

Short Report

Mapping a gene for 46,XY gonadal dysgenesis by linkage analysis

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46,XY gonadal dysgenesis was transmitted as an autosomal-dominant trait in a large family with multiple affected members. Expressivity of the trait was highly variable, ranging from pure to partial gonadal dysgenesis associated with normal female genitalia or sexual ambiguity, to mild hypospadias in otherwise normal males. The phenotypic features of this trait appeared to be confined to the genitourinary system. Multipoint parametric analysis using markers D5S664, D5S633, and D5D2102 yielded an LOD score of 4.47, assuming sex-limited, autosomal-dominant inheritance with a penetrance of 0.6. Because mutation in testis-determining genes leads to gonadal dysgenesis in 46,XY individuals, we postulate that the gene mapped by this study normally plays a role in gonadal differentiation.

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Individuals with 46,XY gonadal dysgenesis have abnormal testicular determination. The form of gonadal dysgenesis is characterized by the histology of the affected gonads (1, 2). Individuals with 46,XY complete or pure gonadal dysgenesis lack testes and thus have bilateral streak gonads, absent Wolffian structures (seminal vesicles, vas deferens, and epididymis), and well-developed Mullerian structures (uterus, fallopian tubes, and upper third of the vagina). Individuals with mixed gonadal dysgenesis have a streak gonad on one side and a normal-appearing or dysgenetic gonad on the opposite side. The development of Mullerian and Wolffian structures usually correlates with the histology of the ipsilateral gonad. Individuals with partial gonadal dysgenesis have

bilateral dysgenetic testes that include poorly formed seminiferous tubules and ovarian-like stroma. The development of Mullerian and Wolffian structures usually correlates with the extent of testes differentiation. For both mixed and partial gonadal dysgenesis, the development of the external genitalia may be ambiguous.

Genetic analysis of individuals with 46,XY gonadal dysgenesis and its counterpart, 46,XX maleness, has been instrumental for identifying testis-determining genes (3). The first gene to be identified, *SRY*, was isolated by positional cloning using the DNA from 46,XX males with a Y-to-X translocation, i.e. individuals with genetic sex reversal (4, 5). Almost all of the other genes in the pathway, including *WT1*, *SOX9*, *DAX1*,

and *DMRT1/2/3* were cloned from the DNA of individuals with cytogenetic deletions or duplications and 46,XY gonadal dysgenesis (6–14). Familial cases of 46,XX maleness and 46,XY gonadal dysgenesis unlinked to mutations in known genes suggests the existence of other, as yet unidentified, sex-determining genes (reviewed in refs 15, 16).

Opportunities for identifying human sex-determining genes by linkage analysis are limited by the rarity of large families with multiple cases of sex reversal. Hence, any family with the power to generate a large, significant LOD score presents a rare and important opportunity for identifying a human sex-determining gene. Previously, such a family was described (17). Here, we describe mapping the location of the gene that accounts for this phenotype by linkage analysis using microsatellite markers.

Subjects and methods

Descriptions of affected individuals

This family originated and resided in central France. No consanguinity is known among its members. The proband (individual 403, Fig. 1 and Table 1) was diagnosed when she was hospitalized at 7 years of age for acute abdominal pain. A gonadal tumor was removed at emergency laparotomy. Based upon its histology, the tumor was diagnosed as a dysgerminoma. The peripheral blood karyotype was 46,XY. At a later time, the left gonad was removed prophylactically and found to be a streak with some seminiferous tubules, but without germ cells or Leydig cells. The Fallopian tube and the uterus appeared normal. These findings led to the diagnosis of pure gonadal dysgenesis.

Because of the possibility of familial gonadal dysgenesis, a family study was performed, including chromosome analyses. These studies are summarized here (Table 1 and Fig. 1). Additional clinical information has been published recently (17). Three other females were found to have gonadal tumors, including teratoma (215), gonadoblastoma (334), and dysgerminoma and gonadoblastoma (402). Individuals 215 and 334 were diagnosed during their evaluations for primary amenorrhea, whereas individual 402 was diagnosed during this family study. Altogether, seven females were identified with a 46,XY karyotype. Of these females, three had pure gonadal dysgenesis (215, 403, and 420) and four had partial gonadal dysgenesis (212, 334, 402, and 428). Two of the females with partial gonadal dysgenesis had clitoral hypertrophy (212 and 215) and one had hirsutism (215).

