

Genetic linkage mapping of the human steroid 5 α -reductase type 2 gene (SRD5A2) close to D2S352 on chromosome region 2p23→p22

J. Morissette, F. Durocher, J.-F. Leblanc, T. Normand, F. Labrie, and J. Simard

MRC Group in Molecular Endocrinology, CHUL Research Center and Laval University, Québec (Canada)

Abstract. Two steroid 5 α -reductase isoenzymes catalyze the conversion of testosterone into dihydrotestosterone, the more bioactive androgen, which is essential for male phenotypic sexual differentiation and for androgen-mediated growth of such tissues and organs as the prostate. Inherited mutations in SRD5A2 cause male pseudohermaphroditism. The SRD5A1 and SRD5A2 genes encoding the steroid 5 α -reductase type 1 and type 2 isoenzymes have been previously assigned by in situ hybridization to 5p15 and 2p23, respectively. To map the SRD5A2 gene by linkage analysis, a novel *RsaI* RFLP detected in exon I and a TA repeat polymorphism found in exon V were genotyped in eight CEPH reference families. A two-point linkage analysis was performed between these polymorphisms and

the chromosome 2 microsatellite markers of Généthon and NIH/CEPH. The closest linkage was observed with D2S352 ($Z_{\max} = 24.06$; $\theta_{\max} = 0.001$) in the region 2p23→p22. To further define the localization of SRD5A2, a framework map, including nine Généthon markers flanking the polymorphic SRD5A2 locus, was built by multipoint linkage analysis. This led to a high-resolution genetic map of the region flanking the polymorphic SRD5A2 gene, including the nine Généthon markers and three NIH/CEPH markers, yielded the following order: tel-D2S48-D2S149-D2S320-D2S171-D2S165-[D2S352/SRD5A2]-D2S367-[D2S19/D2S177]-[D2S391/CALM]-D2S378-cen.

The isoenzymes of the steroid 5 α -reductase family catalyze the 5 α -reduction of 4-androstenedione, testosterone, and progesterone into their corresponding 5 α -dihydro-3-ketosteroids. The best-known role of these isoenzymes is the transformation of testosterone to dihydrotestosterone (DHT), the most potent androgen, which promotes the differentiation of the male external genitalia, urethra, and prostate during fetal development and is responsible for virilization at puberty (Wilson et al., 1993). In addition, increased synthesis of DHT may be involved in the development of both benign and neoplastic growth of the prostate in elderly men (Labrie et al., 1993). It has been suggested that modulation of steroid 5 α -reductase activity may be responsible for some variations in prostate cancer risk among US ethnic groups (Ross et al., 1992).

Two types of human steroid 5 α -reductase, designated type 1 and type 2, have been characterized (Andersson et al., 1990, 1991). The steroid 5 α -reductase type 1 gene (SRD5A1) encodes an isoenzyme that is transiently expressed in newborn skin and scalp and is continuously expressed in liver after birth and in the skin from puberty onward (Thigpen et al., 1993), thus being primarily responsible for virilization and male-pattern baldness. The steroid 5 α -reductase type 2 isoenzyme, encoded by SRD5A2, is also transiently expressed in newborn skin and scalp, as well as in the liver, and is the predominant type found in fetal genital skin, the male accessory sex glands, and the prostate, including both benign prostatic hyperplasia and adenocarcinoma of the prostate (Thigpen et al., 1993; Luu-The et al., 1994).

The steroid 5 α -reductase genes are not syntenic. The SRD5A1 gene has been assigned to band p15 of chromosome 5 (Jenkins et al., 1991), whereas SRD5A2 has been assigned to band 2p23 by somatic cell hybrid and in situ hybridization (Thigpen et al., 1993). Both genes contain five exons and four introns that show identical splicing sites (Jenkins et al., 1991; Labrie et al., 1992; Thigpen et al., 1992b). An inherited muta-

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Request reprints from Dr. Jacques Simard, MRC Group in Molecular Endocrinology, CHUL Research Center, 2705 Laurier Boulevard, Québec, G1V 4G2 (Canada); telephone: 418-654-2264; fax: 418-654-2735.

