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Association of Stearoyl-coA desaturase 1 Activity with Familial Combined Hyperlipidemia

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Abstract

Stearoyl-coA desaturase 1 (SCD1) is the rate-limiting enzyme involved in the synthesis of monounsaturated fatty acids, and in mice SCD1 activity is associated with plasma triglyceride levels.

Objective—We used the fatty acid desaturation index (the plasma ratio of 18:1/18:0), as a marker of SCD1 activity to investigate the relationship of SCD1 to familial combined hyperlipidemia (FCHL).

Methods and Results—The fatty acid desaturation index was measured in 400 individuals from 18 extended FCHL pedigrees. FCHL-affected individuals exhibited increased SCD1 activity when compared to unrelated controls (P<0.0001). The fatty acid desaturation index was found to be highly heritable ($h^2 = 0.48$, $p= 2.2 \times 10^{-11}$) in this study sample. QTL analysis in 346 sibling pairs from 18 FCHL families revealed suggestive linkage of the desaturation index to chromosomes 3p26.1-3p13 (z=2.7, P=0.003), containing the peroxisome proliferator-activated receptor gamma (PPARγ) gene, and 20p11.21-20q13.32 (z=1.7, P=0.04), containing the hepatocyte nuclear factor 4, alpha (HNF4α) gene. A specific haplotype of HNF4α was found to be associated with the desaturation index in these FCHL families (P=0.002).

Conclusion—Our results demonstrate that the fatty acid desaturation index is a highly heritable trait that is associated with the dyslipidemia observed in FCHL.

Keywords

familial combined hyperlipidemia; genetics; Stearoyl-coA desaturase 1; peroxisome proliferator-activated receptor gamma; hepatocyte nuclear factor 4 alpha

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Stearoyl-CoA desaturase (SCD) is the major enzyme which catalyzes the conversion of saturated fatty acids to monounsaturated fatty acids. The primary products of SCD-1, palmitate and oleate, are the most abundant monounsaturated fatty acids of phospholipids, triglycerides, wax esters, and cholesterol esters [1]. Mice homozygous for either a naturally occurring mutation or a targeted disruption of the SCD1 gene exhibit a marked reduction in VLDL triglycerides [2]. $SCD1^{-/-}$ mice on a leptin-deficient *ob/ob* background exhibit reduced adiposity and

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increased fatty acid oxidation when compared to control animals, demonstrating a key role for SCD1 in the regulation of lipid homeostasis [2].

Familial combined hyperlipidemia (FCHL) is characterized by elevated plasma triglyceride and/or cholesterol levels [3]. FCHL often occurs in combination with insulin resistance, central adiposity, altered fatty acid metabolism, and other dyslipidemic phenotypes that predispose to early coronary artery disease (CAD) [4,5]. The observed role of SCD1 in the regulation of VLDL metabolism and its involvement in many of the lipid parameters that are perturbed in FCHL suggest that the activity of this enzyme may be altered in FCHL subjects. In fact, the desaturation index was strongly correlated with both triglyceride and HDL levels in a human cohort of which approximately 60% of the subjects had FCHL [6]. In mice, studies of SCD activity in the HYPLIP mouse model of hyperlipidemia showed that hepatic SCD activity was increased 4-fold when compared to control animals [6]. Taken together these data suggest that SCD1 may contribute to triglyceride levels in FCHL individuals. We analyzed the desaturation index of 400 family members from 18 extended FCHL pedigrees, and performed a genome scan to identify chromosomal regions that may harbor genes that contribute to variation in SCD1 activity.

Methods

Eighteen extended FCHL families of Dutch Caucasian descent were ascertained through probands recruited from the Lipid Clinic of the Utrecht Academic University Hospital, as previously described [7]. The probands met the following criteria: (1) a primary combined hyperlipidemia with a fasting plasma cholesterol >6.5 mmol/L, or >90th percentile for age, defined according to tables from the Lipid Research Clinics, and fasting plasma triglycerides >2.3 mmol/L; (2) at least one first degree relative with a different hyperlipidemic phenotype; and (3) a positive family history of premature CAD defined as a myocardial infarction or cardiovascular disease before 60 years of age. Exclusion criteria for the probands included diabetes, obesity (BMI >30), tendon xanthomas, or type III hyperlipidemia (apoE2/E2). None of the subjects included in the study used exogenous insulin or hypoglycemic agents. All subjects gave informed consent, and the study protocol was approved by the Human Investigation Review Committees of Utrecht University Hospital, the Netherlands, and the University of California, Los Angeles.

