

Linkage Analysis of a Kindred with Inherited 46,XY Partial Gonadal Dysgenesis*

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ABSTRACT

We have reported a kindred in which 46,XY gonadal dysgenesis was inherited in an X-linked (or autosomal dominant sex-limited) manner and in which affected subjects did not have a large duplication of the short arm of the X-chromosome. In the present study we used linkage and sequence analyses to test the role of X-linked and various autosomal genes in the etiology of the familial 46,XY partial gonadal dysgenesis.

For analysis of X-linkage, 28 microsatellite polymorphisms and 1 restriction fragment length polymorphism were studied. The genotypes of informative family members were determined at each locus, and data were analyzed. Despite the large number of loci tested, our studies did not establish linkage between the trait and an X-chromosomal locus.

With respect to the study of autosomal genes, linkage analysis using a polymorphism within the 3'-untranslated region of the WT1 gene excluded involvement of WT-1 in the etiology of the abnormal gonadal differentiation of the family in this study. Similarly, linkage analysis using four microsatellites on the distal short arm of chromosome 9 was not consistent with linkage. Linkage analysis of a locus close to the SOX9 gene as well as analysis of the coding region of the SOX9 gene suggested that this gene was not associated with the trait in the affected subjects we studied. Our data suggest the role of an autosomal gene in the abnormal gonadal differentiation in the family in the study, but do not formally exclude the role of an X-chromosome gene. (*J Clin Endocrinol Metab* 81: 4479-4483, 1996)

THE STUDY of subjects with 46,XY complete gonadal dysgenesis has helped to identify the sex-determining region Y gene (SRY) that encodes the testis-determining factor (1, 2). However, mutations in the SRY gene account for a relatively small number of affected subjects (3-5).

Some evidence for the role of an X-linked gene in testis determination comes from the study of various kindreds in which 46,XY complete gonadal dysgenesis was inherited as an X-linked or sex-limited autosomal dominant trait and in which there was no apparent duplication of the X-chromosome (6-9). Additional evidence comes from cases of 46,XY complete gonadal dysgenesis that have been associated with duplications of the short arm of the X-chromosome between Xp21.2 and Xp22.1 (10-14). Analysis of submicroscopic duplications identified a region termed the dosage-sensitive sex

reversal (DSS) locus. This locus contains the adrenal hypoplasia congenita gene (15). The syndrome of X-linked α -thalassemia/mental retardation has been associated with abnormal testis development and results from mutations in the XH2 gene, located in Xq13.3 (16, 17).

Some cases of 46,XY complete gonadal dysgenesis have been associated with mutations of the Wilms' tumor suppressor (WT1) gene (18) or with mutations of the SOX9 gene; the latter was recently identified as the gene involved in camptomelic dysplasia (19, 20). Deletions of the distal short arm of chromosome 9 have also been detected in cases of 46,XY partial gonadal dysgenesis (21, 22).

In 1993, we reported a kindred in which 46,XY partial gonadal dysgenesis was inherited as an X-linked (or autosomal dominant sex-limited) trait (23). Affected subjects had no other congenital anomalies. The kindred included four affected individuals in two generations. The sequence of the open reading frame of SRY was normal. In addition, Southern analysis was performed using six Xp specific loci to investigate a duplication of the short arm of the X-chromosome. This study was also negative and excluded a large duplication of the short arm of the X-chromosome. The absence of mental retardation and characteristic facial features excluded the α -thalassemia/mental retardation syndrome. In the present study, we used linkage analysis and sequence analysis to test the possible role of X-linked and/or various autosomal genes in the etiology of our previously reported familial 46,XY gonadal dysgenesis.

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Subjects and Methods

Subjects

The pedigree of the kindred with 46,XY partial gonadal dysgenesis is shown in Fig. 1. Affected subjects include three siblings (III-5, -7, and -9), the proband (IV-8), and her brother (IV-10). Subject IV-10 was born after the initial report of the kindred (23). Subjects II-2 and III-10 are obligate carriers of the trait.

