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Genetics of severe hypercholesterolemia in the general population: Insights from the STANISLAS cohort

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ABSTRACT

Background: Severe hypercholesterolemia (SH) is a common condition characterized by increased levels of total and low-density lipoprotein cholesterol (LDLc). **Methods:** The aim of this study is to screen for prevalence of hypercholesterolemia, perform heritability estimation of circulating lipoproteins and study the association between SH cases and surrogate cardiovascular disease markers among participants of STANISLAS cohort. Gene candidate analyses were utilized to investigate the association between lipid levels, SH and polymorphisms from the three commonly reported genes (*APOB*, *LDLR* and *PCSK9*). **Results:** Participants with SH (n=102; 6.9%) were older (58 vs. 51yr), had higher total cholesterol (290 vs. 209mg/dL), LDLc (206 vs. 136mg/dL) and triglycerides (114 vs. 88 mg/dL). Despite smoking less, they had carotid plaques more frequently (21.2 vs. 9.3%), higher cIMT (676 vs. 597µm), and had more frequent family history cardiovascular disease. The circulating lipid levels have an important heritability: LDLc 51.6%, HDLc 66.6%, total cholesterol 49.8%, and triglycerides 41.4%. The SNPs located in *LDLR* gene present the strongest association with LDLc levels: rs55997232, rs17242395, rs1010679, and rs11668477. **Conclusion:** In a healthy cohort, participants with SH had premature vascular damage. LDLc had an important component of heritability and SNPs linked to the *LDLR* gene presented a strong association with LDLc. These findings reinforce the need for an early identification and treatment of SH subjects, which is mostly polygenic.

KEY WORDS: Severe hypercholesterolemia, LDL cholesterol, heritability, genetic, arterial stiffness, target organ damage

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ABBREVIATIONS: SH: Severe Hypercholesterolemia; LDLc: Low Density Lipoprotein cholesterol; HDLc: High density lipoprotein cholesterol; PWV: pulse-wave velocity; cIMT: carotid intima-media thickness; GRM: genetic relatedness matrix

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INTRODUCTION

Hypercholesterolemia is a common but underdiagnosed condition characterized by increased levels of total and low-density lipoprotein cholesterol (LDLc). If left untreated, it may lead to premature cardiovascular events and death^{1,2,3}. Two kinds of hypercholesterolemia have been reported, the familial form (Familial Hypercholesterolemia FH) and the sporadic form. The estimated prevalence of FH is 0.4% (1 person per 250 people) but the true worldwide prevalence of hypercholesterolemia is unknown because most patients remain undiagnosed^{4,5}.

Familial hypercholesterolemia is defined by several criteria (Supplemental Table 1) including genetic mutations in low-density lipoprotein receptor (*LDLR*), apolipoprotein B (*APOB*) and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes^{6,7}. However, beyond the “monogenic causes” responsible of FH, many people with hypercholesterolemia may present a polygenic severe form of hypercholesterolemia (SH, severe Hypercholesterolemia). Many of these polygenic forms remain to be characterized⁸.

The STANISLAS (*Suivi Temporaire Annuel Non-Invasif de la Santé des Lorrains Assurés Sociaux*) cohort is a single-centre familial longitudinal cohort from the Lorraine region of France, where the circulating levels of cholesterol and triglycerides were determined along with a detailed cardiovascular phenotyping⁹. Importantly, the familial design of this cohort allows the estimation of heritability of many variables including circulating lipoproteins.

The objectives of this study are to: 1) screen for prevalence of hypercholesterolemia among the participants, 2) describe the characteristics of the participants with hypercholesterolemia, 3) perform heritability estimation

of circulating lipoproteins, 4) assess for potential familial form of hypercholesterolemia accordingly to genetic characteristics, and 5) study the association between SH cases and surrogate cardiovascular disease markers.

METHODS

Study population

The design of the STANISLAS cohort has been previously published⁹. In brief, the STANISLAS cohort is a single-center familial longitudinal cohort composed of 1,006 families (4,295 subjects) from the Nancy region recruited in 1993–1995 at the Center for Preventive Medicine. The cohort was established with the primary objective of investigating gene-gene and gene-environment interactions in the field of cardiovascular diseases. The study protocols for all examinations were reviewed and approved by the local Ethic Committee of CPP Est 3, France. All participants provided written informed consent to participate in the study. The most recent visit (fourth visit, V4) included 1705 subjects. For the present analysis, we excluded 213 subjects that were treated with a statin, and 17 participants who had missing LDLc values. The study population included a total of 1475 subjects, among whom, 1377 were successfully genotyped (Figure 1).