Genetic Statistical Analyses

To assess the correlation of 18:1/18:0 with 16:1/16:0, Pearson's correlation was assessed using the R statistical package (http://www.r-project.org/). All traits were transformed to normality using the inverse normal transformation option of SOLAR 4.07 [8]. The effect of sex, age, sex by age interaction, triglycerides, total cholesterol, LDL-C, HDL-C, apoB, FFA, glucose, and BMI were tested as covariates of the desaturation index. Potential predictors of the desaturation index were considered significant if the regression coefficient reached $P<0.05$. The contribution of genetic factors to variation in the desaturation index was estimated by calculating the heritability (h^2) using the SOLAR software [8]. Significance of h^2 values was assessed by a likelihood ratio test, in which the likelihood of the model with an additive genetic variance component and covariates was compared with the likelihood model in which the additive genetic variance component was constrained to be 0. However, h^2 may be overestimated because only known covariates are entered into the model and unknown environmental effects are not included in this analysis. ANOVA was used to assess the differences in each FCHL characteristic between affected and unaffected relatives after adjustment for age, sex, and family membership where appropriate using the PROC GLM option of SAS v9.1 [9]. Trait differences were considered significant if the value for this analysis was $P<0.05$. The QTL analysis of the desaturation index trait was conducted using the

nonparametric linkage statistic of the Genehunter 2.1 software[10]. There were 346 sibling pairs included in this analysis.

Genetic association of the desaturation index was tested using the family-based association test (FBAT) program and the haplotype-based association test (HBAT) program [11]. We used the "–o" option of HBAT to assess quantitative association of haplotypes with the desaturation index trait. In order to correct for multiple testing, the "–p" option of HBAT was also used, which performs the Monte-Carlo permutation procedure. The PedCheck program was used to assess pedigree inconsistencies[12]. Pairwise LD was examined using the JLIN program [13].

Results

We measured the desaturation index, the ratio of plasma 18:1 / 18:0 and 16:1 /16:0, in 39 FCHL probands and 70 normolipidemic control individuals and in 400 family members from 18 extended pedigrees for whom plasma samples were available. The results and relevant clinical characteristics for these study samples are listed in Table 1. The mean 16:1/16:0 and 18:1/18:0 desaturation indices in FCHL probands was significantly higher than that of normolipidemic spouse control individuals (0.134 ± 0.060 vs. 0.101 ± 0.030 for 16:1/16:0, $P<0.003$, and 3.95 ± 1.08 vs. 2.93 ± 0.882 for 18:1/18:0, $P\leq0.0001$, in probands (n=39) and spouses (n=70), respectively). A similar increase in desaturation indices was also observed in hyperlipidemic relatives when compared to spouse controls (Table 1).

As expected, there was strong correlation between the 18:1/18:0 and 16:1/16:0 values for the 400 FCHL family members (Pearson correlation = 0.53, $p=2.2\times10^{-16}$), therefore for all subsequent analyses we used the 18:1/18:0 molar ratio to define the desaturation index. To assess whether the desaturation index is quantitatively correlated with total cholesterol, triglycerides, HDL-C, as well as other metabolic parameters, we conducted regression analyses in order to identify covariates that are significantly correlated with the desaturation index in these pedigrees. Both triglyceride ($\beta = 0.34$, $P=2.0 \times 10^{-9}$) and to a lesser extent, HDLC ($\beta = -0.19$, $P=0.0005$) levels were significant predictors of the desaturation index. Although the desaturation index has been associated with measures of body adiposity in other studies [14–18], BMI was not a significant predictor of the desaturation index in these FCHL families ($\beta = -0.002$, $P=0.97$). This finding could be due to the strong correlation of the desaturation index with plasma triglycerides in this population such that it obscures the relationship of SCD1 activity with BMI. We tested this hypothesis by testing the triglyceride- and HDLC-adjusted desaturation index residuals for correlation with BMI, however BMI was still not a significant predictor of the desaturation index ($\beta = -0.001$, $P=0.79$) in these families.

