Supplementary material

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rs11613352 Polymorphism (TT Genotype) Associates With a Decrease of TG and an Increase of HDL in Familial Hypercholesterolemia Patients

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SAFEHEART STUDY AND CARDIOVASCULAR DISEASE IN FAMILIAL HYPERCHOLESTEROLEMIA

Study Design and Recruitment

SAFEHEART is an open, multicenter, long-term, prospective cohort study in a well-defined familial hypercholesterolemia (FH) population, conducted in 19 outpatient lipid clinics in Spain. Recruitment of participants from FH families began in 2004 and is still ongoing. Inclusion criteria are: 1) index cases with genetic diagnosis of FH, 2) relatives older than 15 years with genetic diagnosis of FH, and 3) relatives older than 15 years without a genetic diagnosis of FH (control group). Relatives in the control group with high cholesterol due to other causes were not excluded.

This study was approved by the local ethics committees and all eligible participants gave written informed consent.

The SpAnish Familial HypErcHolEsterolaemiA CohoRt STudy (SAFEHEART), was designed to gain insight into the prognostic factors and mechanisms that influence the development of congenital heart disease and mortality in a well-defined FH population. A Coordinating Centre was created to organize and to implement the follow-up of cases. An active epidemiological surveillance system has been developed for the detection of fatal and nonfatal cardiovascular events, classified according to WHO-MONICA criteria.[1]

Premature congenital heart disease is defined if one of the following events occurs in males before 55 years of age and in females before 65 years of age, 1) Myocardial infarction, proved by at least 2 of the following 3 evidences: classic symptoms (> 15 minutes); specific electrocardiographic changes; and elevated cardiac enzymes (> 2× upper limit of normal); 2) Angina pectoris, diagnosed as classic symptoms in combination with at least one unequivocal result of an exercise test, nuclear scintigram, dobutamine stress ultrasound scan, or > 70%

stenosis on a coronary angiogram. 3) Percutaneous coronary intervention or other invasive procedures and coronary artery bypass grafting.

Measures and Blood Samples

Demographic and clinical characteristics of subjects include age, educational status, medical history focused on cardiovascular disease, classic cardiovascular risk factors (hypertension, type 2 diabetes, smoking status), physical examination, and current treatment for hypercholesterolemia and other risk factors. History of cardiovascular disease is obtained from medical charts provided by the subjects at inclusion. Quality of life (SF-12),[2] food frequency[3], and physical activity[4] surveys are recorded on standardized forms. Physical examination includes weight (kg), height (cm), body mass index (Kg/m2), and waist circumference (cm). Blood pressure is measured twice in supine position with an Omron MX3 sphyngomanometer.

Venous blood samples are taken after 12 hours of fasting. Serum, plasma, and deoxyribonucleic acid (DNA) samples are aliquotted and preserved at -80°C in a biobank located at the Cardiovascular Research Center in Barcelona. DNA is isolated from whole blood using standard methods and the genetic diagnosis of FH is made using a DNA-microarray (Progenika SA, Bilbao, Spain). Serum total cholesterol, triglycerides, and high-density lipoprotein cholesterol levels are measured in a centralized laboratory using enzymatic methods. Serum low-density lipoprotein cholesterol concentration is calculated using the Friedewald formula.[5] Mutations are classified as receptor-negative or receptor-defective, depending on their functional class as reported previously.[6] Those mutations with nonreported functional class in the literature were classified as “unknown”.