Study design

All participants were observed at the *Centre d'Investigation Clinique Plurithématique Pierre Drouin* at the University Center Hospital of Nancy (*CIC-P de Nancy*). Samples were collected early morning after an overnight fasting period. Standardized sample handling procedures enabled the collection of serum and plasma. Blood lipids were determined after plasma centrifugation in the central

SUPPLEMENTAL TABLE 1. Comparison of diagnostic criteria for the FH (from McGowan et al, 2019)

Criteria	MedPed	Dutch	Simon Broome	NLA	AHA
Family history of premature CAD		+	+	+	+
Family history of tendon Xanthomonas		+	+		
Family history of hypercholesterolemia	+	+	+	+	
Patient premature CAD		+		+	
Patient premature PVD		+			
Tendon Xanthomonas		+	+	+	
Corneal arcus		+		+	
Elevated LDLc	+	+	+	+	+
Genetic mutation		+	+	+	+

AHA: American heart Association, CAD: coronary artery disease, FH: familial hypercholesterolemia, LDLc: low density lipoprotein cholesterol, MedPed: make early diagnosis to prevent early death, NLA: National lipid association, PVD: peripheral vascular disease

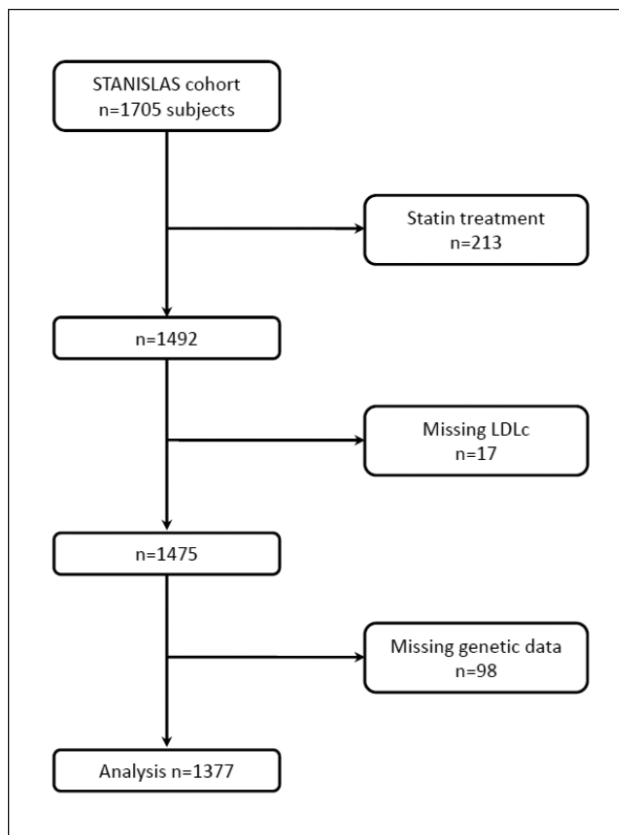


FIGURE 1. Study flow chart.

laboratory of the hospital using the Roche Cobas® lipid panel. LDL cholesterol levels were automatically calculated in the central laboratory using the Friedwald formula, excluding subjects with triglyceride levels superior to 4 g/L in whom LDLc was not calculated. Personal and familial medical history, medications, anthropometric parameters, blood pressure, pulse-wave velocity (PWV), carotid intima-media thickness (cIMT) and echography (including carotid plaque assessment and echocardiography) were recorded¹⁰⁻¹². Participants more likely to have any form of SH were identified on the basis of LDLc levels: LDLc ≥ 190 mg/dL if age ≥ 20yr and LDLc ≥ 160 mg/dL if age < 20yr^{13,14}.

Genetic analyses and heritability estimations

Blood DNA was extracted using Gentra Puregene Blood Kit (Qiagen, Hilden Germany®) and stored at -20°C. Genotyping was conducted at the *Centre National de Recherche en Génomique Humaine* (CNRGH, Evry, France) using two chips: 1) the Illumina Global Screening Array (GSA) which is composed of 687572 intronic and exonic markers, and 2) the Illumina Exome Array, which is constituted of 244330 SNPs, mostly exonic.