Preparation of genomic DNA

Peripheral blood leukocytes were obtained from subjects II-2, III-2, III-5 to -11, IV-1 to -8, and IV-10 and from unrelated normal males and females. Informed consent was obtained in accordance with the investigational review board of the Johns Hopkins University School of Medicine. Genomic DNA was isolated using standard protocols (24).

Southern analysis

Southern blot analysis was performed as previously described (25). Genomic DNA from all family members was digested with *Bgl*III and hybridized with the cloned probe QST-59, which reacts with the DXS319 locus (15). This fragment was obtained from Dr. Giovanna Camerino.

Microsatellite polymorphism analysis

Dinucleotide and trinucleotide repeat polymorphisms from the X-chromosome and chromosomes 9p, 11p, and 17q were analyzed as shown in Tables 1 and 2. DNA fragments including the tandem repeat sequences were amplified using PCR in the presence of [α -³²P]deoxy-CTP. The PCR products were separated on 6% polyacrylamide sequencing gels, which were then exposed to x-ray film. In each case, obligate carriers were screened to determine whether they were heterozygous for the specific locus. If heterozygosity was determined, then all family members were tested.

Screening for mutations in the SOX9 gene

The open reading frame of the SOX9 gene was amplified from genomic DNA of patient III-7 and subjected to single strand conformation polymorphism (SSCP) analysis, as described by Kwok *et al.* (26).

Statistical analysis

Statistical analysis of the data was performed using the MLINK program from the Linkage package (27). The trait was assumed to be 100% penetrant, and the gene frequency was assumed to be 0.001. 46,XY individuals were assumed to be fully penetrant for the sex reversal allele, if inherited, whereas 46,XX individuals were not. For analysis of autosomal markers, this required assigning different liability classes based on genotype (28).

Results

Analysis of loci near DSS

Analysis of DXS989, DXS319, HMG 122/123, DXS1234, DXS1238, and DXS1242 showed one allele at each locus for all affected individuals in the kindred (data not shown).

Linkage analysis of X-chromosome loci

The results of linkage analysis of 29 polymorphic X-chromosome loci using the program Linkage are presented in Table 3. Seventeen of these loci had logarithm of the odds (lod) scores less than -2.0, excluding linkage to the trait. Most of the remaining loci also had negative lod scores, with the exception of DSX7 and MAOA, which were closely linked to each other. Calculation of the lod scores for these loci did not include the genotypes of subjects IV-8 and IV-10, who

