

IMPACT OF VARIANTS WITHIN SEVEN CANDIDATE GENES ON STATIN TREATMENT EFFICACY

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Summary

Statins are the most commonly used drugs in patients with dyslipidemia. Among the patients, a significant inter-individual variability with supposed strong genetic background in statin treatment efficacy has been observed. Genome wide screenings detected variants within the *CELSR2/PSRC1/SORT1*, *CILP2/PBX4*, *APOB*, *APOE/C1/C4*, *HMGCoA reductase*, *LDL receptor* and *PCSK9* genes that are among the candidates potentially modifying response to statins. Ten variants (SNPs) within these genes (rs599838, rs646776, rs16996148, rs693, rs515135, rs4420638, rs12654264, rs6511720, rs6235, rs11206510) were analyzed in 895 (46% men, average age 60.3±13.1 years) patients with dyslipidemia treated with equipotent doses of statins (~90% on simvastatin or atorvastatin, doses 10 or 20 mg) and selected 672 normolipidemic controls (40% men, average age 46.5 years). Lipid parameters were available prior to the treatment and after 12 weeks of therapy. Statin treatment resulted in a significant decrease of both total cholesterol (7.00±1.53 → 5.15±1.17 mmol/L, P<0.0001) and triglycerides (2.03±1.01 → 1.65±1.23 mmol/L, P<0.0005). Rs599838 variant was not detected in first analyzed 284 patients. After adjustment for multiple testing, there was no significant association between individual SNPs and statin treatment efficacy. Only the rs4420638 (*APOE/C1/C4* gene cluster) G allele carriers seem to show more profitable change of HDL cholesterol (P = 0.007 without and P = 0.06 after adjustment). Results demonstrated that, although associated with plasma TC and LDL cholesterol *per se*, variants within the *CELSR2/PSRC1/SORT1*, *CILP2/PBX4*, *APOB*, *APOE/C1/C4*, *HMGCoA reductase*, *LDL receptor* and *PCSK9* genes do not modify therapeutic response to statins.

Abbreviations

APO, apolipoprotein; CELSR2/PSRC1/SORT1, cadherin egl lag seven-pass g-type receptor 2/ proline/serine-rich coiled-coil protein 1/sortilin1; CILP2/PBX4, cartilage intermediate layer protein 2/ pre-b-cell leukemia transcription factor 4; GWA, genome wide association; HMGCoA, 3-hydroxy-3-methylglutaryl coenzyme A; MONICA, MONItoring of CArdiovascular disease; PCSK9, proprotein convertase, subtilisin/kexin-type, 9; SNP, single nucleotide polymorphism;

Introduction

Inhibitors of hydroxy-methylglutaryl coenzyme A reductase (statins) are the most commonly prescribed drugs in cardiovascular prevention both in primary and secondary prevention. Not only ameliorating plasma atherogenic lipoprotein profile, but also possessing a plenty of other pleiotropic effects (for example see Pitha *et al.* 2010) these agents reduce the risk of both cardio- and cerebro-vascular events in a wide spectrum of patients (Sadowitz *et al.* 2010).

However, plasma cholesterol lowering effect of statins significantly differs among individuals – namely, the use of the same dose of the same statin in different patients produces LDL-cholesterol decrease in a wide range from 8 to 55%, and a reduction of triglyceride (TG) concentrations from 7 to 30% (Sever *et al.* 2003). Importantly, also the time to reach maximum decrease differs significantly between individuals (Hachem and Mooradian 2006). The individual response to statins is relative stable over time and, likely, has a genetic background.

So far, no single mutation was described leading to the inefficient statin treatment and it was proved that the genetic determination of statin treatment efficacy is based on polygenic background (Mangravite and Krauss, 2007; Maggo *et al.* 2011). Dozens of individual variants were tested in effort to detect significant genetic determinants of statin treatment efficacy, however the results are so far inconsistent and far from the potential clinical use. Results of genome wide association studies (Kathiresan *et al.* 2008; Sandhu *et al.* 2008) have pointed on several gene regions that significantly influence plasma cholesterol levels per se.