Table I. Two-point linkage analysis between SRD5A2 and markers on chromosome region 2p25 → p15

Marker	D2S48	D2S149	D2S320	D2S171	D2S165	D2S352	D2S367	D2S19	D2S177	D2S391	D2S378
θ_{\max}	0.073	0.149	0.114	0.043	0.024	0.001	0.025	0.081	0.092	0.136	0.220
Z_{\max}	5.25	8.47	12.36	15.69	20.30	24.06	19.42	6.32	12.43	8.15	6.28

tion or mutations in SRD5A2 lead to male pseudohermaphroditism, in which affected males show a normal internal urogenital tract but female external genitalia, while affected females are normal (Andersson et al., 1991; Thigpen et al., 1992a, b; Wilson et al., 1993). The potential role of genetic variability of the SRD5A2 gene in predisposing individuals to prostate cancer has been suggested very recently by analysis of the distribution of a TA repeat polymorphism (Labrie et al., 1992; Davis et al., 1993) in this gene in low-risk Asian-Americans, high-risk African-Americans, and intermediate-risk non-Hispanic Caucasians (Reichardt et al., 1995). In view of the crucial role of the type 2 steroid 5 α -reductase isoenzyme in both normal and pathological conditions of the prostate, the present study was designed to determine the location of the SRD5A2 gene more precisely.

To map the SRD5A2 gene by genetic linkage, we searched for polymorphism(s) within this gene and found a *RsaI* RFLP in exon 1 caused by a transversion of a C for a G converting codon 89 (CTA) encoding a Leu to GTA encoding a Val. This biallelic polymorphism was detected by *RsaI* digestion of a 349-bp PCR fragment amplified with the upstream primer 5'-CGCCTGGTTCTGCAGGAGCT-3' and the downstream primer 5'-GTGAAGGCGCGTCTGTG-3'. PCR amplification was performed in a 100- μ l reaction mixture of 10 mM Tris (pH 8.3), 50 mM KCl, 1.0 mM MgCl₂, 10% DMSO, 0.05% Tween-20, 50 μ M of each dNTP, 0.25 μ M of each primer, and 0.08–0.25 μ g of genomic DNA. After an initial denaturation step at 100°C for 10 min, the samples were cooled to 72°C prior to the addition of 1 U AmpliTaq polymerase (Perkin-Elmer Cetus) and underwent 35 cycles, each consisting of 1 min at 95°C, 1 min at 60°C, and 30 s at 72°C, in a Perkin-Elmer Cetus model 480 thermal cycler. The frequency of the A1 (Leu89) and A2 (Val89) alleles was estimated at 0.34 and 0.66, respectively, from 56 chromosomes obtained from 28 unrelated Caucasian subjects. The observed heterozygosity was 0.39. In addition, we also typed the unusual TA repeat polymorphism located in exon V in the 3' untranslated region of the gene (Labrie et al., 1992; Davis et al., 1993; Reichardt et al., 1995). The frequency of the two alleles, estimated from 56 chromosomes obtained from 28 unrelated Caucasian subjects, was (TA)₀ = 0.875 and (TA)₉ = 0.125. The observed heterozygosity was 0.25. The sizes of the PCR fragments amplified with the upstream primer 5'-CTCGGAAAGCCCTTATTCCATTCATCT-3' and the downstream primer 5'-AATCCCCAGGC-CAGCTGGCAG-3' were 175 and 193 bp for the (TA)₀ and (TA)₉ alleles, respectively. PCR amplification was done in a 100- μ l volume containing 10 mM Tris (pH 8.3); 50 mM KCl; 1.5 mM MgCl₂; 5% DMSO; 200 μ M dCTP, dGTP, and dTTP; 2 μ M dATP; 8 μ M [α -³⁵S]dATP, 0.25 μ M of each primer, and 0.01–0.10 μ g of genomic DNA. The reaction mixture was

cycled through 40 cycles, each consisting of 1 min at 95°C, 1 min at 57°C, 1 min at 72°C. The frequency of the alleles estimated from 56 chromosomes from 28 unrelated Caucasian subjects was as follows: A1(Leu89)-(TA)₀ = 0.32, A2(Val89)-(TA)₀ = 0.54 and A2(Val89)-(TA)₉ = 0.13, with an observed heterozygosity of 0.57.