Four males were identified with genital abnormalities: one had first-degree hypospadias with chordee (329); and three had perineal hypospadias (209, 313, and 427). Two of these individuals had micropenis (209 and 313) and one had cryptorchidism (313). The male with first-degree hypospadias and chordee had normal fertility and went on to father two children: one had perineal hypospadias and chordee (427) and the other had 46,XY partial gonadal dysgenesis (428). No extragonadal abnormalities were observed in any individuals.

In summary, among 76 descendants in four generations, seven women had 46,XY pure or partial gonadal dysgenesis and four men had hypospadias or other anomalies (Table 1 and Fig. 1). Among these affected individuals, four had gonadal tumors. DNA was obtained from the peripheral blood for 58 members of the

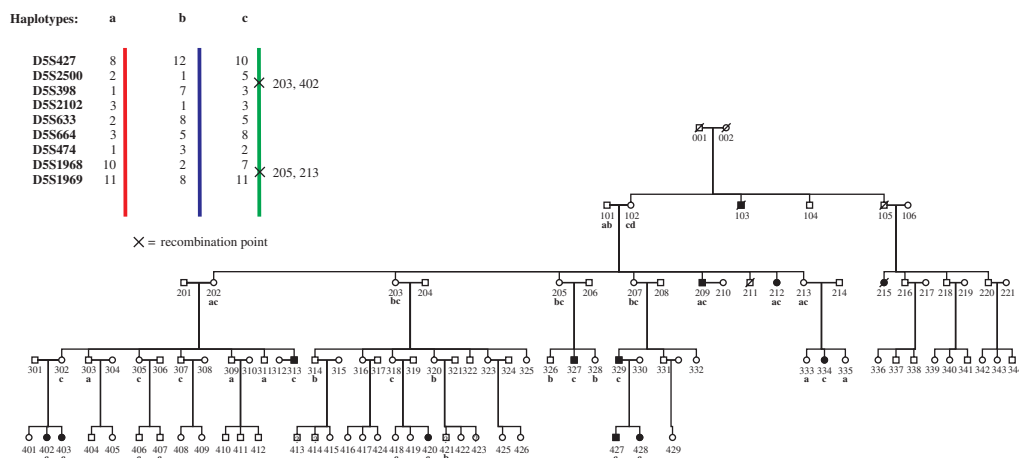


Fig. 1. Pedigree with multiple cases of 46,XY gonadal dysgenesis (indicated by shaded circles) and male pseudohermaphroditism (indicated by shaded squares). This figure also shows the markers associated with a core haplotype, the sites of recombination that define the haplotype, and the individuals in whom these recombinational events occurred.

Table 1. Summary of clinical findings among affected family members

No.	Sex of rearing	Karyotype	Gonadal abnormality	External genitalia	Histology of tumor (if present)
209	Male	46,XY	None observed	Micropenis Perineal hypospadias	No tumor
212	Female	46,XY	Partial gonadal dysgenesis	Clitoral hypertrophy Atrophic vagina Hirsutism	No tumor
215 ^a	Female	?	Pure gonadal dysgenesis	Clitoral hypertrophy	Malignant teratoma
313	Male	46,XY	Cryptorchidism	Micropenis Perineal hypospadias	No tumor
329	Male	46,XY	None observed	First-degree hypospadias Fertile male Cordee	No tumor
334	Female	46,XY	Partial gonadal dysgenesis	Normal	Gonadoblastoma
402	Female	46,XY	Partial gonadal dysgenesis	Normal	Dysgerminoma Gonadoblastoma
403	Female	46,XY	Pure gonadal dysgenesis	Normal	Dysgerminoma
420	Female	46,XY	Pure gonadal dysgenesis	Normal	No tumor
427	Male	46,XY	Normal	Perineal hypospadias Cordee	No tumor
428	Female	46,XY	Partial gonadal dysgenesis	Normal	No tumor

^aNot genotyped.

family, excluding one of the affected, and genotyped using microsatellite markers.

