of 9p associated with male to female sex reversal: definition of the breakpoints at 9p23.3-p24.1. Genomics 41:271–274

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Molecular Analysis of 9p Deletions Associated with XY Sex Reversal: Refining the Localization of a Sex-Determining Gene to the Tip of the Chromosome

To the Editor:

Mammalian sexual development is dependent on chromosomal constitution. The Y chromosome acts as a dominant inducer of testis formation. The choice between testis and ovary equates with the choice of male or female development (sex determination). The differentiation of internal and external genitalia and secondary characteristics are regulated by endocrine functions associated with the gonads (sex differentiation). Any defect along the male and female pathway can result in inconsistencies between karyotypic and phenotypic sex (sex reversal) (Ford et al. 1959; Jacobs and Strong 1959; Jost et al. 1973).

The molecular-genetic analysis of primary (gonadal) sex-reversal syndromes has led to the identification of several sex-determining genes: *SRY,* the only Y-linked gene needed for testis formation; *DSS,* the X-linked dosage-sensitive sex-reversal locus; and the campomelic dysplasia (CD) gene *SOX9*. Functional studies performed in the mouse have identified other genes involved in gonadogenesis, such as the Wilms tumor gene *WT-1,* and the gene encoding for the steroidogenic factor 1 *SF-1* (reviewed by Schafer and Goodfellow 1996). The genes identified to date cannot account for all the sex-reversal cases found in the literature. The reports of both familial forms of gonadal dysgenesis and sporadic forms associated with autosomal anomalies implicate the existence of other loci (Schafer and Goodfellow 1996). Male-to-female primary sex reversal has been reported in patients with terminal deletions of the short arm of chromosome 9: unbalanced translocations and terminal deletions in which breakpoints range from 9p21 to 9p24 (Bennett et al. 1993; Begleiter et al. 1995; McDonald et al. 1997; Ogata et al. 1997; Veitia et al. 1997) (MIM 273350). The patients have normal female or ambiguous external genitalia and varying grades of mixed gonadal dysgenesis, ranging from fibrous streak gonads associated with Wolffian duct remnants to immature hypoplastic testicles. The lack of a correlation between the size of the deletions and the degree of impaired male development excludes the possibility that the deletion has a position effect on the differentiation of the sexual phenotype. It is likely that the interval in the 9p24 region deleted in all patients contains gene(s) involved in the differentiation of the male phenotype.

To better define the chromosome 9 region associated with sex reversal, we have performed a deletion-mapping analysis, by looking for the loss of heterozygosity in a panel of sex-reversed patients with translocations/deletions of chromosome 9p. This study involves four patients (SV, B, RB, and TO) who previously had been reported in the literature (Hoo et al. 1989; Bennett et al. 1993; Begleiter et al. 1995; Ogata et al. 1997) and an additional case (GG). These individuals are monosomic for varying segments of the chromosome 9 short arm. The extent of the sex reversal is variable among them, ranging from an ambiguous intersex to a nearly complete female phenotype. Karyotypes and clinical details are summarized in table 1.

The extent of the 9p deletions in the sex-reversed patients was analyzed by the typing of 20 microsatellite markers localized to an interval of 27.1 cM in 9p23-9pter. The heterozygosity of the markers tested is >70%. Their relative order on the chromosome was derived from an integrated map of 9p23-9pter, which combines genetic-mapping data on the above-mentioned loci with radiation hybrid–mapping data (fig. 1) (Bouzyk et al. 1996; Dib et al. 1996).

Loss of heterozygosity at the microsatellite loci was scored as potential loss of one allele. For patients TO, GG, and SV, it was possible to genotype the parents. This allowed the ascertainment of true hemizygosity for some of the markers tested. The results from this analysis are summarized in table 2. The microsatellite analysis allowed us to determine the potential maximum size of all the deletions but did not give any information on the minimum size of the deletions in patients RB, B, and SV. In the case of SV, the haplotype analysis of the parents' DNA for the potentially deleted markers was not informative. In the case of RB and B, parental DNA was not available. To better define the breakpoints in patients SV, RB, and B, FISH analysis also was performed, with genomic clones from 9p24 (for details, see the legend to fig. 1). All the results obtained from microsatellite typing and the FISH experiments were analyzed in relation to the integrated map of 9p23-9pter and were converted into a map that represents the maximum deletions potentially present in each patient (fig. 1, *bars on right side*):

Patient GG.—The maximum deletion extends from the telomere to D9S256-D9S269. The proximal break-

Table 1

Karyotype and Clinical Features of the 9p Patients Studied

	SV	RB	B	TO	GG
Karyotype	46,XY,−(9),+der(9) t(7;9)(q21.1;p24), maternal	46,XY,−9,+der(9) t(2;9)(p11;p24), de novo	46,XY,del(9) (p23.05), de novo	46,XY,del(9) (p23;p24.3), de novo	46,XY,del(9) (p23), de novo
External genitalia	Ambiguous	Female	Female	Ambiguous	Ambiguous
Wolffian structures	Unknown	Unknown	Epididymes, Wolffian duct remnants	Epididymes, vasa deferentia	Rudimentary vasa deferentia on the right, normal on the left
Müllerian structures	Rudimentary uterus	Uterus	Uterus, fallopian tubes	Unknown	Fallopian tubes on the right side
Gonads	Testes-like gonads, no histology data	Bilateral gonads, no biopsy	Streak gonads	Hypoplastic testes	Small immature right testis, normal left testis

point lies in a region of 6 cM, between D9S286 and D9S256-D9S269.