These new genes or newly detected variants within the well known and characterized genes include *CELSR2/PSRC1/SORT1* (rs646776 and rs599838), *CILP2/PBX4* (rs16996148), *APOB* (rs693 and rs515135), *APOE/C1/C4* gene cluster

(rs4420638), *HMGCoA reductase* (rs12654264), *LDL receptor* (rs6511720) and *PCSK9* (rs11206510 and rs6235).

To evaluate the potential role of gene variants within these newly identified gene regions in the modification of individual treatment response to statins (modulation of the final impact of statin treatment on lipoprotein levels in the plasma) we conducted a retrospective study in a cohort of lipid clinic patients treated with statins.

Methods

Patient selection

Patients with primary dyslipidemia indicated for statin treatment were selected at i/ Lipid Clinics of the 3rd Department of Internal Medicine of the 1st Faculty of Medicine, Charles University and ii/ at the Institute for Clinical and Experimental Medicine, Prague, the Czech Republic.

Eight hundred ninety five adult patients were included, average age 60.3 ± 13.1 years (411 males, aged 60.8 ± 12.8 years and 484 females, aged 59.7 ± 13.5 years). All patients received a standardized lifestyle advice at their first visit to the clinics and were instructed to maintain low-cholesterol/low fat diet according to the standardized education provided by an experienced dietitian. Table 1 shows the baseline characteristics of the study group. We compared the pre-treatment lipid levels with the first values obtained after initiation of statin treatment, usually after 12 weeks (range 10 to 13 weeks) of therapy. Patients taking simvastatin (32%), atorvastatin (47%) and rosuvastatin (21%) in doses of 10 or 20 mg/day were enrolled in the study. The treatment effects were evaluated after adjusting for differential potency of individual molecules and for the dose. We did not include subjects on

combination lipid-lowering therapy (e.g. statin-fibrate, statin-ezetimibe) and those who experienced weight loss of more than 5% between visits suggesting a substantial impact of lifestyle changes. Also, the individuals fulfilling the clinical and laboratory criteria of familial hypercholesterolemia were not included in the study.

Controls selection

A subset of 672 healthy individuals (282 males and 390 females) selected from the Central European Czech post-MONICA study (2,559 individuals of Caucasian ethnicity, 1,191 males, average age 49.0 ± 10.7 years) was used (Thunsdall-Pedoe et al. 2003). The criteria for the selection were i) no history of (self reported) cardiovascular disease, ii) no lipid-lowering treatment (self reported) and iii) the plasma lipid values below 5.2 mmol/L for total cholesterol, below 2.0 mmol/L for plasma TG and over 0.7 mmol/L (for males) or 0.8 mmol/L (for females) for HDL-cholesterol (Table 1).

Written informed consent was obtained from all the study participants and the local ethics committee approved the design of the study according to the Declaration of Helsinki of 2000.

Genotype analysis

Three millilitres of whole blood collected into EDTA tubes for DNA isolation were stored at -20°C . The DNA was isolated using the standard salting out method (Miller *et al.* 1988) and individual variants within the seven genes (rs646776 and rs599838 - *CELSR2/PSRC1/SORT1*, rs16996148 - *CILP2/PBX4*, rs693 and rs515135 - *APOB*, rs4420638 - *APOE/C1/C4* gene cluster, rs12654264 - *HMGCoA reductase*, rs11206510 and rs6235 - *PCSK9* and rs6511720 - *LDL receptor*) were

genotyped using polymerase chain reaction (PCR) and restriction analysis. A PCR device DYAD (MJ Research, Waltham, MA) was used to perform the PCR reaction in a total volume of 25 μ L. DNA was amplified under the following conditions: initial denaturation of 96°C for 3 min, followed by 35 cycles of 95°C for 15 sec, appropriate annealing temperature for 30 sec and 72°C for 30 sec with the last amplification step was extended for 3 min at 72°C. A ten μ l of PCR product was digested in a total volume of 25 μ l with an appropriate restriction enzyme at 37°C overnight in the buffer provided by the manufacturer (Fermentas, Burlington, Canada). For more details regarding the PCR conditions, oligonucleotides and restriction enzymes used, see Table 2. Restriction fragments were separated on 10% PAA gel using the high capacity MADGE technique (Day and Humphries 1994).