Genotyping

A total of 324 microsatellite markers spaced at ~10 cm intervals on 22 autosomal chromosomes were typed for 42 family members who were informative for the phenotype or its transmission. Unaffected siblings and their unaffected offspring were initially not typed. The markers were selected from the Marshfield Clinic Set8A Combo List using standard conditions for this panel (<http://research.marshfieldclinic.org/genetics/sets/combo.html>). The X chromosome was not typed owing to incompatibility with X-linked transmission in this family (transmission from 329 to 428). An additional 18 markers were added for all available DNAs ($n = 62$) in the region of apparent linkage on chromosome 5, from the GeneMap99, Marshfield, Genome, and Celera databases (Fig. 2). Markers were pooled according to fluorescence label (6-FAM, HEX, and NED) and size, and electrophoresed on a 3700 DNA Analyzer (Applied Biosystems, Foster City, CA).

Data analysis

Semiautomated sizing of alleles was performed using the GENESCAN Analysis program, version 2.1 (Applied Biosystems), and individual genotypes were assigned with the help of the GENOTYPER program, version 2.1 (Applied Biosys-

tems). To ensure accuracy, two individuals checked each genotype manually. Parametric linkage analysis was carried out at 324 markers on 22 autosomal chromosomes using the LINKAGE computer programs (<http://linkage.rockefeller.edu>) (18). Affected-only, non-parametric analysis was performed to reduce the impact from non-penetrant gene carriers, if the trait did not follow typical Mendelian rules. Marker allele frequencies were calculated from genotyped individuals. For parametric analysis, the disease allele frequency was set to be 0.001. Initially, the trait was assumed to be a fully penetrant, Mendelian phenotype; therefore, a model of sex-limited inheritance with a penetrance of 0.99 in 46,XY individuals, and a penetrance of 0.005 in 46,XX individuals, was used. For these markers, a core haplotype was developed (using the SIMWALK2 program) which was found in all females with 46,XY gonadal dysgenesis and all males with hypospadias (19). However, this core haplotype also segregated in three 'normal' male family members (307, 406, and 407), which prompted reconsideration of the monogenic assumption in this family. Another genome-wide screen was performed with the assumption of a reduced penetrance (0.6) in all 46,XY individuals. This penetrance figure was applied to linkage analysis for other markers on chromosome 5.

Results

A genome-wide scan with the penetrance of 0.6 demonstrated linkage at three consecutive