For heritability estimation, we used a linear mixed model, which allowed to simultaneously include additive genetic effects across the genome, common environment effects shared by nuclear family and fixed effects (sex and age). The additive genetic effects were assessed using the Genetic Relatedness Matrix (GRM) which has been computed using polymorphic SNPs of included subjects and which is based on genotype correlations. The use of the GRM allowed a better inference of relatedness between siblings, instead of an expected average¹⁵.

Gene candidate association analyses were utilized in order to investigate the association between lipid levels, SH and genetic polymorphisms from the three commonly reported genes (*APOB*, *LDLR* and *PCSK9*). For each gene, we defined an interval that encompassed their boundaries (±20kb) based on the reference genome built 37 from the Ensembl database (<http://grch37.ensembl.org>). Then we selected the SNPs from the two chips and performed usual quality control steps (Supplemental Table 2). A total of 7 SNPs were excluded for monomorphism, no SNP had >5% of missing data and no SNP deviated from the Hardy-Weinberg equilibrium at a threshold of $p < 1.10^{-8}$. Then 29 other SNPs were excluded because they were on linkage disequilibrium ($r^2 < 0.9$).

Genetic analyses were performed using linear mixed-effect model in order to take into account pedigree data, under an additive genetic model (with age and sex as covariates). Results were considered statistically significant at $p < 0.05$ after correction for multiple testing with a 1% false discovery rate. These analyses were performed with R (version 3.5.0) using the R package Gaston (gaston:

SUPPLEMENTAL TABLE 2. Selection and characteristics of SNP included in the study

Gene	Chromosome	Range	SNP GSA chip	SNP Exom chip	Exclusion criteria					SNP included
					Common	Monom.	Callrate (<0.95)	HWeq (P > 10 ⁻⁸)	LD (r ² < 0.9)	
<i>PCSK9</i>	1	55485221 - 55550525	24	12	3	2	0	0	3	28
<i>LDLR</i>	19	11180138 - 11264496	43	8	6	3	0	0	14	28
<i>APOB</i>	2	21204301 - 21286945	43	47	23	2	0	0	12	53

Monom.: monomoprphic; HWeq: hardy-Weinberg equilibrium; LD: linkage disequilibrium

Genetic Data Handling & Linear Mixed Models) for heritability estimation and GWAS analysis.

Statistical analyses

Continuous variables are expressed as means \pm standard deviation (SD) or median (interquartile range), categorical variables as frequencies (percentages), and odd ratios (ORs) as the point estimate and the respective 95% confidence interval (95% CI). Comparisons of characteristics were performed using Student's *t*-test or

Wilcoxon non parametric tests for continuous variables and χ^2 or exact Fisher tests for categorical variables. Factors associated with SH were assessed using both univariate and multivariate logistic regression with SH as binary outcome variable. First, univariate logistic regression was performed between SH and each variable significantly associated with SH with a $p < 0.01$ in Table 1. Second, multivariable logistic regression was performed using a stepwise forward selection process, retaining only the variables associated with SH with a $p < 0.05$ in

TABLE 1. Characteristics of the participants in the overall population and according to SH status