In order to estimate the heritability of the desaturation index, we used variance components analysis in all 400 individuals that were measured for the desaturation index. Unadjusted heritability was estimated to be 0.47 ($P= 1.0 \times 10^{-9}$). After adjustment for sex ($\beta=0.18$,$P=0.05$), age ($\beta=-0.004$, $P=0.2$), triglyceride ($\beta=0.34$, $P= 2.0\times10^{-9}$) and HDL-C ($\beta = -0.19$, $P= 0.0005$) levels, the estimated heritability (h^2) of the desaturation index increased slightly to $h^2=0.48$ ($P=2.2 \times 10^{-11}$), indicating that the desaturation index is a highly heritable trait in this FCHL cohort even after adjustment for covariates.

Given the evidence that genetic factors contribute to the desaturation index, we performed quantitative nonparametric sibpair analysis to identify regions of the genome that may harbor genes that contribute to variation in the desaturation index. We performed the analysis using both age- and sex- adjusted and unadjusted desaturation index values. No evidence of genome-wide significant linkage of the desaturation index was obtained in this nonparametric genome scan. We did, however, obtain suggestive evidence for linkage to chromosome 3p26.1-3p13 (z=2.7, P=0.003), 7p22.2-p15.3 (z=2.1, P=0.02), and 20p11.21-20q13.32 (z=1.7, P=0.04). All

regions exhibiting a z-score >1.64 (P≤0.05) are shown in Table 2 and Figure 1. Two of these regions, 3p26.1-3p13 and 20p11.21-20q13.32 are located near genes which have been previously associated with either free fatty acid or triglyceride levels in FCHL cohorts [19,20]. No evidence for linkage was obtained for the region containing the SCD1 gene on chromosome 10 (Figure 1). Additional adjustment of the desaturation index for triglyceride and BMI levels did not significantly alter the results (Figure 1). Linkage of each region to 16:1/16:0 was consistent with the linkage results of 18:1/18:0, although the overall linkage scores were lower for 16:1/16:0.

The strongest evidence for linkage was located on chromosome 3 near the PPARγ gene (z=2.7, P=0.003). The empirical p-value for this linkage region was P=0.01, indicating that there is a 1% chance that the linkage of the desaturation index to this region is a false positive. We identified tagging SNPs in the 148.5 kb genomic region containing the PPARγ gene (+/− 1kb) using HAPMAP data from 30 CEPH trios (Figure 1). Seven tagging SNPs and two coding SNPs (rs1801282, also known as the Pro12Ala polymorphism and rs3856806, also known as C161T), which had previously been studied in our case-control set, were genotyped in our FCHL families [19]. These nine SNPs span a total of 141 kb. Pairwise linkage disequilibrium (LD) was similar between FCHL probands and normolipidemic spouses for al SNPs tested (Figure SI). The D' statistic suggests that there is strong LD among all 9 of the PPARg SNPs in both probands and spouses, whereas the r^2 statistics suggest that there is little LD in the region. Since the D' statistics are not dependent on allele frequencies, the measures of LD indicated by the D' statistic may be inflated given the small size of our case-control cohort.