Analysis of plasma lipids

The lipoprotein parameters in fasting plasma samples were assessed using autoanalyzers and conventional enzymatic methods with reagents from Boehringer Mannheim Diagnostics and Hoffmann-La Roche in CDC Atlanta accredited local laboratories.

Statistical analysis

The Hardy-Weinberg test (<http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls>) was applied to confirm the independent segregation of the alleles. Chi-square test, ANOVA and ANCOVA for adjustments (for sex, age and untreated plasma levels) were used for statistical analysis. All tests were two tailed and the significance level $P < 0.05$ was considered to be significant at primary analysis. Further, Bonferroni correction for multiple testing

(nine variants were analyzed) was applied. The changes of plasma lipids were compared between subjects with different genotypes for individual polymorphisms. If there were less than 5% of the carriers of one genotype, these individuals were pooled with heterozygotes and analysed together.

Results

Basic characteristics

There was a significant decrease in total cholesterol ($7.00 \pm 1.53 \rightarrow 5.15 \pm 1.17$ mmol/L, $P < 0.0001$) and triglycerides ($2.03 \pm 1.01 \rightarrow 1.65 \pm 1.23$ mmol/L, $P < 0.0001$) as a response on statin treatment (Table 1). Plasma levels of HDL cholesterol did not change significantly ($1.43 \pm 0.48 \rightarrow 1.41 \pm 0.40$ mmol/L, $P = 0.08$).

The call rates for the individual polymorphisms varied between 100% for the rs1264264 within the HMGCoA reductase gene in patients and 93.6% for the rs4420638 within the *APOE/C1/C4* gene cluster in patients. Rs599838 variant was not detected within the 284 patients and, thus, was not genotyped in the entire groups of patient and controls.

In the analyzed groups, the genotype frequencies of individual polymorphisms were similar to the so far published frequencies in other Caucasian populations. Hardy-Weinberg test confirmed the independent segregation of individual alleles with two exceptions ($P < 0.0005$ for the rs16996148 in controls and $P < 0.01$ for the rs11206510 in patients). These differences could likely be explained as the groups do not represent general population samples, but preselected population subgroups. No gender differences in genotype frequencies were observed either in the patients or in controls (data not shown in details).

Genotype differences between the analyzed groups

The detected genotype frequencies were significantly different between the patients and controls in most of the analyzed variants (Table 3). The largest differences ($P < 0.0001$) were observed for the rs646776 variant within the *CELSR2* gene cluster and rs6511720 variant within the *LDL receptor* gene. Also other variants (with two exceptions of rs515135 and rs6235) differed between the analysed groups and, thus, certain role of these SNPs in determination of plasma lipid levels can be expected.

Associations between the SNPs and statin treatment efficacy

The carriers of minor rs4420638 G allele within the *APOE/C1/C3* cluster had significantly lower response to statin treatment compared to common homozygotes if HDL cholesterol was analyzed ($\Delta + 12.5\%$ vs. $\Delta + 6.1\%$, $P = 0.007$ adjusted for age, sex and untreated TG levels; $P = 0.065$ after adjustment for multiple testing). Similar difference was obtained for plasma TG levels. However, in this case the difference was below the significance threshold after correction for multiple testing (Table 4). This variant did impact on-treatment total cholesterol levels.

For any of the other variants analyzed, we did not find a significant association between the genetic polymorphism and changes of plasma lipid levels induced by statin therapy (decrease of total cholesterol or triglycerides; increase of HDL-cholesterol) (data not shown in details).

Discussion

In a large group of adult dyslipidemic patients of Slavonic Caucasian descent we have extended our previous report (Hubacek *et al.* 2012) focused on analysis of

the genetic determination of statin treatment efficacy. In this extended group of patient, we did not confirm our previous finding suggesting a significant effect of the SNP within the *CELSR2/PSRC1/SORT1* gene cluster on statin treatment efficacy. No significant effect of the variants within genes/gene clusters for *CILP2/PBX4*, *PCSK9*, *HMGCoA reductase*, *LDL receptor* or *APOB* on statin mediated lipid decrease was observed. At first time, we have detected a possible effect of the rs4420638 SNP within the *APOE/C1/C3* gene cluster on statin induced changes of plasma triglycerides and HDL cholesterol. Presence of the minor allele was associated with profitable changes of TG levels and HDL-C. However, the major statin targets, total and LDL-cholesterol concentrations, decreased independently of this variant.

