measured by fluorescence polarization immunoassay (Abbott Laboratories, Abbott Park, Ill.). Plasma aldosterone, corticotropin, and renin activity were measured by radioimmunoassay by Mayo Medical Laboratories (Rochester, Minn.).

**RESULTS**

The proband and his affected brother both had an adenine substituted for guanine in exon 1 of the *DAX1* gene (Fig. 2). This nonsense mutation introduces a stop codon at position 172. The boys' mother was heterozygous for this nonsense mutation, indicating a carrier state. Thus, as expected for an X-linked disorder, the mother was heterozygous and unaffected, whereas her affected sons were hemizygous for the mutation.

The proband's aunt had hypogonadotropic hypogonadism, but not adrenal insufficiency. She had only the nonsense mutation, with no wild-type sequence, on direct sequencing of PCR-amplified peripheral-blood DNA. To determine whether she was homozygous (with two alleles each bearing the nonsense mutation) or hemizygous (one allele with a single copy of the mutation), we performed further analyses. The peripheral-blood karyotype was normal (46,XX), with no evidence of structural abnormalities or mosaicism in 100 cells. A polymorphic marker was identified 18 bp upstream from the mutation. The aunt was heterozygous for this polymorphism.

We then subcloned the aunt's genomic DNA obtained from lymphocytes. All 15 colonies had the mutation, and 6 had both the mutation and the polymorphism, confirming that two alleles were present in this XX female. One *DAX1* allele had both the mutation and polymorphism, whereas the other allele had only the mutation (Fig. 1).

Molecular analysis of *DAX1* was also performed in the boys' maternal grandparents. Their grandfather carried the mutation but not the polymorphism, whereas their grandmother was heterozygous

**Figure 2.** Sequence Analysis of Exon 1 of the *DAX1* Gene in the Proband and His Mother, Maternal Aunt, and Maternal Grandparents.

Sequence analysis revealed the substitution of adenine for guanine (G→A) (solid arrow) in the proband's DNA. His mother was heterozygous for this nonsense mutation, indicating a carrier state. Only the mutation (no wild-type sequence) was found in his aunt, who was also heterozygous for a polymorphism 18 bp upstream from the mutation (open arrow). His grandmother did not have the nonsense mutation and was heterozygous for the polymorphism, whereas his grandfather had the mutation, but not the polymorphism. Automated fluorescence sequencing was performed with the use of PCR products as templates.

for the polymorphism and did not have the mutation (Fig. 1). To search for evidence of mosaicism, we obtained genomic DNA from other tissues in the aunt, grandmother, and grandfather. In the aunt, analysis of lymphocytes, urinary sediment, and skin all revealed homozygosity for the *DAX1* mutation and heterozygosity for the polymorphism. There was no evidence of mosaicism. In the grandmother and grandfather, the results of mutational analysis of DNA from urinary sediment were similar to those in leukocyte DNA. Subcloning analysis of the grandmother's genomic DNA was also performed; eight colonies did not have the nonsense mutation, and two had the polymorphism. In addition, no other base-pair substitutions were detected in the coding region and exon-intron boundaries of *DAX1* in any of the family members studied.

To search for further evidence of mosaicism, we used sequence-specific oligonucleotide hybridization to analyze DNA samples from all available family members (including lymphocytes, urinary sediment, and skin from the aunt). In this technique, DNA is bound to nitrocellulose filters and hybridized under stringent conditions to radiolabeled oligonucleotide probes that either match the wild-type sequence or contain a specific single-base-pair substitution. We found no evidence of mosaicism in any of the family members (data not shown).

### DISCUSSION

We describe two boys with typical adrenal hypoplasia congenita (neonatal adrenal insufficiency with hypogonadotropic hypogonadism) and a nonsense mutation in the *DAX1* gene. This mutation has previously been described in two related males with hypogonadotropic hypogonadism in whom adrenal insufficiency was diagnosed at six weeks and five years of age.<sup>1</sup> As expected, our patients' mother was an unaffected carrier.

