Dehydroepiandrosterone sulfotransferase gene (STD): localization to human chromosome band 19q13.3

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Abstract. Dehydroepiandrosterone (DHEA) sulfotransferase (ST) catalyzes the sulfate conjugation of DHEA and other steroid compounds. The human gene for DHEA ST (STD) was mapped by the polymerase chain reaction to chromosome 19

using human × rodent somatic cell hybrid panels. Fluorescence in situ hybridization was then used to localize the STD gene to the region 19q13.3.

Dehydroepiandrosterone (DHEA) sulfotransferase (ST) is one of four well-defined cytosolic ST enzymes known to be present in human liver (Aksoy et al., 1994b; Weinshilboum and Otterness, 1994). DHEA ST catalyzes the sulfate conjugation of DHEA, other steroid hormones (e.g., estrogens), bile acids, and cholesterol (Radomska et al., 1990; Weinshilboum and Otterness, 1994). DHEA and its sulfate conjugate play important roles in the hormonal regulation of pregnancy (Warren and Timberlake, 1964), and epidemiologic data also suggest that plasma levels of DHEA sulfate might be related to the risk of cardiovascular disease (Nafziger et al., 1991). In human liver, the organ in which DHEA ST is most highly expressed (Otterness and Weinshilboum, 1994), 20–25% of subjects are included in a high-enzyme-activity subgroup, raising the possibility that the activity of DHEA ST might be controlled by a genetic polymorphism (Aksoy et al., 1993).

We recently cloned and expressed a cDNA for human liver DHEA ST (Otterness et al., 1992) and then used that cDNA to isolate genomic DNA clones (Otterness and Weinshilboum, 1994). The HUGO Nomenclature Committee has assigned the symbol STD to the human DHEA ST gene. Our characterization of the STD genomic clones provided information that

made it possible to use the polymerase chain reaction (PCR) to map the STD gene to human chromosome 19, and, subsequently, to use fluorescence in situ hybridization (FISH) to determine its location on that chromosome.

Materials and methods

PCR analysis

Nucleotide sequence information from a partial genomic clone for STD (Otterness and Weinshilboum, 1994) was used to design two sets of PCR primers for determination of the chromosomal localization of the gene with DNA obtained from NIGMS Human/Rodent Somatic Cell Hybrid Mapping Panels 1 and 2 (Coriell Institute for Medical Research, Camden, NJ) as template. To avoid amplification of possible homologous genes that might be present in rodent DNA, one primer of each set was based on noncoding sequence, while the other was based on sequence from exon 1. The sense primer for the first primer set consisted of the sequence 5'-GGAGGTA-TAATTGTGTGATACTTC-3'. This sequence represented nucleotides -680 to -657 of the gene (numbered with respect to the "A" in the ATG translation initiation codon). The antisense primer for the first primer set had the sequence 5'-CCGACATGATGATGACCTC-3', representing nucleotides +7 to -12. The PCR amplification product generated by this pair of primers was expected to be 687 nucleotides in length. The second primer set included sense primer 5'-GTACGTGATGAGTTCGTG-3', representing nucleotides +76 to +93, and antisense primer 5'-CACTCTGTGGATTCTCTAA-TACC-3', representing intron 1 nucleotides 226 to 202. The amplification product generated with these primers was expected to be 287 nucleotides in length. The PCR was performed in a Perkin Elmer Cetus DNA thermal cycler in a 100-μl reaction volume that included 77–150 ng of template DNA, 100 ng of each primer, 50 μM of each dNTP, 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01 % gelatin, and 1 U *Thermus aquaticus* DNA polymerase (AmpliTaQ; Perkin Elmer Cetus). The reaction conditions used, after initial denaturation for 10 min at 95 °C, were 35 cycles of 1 min at 94 °C, 2 min at 50 °C, and 3 min at 72 °C, followed by a final 10-min incubation at 72 °C. Amplification products were then analyzed on 1 % agarose gels.

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Fig. 1. Chromosomal localization of the human STD gene on the basis of performance of the polymerase chain reaction (PCR) with NIGMS Human/Rodent Hybrid Cell Panel 2 DNA as a template. The primers used were designed to amplify a portion of the 5'-flanking region of the human STD gene. Results are shown for a monochromosomal hybrid cell line that contained only human chromosome 19, as well as mouse, hamster, and human total genomic DNAs and a non-DNA-containing control.

FISH

The open reading frame of the human liver DHEA ST cDNA was radioactively labeled by random priming with [α - 32 P]dCTP and was used to screen a high-density arrayed human chromosome 19-enriched cosmid library (deJong et al., 1989; Olsen et al., 1993), as described by Tynan et al. (1993). This library contained approximately four copies of human chromosome 19. Ten positive cosmids were identified. Fluorescence in situ hybridization of one of the STD-specific cosmids (29697) to peripheral blood lymphocyte metaphase chromosomes was carried out as described by Trask et al. (1993).