Characteristic	Overall (n=1475)	Unlikely SH (n=1373)	Possible SH (n=102)	P-value*
Age (years)	53 (33 - 59)	51 (33 - 59)	58 (50 - 61)	<0.0001
Male sex	692 (46.7 %)	646 (46.8 %)	46 (45.1 %)	0.76
Current smoker	327 (22.1 %)	313 (22.7 %)	14 (13.9 %)	0.046
BMI (Kg/m ²)	25 (22 - 28)	25 (22 - 28)	26 (24 - 28)	0.008
Diabetes	45 (3.1 %)	43 (3.2 %)	2 (2.0 %)	0.76
Glycated hemoglobin (%)	5.5 (5.3 - 5.8)	5.5 (5.3 - 5.8)	5.7 (5.5 - 5.9)	<0.0001
Hypertension history	233 (15.8 %)	209 (15.2 %)	24 (23.8 %)	0.033
Mean 24h SBP (mmHg)	119 (113 - 126)	119 (113 - 126)	120 (114 - 127)	0.36
Mean 24h DBP (mmHg)	74 \pm 7	74 \pm 7	74 \pm 8	0.84
Heart rate (bpm)	63 (57 - 69)	63 (57 - 69)	62 (58 - 68)	0.51
eGFR (ml/min/1.73m ²)	98 (88 - 110)	98 (88 - 110)	94 (85 - 100)	0.0005
Microalbuminuria (mg/L)	6.0 (3.5 - 9.7)	6.0 (3.5 - 9.8)	6.0 (3.6 - 9.0)	0.68
C-reactive protein (mg/L)	1.4 (0.7 - 3.2)	1.4 (0.7 - 3.2)	1.5 (0.8 - 2.2)	0.49
Total cholesterol (mg/dL)	215 \pm 39	209 \pm 35	290 \pm 18	<0.0001
HDLc (mg/dL)	58 \pm 14	58 \pm 14	60 \pm 13	0.15
LDLc (mg/dL)	136 \pm 34	131 \pm 29	206 \pm 13	<0.0001
Triglycerides (mg/dL)	88 (65 - 122)	86 (64 - 119)	114 (88 - 137)	<0.0001
IMT (μ m)	600 (512 - 703)	597 (510 - 694)	676 (560 - 778)	<0.0001
PWV (m/s)	8.0 (7.2 - 9.1)	8.0 (7.2 - 9.1)	8.1 (7.5 - 9.2)	0.014
Carotid plaque	146 (10.1 %)	125 (9.3 %)	21 (21.2 %)	0.0007
CVD history	150 (10.2 %)	140 (10.2 %)	10 (9.9 %)	1.00
Family history of:				
Myocardial infarction	226 (15.4 %)	199 (14.6 %)	27 (26.5 %)	0.003
Stroke	247 (16.8 %)	218 (16.0 %)	29 (28.4 %)	0.002
Hyperlipidemia	603 (48.2 %)	544 (46.9 %)	59 (66.3 %)	0.0004

Legend: SH, Severe Hypercholesterolemia; BMI, body mass index; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; CVD, cardiovascular disease including any of myocardial infarction, stroke or peripheral artery disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; IMT, intima-media thickness; PWV, pulse-wave velocity; eGFR, estimated glomerular filtration rate.

*Comparisons of characteristics were performed using Student's *t*-test or Wilcoxon non parametric tests for continuous variables and χ^2 or exact Fisher tests for categorical variables

the multivariable model. Assumptions of log linearity for continuous variables were checked using restricted cubic spline analyses. When log linearity hypothesis was not respected, continuous variables were categorized according to the shape of the spline curve. Accordingly, triglycerides, glycated hemoglobin and body mass index were expressed as binary covariates (\leq vs. >88 mg/dL, \leq vs. $>5.5\%$ and \leq vs. >25 kg/m², respectively). These analyses were performed using SAS version 9.4.6 (SAS Institute Inc., Cary, NC, USA) and the R software (version 3.5.0). A two-tailed significance level $p < 0.05$ was used.

RESULTS

Characteristics of the study population

The comparison of the population characteristics by SH status is depicted in the Table 1. Participants with possible SH ($n=102$; 6.9%) were older (58 vs. 51yr), had higher total cholesterol (290 vs. 209mg/dL), LDL cholesterol (206 vs. 136mg/dL) and triglycerides (114 vs. 88 mg/dL), higher glycated hemoglobin (5.7 vs. 5.5%), had higher BMI (26 vs. 25 kg/m²), had more often hypertension (23.8 vs. 15.2%), had lower eGFR (94 vs. 98 mL/min/1.73m²), had carotid plaques more often (21.2 vs. 9.3%), higher cIMT (676 vs. 597 μ m), and higher PWV (8.1 vs. 8.0m/s) despite smoking less (13.9 vs. 22.7%), and had more frequent family history of myocardial infarction (26.5 vs. 14.6%), stroke (28.4 vs. 16.0%), and hyperlipidemia (66.3 vs. 46.9%) ($p < 0.05$ for all). The HDLc levels did not differ between groups. Table

1. In the multivariable logistic model higher cIMT, glycated hemoglobin and triglyceride levels, and family history of hyperlipemia remained independently associated with SH. Supplemental Table 3.