Two family members had unusual genotypic and phenotypic findings. The boys' unaffected grandfather was hemizygous for the nonsense mutation, indicating a lack of penetrance of the mutation. Their maternal aunt, who had isolated hypogonadotropic hypogonadism, was homozygous for the mutation and heterozygous for a nearby polymorphic marker. The presence of the polymorphism indicates that she inherited two different *DAX1* alleles, one from each of her parents, and that the nonsense mutation in her paternal allele was probably introduced into the maternal allele through spontaneous gene conversion early in embryogenesis.

That the same mutation could result in two brothers with the complete syndrome, an unaffected grandfather, and an aunt with only hypogonadotropic hypogonadism is a remarkable discrepancy in genotype-phenotype relations. This discrepancy might be explained by the presence of undetected mosaicism in the grandfather and aunt. We cannot determine whether mosaicism explains the phenotype of the grandfather and aunt because we were not able to obtain adrenal, hypothalamic, or pituitary tissue from them. Alternatively, this variable expressivity might reflect other proteins that serve as *DAX1* surrogates during development or epigenetic phenomena that modulate the phenotype of patients with *DAX1* mutations.

Although gene conversion during mitosis of somatic cells should result in mosaicism, conversion early in embryogenesis could produce a person without mosaicism. At approximately three to four days of gestation, the majority of cells differentiate into the trophoblasts destined to become extraembryonic tissue, whereas less than 40 percent of cells evolve into the inner cell mass, which is destined to become embryonic tissue.<sup>16</sup> Gene conversion before this unequal division of cells could produce a person without mosaicism.

Gene conversion represents one mechanism by which an X-linked recessive disease may occur in a female. Interallelic gene conversion has been described as a source of allelic diversity at the HLA loci, with a frequency of approximately 1 in 10,000 sperm.<sup>17</sup> Gene conversion during mitosis involving the transfer of genetic material between maternal and paternal alleles, as may have occurred in our patient, has been described for the autosomal genes causing epidermolysis bullosa<sup>18</sup> and Fanconi's anemia.<sup>19</sup>

Isolated hypogonadotropic hypogonadism may occur in families or sporadically and is five to seven times as common in males as in females.<sup>20</sup> Males with this condition may have Kallmann's syndrome owing to a mutation of another X-chromosome gene, *KALI*.<sup>21</sup> Defects in *KALI* have been found in 52 percent of patients with X-linked cases of isolated hypogonadotropic hypogonadism<sup>22</sup> and in 5 percent of patients with sporadic cases.<sup>23</sup> Although *DAX1* mutations have been thought to cause both adrenal insufficiency and hypogonadotropic hypogonadism, our findings suggest that alterations in the *DAX1* gene or functionally related genes may cause isolated hypogonadotropic hypogonadism.

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### REFERENCES

1. Muscatelli F, Strom TM, Walker AP, et al. Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature* 1994;372:672-6.
2. Guo W, Mason JS, Stone CG Jr, et al. Diagnosis of X-linked adrenal hypoplasia congenita by mutation analysis of the DAX1 gene. *JAMA* 1995; 274:324-30.
3. Yanase T, Takayanagi R, Oba K, Nishi Y, Ohe K, Nawata H. New mutations of DAX-1 genes in two Japanese patients with X-linked congenital adrenal hypoplasia and hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1996;81:530-5.
4. Nakae J, Tajima T, Kusuda S, et al. Truncation at the C-terminus of the

DAX-1 protein impairs its biological actions in patients with X-linked adrenal hypoplasia congenita. *J Clin Endocrinol Metab* 1996;81:3680-5.

