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Supplement material

A common variant in low density lipoprotein receptor-related protein 6 gene (LRP6) is associated with LDL-cholesterol

Maciej Tomaszewski et al.

Phenotyping in Silesian Cardiovascular Study

Basic demographic and clinical information was collected from each subject using standardized, coded questionnaires. Each participant underwent a physical examination and standard anthropometric measurements, as reported before (1-4). Blood pressure was measured in triplicate according to the guidelines (5) using an oscillometric method. Fasting blood samples were secured for further biochemical phenotyping and DNA analysis.

Hypertension was defined as blood pressure ≥140/90 mmHg measured on at least 2 separate occasions and/or taking antihypertensive medication. Diagnosis of coronary artery disease was based on at least one of the following criteria: at least 70%-stenosis of the luminal diameter in one of the main epicardial coronary arteries on angiography, clinically documented history of myocardial infarction or reperfusion/revascularization therapy, angina confirmed by a physician and/or taking antianginal medication.

The following thresholds of circulating concentrations of TC, HDL-C and triglycerides were used to classify subjects as affected in secondary family-based analysis:

• total cholesterol ≥5.18 mmol/L (6),

• triglycerides ≥1.7 mmol/L (6),

• HDL-cholesterol ≤1.03 mmol/L in men and ≤1.3 mmol/L in women

or for subjects aged <19 years - sex and age-specific cut-off points [as per (7)].

In addition, subjects on lipid-lowering medication were also defined as affected, irrespective of their baseline lipids levels.

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Extended Figure Legend

Figure 1. LRP6 gene and LDL-C in Silesian Cardiovascular Study – family-based association analysis

Top panel. -log transformed p-values from the family-based test of association between 12 LRP6 single nucleotide polymorphisms and LDL-C as a qualitative phenotype. Of 12 genetic markers examined, only one (rs10845493 – blue dot) was associated with LDL-C (p=0.0053) after applying a correction for multiple testing (the dashed horizontal line corresponding to -log of p=0.0085).

Middle panel. Structure of LRP6 gene. Green vertical lines correspond to exons.

Bottom panel. LRP6 spans approximately 150,000 bp within the short arm of chromosome 12. The linkage disequilibrium (LD) map of 12 LRP6 SNPs genotyped

in Silesian Cardiovascular Study families is based on r^2-coefficients (whereby dark red corresponds to r^2=1 – maximal LD and white to r^2=0 – no LD) and was created using LocusView software.

Supplementary Tables

Supplementary Table I. LRP6 SNPs and LDL-cholesterol – the results of family-based association analysis in Silesian Cardiovascular Study

SNP No	SNP	Alleles	MA	HWE-parents	MAF-parents (%)	Over-transmitted allele	P-value in FBAT
1	rs12313200	A/G	A	0.327	16.6	G	0.7293
2	rs2075241	C/G	C	0.070	20.8	G	0.7495
3	rs7980903	C/T	C	0.987	16.8	C	0.1486
4	rs11054704	A/G	A	1.0	14.3	A	0.7856
5	rs2302685	C/T	C	0.983	16.6	C	0.3377
6	rs11609634	C/T	T	0.754	49.7	T	0.931
7	rs2417085	C/T	C	0.983	44.7	C	0.3128
8	rs10845493	C/T	T	0.735	10.5	T	0.0053
9	rs10492120	C/T	C	0.895	37.1	C	0.3771
10	rs17302049	A/G	G	0.470	12.9	A	0.9449
11	rs11054738	A/T	A	0.807	16.9	A	0.1089
12	rs10743980	C/T	T	1.0	42.6	T	0.2575

SNP – single nucleotide polymorphisms, MA – minor allele, HWE – the p-value from Hardy-Weinberg equilibrium chi^2 test in the parental generation, MAF – minor allele frequency in the parental generation, FBAT – family-based association test

Supplementary Table II. Association between LRP6 and LDL-C – family-based haplotype analysis.

Haplo	Rs12313200	rs2075241	rs7980903	rs2417085	rs10845493	rs17302049	Frequency	P-value
h1	G	G	T	T	C	A	0.367	0.4743
h2	G	C	T	C	C	A	0.211	0.5838
h3	G	G	T	T	C	G	0.108	0.9490
h4	G	G	C	C	T	A	0.089	0.0887
h5	A	G	T	C	C	A	0.07	0.7850
h6	A	G	T	T	C	A	0.054	0.2948
h7	G	G	C	C	C	A	0.053	0.4863
h8	G	G	C	T	C	A	0.011	0.9344
h9	G	G	T	C	C	A	0.008	0.8511
h10	G	G	T	C	T	A	0.007	0.4468
h11	A	G	T	C	T	A	0.006	0.5297
h12	G	G	C	T	C	G	0.006	0.8280
h13	G	C	T	T	C	A	0.004	0.4437
h14	G	C	T	T	C	G	0.001	0.2194
h15	G	C	T	C	T	A	0.001	0.5151
whole marker permutation test (*minimal p*) p=0.5235								

Haplo – haplotype; rs12313200, rs2075241, rs7980903, rs2417085, rs10845493 and rs17302049 represent 6 effective independent markers that explain 92.3% variance in LRP6 locus in SCS families; p-value – individual p-value for each haplotypic combination

Supplementary Table III. LDL-C levels in 218 SCS replication panel subjects with available genotype and phenotype information and no history of lipid-lowering medication – stratification based on rs10845493 genotype.

	TT genotype	CT genotype	CC genotype
Count (%)	6 (2.7)	42 (19.3)	170 (78.0)
LDL-C (mmol/L)	3.9 ±0.8	4.0±1.0	3.6±1.1

Data are means and standard deviations

Supplementary Table IV. LDL-C levels in 1138 YMCA subjects with available genotype and phenotype information – stratification based on rs10845493 genotype.