These two markers in the SRD5A2 gene were genotyped in the eight largest reference CEPH families (102, 884, 1331, 1332, 1347, 1362, 1413, 1416), taking advantage of the existing framework maps of Génethon (Gyapay et al., 1994) and NIH/CEPH (NIH/CEPH Collaborative Mapping Group, 1992; Murray et al., 1994).

A two-point linkage analysis was performed between the SRD5A2 polymorphisms and markers on chromosome 2 from the Génethon (Gyapay et al., 1994) and NIH/CEPH (NIH/CEPH Collaborative Mapping Group, 1992; Murray et al., 1994) databases (Table I). Linkage analysis was performed using version 5.2 of the LINKAGE package of computer programs (Lathrop and Lalouel, 1984). Pairwise lod scores between the SRD5A2 polymorphisms and the other chromosome 2 markers were calculated with the MLINK program, whereas θ_{\max} and Z_{\max} were calculated with CLODScore program.

No recombinant was obtained between the SRD5A2 marker and D2S352 (θ_{\max} = 0.001 and Z_{\max} = 24.06; 1 lod unit confidence interval: $0 < \theta \leq 0.028$) (Table I). The θ_{\max} and Z_{\max} values of 11 selected markers in the region 2p25 → p15 are indicated in Table I. The map area containing these markers has been confirmed using the most recent YAC contig map (Chumakov et al., 1995).

A framework map was then constructed by multipoint linkage analysis using a subset of nine Génethon microsatellite reference markers to determine their order and location relative to the SRD5A2 locus via CILINK. Genotyping error detection was done by checking for double recombinants before map construction based on both pairwise and multilocus recombinant analysis as described elsewhere (Lathrop et al., 1988; Durocher et al., 1995a, b; Morissette et al., 1995). The RTH and REC-STAT programs from M. Lathrop were used to haplotype the markers on CEPH families and count recombinants.

The SRD5A2 locus within the fixed map, based on segregation analysis in the CEPH pedigrees, is in the interval flanked by D2S165 and D2S367 (Fig. 2) and does not recombine with D2S352. The order of the SRD5A2 gene and selected markers is as follows: tel–D2S48 (θ = 0.20)–D2S149 (θ = 0.05)–D2S320 (θ = 0.07)–D2S171 (θ = 0.02)–D2S165 (θ = 0.03)–D2S352 (θ = 0.001)–SRD5A2 (θ = 0.03)–D2S367 (θ = 0.06)–D2S177 (θ = 0.10)–D2S391 (θ = 0.06)–D2S378–cen. The most likely order was supported by odds of 1,000:1 against any permutation of two adjacent markers (Fig. 2).