Heritability estimations

The circulating lipid levels have an important heritability component: LDLc 51.6%, HDLc 66.6%, total cholesterol 49.8%, and triglycerides 41.4%. The variance in the lipid levels explained by environmental factors shared by nuclear family was low, especially for LDL and total cholesterol: LDLc 3.9%, HDLc 14.7%, total cholesterol 4.8%, and triglycerides 13.1%. About 45% of the variance of LDLc, triglycerides and total cholesterol levels remain unexplained neither by genetic nor by common environment effects, except for HDLc for which unexplained variance represent only 18.7%. Table 2.

Gene candidate analyses

Gene candidate analysis for the 3 genes implicated in definition of familial form of hypercholesterolemia is shown in the Table 3. All allele frequencies are in concordance with those from European population panels of reference. Compared with the *APOB* and *PCSK9* SNPs, those located in *LDLR* gene present the strongest association with LDLc levels. After correction for test multiplicity, the *LDLR* SNPs rs55997232, rs17242395, rs1010679, and rs11668477, and the *APOB* SNPs rs1367117, rs6548010, rs6754295, rs481069, and rs61743299, remained associ-

SUPPLEMENTAL TABLE 3. Logistic regression (stepwise forward) of all factors in Table 1 with a $p < 0.05$ to study those independently associated with possible SH

Characteristic	Univariable		Multivariable	
	OR (95%CI)	P-value	OR (95%CI)	P-value
Age (per year)	1.04 (1.02 - 1.06)	<0.0001		
Current smoker	0.55 (0.31 - 0.97)	0.041		
Carotid plaques	2.64 (1.58 - 4.42)	0.0002		
BMI (per Kg/m ²)	1.51 (1.01 - 2.27)	0.047		
Hypertension history	1.74 (1.07 - 2.81)	0.024		
IMT (per 10 μ m)	1.03 (1.02 - 1.04)	<0.0001	1.02 (1.01 - 1.04)	0.002
PWV (per m/s)	1.14 (1.02 - 1.27)	0.024		
Glycated hemoglobin (>5.5%)	2.51 (1.63 - 3.87)	<0.0001	2.04 (1.25 - 3.34)	0.004
Triglycerides (>88mg/dL)	3.09 (1.96 - 4.86)	<0.0001	2.60 (1.57 - 4.29)	0.0002
Family history of:				
Myocardial infarction	2.10 (1.32 - 3.35)	0.002		
Stroke	2.09 (1.33 - 3.29)	0.001		
Hyperlipidaemia	2.23 (1.42 - 3.51)	0.0005	2.01 (1.25 - 3.21)	0.0037

Legend: SH, Severe hypercholesterolemia; BMI, body mass index; IMT, intima-media thickness; PWV, pulse-wave velocity.

TABLE 2. Variance decomposition and heritability estimations

Blood lipids	Heritability (%)	Common environment effect (%)	Residual effects (%)
LDL cholesterol	51.6	3.9	44.4
HDL cholesterol	66.6	14.7	18.7
Total cholesterol	49.8	4.8	45.4
Triglycerides	41.4	13.1	45.5

Legend: HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Sex and age are used as covariates

TABLE 3. Association between SNP from the 3 gene candidates, the LDLc levels and SH