	TT genotype	CT genotype	CC genotype
Count (%)	21 (1.8)	289 (25.4)	828 (72.8)
LDL-C (mmol/L)	2.9 ±1.2	2.6±0.8	2.5±0.9

Data are means and standard deviations

Supplementary Table V. Other clinical characteristics of 218 SCS replication panel subjects with available genotype and phenotype information – stratification based on rs10845493 genotype

Phenotype	All	CC genotype	CT+TT genotypes	p-value
N	218	170	48	
Age (years)	52.4±14.6	52.7±15.3	51.7±12.3	0.643
Sex (M/F)	116/102	99/71	17/31	0.006
BMI (kg/m^2)	27.0±4.4	26.7±4.4	27.7±4.4	0.189
SBP (mmHg)	132.0±19.3	131.6±19.6	133.8±18.3	0.476
DBP (mmHg)	75.6±10.8	75.0±11.3	78.0±8.5	0.085
Hypertension (%)	140 (64.2)	107 (62.9)	33 (68.8)	0.499
Antihypertensive treatment (%)	104 (47.7)	78 (45.9)	26 (54.2)	0.330
CAD (%)	95 (43.6)	78 (45.9)	17 (35.4)	0.248
TC (mmol/L)	5.4±1.2	5.3±1.2	5.8±1.2	0.020
↑ TC (%)	119 (54.6)	87 (51.2)	32 (66.7)	0.071
Triglycerides(mmol/L)	1.5 (1.0-2.0)	1.5 (1.0-2.0)	1.4 (0.9-1.9)	0.430
↑ Triglycerides (%)	87 (39.9)	71 (41.8)	16 (33.3)	0.321
HDL-C (mmol/L)	1.0±0.3	1.0±0.3	1.1±0.3	0.521
↓ HDL-C (%)	153 (70.2)	117 (68.8)	36 (75.0)	0.479

Data are means and standard deviations, medians and 25%-75% inter-quartile ranges or counts and percentages, BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, CAD – coronary artery disease, TC – total cholesterol, HDL-C – HDL-cholesterol, ↑ TC, ↑ Triglycerides, ↓ HDL-C were defined as serum levels of respective lipid fractions over thresholds defined in the Methods and Supplement material

Supplementary Table VI. Other clinical characteristics of 1138 YMCA subjects with available genotype and phenotype information – stratification based on rs10845493 genotype.

Phenotype	All	CC genotype	CT genotype	TT genotype	p-value
n	1138	828	289	21	-
Age (years)	19.0 (18.0-19.0)	19.0 (18.0-19.0)	19.0 (18.0-19.0)	19.0 (17.0-19.0)	0.378
Sex (M/F)	1138/0	828/0	289/0	21/0	-
BMI (kg/m^2)	22.5 (20.7-24.4)	22.5 (20.8-24.4)	22.2 (20.5-24.3)	22.8 (21.0-24.1)	0.490
SBP (mmHg)	118.3 (108.3-126.7)	118.3 (108.3-126.7)	118.3 (108.3-126.7)	116.7 (110.0-129.2)	0.787
DBP (mmHg)	73.3 (70.0-80.0)	73.3 (70.0 -80.0)	73.3 (10.0-80)	76.7 (70.0-81.7)	0.301
Hypertension (%)	117 (10.3)	86 (10.4)	29 (10.0)	2 (9.5)	0.838
Antihypertensive treatment (%)	17 (1.5)	10 (1.2)	7 (2.4)	0 (0.0)	0.194
TC (mmol/L)	4.2 (3.6-4.8)	4.1 (3.6-4.8)	4.3 (3.7-4.8)	4.6 (3.8-5.5)	0.062
↑ TC (%)	193 (17.0)	136 (16.4)	50 (17.3)	7 (33.3)	0.208
Triglycerides (mmol/L)	1.0 (0.8-1.4)	1.0 (0.8-1.4)	1.0 (0.8-1.4)	1.3 (0.8-2.1)	0.111
↑ Triglycerides (%)	171 (15.0)	122 (14.7)	41 (14.2)	8 (38.1)	0.222
HDL-C (mmol/L)	1.1 (1.0-1.3)	1.1 (1.0-1.3)	1.2 (1.0-1.3)	1.1 (0.9-1.4)	0.879
↓ HDL-C (%)	397 (34.9)	291 (35.1)	99 (34.3)	7 (33.3)	0.755

Data medians and 25%-75% inter-quartile ranges or counts and percentages, BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, TC – total cholesterol, HDL-C – HDL-cholesterol, ↑ TC, ↑ Triglycerides, ↓ HDL-C were defined as serum levels of respective lipid fractions over thresholds defined in the Methods and Supplement material

Supplementary Table VII. Association between rs10845493 and age-, sex-, and BMI-adjusted LDL-C in 218 subjects from SCS replication panel - dominant and recessive model of inheritance

Model	SNP	Minor allele	β-coefficient	Standard error	P-value
Dominant	rs10845493	T	0.40	0.18	0.0313
Recessive	rs10845493	T	0.24	0.46	0.6076

SNP – single nucleotide polymorphism; included were subjects with available genotype and phenotype information and no history of lipid-lowering medication

Supplementary Table VIII. Association between rs10845493 and age-, and BMI-adjusted LDL-C in 1138 subjects from YMCA cohort - dominant and recessive model of inheritance

Model	SNP	Minor allele	β-coefficient	Standard error	P-value
Dominant	rs10845493	T	0.09	0.06	0.1082
Recessive	rs10845493	T	0.37	0.19	0.0501

SNP – single nucleotide polymorphism; included were subjects with available genotype and phenotype information and no history of lipid-lowering medication