We observed only modest association of the desaturation index with four SNPs (rs2972164, rs10510418, rs2938395, rs709157) out of the nine that were genotyped (Table SI, P<0.05), although these results do not remain significant after correction for multiple testing. We tested for haplotype association using the four moderately associated SNPs (P≤0.05 before correction for multiple testing). We observed no significant association of the four-SNP haplotype; however, haplotypes comprised of three SNPs (rs2972164, rs10510418, and rs2938395) defined both a risk and a protective haplotype for the desaturation index trait (Table 3). The most significant association was observed for haplotypes composed of two of these SNPs rs2972164 and rs2938395. Under a dominant model, we observed marginal evidence of association of the T-T haplotype of rs2972164 and rs2938395 with the desaturation index (Table 3, P=0.02), and under an additive model, the C-C haplotype of rs2972164 and rs2938395 was also marginally associated with the desaturation index (P=0.01). The effect of each haplotype can be interpreted by evaluating the sign of the FBAT statistic. For the T-T haplotype, the sign of the FBAT statistic was positive, suggesting that the effect of the haplotype is to raise the desaturation index value. We have therefore designated this haplotype as the risk haplotype. Similarly, the negative FBAT statistic obtained for the C-C haplotype indicates that the effect of the haplotype is to decrease the desaturation index value, and it is therefore designated as a protective haplotype. Although the association of PPARγ with the desaturation index is only weakly significant, linkage analysis of families which contribute to the association signal revealed an increase in the linkage signal to 3.15, suggesting that subsetting families on the basis of PPARg haplotypes strengthens the linkage signal at the chromosome 3 locus. These data suggest that PPARγ haplotypes may contribute to the variation in desaturation indices in FCHL families; however confirmation of these findings in an independent population is warranted.

We also observed suggestive evidence of linkage of the desaturation index to chromosome 20p11.21-20q13.32 (p= 0.01), a region previously linked to triglycerides and HDL-C in Finnish FCHL and low-HDL families respectively [21,22]. The chromosome 20 linkage peak is located directly over the HNF4α gene which has been recently associated with elevated serum lipids in Finnish and Mexican FCHL families [20]. We genotyped five SNPs (rs2144908, rs6031558,

rs2425640, rs745975, rs3212198) that were associated individually or as haplotypes with triglycerides in the Finnish FCHL cohort and with glucose levels in the Mexican FCHL cohort, in our extended FCHL pedigrees (Table SII). Pairwise LD patterns for the five SNPs tested did not differ from those obtained by Weisglas-Volkov et al. (Figure S1) [20]. We observed only marginal association of rs3212198 with the desaturation index in our FCHL families (P=0.03), which does not remain significant after correction for multiple testing. In the study by Weissglas-Volkov et al., a risk haplotype comprised of rs6031558, rs745975, and rs3212198 was significantly associated with serum triglycerides in Finnish and Mexican FCHL families. We tested for association of this haplotype with the desaturation index in our FCHL cohort. We observed quantitative association of the G-G-A haplotype of rs6031558 - rs745975 - rs3212198 with the desaturation index (P=0.01, Table 3) in our FCHL families, although most of the association signal seems to come from rs6031558 - rs745975 (P=0.002). The G-G-A haplotype is the same risk haplotype that was found to be associated with triglycerides in Finnish and Mexican FCHL families[20], supporting the hypothesis that specific HNF4α haplotypes contribute to the development of the FCHL phenotype. Subset analysis of the HNF4a associated families revealed that these families provide no evidence for linkage to the peak marker D20S481, however, the linkage signal in the non-associated families remains and even increases to 1.9. These data suggest that although HNF4a haplotypes are associated with the desaturation index, this association does not account for the linkage of the desaturation index to this chromosome 20 region.

Discussion

FCHL individuals present with elevated plasma triglyceride and cholesterol levels, often in conjunction with insulin resistance and altered fatty acid metabolism [23]. Identification of biochemical markers of FCHL may help unravel the genetic complexities which underlie the disease [5]. Given its central role in the regulation of lipid homeostasis, we examined the role of SCD1 to FCHL and evaluated its correlation with the FCHL phenotype. The results indicate that the desaturation index is heritable and is associated with the dyslipidemic profile of FCHL individuals. Additionally, we provide evidence that this association may be due, at least in part, to common variants HNF4α.

We observed correlations of SCD1 activity with plasma triglyceride and HDL-C levels. BMI was not a significant predictor of the desaturation index in this cohort, as has been reported in studies of metabolic syndrome and obesity [16,17] 14,[15]. This finding could be due to the differences in ascertainment criteria for metabolic syndrome, obesity, and FCHL. Although BMI is increased in FCHL (the mean BMI of hyperlipidemics is 26.3 ± 3.53, Table 1), the hyperlipidemic individuals are not obese (defined as BMI>30). The strong correlation of the desaturation index with plasma triglycerides and HDL-C confirms the findings of Attie et al. who reported that the desaturation ratio accounted for more than 40% of the variation in plasma triglycerides, although in our FCHL families the desaturation index accounts for about 14% ($P=1.7 \times 10^{-14}$) of the variation in triglyceride levels [6].