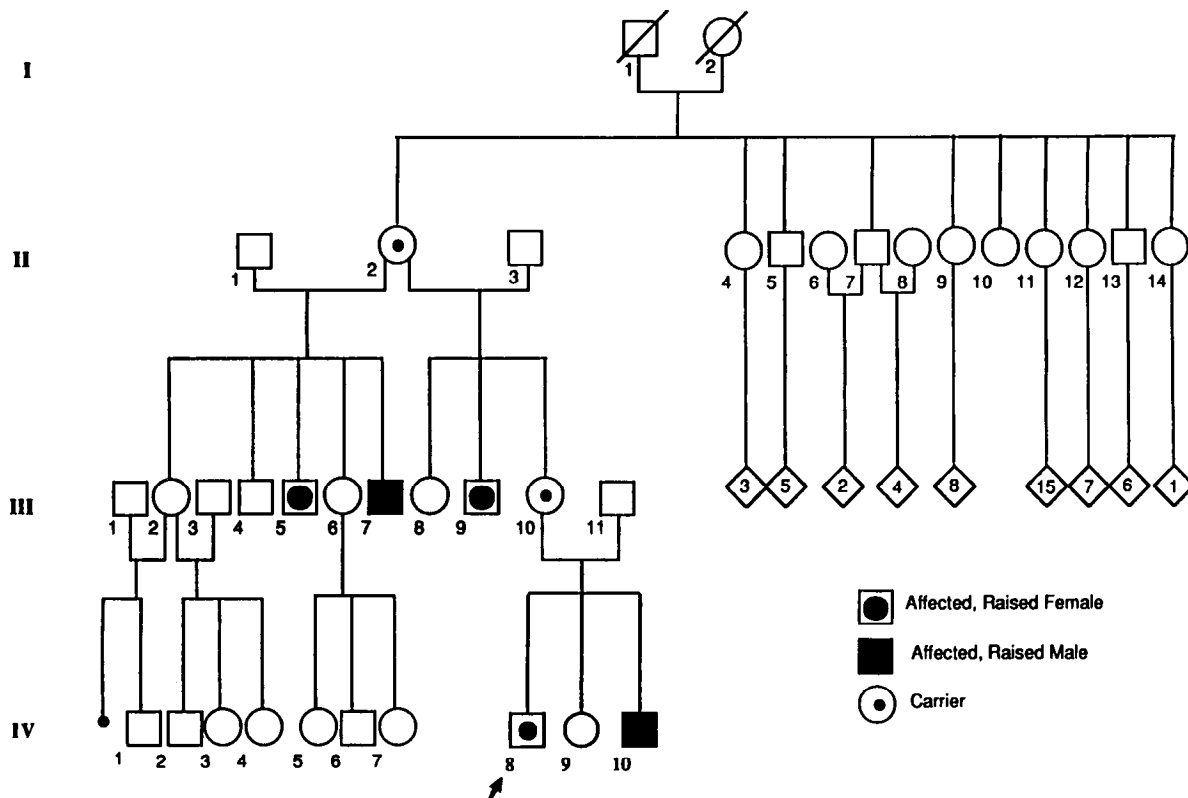


FIG. 1. Pedigree of a kindred with 46,XY partial gonadal dysgenesis. The arrow indicates the proband.

TABLE 1. X Chromosomal loci

Locus	Ref. no.
DXS996	29
KAL	30
DXS207	31
DXS999	29
DXS451	32
DXS989	29
DXS319	33
HMG122/123	Giovana Camerino, personal communication
DXS1234	34
DXS1238	35
DXS1242	36
DXS993	29
DXS7	37
MAOA	38
MAOA	39
MAOB	40
ALAS2	41
AR	42
DXS453	29
DXS56	43
DXS456	44
DXS3	45
DXS178	46
DXS424	44
DXS1114	47
DXS294	48
DXS297	49
DXS1113	50
F8	51

TABLE 2. Autosomal loci

Locus	Chromosome	Ref. no.
VLDLR	9p	52
D9S143	9p	53
D9S144	9p	53
D9S129	9p	54
WT1	11p	55
D17S949	17q	29

were noninformative at these loci. When two closely linked flanking markers, DXS993 and MAOB, were analyzed, lod scores were no longer positive, although they were not sufficiently negative for exclusion.

Linkage analysis of autosomal loci

Analysis of four polymorphic loci located on distal chromosome 9p excluded linkage between the trait and D9S129 and D9S144. In addition, negative lod scores were calculated for the remaining two loci (Table 4). Analysis of polymorphic loci in the WT1 gene and at D17S949 excluded linkage between these loci and the partial gonadal dysgenesis of this kindred (Table 4).

SSCP analysis of the SOX9 gene of subject III-7 was normal.

Discussion

In the present study, we considered possible causes of the 46,XY partial gonadal dysgenesis in the family that we previously reported (23). In this study, we investigated the possibility of a small duplication of the X-chromosome by searching for heterozygosity of six microsatellite polymor-

TABLE 3. Logarithm of the odds values at three recombination frequencies for X chromosomal loci