Gene	ID	Chr.	Position	A1	A2	Freq. A2	Beta	SD	Association with LDLc levels		Association with SH	
									P-value	P BH	P-value	P BH
<i>LDLR</i>	rs55997232	19	11188117	T	C	0.867	0.090	0.018	9E-07	3E-5	0.019	0.28
	rs17242395	19	11206969	A	G	0.871	0.087	0.019	3E-06	4.3E-5	0.034	0.28
	rs1010679	19	11207102	C	T	0.780	0.062	0.015	4E-05	3.4E-4	0.047	0.28
	rs11668477	19	11195030	G	A	0.785	0.062	0.015	5E-5	3.4E-4	0.039	0.28
	rs17243011	19	11242215	A	G	0.996	0.203	0.097	0.037	0.21	NS	
	rs72658879	19	11244008	A	G	0.959			NS		0.049	0.28
<i>PCSK9</i>	rs540796	1	55524197	A	G	0.834	0.051	0.017	0.002	0.06	0.002	0.06
	rs505151	1	55529187	G	A	0.967	-0.090	0.035	0.011	0.15	NS	
	rs11591147	1	55505647	T	G	0.984	0.103	0.049	0.035	0.20	NS	
	rs2479394	1	55486064	G	A	0.707	-0.028	0.014	0.036	0.20	NS	
	rs7552841	1	55518752	T	C	0.610	-0.026	0.013	0.041	0.20	0.035	0.20
	rs28362277	1	55527222	C	A	0.999			NS		0.012	0.15
	rs487230	1	55541174	A	G	0.783			NS		0.036	0.20
	rs28362263	1	55523855	A	G	0.999			NS		0.041	0.20
<i>APOB</i>	rs1367117	2	21263900	A	G	0.690	-0.045	0.013	0.0007	0.04	0.017	0.45
	rs6548010	2	21277922	G	A	0.683	-0.042	0.013	0.002	0.05	0.048	0.51
	rs6754295	2	21206183	G	T	0.759	0.044	0.015	0.003	0.05	NS	
	rs481069	2	21281856	G	A	0.838	0.050	0.017	0.004	0.05	NS	
	rs61743299	2	21225597	T	A	0.999	-0.521	0.185	0.004	0.05	0.034	0.45
	rs515135	2	21286057	T	C	0.790	0.042	0.015	0.006	0.06	NS	
	rs1801695	2	21224853	T	C	0.968	0.098	0.037	0.008	0.06	NS	
	rs1042034	2	21225281	C	T	0.783	0.038	0.015	0.013	0.09	NS	
	rs12720796	2	21261998	C	A	0.979	-0.086	0.043	0.046	0.27	NS	
	rs1801701	2	21228827	T	C	0.911			NS		0.024	0.45
rs1042023	2	21229446	C	G	0.983			NS		0.033	0.45	

Legend: SH, severe hypercholesterolemia; LDLc, low-density lipoprotein cholesterol; Chr., chromosome; A1, allele 1; A2, allele 2; beta, effect per A2 under additive genetic model; SD, standard deviation; BH, Benjamini-Hochberg corrected p-value; NS, association non-significant. Sex and age are used as covariates

ated with LDLc. No SNPs located on PCSK9 gene reach the significant threshold after correction for test multiplicity. Table 3. Several *LDLR*, *PCSK9*, and *APOB* SNPs were associated with SH, but none remained significant after correction for test multiplicity, they are almost the same than those associated with LDLc levels. Table 3. We did not find monogenic mutations of familial hypercholesterolemia, but all subjects with SH showed at least one mutated polymorphism from one of the 3 genes of interest.

Sensitive analysis are shown in Supplemental Table 4, where analyses without subjects that were not fasting (n=30) have been run, results are similar than those in the Table 3, and in Supplemental Table 5, association tests between subjects taking statin or not taking statin for the 3 candidate genes implicated in familial form of hypercholesterolemia occurrence are shown, we find some polymorphisms in *APOB* gene linked with taking statin. However, results for association with LDL levels are similar with or without taking into account subjects with statin treatment (data not shown).

DISCUSSION

The present study shows that in a generally healthy populational cohort not taking statins, the proportion of participants with possible polygenic hypercholesterolemia was nearly 7%; these participants had more frequently a family history of cardiovascular disease and carotid plaques, and also had higher cIMT and PWV, indicating more advanced arterial ageing and vascular damage.

The LDLc had an important heritability with more than 50% of the variance explained by additive genetic effects, however less than 4% of the variance resulted from common environmental effects, that may support the notion that lifestyle intervention has little impact on the LDLc levels or that we may have missed to take into account some other shared environmental factors. Moreover, in the GWAS analysis, SNPs located on the *LDLR* gene presented a strong association with the plasmatic LDLc levels. These findings of a main genetic origin reinforce the need for an early identification and treatment of individuals with SH. The prevalence of polygenic forms of SH in the general

SUPPLEMENTAL TABLE 4. Association between SNP from the 3 gene candidates and the LDLc levels when excluding subjects without fasting blood sample