In the present study, we demonstrate that variation in the desaturation index is highly heritable in FCHL families. We are the first to directly investigate whether or not SCD1 activity is a heritable trait in humans, although some evidence of the genetic determination of SCD1 in humans has been reported [14]. In an expression array study of muscle from lean and obese humans, elevated SCD1 expression levels were elevated 3 fold in the muscle of obese subjects when compared to that of lean subjects. The increased SCD1 expression was retained in cultured primary skeletal myocytes obtained from obese and lean individuals, indicating that isolated myocytes cultured under identical environment and stimuli retain SCD1 expression differences in vitro [14]. These data are consistent with the notion that SCD1 activity is a genetically driven trait.

The strongest evidence for linkage was obtained for a region on chromosome 3 near the PPARγ gene. PPARγ is a key regulator of lipogenic gene expression, and studies in humans and in tissue culture suggest that the SCD1 gene is up-regulated in response to PPARγ activation[24–26]. In two independent studies, PPARγ SNPs were marginally associated with free fatty acid and glycerol levels as well as decreased fasting insulin levels in FCHL probands [19,27]. We identified two common PPARγ haplotypes, one risk and one protective haplotype, which were associated with the desaturation index, suggesting that common variants of PPARγ may contribute to variation in SCD1 activity, although the observed association was relatively weak. Replication of this finding is warranted.

We also observed linkage of the desaturation index to a locus on chromosome 20 which had previously been linked to triglycerides in FCHL families [28]. HNF4α, a highly conserved nuclear receptor, is known to be activated and repressed by the binding of saturated and unsaturated fatty acids respectively, consistent with the hypothesis that HNF4a is involved in the regulation of gene expression by fatty acids[29]. The role of HNF4a in lipid and glucose homeostasis is highlighted by the identification of loss of function mutations in maturity-onset diabetes of the young (MODY1)[30], and by the association of common alleles and haplotypes of HNF4a with T2DM [31] and FCHL[20]. We observed significant association of an HNF4α haplotype, comprised of SNPs rs6031558-rs745975-rs3212198 with the desaturation index, which was the same triglyceride-associated haplotype originally identified by Weissglas-Volkov et al. These findings suggest that the same genetic variants which contribute to elevated serum triglyceride levels in FCHL may also contribute to increased SCD1 activity.

A recent study by Warensjö et al., reported evidence of association of SCD1 SNPs with the desaturation index. We found no evidence for linkage of the desaturation index trait to the Chromosome 10 region in which the SCD1 gene resides. Since association is generally more powerful than linkage [32], it is possible that the effect of the SCD1 locus on the desaturation index may not be detectable by linkage.

FCHL is genetically complex and is most likely due to the action of genetic and environmental factors, as well as their interactions [23]. Identification of new biochemical markers of FCHL could increase our understanding of the underlying mechanisms of the disease. The desaturation index is an attractive candidate marker for FCHL because of the demonstrated effects of SCD1 on VLDL production and fatty acid oxidation [33,34]. SCD1 undergoes rapid turnover in response to a variety of nutritional and hormonal signals, and at the transcriptional level it is regulated by a number of factors including the sterol regulatory binding protein-1 (SREBP1) and peroxisome proliferator-activated receptor, alpha (PPARα) [35]. Lack of SCD1 expression in mice reduces tissue lipid content and protects against both diet-induced and genetically-induced obesity [2]. Recent studies demonstrate that the protection from adiposity is at least partially due to increased fatty acid oxidation in the liver and muscle [34,36]. These studies suggests that increased SCD1 activity in humans could result in increased rates of triglyceride synthesis, and decreased rates of fatty acid oxidation. Such metabolic perturbations could lead to increased deposition of lipids in peripheral tissues leading to insulin resistance in the muscle and to steatosis in the liver. Altered fatty acid metabolism, insulin resistance, and hepatic steatosis often occur in combination with FCHL, and SCD1 may contribute to the development of these phenotypes [37–39]. Therefore, identification of genes which modulate SCD1 activity in humans could provide new clues to the altered metabolic pathways involved in FCHL and its overlapping syndromes. Our results demonstrate that the desaturation index is significantly associated with FCHL and furthermore suggest that the dyslipidemia observed in FCHL families may be partially due to genetic variations which affect SCD1 activity.