Patient TO.—The maximum deletion extends from the telomere to D9S168. The haplotype analysis of the parents' DNA enabled us to refine the localization of the proximal boundary to a region of 2.4 cM, between D9S144 and D9S168.

Table 2

Summary of Results Obtained by Typing Microsatellites from 9p24, on DNA from SV, RB, B, TO, and GG

	No. of Alleles[a]				
Marker	SV	RB	B	TO	GG
D9S1779	1	1	1	1[b]	1[b]
D9S1858	1	1	1	1	1
D9S129	1	1	1	1	1[b]
D9S143	2	1	1	1	1
D9S54	2	1	1	1[b]	1
D9S1871	2	1	1	1[b]	1
D9S178	1	1	1	1[b]	1
D9S288	2	1	1	1[b]	1[b]
D9S132	2	1	1	1[b]	1
D9S1810	2	2	1	1	1
D9S1852	ND	1	1	1	1[b]
D9S281	2	2	1	1[b]	1[b]
D9S286	1	2	1	1[b]	1[b]
D9S144	ND	2	1	1[b]	ND
D9S168	2	2	2	2	1
D9S256	2	2	2	1	2
D9S269	2	2	2	1	1
D9S267	2	2	2	1	2
D9S268	1	2	1	2	2
D9S274	2	1	1	2	1

[a] ND = no data.
[b] Confirmed hemizygosity.

Patient B.—The maximum deletion extends from the telomere to D9S168. FISH analysis with probe 4 allowed us to refine the proximal breakpoint to a region of 10 cM, between D9S1810 and D9S168. An additional FISH probe (probe 1) was used on metaphase spreads from B, to assess presence or absence of the most terminal microsatellite marker. The patient was found to have one copy deleted.

Patient RB.—The maximum deletion extends from the telomere to D9S1810. FISH analysis with probe 3 allowed us to refine the proximal breakpoint to a region of 2 cM, between D9S288/D9S178 and D9S1810.

Patient SV.—The maximum deletion extends from the telomere to D9S143. FISH analysis with probe 2 confirmed the localization of the breakpoint to a region of 1.9 cM, between D9S129 and D9S143.

The haplotype analysis of the typing data shows that SV, TO, and GG have lost the paternally derived alleles. Although it was not possible to verify whether all the deletions are terminal or interstitial, the FISH data together with the microsatellite data show that all the deletions extend at least up to the most telomeric marker D9S1779.

The correlation between the portion of 9p that is deleted and the phenotypic sex of the five patients defines the minimum region deleted in all the patients as being the 9p sex-reversal interval critical for male sexual development. This region extends from a distal boundary located between the telomere and D9S1779 to a proximal boundary located between D9S129 and D9S143. The size of this interval, on the integrated map, is 3–5 cM. This result, compatible with all the molecular data published to date on 9p deletions associated with sex reversal, supports the evidence for the localization of a