Results and discussion

Both sets of STD-specific primers were used to screen NIGMS Human/Rodent Somatic Cell Hybrid Mapping Panel 1. Use of the primer set that had been designed to amplify a portion of the 5'-flanking region of the STD gene resulted in amplification of a product of the appropriate length, 687 nucleotides, with template DNA from hybrid cell lines 9925, 9926, 9927, 9928, 9933, and 9936 (Table I). Furthermore, use of the primer set designed to amplify a portion of intron 1 of the gene resulted in amplification of a product of the anticipated size, 287 nucleotides, with DNA from the same cell lines, plus line 9932 (Table I). All of these cell lines contained human chromosome 19, but line 9932 contained 2% or fewer cells with that chromosome, perhaps accounting for the variable results obtained with this line. Cell line 9938, which was discordant for both sets of PCR primers, contained 4% or fewer cells with chromosome 19. Based on high concordance (83% and 89%) and low discordance (17% and 11%) ratios for chromosome 19, these experiments performed with Panel 1 suggested that the STD gene was localized on that chromosome. However, chromosomes 1 and 13 could not be ruled out on the basis of the

Table I. Segregation of human STD-specific PCR products and human chromosomes in NIGMS Human/Rodent Somatic Cell Hybrid Mapping Panel 1

Hybrid	Human chromosome ^a																								Human STD PCR products ^b	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	687 bp	287 bp
09925	74	24	0	74	76	60	82	78	0	0	4	68	6	86	78	14	98	96	46	84	0	76	0	0	+	+
09926	69	75	75	65	2	88	85	69	0	68	0	2	77	73	93	2	81	75	84	96	2	4	2	0	+	+
09927	69	83	75	77	0	93	79	73	0	82	0	0	77	79	90	0	81	73	87	89	0	0	0	0	+	+
09928	0	84	58	0	48	32	0	66	0	2	0	0	4	76	92	0	98	0	28	0	70	82	0	78	+	+
09929	0	0	61	59	0	43	2	49	0	0	33	49	0	59	2	0	96	0	2	31	0	0	2	0	-	-
09930A	0	34	62	4	12	0	26	4	0	0	6	22	56	82	12	0	86	78	0	22	82	76	6	8	-	-
09931	0	0	0	0	26	0	78	0	0	46	0	64	0	100	0	0	100	0	0	78	90	0	0	14	-	-
09932	0	0	0	68	86	46	0	80	0	2	28	26	0	0	0	0	96	0	2	0	92	0	0	0	-	+
09933	50	0	84	16	54	76	92	54	0	6	0	50	84	78	92	0	88	70	80	32	94	88	0	32	+	+
09934	0	50	0	0	83	79	4	87	0	0	77	87	0	2	89	0	90	89	0	91	89	2	0	0	-	-
09935A	0	0	52	10	28	12	0	0	0	8	0	22	74	72	0	0	93	59	0	9	91	71	0	0	-	-
09936	0	0	0	18	0	46	70	10	0	16	34	0	2	88	2	0	100	0	44	24	0	18	0	0	+	+
09937	0	0	54	38	0	62	54	70	0	4	0	42	0	70	60	0	96	66	0	0	0	0	0	0	-	-
09938	0	0	2	88	60	88	86	4	0	0	36	92	0	80	4	0	92	0	4	80	76	60	0	2	-	-
10340	0	0	46	0	0	0	84	62	0	0	0	0	0	0	62	0	100	0	0	0	0	0	0	0	-	-
10324	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	90	0	-	-
10567	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98	0	0	0	0	0	0	0	0	-	-
10611	0	0	0	0	0	0	0	0	69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
% Discordance																										
687 bp	11	22	44	39	44	33	44	39	39	28	50	61	11	39	33	28	50	33	17	39	50	28	44	39		
287 bp	17	28	50	33	39	28	50	33	44	22	44	56	17	44	39	33	44	39	11	44	44	33	50	44		

^a Percentage based on cytogenetic analysis of at least 25 cells examined both at first passage and at final harvest.

^b + = Specific PCR products present; - = specific PCR products absent.

Panel 1 data. Therefore, NIGMS Human/Rodent Somatic Cell Hybrid Mapping Panel 2, a panel of hybrid cell lines containing only one or two human chromosomes per line, was also screened by performing the PCR. The results when the PCR was performed with both sets of primers with Panel 2 were compatible with the conclusion that the STD gene mapped to human chromosome 19, since only that DNA sample yielded amplification products of the appropriate lengths (Fig. 1).

FISH was then used to localize the STD gene on chromosome 19 (Fig. 2). In 17 of the 20 cells scored, a fluorescent signal was present on both chromatids of chromosome 19 at a position corresponding to 19q13.3, whereas signal was present on only one chromatid at this position in the remaining three cells. No significant site of hybridization was noted on other chromosomes. Assignment of the STD gene to this region by in situ hybridization provided additional confirmation of the localization of the gene to chromosome 19 as determined by PCR analysis of the human $\times$ rodent somatic cell hybrid DNA.