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Disclosure

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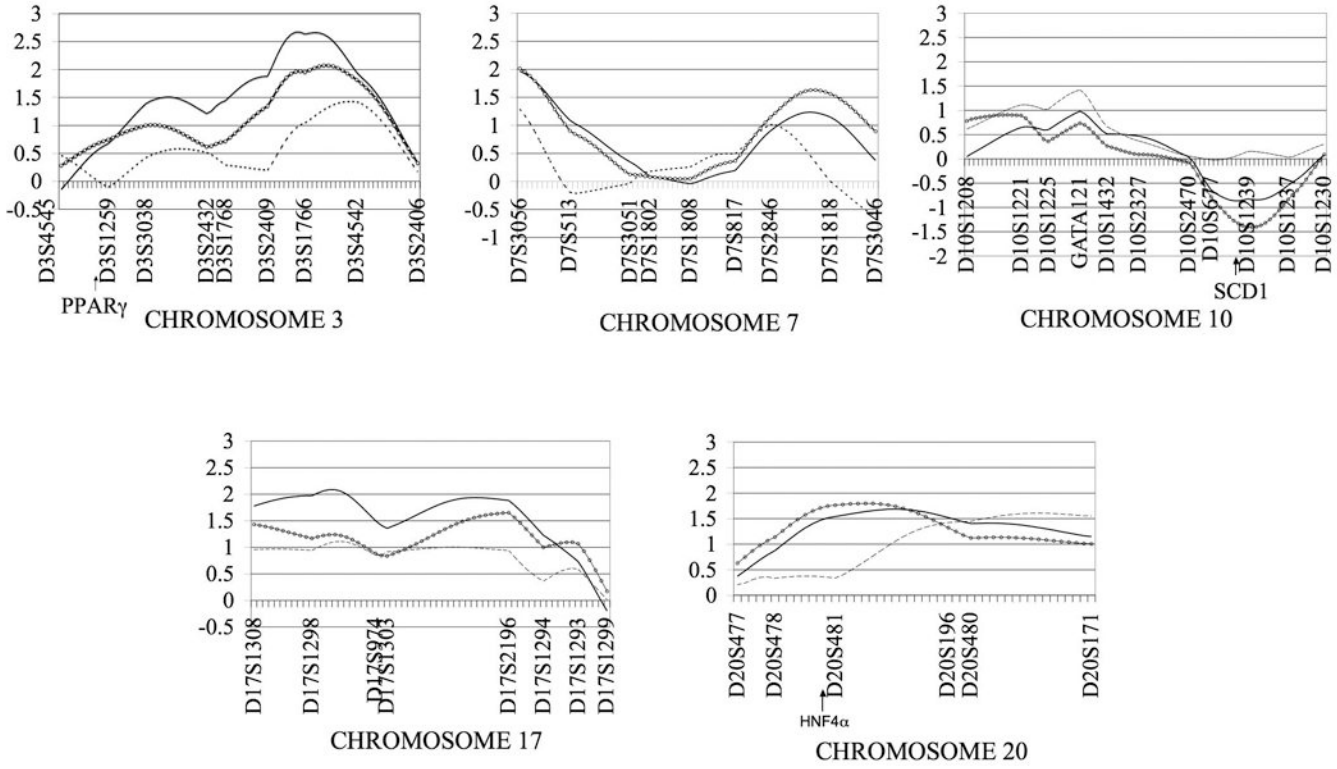

Figure 1.

