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Familial Case with Sequence Variant in the Testis-determining Region Associated with Two Sex Phenotypes

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Summary

The human Y chromosome encodes a testis-determining factor (TDF) which is responsible for initiating male sex determination. Recently a region of the Y chromosome (SRY) was identified as part of the TDF gene. We have identified a three-generation family (N) in which all XY individuals have a single base-pair substitution resulting in a conservative amino acid change in the conserved domain of the SRY open reading frame. Three individuals are XY sex-reversed females, and two are XY males. Several models are proposed to explain association between a sequence variant in SRY and two sex phenotypes.

Introduction

The Y chromosome contains at least one testis-determining factor (TDF). Recently, a region termed "SRY" was identified as belonging to a testis-determining gene (Sinclair et al. 1990). The SRY open reading frame (SRY-orf) has a conserved motif which shares homology with domains found in the high-mobility-group proteins (Wright and Dixon 1988), the T lymphocyte–specific transcription factor TCF-1 (van de Wetering et al. 1991), and the human nucleolar transcription factor hUBF (Jantzen et al. 1990). The conserved domain has been postulated to have a DNA-binding function (Jantzen et al. 1990). Evidence supporting the role of SRY in testis determination was obtained from analysis of XY females with gonadal dysgenesis who were found to have de novo mutations in the SRY-orf: a single base-pair change (Berta et al. 1990) and a 4-bp deletion (Jäger et al. 1990). Both cases of XY sex reversal associated with de novo mutations in the SRY-orf strongly infer that the SRY-orf is part of the TDF gene. In the same analysis, a single mutation was found in an XY female (JN) and also in her father (Berta et al. 1990).

In this report we describe the molecular structure of the SRY-orf in a family (fig. 1) which contains three XY females (including JN) and two XY males. All five XY members had a single base-pair change resulting in a conservative amino acid change in the SRY-orf.

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Material and Methods

Molecular Analysis

DNA was isolated from 40 ml of peripheral blood according to a method described elsewhere (Damiani et al. 1990). Relatedness of the five XY members of the family was checked using minisatellite DNA probes (Jeffreys et al. 1985). DNA amplification of the conserved domain of the SRY-orf was performed using oligonucleotide primers which bordered the conserved region of the SRY-orf: GGAATTCCCTAACTCTA-AGTATCAGTGT (bases 529–548 (Sinclair et al. 1990), including a terminal *Eco*RI site) and GGAAT-TCCGCAAACTGCAATTCTTCGGC (bases 843–823, including a terminal *Eco*RI site). Amplified DNA fragments were sequenced directly, essentially according to a method described by Gyllensten and Erlich (1988).

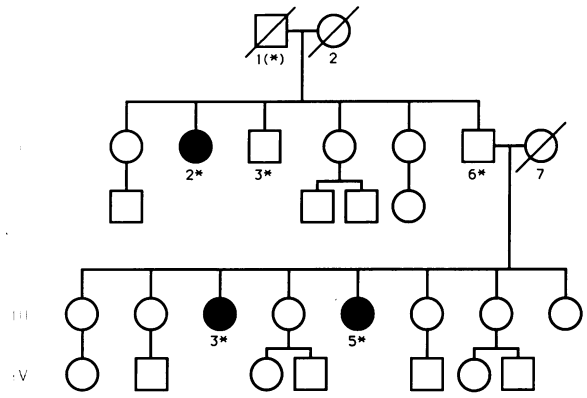

Figure 1 Pedigree of family N. Blackened symbols indicate XY females. An asterisk indicates an individual with a single base-pair change in the SRY-orf. Roman numerals denote generation, and arabic numerals denote individuals described in the text. Individuals II-6 and III-3 have been described elsewhere (Berta et al. 1990).

Histology

A right-gonad biopsy was done and fixed in 10% formalin overnight. After dehydration in a graduated series of alcohol and toluene solutions, the specimen was embedded in paraffin. Tissue sections, 5 mm thick, were cut by a microtome (Leitz), were stained with hematoxylin and eosin, were dehydrated, and were mounted in entellan (rapid mounting media for microscopy, containing xylene and allylcrylate; Merck).

Cytogenetics

Chromosome analysis using Q- and R-banding was performed on peripheral blood lymphocytes. Karyotypes were performed on 40–50 metaphases from each patient.

Results

Case Reports

Propositus III-3 is a phenotypic female who presented at 17 years old with primary amenorrhea. Somatic examination showed a tall, macroskeletal female (height 169 cm, weight 51 kg) with a slight bilateral cubitus valgus and a tuberous nevus in the middle of the lumbar region. No dysmorphy was noted. Vagina and vulva were normal. The laparotomy revealed bilateral streak gonads, which were removed, and normal size uterus and Fallopian tubes. The histological study of the streak gonads showed an ovarian sarcomatoid stroma with some cystic serous formations. Follicles, ova, and neoplastic tissues were not observed. Cytogenetic analysis revealed a 46,XY karyotype in all mitotic cells examined from peripheral blood. The diagnosis inferred from these studies was XY female with pure gonadal dysgenesis.