Gene	ID	Chr.	Position	A1	A2	Freq. A2	Beta	SD	P-value	P BH
<i>LDLR</i>	rs55997232	19	11188117	T	C	0.866	0.093	0.019	8.62E-07	2.41E-05
	rs17242395	19	11206969	A	G	0.870	0.089	0.019	2.67E-06	3.74E-05
	rs11668477	19	11195030	G	A	0.783	0.063	0.016	4.94E-05	4.57E-04
	rs1010679	19	11207102	C	T	0.778	0.061	0.015	6.52E-05	4.57E-04
<i>PCSK9</i>	rs540796	1	55524197	A	G	0.834	0.051	0.017	0.002	0.061
	rs505151	1	55529187	G	A	0.968	-0.089	0.035	0.011	0.151
	rs11591147	1	55505647	T	G	0.984	0.103	0.049	0.035	0.202
	rs2479394	1	55486064	G	A	0.708	-0.028	0.014	0.036	0.202
<i>APOB</i>	rs7552841	1	55518752	T	C	0.610	-0.026	0.013	0.041	0.202
	rs1367117	2	21263900	A	G	0.691	-0.049	0.014	0.0004	0.022
	rs6548010	2	21277922	G	A	0.684	-0.045	0.014	0.001	0.025
	rs6754295	2	21206183	G	T	0.756	0.046	0.015	0.002	0.032
<i>APOB</i>	rs481069	2	21281856	G	A	0.839	0.053	0.018	0.002	0.032
	rs515135	2	21286057	T	C	0.790	0.046	0.016	0.004	0.040
	rs61743299	2	21225597	T	A	0.999	-0.521	0.186	0.005	0.044
	rs1801695	2	21224853	T	C	0.967	0.099	0.037	0.007	0.052
	rs1042034	2	21225281	C	T	0.781	0.040	0.016	0.010	0.064
	rs1042023	2	21229446	C	G	0.982	-0.101	0.048	0.035	0.209
	rs12720796	2	21261998	C	A	0.979	-0.089	0.044	0.042	0.213
	rs13392272	2	21217490	T	C	0.551	-0.026	0.013	0.044	0.213

Legend: LDLc, low-density lipoprotein cholesterol; Chr., chromosome; A1, allele 1; A2, allele 2; beta, effect per A2 under additive genetic model; SD, standard deviation; BH, Benjamini-Hochberg corrected p-value. Sex and age are used as covariates

SUPPLEMENTAL TABLE 5. Genetic comparisons between individuals taking Statins or not for the 3 candidate genes associated with SH occurrence using association tests for binary variables

	P value	P BH
PCSK9		
Rs28385710	0.005	0.16
Rs72646509	0.02	0.38
Rs634272	0.05	0.48
LDLR		
Rs17242395	0.002	0.06
Rs55997232	0.004	0.06
APOB		
Rs61742247	0.001	0.05
Rs13392272	0.003	0.05
Rs515135	0.004	0.05
Rs12713956	0.004	0.05

Sex and age are used as covariates

For this table only, the 213 subjects that were using statins have been included in the dataset.

population is high, easy to identify, and the cardiovascular damage is potentially preventable.

In our cohort the prevalence of participants with SH was higher than the prevalence usually described in the literature^{4,5}. It should be noted that if these individuals had not been included in our cohort they would otherwise have not been identified. These findings are reflective of the “real-world” where a low proportion of people are diagnosed and treated. Supporting our findings, the prevalence of LDLc ≥ 190 mg/dL among American adults free of coronary artery disease was also 7%, and they had a multiple fold higher risk of cardiovascular events¹⁶. Moreover, a familial hypercholesterolemia monogenic mutation identified by gene sequencing was rare (<2% among those with a LDLc ≥ 190 mg/dL)¹⁶. To date, there is no gold standard for the clinical diagnosis of SH. Nonetheless, the first step is to assess the cholesterol levels and to perform a thorough clinical examination looking for features of hypercholesterolemia (e.g., xanthomas, xanthelasmas) and premature cardiovascular disease (e.g., angina pectoris, intermittent claudication, erectile dysfunction)¹⁷. Although these features may be useful in the case of severe autosomal dominant hypercholesterolemia, they are of little utility for the great majority of people with polygenic forms of hypercholesterolemia. In our cohort we did not systematically evaluate the presence of xanthomas or xanthelasmas, and the proportion of patients with any of

myocardial infarction, stroke, or peripheral artery disease was low ($\leq 10\%$) is not different between people with SH or not. On the other hand, a simple LDLc measurement could identify patients with carotid plaques, signals of premature vascular ageing (as supported by higher PWV and cIMT), and family history of premature cardiovascular disease^{18,19}. Given the important hereditary component of LDLc, this simple and inexpensive laboratorial measurement (i.e. Blood measurement of LDLc) should be used for screening of SH. As supported by our findings, many of these patients will not present an identifiable gene mutation, but rather present polygenic profiles that will make them more prone to have elevated LDLc.