Non-parametric z-scores > 1.64 ($p<0.05$) obtained for age- and sex- adjusted (solid black line), age- , sex-, tg-, and hdlc- adjusted (open circles) 18:1/18:0 desaturation index values and for 16:1/16:0 (dashed lines) in 18 extended FCHL families. Z-scores for the SCD1 gene locus (Chromosome 10) is also shown.

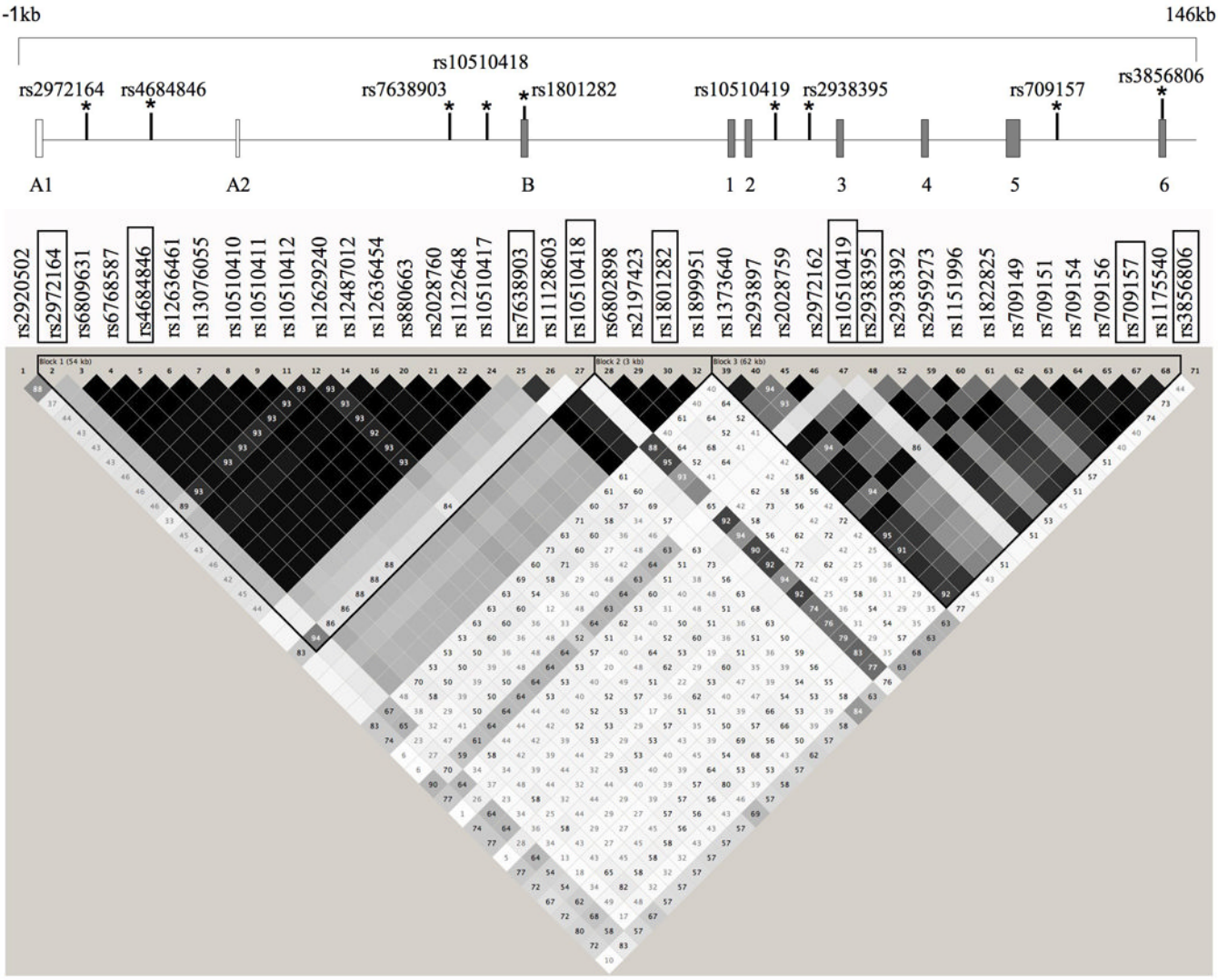

Figure 2.