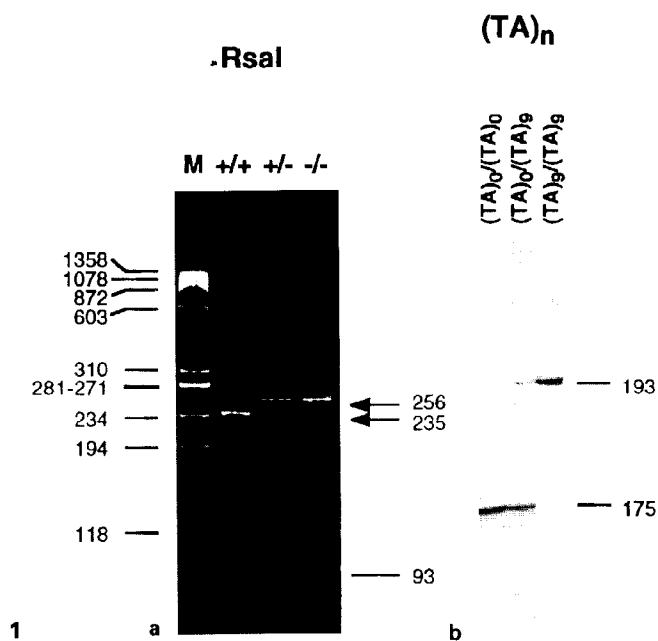
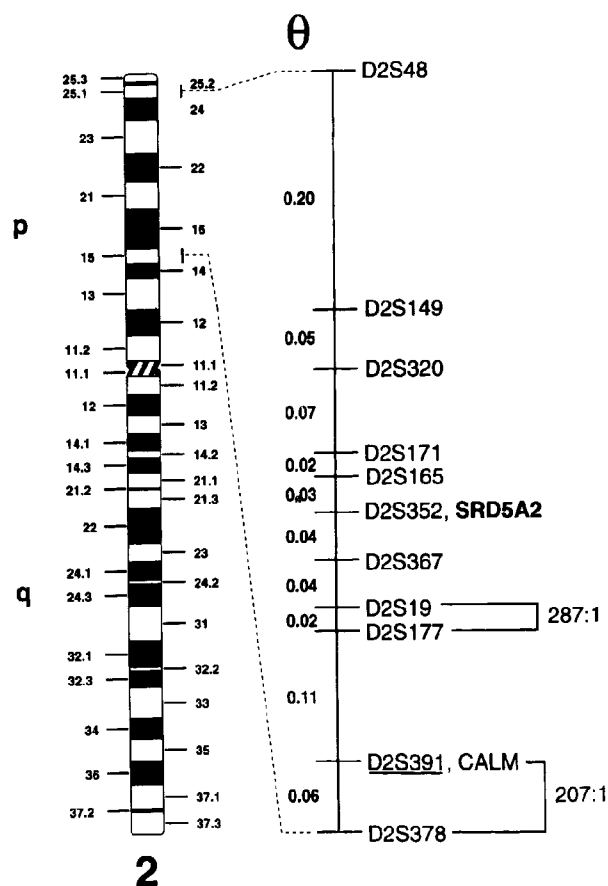
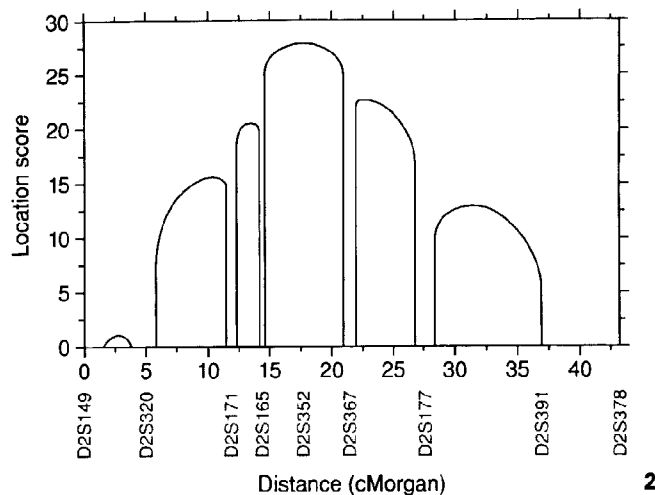


Fig. 1. Detection of *RsaI* polymorphism in exon 1 (a) and TA repeat alleles in exon V (b) in the SRD5A2 gene. (a) The region leading to the 349-bp PCR fragment is polymorphic and has two alleles. Homozygotes for the *RsaI* site (+/+) have two fragments of 93 and 236 bp; heterozygotes for this RFLP (+/-) have three fragments of 93, 236, and 256 bp; and homozygotes without the *RsaI* RFLP have two fragments of 93 and 256 bp. The digested PCR fragments were analyzed on a 6% polyacrylamide nondenaturing gel. Numbers at the left: *HaeIII*-digested ϕ X174RF DNA. (b) PCR reactions of this exon V region reveal two alleles: those with (TA)₀ and those with (TA)₉. This autoradiograph illustrates the pattern seen with a homozygote for (TA)₀, for a heterozygote (TA)₀/(TA)₉, and for a homozygote (TA)₉. PCR products were resolved on a 5% denaturing polyacrylamide gel.