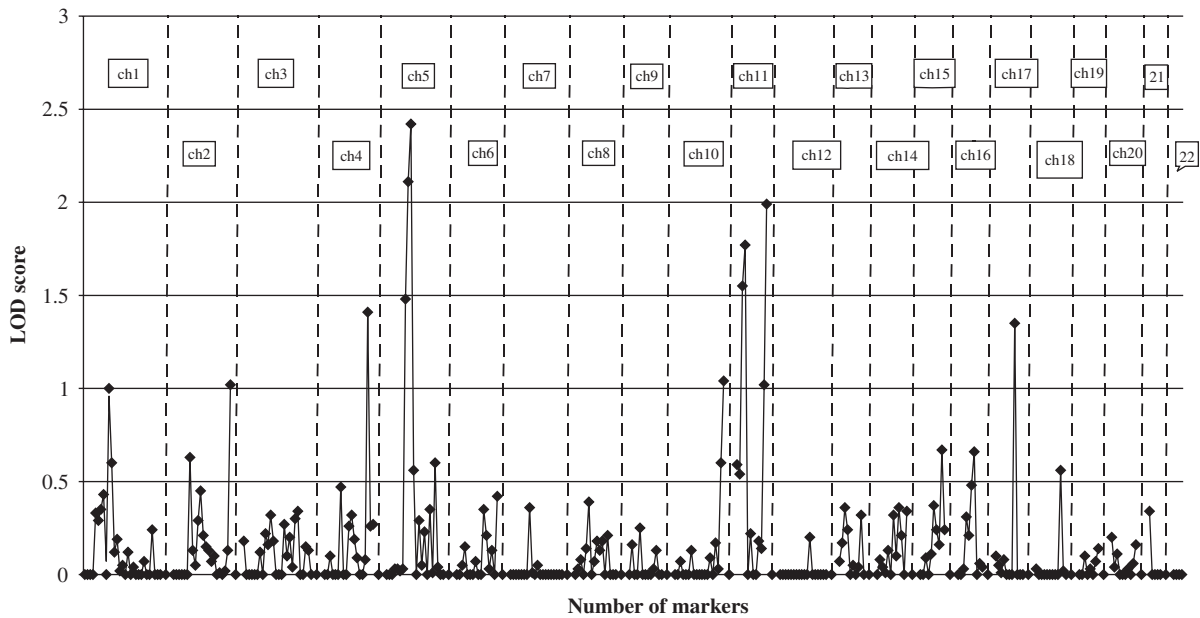


Fig. 2. Maximum LOD scores observed for genome-wide scan for linkage.

markers on chromosome 5, with LOD scores of 2.42 ($\theta=0.05$) at D5S2500, 1.48 at D5S1470 ($\theta=0.10$), and 2.11 at D5S1457 ($\theta=0.10$) in the pericentromeric region of chromosome 5 (Table 2 and Fig. 2). Multipoint linkage analysis, including these three markers, demonstrated an LOD score of 3.42 between D5S1457 and D5S2500 (approximately 3.6cM distant from D5S2500), suggesting that the gene of interest was probably

in this region of chromosome 5. Results from the genome-wide screen using affected-only non-parametric analysis were generally consistent with the results from parametric analysis (data not shown), but less significant than parametric results. The non-parametric LOD scores at D5S1470, D5S1457, and D5S2500 were 0.96, 1.57, and 1.30, respectively. Although several regions had LOD scores of > 1 and one region

Table 2. Linkage analysis of familial gonadal dysgenesis to polymorphic markers in the pericentromeric region of chromosome 5

Marker	Maximum LOD at penetrance = 0.60	Non-parametric LOD	Distance cM (Marshfield) ^a	Distance Mb (Genome) ^b
<u>D5S1470</u>	1.48 (0.10)	0.96	45.34	34177030
D5S674	0.16 (0.35)	0.29	47.09	34686584
D5S426	0.96 (0.15)	0.9	51.99	36498354
D5S1964	0.33 (0.20)	0.3	54.79	39718821
D5S1490	0.14 (0.30)	0.19	57.30	41337159
<u>D5S1457</u>	2.10 (0.10)	1.57	59.30	42872645
D5S634	2.35 (0)	2.34	59.85	43167742
D5S2087	0.43 (0.20)	0.32	59.85	46632226
D5S623	2.26 (0.10)	1.83	60.92	54017187
D5S1969	1.33 (0.15)	1.10	60.92	54984363
D5S1968	3.12 (0)	2.93	60.92	55284154
D5S474	2.49 (0)	1.68	63.60	56707152
D5S664	3.32 (0)	2.51	63.60	56776287
D5S633	3.53 (0)	3.19	64.14	57747520
D5S2102	2.25 (0)	2.04	66.81	58941870
D5S398	3.33 (0)	3.14	68.03	59879360
D5S2500	2.40 (0.05)	1.30	69.23	61035795
D5S624	2.00 (0)	1.80	69.23	62836156
D5S427	2.33 (0.10)	2.02	69.23	69798782
D5S1956	0.71 (0.15)	0.64	70.44	69582824
D5S2089	1.92 (0.15)	1.77	73.35	68632342

Three markers used in the genome-wide screen are underlined. Recombination fractions are indicated in parentheses. Markers that are part of the core haplotype are shaded.