Locus	Recombination frequency		
	0.01	0.05	0.10
DXS996	-3.40	-2.00	-1.40
KAL	-3.91	-1.91	-1.12
DXS207	-2.51	-1.18	-0.67
DXS999	-2.51	-1.16	-0.64
DXS451	-1.11	-0.46	-0.23
DXS989	-3.40	-2.00	-1.40
DXS319	-2.51	-1.18	-0.67
HMG122/123	-4.21	-2.19	-1.38
DXS1234	-2.51	-1.18	-0.67
DXS1238	-4.21	-2.18	-1.37
DXS1242	-3.40	-2.00	-1.40
DXS993	-1.40	-0.72	-0.44
DXS7	1.18	1.07	0.93
MAOA	1.18	1.07	0.93
MAOA	0.08	0.65	0.79
MAOB	-1.40	-0.72	-0.44
ALAS2	-1.40	-0.72	-0.44
AR	-2.22	-0.93	-0.46
DXS453	-0.52	-0.09	0.28
DXS56	-1.92	-0.63	-0.17
DXS456	-0.53	0.05	0.20
DXS3	-2.51	-1.18	-0.67
DXS178	-3.40	-2.00	-1.40
DXS424	-3.91	-1.91	-1.12
DXS1114	-1.40	-0.72	-0.44
DXS294	-1.40	-0.72	-0.44
DXS297	-0.52	-0.09	0.28
DXS1113	-3.91	-1.91	-1.12
F8	-4.22	-2.21	-1.41

phisms near the DSS locus. The polymorphism defined by the HMG 122/123 locus is, in fact, located within the DSS locus (Camerino, G., personal communication). Two other loci (DXS989 and DXS319) are located within a region approximately 5 centimorgans telomeric to DSS, and the three additional loci are located within a region 12 centimorgans in length on the centromeric side of the DSS locus (56). We were unable to find evidence of microduplication in the DSS locus, but our studies do not completely exclude it.

We subsequently investigated the possibility that the abnormality in the family that we studied was related to another X-linked gene. We performed an extensive analysis of 29 polymorphic loci distributed along the X-chromosome at 5- to 15-centimorgan intervals. These loci were selected because of their high rates of heterozygosity in the general population. Considering the number of informative meioses within the pedigree, we could expect a maximum lod score of 2.7, which in itself would have been sufficient to establish linkage for an X-linked trait. However, the lod scores obtained suggest that an X-chromosome gene was not responsible.

Several problems may have influenced our study. First, it is possible that a new mutation in an allele at a given X-chromosome locus may have produced an alteration in the number of tandem repeat units, thereby obscuring linkage between that locus and the trait. However, such an event is considered to be rare (57). Second, as the number of family members presented here is relatively small, the statistical power of our study is somewhat limited. A single recombination event between a weakly linked locus and the trait

TABLE 4. Logarithm of the odds values at three recombination frequencies for autosomal loci

Locus	Recombination frequency		
	0.01	0.05	0.10
VLDLR	-1.40	-0.72	-0.44
D9S129	-4.21	-2.16	-1.33
D9S143	-1.78	-1.01	-0.65
D9S144	-2.22	-0.91	-0.42
WT1	-2.97	-1.58	-0.99
D17S949	-2.58	-1.24	-0.72

would result in a reduction of the lod score from a maximum of 2.7 to a value that would be statistically insignificant. We tried to minimize this possibility by studying loci that were separated by narrow intervals. This problem could be minimized in the future by the combination of this family and several similar families. As no other kindreds with 46,XY partial gonadal dysgenesis have been reported, this is not currently possible.

In subsequent studies, we investigated the role of the WT1 gene, the putative sex-determining gene on chromosome 9, and the SOX9 gene. With respect to WT1, we examined a microsatellite polymorphism within the 3'-untranslated region of the WT1 gene (55) and demonstrated that this gene was not linked to 46,XY partial gonadal dysgenesis in the family under study. We examined four loci located on the short arm of chromosome 9 from the terminal region of 9p to 9p23. Our data excluded linkage between two of these loci and the trait and suggested that the other two loci were also unlinked. We also studied a polymorphic locus in the same region as the SOX9 gene on chromosome 17q and screened the open reading frame of the SOX9 gene using SSCP analysis. These data suggested that an abnormality of the SOX9 gene was not responsible for the trait in the kindred we studied.

Our studies strongly suggest that the gene responsible for the abnormality in the family we studied is not on the X-chromosome, but they do not formally exclude it. They imply a role of an autosomal gene, but suggest that the affected gene is not WT1, the putative gene on the short arm of chromosome 9, or the SOX9 gene.

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