5. Habiby RL, Boepple P, Nachtigall L, Sluss PM, Crowley WF Jr, Jameison JL. Adrenal hypoplasia congenita with hypogonadotropic hypogonadism: evidence that DAX-1 mutations lead to combined hypothalamic and pituitary defects in gonadotropin production. *J Clin Invest* 1996;98:1055-62.

6. Meloni A, Meloni A, Cao A, Rosatelli MC. New frameshift mutation in the DAX-1 gene in a patient with X-linked adrenal hypoplasia and hypogonadotropic hypogonadism. *Hum Mutat* 1996;8:183-4.

7. Schwartz M, Blichfeldt S, Muller J. X-linked adrenal hypoplasia in a large Greenlandic family: detection of a missense mutation (N4401) in the DAX-1 gene: implication for genetic counselling and carrier diagnosis. *Hum Genet* 1997;99:83-7.

8. Kinoshita E, Yoshimoto M, Motomura K, et al. DAX-1 gene mutations and deletions in Japanese patients with adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Horm Res* 1997;48:29-34.

9. Nakae J, Abe S, Tajima T, et al. Three novel mutations and a de novo deletion mutation of the DAX-1 gene in patients with X-linked adrenal hypoplasia congenita. *J Clin Endocrinol Metab* 1997;82:3835-41.

10. Takahashi T, Shoji Y, Shoji Y, Haraguchi N, Takahashi I, Takada G. Active hypothalamic-pituitary-gonadal axis in an infant with X-linked adrenal hypoplasia congenita. *J Pediatr* 1997;130:485-8.

11. Zanaria E, Muscatelli F, Bardoni B, et al. An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature* 1994;372:635-41.

12. Lalli E, Bardoni B, Zazopoulos E, et al. A transcriptional silencing domain in DAX-1 whose mutation causes adrenal hypoplasia congenita. *Mol Endocrinol* 1997;11:1950-60.

13. Ikeda Y, Swain A, Weber TJ, et al. Steroidogenic factor 1 and DAX-1

colocalize in multiple cell lineages: potential links in endocrine development. *Mol Endocrinol* 1996;10:1261-72.

14. Blin N, Stafford DW. A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Res* 1976;3:2303-8.

15. Smith LM, Sanders JZ, Kaiser RJ, et al. Fluorescence detection in automated DNA sequence analysis. *Nature* 1986;321:674-9.

16. Hartshorne GM, Edwards RG. Early embryo development. In: Adashi EY, Rock JA, Rosenwaks Z, eds. Reproductive endocrinology, surgery and technology. Philadelphia: Lippincott-Raven, 1996:436-50.

17. Zangenberg G, Huang M, Arnheim N, Erlich H. New HLA-DPB1 alleles generated by interallelic gene conversion detected by analysis of sperm. *Nat Genet* 1995;10:407-14.

18. Jonkman ME, Scheffer H, Stulp R, et al. Revertant mosaicism in epidermolysis bullosa caused by mitotic gene conversion. *Cell* 1997;88:543-51.

19. Lo Ten Foe JR, Kwee ML, Rooimans MA, et al. Somatic mosaicism in Fanconi anemia: molecular basis and clinical significance. *Eur J Hum Genet* 1997;5:137-48.

20. Jones JR, Kemmann E. Olfacto-genital dysplasia in the female. *Obstet Gynecol Annu* 1976;5:443-66.

21. Legouis R, Hardelin JP, Levilliers J, et al. The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell* 1991;67:423-35.

22. Hardelin JP, Levilliers J, Blanchard S, et al. Heterogeneity in the mutations responsible for X chromosome-linked Kallmann syndrome. *Hum Mol Genet* 1993;2:373-7.

23. Georgopoulos NA, Pralong FP, Seidman CE, Seidman JG, Crowley WF Jr, Vallejo M. Genetic heterogeneity evidenced by low incidence of KAL-1 gene mutations in sporadic cases of gonadotropin-releasing hormone deficiency. *J Clin Endocrinol Metab* 1997;82:213-7.

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