We found that variants located on *LDLR* gene (rs55997232, rs17242395, rs1010679, and rs11668477) were those with stronger association with LDLc levels in our cohort. However, none of these 4 SNPs have been identified in previous studies. Variants located on the *APOB* gene (rs1367117, rs6548010, rs6754295, rs481069, rs61743299 and rs515135) presented lower association with LDLc. The variant rs1367117 had already been previously associated with higher concentrations of LDLc²⁰. Furthermore, some SNPs located on *PCSK9* gene, which did not reach the statistically significant threshold in our study, are known for their associations with LDLc²¹⁻²⁵.

Despite the fact that our findings are not in total concordance with those already reported in other cohorts and populations of different ethnic backgrounds^{8,14,26}, the density of our genotyping is not sufficient to find a mutation of one of the 3 genes commonly implicated in familial form; if we consider a 0.5% or lower prevalence of hypercholesterolemia-associated variants, it is very plausible that we had found none among our 102 patients more likely to have any form of hypercholesterolemia. In this regard, the heritability of LDLc was important, highlighting the need for an early detection and treatment of hypercholesterolemia, thus limiting the lifetime cumulative exposure to elevated LDLc^{16,27}. For limiting the exposure to excessive LDLc levels in this high-risk population, and beyond lifestyle changes, an early initiation of statins is required (at least in the great majority of patients who have any form of SH). Early (during childhood and adolescence) statin initiation with the goal of lowering LDLc below 100 mg/dL may delay the progression of vascular damage and reduce the incidence of cardiovascular events^{19,28,29}. In addition to statins, PCSK9 inhibitors and Ezetimibe may be considered in people unable to tolerate intensive statin therapy or in whom statins are ineffective to lower LDLc to the desired target. Using the STANISLAS cohort, we have previously shown that the circulating PCSK9 levels and

the missense mutation coding the SNP rs562556 were associated with the advent of carotid arterial plaques³⁰. This SNP was not associated with the LDLc levels in the present report. Whenever required other lipid-lowering strategies can also be tried (e.g., ezetimibe and bile acid sequestrants)¹⁴.

Our findings add to the literature that milder polygenic forms of SH may be much more prevalent than initially thought. An important proportion of people carrying these SH forms develop premature vascular damage and may have increased risk of cardiovascular events. Both the diagnosis and effective treatment are relatively easy to achieve (e.g., family history and LDLc measurement), but need to be performed early in life to avoid lifetime exposure to high LDLc levels and reduce the incidence of cardiovascular events.

LIMITATIONS

This study has several limitations. First, this is an observational study and no causality can be established. Second, our sample size might be underpowered to detect genetic mutations and monogenic alterations and the STANISLAS cohort had participants mostly from the Lorraine region of France. Therefore, the low incidence of monogenic familial hypercholesterolemia found herein cannot be generalized to other populations. Third, this is a cohort of mostly healthy people and the impact of LDLc in major adverse cardiovascular events cannot be determined. Fourth, family history was reported by the participants and not confirmed using medical records. Fifth, the presence of xanthomas and xanthelasmas was not systematically assessed. Sixth, we used age and LDLc for SH classification. These criteria are sensitive but less specific i.e., they may incorporate people with sporadic forms of hypercholesterolemia; notwithstanding, these forms likely have an important hereditary component (as here described) and more attention should be paid to them.

CONCLUSIONS

In a generally healthy populational cohort not taking statins, the proportion of participants with SH was nearly 7%. These participants had much higher LDL and total cholesterol levels, family history of cardiovascular disease, and premature vascular damage including carotid plaques. LDLc had an important component of heritability with little environmental effect. SNPs linked to the *LDLR* gene presented a strong association with the blood LDLc levels. These findings reinforce the need for an early identification and treatment of individuals with possible FH as identified by elevated LDLc levels.

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Conflict of Interest

The authors declare that they have no competing interests

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