The detected effect of this SNP on plasma lipid changes after statin treatment is of potential interest, especially because major genetic determinants of statin treatment efficacy detected so far are two SNPs located within the same gene cluster, namely in the *APOE* gene (Hubacek and Vrablik 2011). *APOE4* allele (rs429358) of the same gene seems to be the most powerful genetic risk factor for cardiovascular disease development (Angelopoulos and Lowndres, 2010; Poledne et al. 2010). Carriers of this *APOE4* allele tend to have higher, while persons carrying the *APOE2* allele lower total cholesterol levels if compared to *APOE3E3* homozygotes. This association is partially influenced by sex, diet or physical activity (reviewed by Hayman, 2000) but has been consistently described in all populations analysed so far.

However, there are some points which need to be taken into account. First, after correction for multiple testing, the difference did not reach the arbitrary significance level and thus the effect needs to be confirmed in second independent population. Second, primary statin targets, total and LDL-cholesterol remained

completely unaffected by this SNP. Finally, despite the statistical significance ($P = 0.007$, but 0.06 after correction for multiple testing), the biological significance in the HDL-cholesterol change is speculative. The achieved difference between the genotypes was less than $\sim 10\%$, what represents about 0.12 mmol/L .

With two exceptions (*PCSK9* and *APOB* regions), we have detected differences in genotype frequencies between analyzed groups of patients and controls. This confirms that most of the analyzed variants are important determinants of plasma lipid levels not in West European populations only, but also in the central European Slavonic population.

The genotype frequencies of two variants within the two genes (*APOB* and *PCSK9*) were almost identical among the dyslipidemic patients and normolipidemic controls. This underlines the importance of the confirmatory studies and points to the possibility, that even genome wide analyses (GWAs) could detect a variant without any general effects on plasma lipids and even the chance of the false positive results could not be excluded. This is not the first time that GWAs results were not confirmed in population(s) of different origin. For example, we were not able to confirm the association between the powerful genetic determinants of plasma TG levels (Kooner *et al.* 2008) within the *MLXIPL* gene. In a study with sufficient power (Vrablik *et al.* 2008) this variant was neither associated with plasma TG levels in the population, nor were the genotype frequencies different between the individuals with low and high plasma TG levels.

One of the explanations may be the different genetic or, more likely, different environmental and lifestyle background between the west European/German and central European/Slavonic populations. Some variants likely effect plasma lipid levels

regardless on dietary habits or physical activity, effects of others probably need to be triggered by unfavorable lifestyle.

Loci we have studied include not only the well known genes with a clear link to plasma lipid values as *APOB*, *APOE*, *LDL receptor*, *PCSK9* and *HMGCoA reductase*, but also the gene clusters with rather unclear mechanisms affecting plasma lipid concentrations - the variants being located within the intergenic regions of the *CELSR2* (Waterworth *et al.* 2010) and *CILP2* (Seki *et al.* 2005) gene clusters. At time of their discovery, the gene products of these clusters had no known association with plasma lipid metabolism, but later, thanks to the GWAs results, *SORT1*, a member of the *CELSR2* gene cluster, was recognized to be an intracellular receptor interacting with *APOB* at the apparatus of Golgi facilitating the hepatic transport of *APOB* containing lipoproteins (Kjolby *et al.* 2010).

Variation of the new gene loci contributing to the development of dyslipidemia (Kathiresan *et al.* 2008; Sandhu *et al.* 2008; Aulchenko *et al.* 2009), did not influence therapeutic effect of statins and their contribution to interindividual variability of the final impact of statin therapy on lipoprotein concentrations seems to be neglectable.

