Assignment¹ of Acyl-CoA:cholesterol acyltransferase 1 and 2 (SOAT1, SOAT2) and Diacylglycerol O-acyltransferase 1 (DGAT1) to *M. fascicularis* chromosome band 1p32, 12q13, 8qter; *C. aethiops sabaesus* 13q22, 3q12, 1qter; *S. sciureus* 19q22, 15q21, 16qter by in situ hybridization

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¹ To our knowledge this is the first time these genes have been mapped.

Rationale and significance

SOAT1, SOAT2 (also known as ACAT1 and ACAT2) and DGAT1 belong to the acyl-CoA: cholesterol acyltransferase gene family, members of which esterify cholesterol in Kupffer (SOAT1) and hepatic cells (SOAT2, DGAT1) of the liver (Lee et al. 2000; Buhman et al. 2001). Non-human primates fed monounsaturated fat responded with induction of SOAT2 enzyme activity in the liver, resulting in increased levels of cholesteryl ester containing lipoproteins in plasma followed by development of atherosclerosis (Rudel et al. 2002). Absence of SOAT2 reduced plasma concentrations of cholesteryl ester levels and the incidence of atherosclerosis in knockout mice (Willner et al., 2003). It is not established whether absence of one or more of these enzymes has a similar protective effect in primates. We localized the three genes for lipid esterification in this gene family to chromosomes of two Old World monkeys: *Macaca fascicularis* (macaque, MFA), *Cercopithecus aethiops sabaesus* (African green monkey, CSA) and a New World monkey *Saimiri sciureus sciureus* (Squirrel monkey, SSC). Each of these species has been used as a model for diet-induced atherosclerosis as occurs in humans (HSA) (Rudel and Lofland, 1976; Rudel et al., 2002).

Materials and methods

Karyotyping and nomenclature

Peripheral blood from single male CSA and MFA in the primate colony at the WFU medical center was obtained via venipuncture under ketamine anesthesia. Cultures were set up in RPMI 1640 with PHA and 10% FBS for 72 h at 37°C. Blood from SSC was donated by Lawrence Williams of the University of South Alabama at Mobile. The lymphocytes were growth stimulated in RPMI 1640 and 15% FBS with 30 µg/ml of Con A (Sigma). Twenty metaphases from each species were G banded and karyotyped according to published ideograms from the literature (Pearson et al., 1979 for MFA; Finaz et al., 1976 for CSA; Lau and Arrighi, 1976 for SSC). Band numbering of non-human primate chromosomes followed the recommendations in ISCN (1985).

Fluorescence in situ hybridization (FISH)

Full length cDNA's of SOAT1, SOAT2 and DGAT1 from African green monkey were generated using standard cloning techniques. A commercially prepared (Invitrogen, Carlsbad, CA) cDNA library in the pcDNA1 plasmid vector was screened with riboprobes made from a 3' cDNA fragment obtained by RT-PCR from African green monkey liver mRNA. Functional gene activity of the clones was confirmed by expression in a hamster AC-29 cell line (Anderson et al., 1998). After agarose electrophoresis the cDNA inserts (approximately 2 kb) were cut out of the gel, labeled and hybridized for FISH according to von Kap-herr et al. (2000). Human multi-color Spectravision probes (Vysis, Downer's Grove, IL) and human painting probes 1, 8 and 12 (Vysis), identified non-human primate chromosomes that were homologous to their human counterparts.

Probe names: SOAT1, SOAT2, DGAT1

Probe type: cDNA

Insert size: 2 kb, 2.2 kb, 2 kb

Vector: CMV, pcDNA3, pcDNA3

Proof of authenticity: DNA sequencing

Gene reference: GenBank Accession No. AF053336, AF053234, AF236018

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Fig. 1. FISH of SOAT1, SOAT2, DGAT1 cDNA probes to HSA and CSA chromosomes. Left to right: Ideogram of HSA, G-banded chromosome, human M-FISH probes hybridized to HSA and CSA followed by CSA ideogram, G-banded chromosome, CSA chromosome hybridized with the cDNA probe (green) and painting probes from the homologous human chromosome (red). Top row: Location of SOAT1 to HSA1q25 and CSA13q22 (arrow). The ideogram points out a paracentric inversion in CSA relative to HSA (arrowheads). Middle row: Location of DGAT1 to HSA8q24.3 and CSA 1qter. Bottom row: Location of SOAT2 to HSA12q13.1 and CSA3q12.

Results

Mapping data

Most precise location:

Gene	MFA	CSA	SSC	HSA
SOAT1	1p32	13q22	19q22	1q25.2
SOAT2	12q13	3q12	15q21	12q13.13
DGAT1	8qter	1qter	16qter	8q24.3

Number of cells examined:

Gene	MFA	CSA	SSC	HSA
SOAT1	11	10	31	12
SOAT2	14	13	15	15
DGAT1	16	17	22	20

Number of cells with specific signal:

	SOAT1				SOAT2				DGAT1			
	1	2	3	4	1	2	3	4	1	2	3	4
MFA	-	2	-	9	-	4	-	10	-	9	-	7
CSA	-	-	-	10	-	3	-	10	-	7	-	10
SSC	-	20	-	11	-	6	-	9	-	13	-	9

Location of background signals (sites with >2 signals): none

The location of the non-human primate cDNA's for SOAT1, SOAT2 and DGAT1 on human chromosomes (Fig. 1, Table) was consistent with the human gene reference locations (UCSC genome browser, June 2002 Freeze, <http://genome.ucsc.edu>). The cDNA probes were located to non human primate chromosomes which had homology by human painting probes to human chromosomes 1, 8 and 12. To illustrate, SOAT1 (Fig. 1, top row) is located on 13q22 in CSA, homologous to HSA1q and SOAT2 (bottom row) at 3q12, was homo-

gous to HSA12. DGAT1 (middle row) is located to the terminal q arm of CSA1, similar to its position on HSA8.

Genes linked to these three cDNA's in human, according to a comparative mapping database, are located to the same chromosomes in CSA and MFA to which we localized SOAT1, SOAT2 and DGAT1 (Graves et al., 1996).

Localization of these genes may be helpful in attempts to use gene therapy in atherosclerosis (SOAT's) or obesity (DGAT1), or to the design of transgenic monkeys (Chan et al., 2001; Nilsson et al., 1994) containing mutant copies of these genes.

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