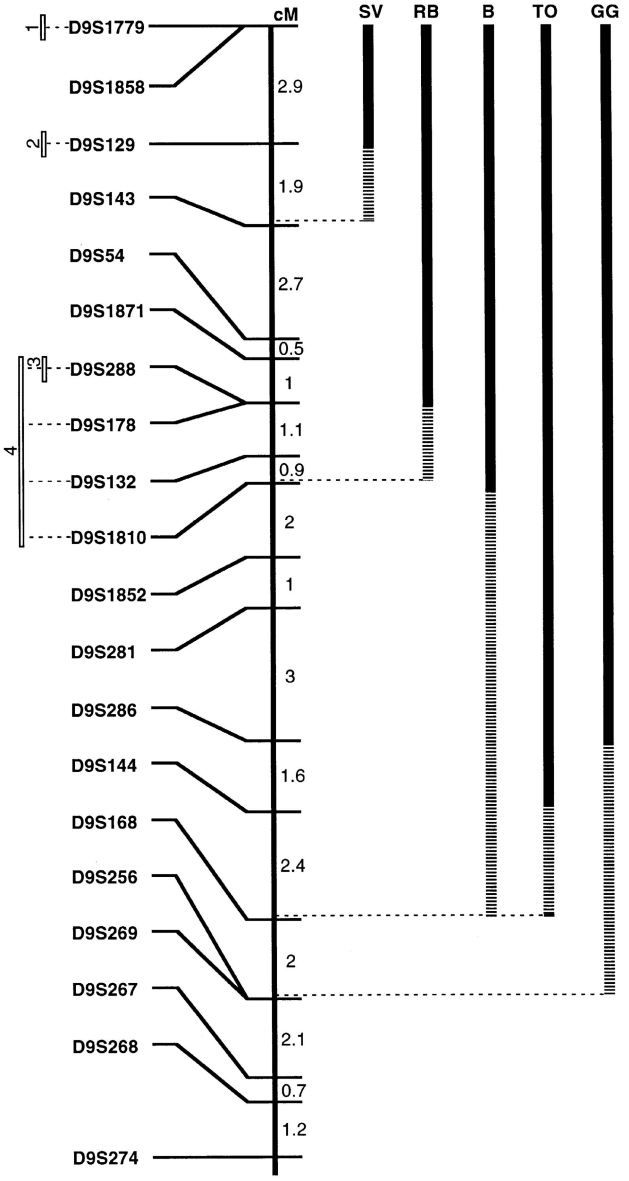

Figure 1 Maximum potential deletions in the XY sex-reversed patients under study, on the basis of results from microsatellite typing and FISH analysis. The data have been analyzed in relation to an integrated map of chromosome 9p24. This map contains the microsatellite loci studied and has been derived from the integration of the Généthon genetic map, as a framework map (Dib et al. 1996), with markers from radiation-hybrid mapping (Bouzyk et al. 1996). The primer sequences for microsatellite typing were obtained from Genome Database and the Généthon World Wide Web pages. The maximum deletions are represented by the bars on the right side of the integrated map: the portions confirmed as truly deleted are black; the portions potentially deleted but not confirmed are horizontally hatched. The bars on the left side of the integrated map represent the genomic clones used as probes in the FISH experiments. Their localization has been deduced on the basis of their marker content. FISH experiments were as follows: probe 1 tested on patient B, probe 2 on patient SV, probe 3 on patient RB, and probe 4 on patient B. All probes were found deleted of one copy in the patients tested. Probe codes are as follows: 1 = PAC clone LLNLP704K1593Q13, 2 = CEPH YAC 798_B_10, 3 = cosmid clone LL09NC01-304d5, and 4 = CEPH YAC 763_G_5).

putative sex-determining gene to the tip of chromosome 9p. Moreover, our data refine the chromosomal assignment of the putative 9p "sex-reversal" gene, significantly narrowing the critical interval, from ~21 cM (D9S144-9pter) (Veitia et al. 1997) to 3–5 cM (D9S143-9pter; present study). This region is perhaps too large for any attempt to clone the gene directly, but it does provide an excellent starting point for a search, by microsatellite typing and Southern blot/dosage analysis, for microdeletions in 46,XY sex-reversed patients with a normal *SRY* gene. Additionally, the availability of detailed physical maps of all of the human genome makes the cloning of a 3–5-Mb region in YAC contigs quite feasible. This will allow the exploitation of the huge amount of expressed sequence tags (ESTs) data now available. Testing the genes/ESTs grossly mapped in 9p23-9p24, for presence/absence inside the deletion, can provide a preliminary screen for potential candidate genes. For example, it has been demonstrated recently that a *Caenorhabditis elegans* homologue of the *Drosophila melanogaster* gene *doublesex* is implicated in sexual development in the worm (Raymond et al. 1998). A human EST homologue to both the *D. melanogaster* and *C. elegans doublesex* gene has been mapped to chromosome 9p24 (Raymond et al. 1998). The gene is contained within the minimum deletion defined in the present study (data not shown), making it an obvious candidate to test further for a role in sex determination and differentiation.

All the 9p monosomies associated with sex reversal that have been described so far have an XY karyotype and variable differentiation of the sexual phenotype. Histological examination of the gonads, which has been performed in a few cases, has revealed the presence of complete or mixed gonadal dysgenesis, suggesting defective testis development (Bennett et al. 1993; McDonald et al. 1997; Ogata et al. 1997; Veitia et al. 1997). The consequent deficiency of the Müllerian inhibiting substance (MIS) and testosterone produced by the testis at the crucial time of sexual differentiation would then be responsible for the defective differentiation of internal and external genitalia. Moreover, sex reversal is not a constant feature of the 9p-XY patients (Huret et al. 1988). It has been proposed that the aberrant sexual phenotype is a recessive character and that the deletion uncovers a mutated allele on the karyotypically normal chromosome. In this case, the variable expression of the sexual phenotype of 46,XY individuals may result primarily from factors other than the mutations, such as differences in genetic background. Alternatively, sex reversal could be the result of a dominant effect of the deletion causing haploinsufficiency for a gene(s) with variable penetrance. The latter hypothesis is more likely. A wide variation of phenotypes, similar to that seen in these patients, is often found in systems, such as sex determination, that are subject to dosage sensitivity. In

human, for example, *DSS* causes male-to-female sex reversal, with varying degrees of masculinization, when present in two copies in 46,XY individuals (Bardoni et al. 1994). *SOX9* heterozygous loss-of-function mutations cause campomelic dysplasia and lead to sex reversal in two-thirds of the 46,XY individuals (Kwok et al. 1995; Meyer et al. 1997). Cloning of the putative gene will answer these questions.

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Electronic-Database Information

Généthon, http://www.genethon.fr
Genome Database, http://gdbwww.gdb.org
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim

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