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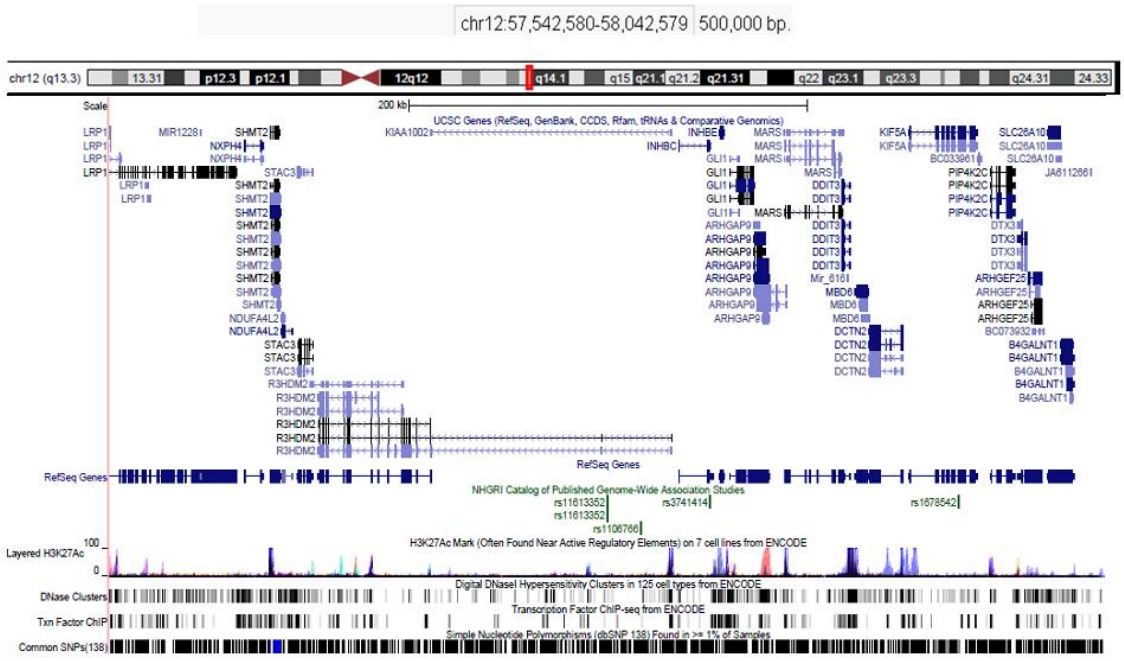
Table 1 Supplementary Material

Plasma Lipid Profiles and Anthropometric Characters by Single Nucleotide Polymorphism Genotypes

rs11613352	CC + CT 580	TT 21	*P*
Sex (% males)	305 (52.6)	8 (38.1)	.266
Age	48.7 (47.6-49.8)	54.4 (48.2-60.7)	.07
BMI (Kg/m^2)	27.1 (26.7-27.5)	25.9 (24.5-27.6)	.265
Waist circ. (cm)	90.1 (87.7-92.5)	86.4 (80.1-92.6)	.651
WTH	0.87 (0.86-0.88)	0.88 (0.84-0.92)	.4
Arcus cornealis (%)	230 (39.6)	11 (52.4)	.265
Xanthomas (%)	126 (21.7)	0	.012
Diabetes (%)	22 (3.8)	1 (4.8)	.562
CVD (%)	111 (19)	2 (9.5)	.396
TC (mg/dL)	245.6 (240.2-251)	233.5 (212.8-254.1)	.249
LDL (mg/dL)	177.5 (172.3-182.7)	161.6 (141.3-181.8)	.255
HDL (mg/dL)	48.6 (47.5-49.7)	56.5 (49.1-63.8)	.007
TG (mg/dL)[a]	90.1 (87-93.3)	67.5 (55.3-82.2)	.003[a]
TC/HDL	5.4 (5.2-5.6)	4.5 (3.6-5.5)	.107
ApoA-1 (mg/dL)	138.6 (136.3-140.9)	146.7 (134.7-158.7)	.162
ApoB (mg/dL)	117.5 (114.6-120.5)	102.1 (89.4-114.8)	.06

ApoA, apolipoprotein A; ApoB, apolipoprotein B; BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoproteins; LDL, low-density lipoproteins; TC, total cholesterol; TG, triglycerides; Waist circ., waist circumference; WTH, waist to heap ratio.

Results are shown as mean (95%CI) or n and percentages (%). Statistical significance for categorical data was obtained by Fisher exact test and by Student t-test for quantitative data. [a] T-test on log-transformed data. Statistical significance was set at $P<.05$.

Figure 1

Visualization of 500 Kb around rs11613352 (in green) generated using UCSC genome browser[7] showing the chromosome band localization, genes and transcripts, marks of histone acetylation (layered H3K27Ac), DNase sensitivity clusters, and transcription factor sites by chromatin immunoprecipitation sequencing (ChIP-seq) results from the Encyclopedia of DNA Elements (ENCODE)[8] and common SNPs (>1%) found in the region (synonymous variation in green and non–synonimous in red; untranslated in blue; unknown or intron localization in black).