^ahttp://www.marshfieldclinic.org/research/genetics/Map_Markers/maps/IndexMapFrames.html.

^b<http://genome.ucsc.edu/goldenPath/decTracks.html>.

on chromosome 11 had an LOD score of 2 in the parametric analyses, the peak on chromosome 5 was most interesting, given that three consecutive markers had LOD scores of > 1 . As a result, we decided to investigate this region more closely. For the fine mapping, 18 additional markers were typed and six (D5S398, D5S2102, D5S633, D5S664, D5S474, and D5S1968) defined a non-recombinant haplotype with parametric LOD scores of ≥ 2 . The highest parametric LOD score was 3.53 at D5S633. Multipoint parametric analysis using markers D5S664, D5S633, and D5D2102 yielded an LOD score of 4.47.

Discussion

This study is the first to map a gene presumed for testis determination using linkage analysis. This assumption seems valid, because other genes for testis determination have been identified by studying individuals with genetic sex reversal, albeit using different methods. The observation of chromosomal translocations, deletions, and duplications in sex-reversed individuals was instrumental for the positional cloning of *SRY*, *SOX9*, *WT1*, and *DAX1*, or, in the case of *WNT4A*, for demonstrating its role in testis determination (4–7, 9, 20–23). Cloning by protein–DNA interaction was required for the identification of *SFI* (24). The observation of an extended phenotype for the alpha thalassemia–mental retardation syndrome that caused 46,XY partial gonadal dysgenesis, assigned a role for *XH2* in the testicular-determining process (25–27). Forty-six genes are predicted to reside within the critical interval defined by the markers D5S664 to D5S1956, of which 16 are hypothetical genes (<http://genome.ucsc.edu/goldenPath/decTracks.html>).

Based on the fact that multiple females without demonstrable phenotypic abnormalities (individuals 202, 203, 205, 207, 213, 302, and 318) are obligatory carriers for this gene suggests that the associated trait is expressed in a sex-limited autosomal-dominant manner. This may occur because the expression differs between the developing testis and ovary, as has been described for most other sex-determining genes, or because development of the ovary is not dependent on normal expression of this gene product.

Although there are numerous affected family members, the pattern of inheritance is probably more complicated than simple monogenic diseases, because individuals 307, 406, and 407 shared the core haplotype, but did not have obvious clinical abnormalities. We propose that in 46,XY carriers, this mutant gene appears to be

highly penetrant, but can be modified by a second locus. Among affected individuals, the associated phenotypes can be highly variable, ranging from mild hypospadias without impairment of fertility, to partial, or even pure, gonadal dysgenesis. In this family, a male affected with hypospadias and cordee had two offspring – one with perineal hypospadias and cordee, and the other with partial gonadal dysgenesis (427 and 428, respectively). These findings are similar to data reported for fathers carrying mutations in their *SRY* gene that alter the physical properties of the encoded protein and who transmit this gene to daughters with pure or partial gonadal dysgenesis (28–31). They suggest that, as is the case with the *SRY* gene, expression of this gene is neither necessary nor sufficient for testicular determination to occur and that its associated phenotype can be influenced by modifier genes.

This family demonstrates that affected relatives may be identified only after a proband has been found in a family with sex reversal and that once identified, these affected individuals may have gonadal tumors, some of which may undergo malignant degeneration (15). Likewise, the siblings may not go into puberty on their own or, if they have partial gonadal dysgenesis, could become virilized at puberty, for example, as occurred with individual 212 in this family. Affected individuals may develop osteoporosis later in life from inadequate exposure to estrogens. With any new case of 46,XY gonadal dysgenesis, the physician should be alerted to the possibility that it may be familial in nature and should ask about other family members. For those who are deemed to be at risk, screening should be performed by karyotype analysis and pelvic ultrasound. For those found to have 46,XY gonadal dysgenesis, prophylactic gonadectomy should be performed and supplemental cyclic estrogens and progestins should be offered from the time of puberty onwards.

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