Fig. 2. Multipoint location scores (base 10) for linkage of the SRD5A2 locus to the framework marker map calculated using the CMAP program. The curve indicates the likelihood that the SRD5A2 locus is at the map location with respect to adjacent markers, the positions of which are given by the vertical labels at the bottom of the x axis. The recombination fractions between the framework markers were calculated using the CILINK program and converted to genetic distances using the Haldane mapping function. The relative genetic position of D2S149 was arbitrarily placed at zero.

Fig. 3. Map of chromosome region 2p25→p15. The map gives the best supported order of the markers and sex-averaged recombination fractions (θ) between adjacent markers. The order of the markers was supported by odds of $\geq 1000:1$ against adjacent markers, with the exception of D2S19 and D2S177 (287:1) and D2S391 and D2S378 (207:1).



To determine the location of SRD5A2 within the region 2p25→p15 with greater precision, we constructed a high-resolution genetic map of the region flanking the SRD5A2 gene, including—in addition to the nine Génethon markers previously used—the NIH/CEPH triallelic polymorphism D2S48 (pEFD122), the biallelic marker D2S19 (CRI-L301/*TaqI*), and the *HindIII* RFLP in the CALM gene (NIH/CEPH Collaborative Mapping Group, 1992; Murray et al., 1994). The GMS program (kindly provided by M. Lathrop) was used to calculate

and validate the marker distances and order in the genetic map (Lathrop et al., 1988). As illustrated in Fig. 3, the order of the markers is tel-D2S48-1.88 × 10¹⁰-D2S149-2.50 × 10⁴-D2S320-3.84 × 10¹⁹-D2S171-4.63 × 10⁵-D2S165-1.16 × 10⁷-[D2S352/SRD5A2]-2.95 × 10⁴-D2S367-1.17 × 10³-[D2S19-2.87 × 10²-D2S177]-8.55 × 10¹¹-[CALM/D2S391-2.07 × 10²-D2S378]-cen. Thus, D2S352 does not recombine with SRD5A2, and D2S391 does not recombine with CALM. We also obtained the same order for these markers by using

CRI-MAP's FLIP 3 program, the values for D2S19 and D2S177 (250:1) and D2S391 and D2S378 (223:1) being similar to those obtained with GMS. The location of the SRD5A2 gene was estimated to lie within the region 2p23→p22, based on the location of D2S352 at position 0.51 on the YAC contig map, which is between D2S165 (located at position 0.47, which has been mapped by FISH to 2p23.3→p22.3) and D2S367 (located at position 0.54, which has also been mapped by FISH to the same region) (Chumakov et al., 1995). This localization is in good agreement with the previous mapping of SRD5A2 by FISH to band 2p23 (Thigpen et al., 1992b). The location of the CALM gene, which encodes the ubiquitous Ca⁺⁺-binding protein calmodulin, was estimated to lie within band 2p16, based on the location of D2S391 at position 0.70 on the YAC contig map mapped by FISH to 2p16.3→p16.1 (Chumakov et al., 1995).

Mapping of the SRD5A2 locus should prove useful for genetic linkage analyses in various pathophysiological conditions, including male pseudohermaphroditism resulting from steroid 5 α -reductase type 2 deficiency and prostate cancer in patients having a positive family history. The characterization of the novel *RsaI* polymorphism, coupled with the localization of the SRD5A2 locus relative to other highly polymorphic flanking markers, provides the tools necessary to determine if genetic variability of the SRD5A2 gene could explain the significant racial and ethnic variability in prostate cancer risk, as recently suggested by Reichardt and coworkers (1995).

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