III-5 is a younger sister of propositus III-3. She presented at 20 years of age with primary amenorrhea and no developed secondary sexual characteristics. She was bony and tall (height 168 cm, weight 50 kg). Laparotomy revealed a hypoplasic uterus and filiform Fallopian tubes. No left ovary was detected, and therefore histology was performed only on the right gonad. The right gonad appeared as a small cystic mass whose wall contained an area of ovarian-type cortical stroma with elongated and wavy plump cells. No mature or germ cells were observed. III-5's karyotype was also 46,XY, on all the mitoses examined. The diagnosis was therefore XY female with pure gonadal dysgenesis.

The propositus' aunt (II-2) was a phenotypic female. She had primary amenorrhea associated with sterility and underwent surgery for a left-ovarian gonadoblastoma at 36 years of age. Her karyotype was 46,XY, which implied that she also was an XY female.

Basal serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were determined for individuals III-3, III-5, and II-2. The levels of both FSH and LH were elevated in these three individuals compared with a normal female (III-3, FSH 60 mUI/ml and LH 80 mUI/ml; III-5, FSH 80 mUI/ml and LH 80mUI/ml; and II-2, FSH 50 mUI/ml and LH 49.9 mUI/ml; normal female, FSH <10 mUI/ml and LH <10 mUI/ml).

The propositus' father (II-6) was a fertile XY male. The propositus' uncle (II-3) was an XY male who had no children. Clinical examination showed that both individuals were phenotypically normal males, with normal penis and two gonads in the scrotum. Both individuals had a normal sexual life.

The ethnic background of the family was French, and there was no indication of inbreeding. There was also no indication of other XY female relatives on either the paternal or maternal side of the family.

Molecular Genetics

The DNA sequence of the conserved motif of the SRY-orf of each of the five XY family members was analyzed. In all five cases the same base-pair substitution (G to C at position 588 of pY53.3; Sinclair et al. 1990), resulting in a valine-to-leucine amino acid

change, was found (data not shown). No other base-pair change was observed in the 240-bp conserved region. This allelic form of the TDF-orf is termed "TDF*."

Genealogy Pedigree

TDF* DNA sequences were found in five members of the family who each received a Y chromosome (fig. 1). Checking of the family relatedness by using minisatellite DNA probes (Jeffreys et al. 1985) showed results compatible with parental relationships (data not shown).

Discussion

A single base-pair substitution in the SRY-orf, resulting in a conservative amino acid change, was found in five members of family N, who each had a Y chromosome. Two were apparently normal males (one was known to be fertile), and three were XY females. Several hypotheses could explain the association between a sequence variant in the SRY-orf and two sexual phenotypes. First, the gene-product activity of TDF* could have been around a critical threshold level such that either the physiological state or the environmental condition at the proper moment during development may have altered SRY gene-product activity favoring either male or female development.

Second, the sexual phenotypes observed in family N may have involved sex-determining genes other than TDF. The TDF* allele may be fortuitously present in the family, as an inconsequential SRY polymorphism. Previous studies have suggested the presence of X-linked recessive genes (Wachtel 1983) and of male-limited autosomal recessive genes (Simpson et al. 1981) in families with sex-reversed XY individuals.

Third, since the SRY-orf sequence is highly conserved in a population of more than 50 normal males and patients (Berta et al. 1990; authors' unpublished data), it is likely that the presence of the TDF* allele influences sex reversal. The TDF* allele could generate two phenotypes through interaction with two independently segregating alleles of a second gene. Evidence in support of this hypothesis comes from studies of autosomal alleles involved in mouse testis determination (Eicher and Washburn 1986). Crosses between different mice strains indicated that three autosomal recessive alleles (Tas, Tda-1, and Tda-2) interacted with alleles on the Y chromosome, which resulted in the generation of both XY females and hermaphrodites (Eicher and Washburn 1986).

In both the second and the third hypotheses, the mutated locus cannot be linked to the TDF* region, because of the presence of two different sexual phenotypes in TDF* individuals in two generations of the family. No descendant of II-6 will have TDF*, since his two XY siblings are sterile XY females. Therefore, if cases of XY females with gonadal dysgenesis occur in future descendants of family N, the indication will be that neither the "threshold level" nor the "interaction" hypothesis is valid. This would imply that only the male-limited autosomal recessive gene model is valid.

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