Genomic Structure and Linkage Disequilibrium Map of PPARγ. A) Positions of genotyped SNPs selected for analysis. B) The PPARγ gene +/− 1kb in 30 CEPH trios using the HAPMAP data (release no. 16c.1/phaseI, June 2005). Linkage disequilibrium is displayed using the Haploview Program v. 3.2 (available at www.broad.mit.edu/mpg/haploview/). Genotyped SNPs are boxed. D' values are shown.

Table 1

Clinical Characteristics of the 18 FCHL families

Trait	*Hyperlipidemic Individuals*[†] *(n=189)*	*Normolipidemic Individuals (n=226)*	*Spouses (n=181)*	*P-value HL vs.NL*	P-value HL vs. S
Male/Female, n	107/82	105/121	76/105	0.05	0.01
Age, y	46 ± 16	32 ± 13	46 ± 15	<0.0001	NS
Body mass index (BMI), kg/m2	26.3 ± 3.53	23.3 ± 3.25	25.2 ± 3.83	<0.0001	<0.0001
Triglycerides, mmol/L	3.74 ± 7.49	1.25 ± 0.416	1.64 ± 1.09	<0.0001	<0.0001
Total cholesterol, mmol/L	7.51 ± 2.53	4.92 ± 0.812	5.66 ± 1.09	<0.0001	<0.0001
LDL-C, mmol/L	4.74 ± 1.32	3.11 ± 0.728	3.70 ± 1.01	<0.0001	<0.0001
HDL-C, mmol/L	1.13 ± 0.328	1.24 ± 0.311	1.25 ± 0.346	0.003	0.013
Desaturation Index[†]					
18:1/18:0	3.40 ± 0.835	3.11 ± 0.728	3.02 ± 0.845	0.02	<0.0001
16:1/16:0	0.116 ± 0.043	0.098 ± 0.003	0.102 ± 0.029	0.001	0.0001

Non-transformed trait values are expressed as mean ± SD.

[*] Includes 18 probands and 171 hyperlipidemic relatives

[†] The desaturation index was measured in 148 hyperlipidemic individuals, 173 normolipidemics, and 79 spouses.

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Table 2

Chromosomal Locations of Desaturation Index QTL in 18 FCHL Pedigrees

Band	Location of Peak*	Peak Z-score	P-value
3p26.1-3p13	76	**2.7** (2.1)	0.003 (0.02)
7p22.2-7p15.3	pter	**1.9** (2.0)	0.03 (0.02)
7p14.3-q32.3	49	**1.6** (1.9)	0.02 (0.03)
17p13.3-q21.32	14	**2.1** (1.2)	0.03 (0.1)
20p11.21-20q13.32	69	**1.7** (1.8)	0.04 (0.04)

Z-scores and P-values for age- and sex-adjusted (bold) and for age-, sex-, tg-, and hdlc-( ) adjusted desaturation index trait.

*From the p-terminus in centiMorgans

Table 3

Haplotype Association of PPARγ and HNF4α with desaturation index in FCHL families.

PPARγ				
Markers	***Implicated Haplotype***	***Freq.***	***P-value****	***P-value†***
rs2972164 - rs10510418 - rs2938395- rs709157	C-A-C-A	0.476	0.09	0.07
‡*rs2972164 - rs10510418 - rs2938395*	T-C-T (risk)	0.254	0.04	0.04
§*rs2972164 - rs10510418 - rs2938395*	C-A-C (protective)	0.480	0.03	0.03
‡*rs2972164- rs2938395*	T-T (risk)	0.258	0.01	0.01
§*rs2972164- rs2938395*	C-C (protective)	0.477	0.03	0.03
HNF4α				
§*rs6031558- rs745975-rs3212198*	G-G-A (risk)	0.338	0.01	0.008
§*rs6031558- rs745975*	G-G (risk)	0.463	0.002	0.002

*P-value listed is for the -o option of HBAT

†P-value listed is for the -p option of HBAT (*see Methods*)

‡Dominant Model

§Additive Model