DHEA ST catalyzes the sulfation of DHEA, other steroid hormones, bile acids, and cholesterol (Falany et al., 1989; Radominska et al., 1990). DHEA ST is only one member of an ST gene superfamily. At this moment, cDNAs for 19 eukaryotic cytosolic ST enzymes have been reported. Comparison of the amino acid sequences encoded by these cDNAs makes it possible to group these proteins into three "families," members of which display 45% or greater amino acid sequence identity: the hydroxysteroid ST family, of which DHEA ST is a member; the phenol and estrogen ST family; and the flavonol ST family (Otterness and Weinshilboum, 1994; Weinshilboum and Otterness, 1994). Genes for the two human phenol ST (PST) enzymes, the so-called thermostable, or phenol-preferring ("P"), form of PST, and the thermolabile, or monoamine-preferring ("M") form (gene symbols STP and STM, respectively) have been mapped to human chromosome 16 (Dooley et al., 1993; Aksoy et al., 1994a). Our studies have demonstrated that STD is localized on human chromosome region 19q13.3. It will be of interest to determine the chromosomal localization of the

Fig. 2. FISH mapping of the human STD gene. An STD-positive cosmid was mapped to metaphase chromosomes as described in the Materials and methods. The solid symbols indicate the presence of fluorescent signal on both chromatids, whereas the open symbols indicate the presence of fluorescent signal on only a single chromatid.

gene for the single remaining known cytosolic human ST enzyme, estrogen ST (Aksoy et al., 1994b).

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References

- Aksoy IA, Callen DF, Apostolou S, Her C, Weinshilboum RM: Thermolabile phenol sulfotransferase (STM): localization to human chromosome 16p11.2. *Genomics* 23:275-277 (1994a).
- Aksoy IA, Sochorová V, Weinshilboum R: Human liver dehydroepiandrosterone sulfotransferase: nature and extent of individual variation. *Clin Pharmacol Ther* 54:498-506 (1993).
- Aksoy IA, Wood TC, Weinshilboum RM: Human liver estrogen sulfotransferase: cDNA cloning, expression and biochemical characterization. *Biochem Biophys Res Commun* 200:1621-1629 (1994b).
- deJong PJ, Yokabata K, Chen C, Lohman F, Pederson L, McNinch J, Van Dilla M: Human chromosome-specific partial digest libraries in lambda and cosmid vectors. *Cytogenet Cell Genet* 51:985 (1989).
- Dooley TP, Obermoeller RD, Leiter EH, Chapman HD, Falany CN, Deng Z, Siciliano MJ: Mapping of the phenol sulfotransferase gene (STP) to human chromosome 16p12.1→p11.2 and to mouse chromosome 7. *Genomics* 18:440-443 (1993).
- Falany CN, Vazquez ME, Kalb JM: Purification and characterization of human liver dehydroepiandrosterone sulphotransferase. *Biochem J* 260:641-646 (1989).
- Nafziger AN, Herrington DM, Bush TL: Dehydroepiandrosterone and dehydroepiandrosterone sulfate: their relation to cardiovascular disease. *Epidemiol Rev* 13:267-293 (1991).
- Olsen AS, Combs J, Garcia E, Elliot J, Amemiya C, deJong P, Threadgill G: Automated production of high density cosmid and YAC colony filters with a robotic workstation. *BioTechniques* 14:116-123 (1993).
- Otterness DM, Weinshilboum R: Human dehydroepiandrosterone sulfotransferase: molecular cloning of cDNA and genomic DNA. *Chem-Biol Interact* 92:145-159 (1994).
- Otterness DM, Wieben ED, Wood TC, Watson RWG, Madden BJ, McCormick DJ, Weinshilboum RM: Human liver dehydroepiandrosterone sulfotransferase: molecular cloning and expression of cDNA. *Mol Pharmacol* 41:865-872 (1992).
- Radominska A, Comer KA, Zimniak P, Falany J, Iscan M, Falany CN: Human liver steroid sulphotransferase sulphates bile acids. *Biochem J* 272:597-604 (1990).
- Trask B, Fertitta A, Christensen M, Youngblom J, Bergmann A, Copeland A, deJong P, Mohrenweiser H, Olsen A, Carrano A, Tynan K: Fluorescence in situ hybridization mapping of human chromosome 19: cytogenetic band location of 540 cosmids and 70 genes or DNA markers. *Genomics* 15:133-145 (1993).
- Tynan K, Hoffman SMG, Tsujimoto S, Brandriff B, Gordon L, Carrano AV, Mohrenweiser HW: Organization of the multiple polymorphic sites of the D19S11 locus within a 650-kb cosmid contig. *Genomics* 17:316-323 (1993).
- Warren JC, Timberlake CE: Biosynthesis of estrogens in pregnancy: precursor role of plasma dehydroisoandrosterone. *Obstet Gynecol* 23:689-698 (1964).
- Weinshilboum RM, Otterness DM: Sulfotransferase enzymes, in Kauffman FC (ed): *Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity. Handbook of Experimental Pharmacology*, Vol 112, pp 45-78 (Springer-Verlag, Berlin/Heidelberg/New York 1994).