The reason for the observed lack of association could be the fact, that these genes do not alter the pathway(s) involved in the metabolism or transport of statins. In general, these pathways are supposed to be more likely affecting statin treatment efficacy (or there is a greater chance to detect such an effect), as they are less prone to environmental modifications. Variations we have studied potentially impact pathways that involve transport proteins or enzymes directly linked to processing of different lipoprotein subpopulations (mostly LDL and TG-rich particles) and not to the metabolism or transport of statins.

Another possibility might be only modest modifying effect, which could not be recognized due to the relatively small sample size and patient heterogeneity. We want to underline, that the observed differences between the genotypes were mostly minimal. Thus, it is unlikely that even a substantially increased sample size would enable identification of any modifying effects of clinical significance.

The increasing popularity of genome wide association studies (Rosenberg *et al.* 2010) has also its pitfalls. Most importantly, there is a substantial lack of the replication studies (Munafò, 2009) performed (or published) and thus some publication bias needs to be expected and the real effect in different populations could be over- or under- estimated. Further, also detailed analyses of interethnic or even international differences or possible gene-gene or gene-environment interactions have never been analyzed in these studies.

It is of outstanding interest to understand the genetic puzzle of the drug efficacy, as we do not have any other kind of tests to predict the effects of pharmacotherapy and prediction of the individual efficacy of a drug can be done only by methods of genetic analysis. Such test would have a potential to detect the high- and hypo- responders and, moreover, identification of those at high risk of side effects. The genetic analyses could allow us to select the most effective and, more importantly, safer treatment alternative for an individual patient. The economical and health benefits of this approach are evident, but we are at the very beginning to understand the genetic determination of statin treatment efficacy (Mangravite *et al.* 2010; Ordovas and Mooser, 2002).

The impact of genetic polymorphisms on statin treatment efficacy has been analyzed in several clinical trials. So far, variants in more than 30 different genes have been examined (Mangravite and Krauss 2007, Maggo *et al.* 2011) but the

results were not replicated in larger patient groups and also the magnitude of impact on statin efficacy was small. Interestingly only the impact of the *APOE* gene on statin treatment efficacy was analyzed in more studies (reviewed by Hubacek and Vrablik 2011) and despite the results are far from being consistent, *APOE4* allele is associated with poorer response to statin treatment. Other genes analyzed include for example apolipoprotein A5 (Hubacek *et al.* 2009) or cholesterol 7-alpha hydroxylase (Kajinami *et al.* 2005) The expanding knowledge in this field is quickly growing, but, so far, it is not sufficient to be used in clinical practice. Only comprehensive interdisciplinary biomedical research (Berger 2011) in large populations focused on impact of gene - gene - environment interactions will enable to broaden our understanding of the determination of statin treatment efficacy.

Our pilot study was focused on the analysis of the new role of gene loci identified through genome wide association studies in pharmacogenetic of statin treatment. We have detected a potential of the rs4420638 variant within the *APOE/C1/C3* gene cluster (but not within *CILP2/PBX4*, *PCSK9*, *LDL receptor*, *HMGCoA reductase*, *CELSR2/PSRC1/SORT1* and *APOB* gene loci) to significantly impact on statin treatment efficacy. If this variant could be of clinical importance needs to be analyzed in a large clinical trial.

Conflict of interest

There is no conflict of interest.

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References

- ANGELOPOULOS TJ, LOWNDES J. ApoE genotype: impact on health, fitness and nutrition. *World Rev Nutr Diet* **98**: 77-93, 2008.
- AULCHENKO YS, RIPATTI S, LINDQVIST I, BOOMSMA D, HEID IM, PRAMSTALLER PP, PENNINX BW, JANSSENS AC, WILSON JF, SPECTOR T, MARTIN NG, PEDERSEN NL, KYVIK KO, KAPRIO J, HOFMAN A, FREIMER NB, JARVELIN MR, GYLLENSTEN U, CAMPBELL H, RUDAN I, JOHANSSON A, MARRONI F, HAYWARD C, VITART V, JONASSON I, PATTARO C, WRIGHT A, HASTIE N, PICHLER I, HICKS AA, FALCHI M, WILLEMSSEN G, HOTTENGA JJ, DE GEUS EJ, MONTGOMERY GW, WHITFIELD J, MAGNUSSON P, SAHARINEN J, PEROLA M, SILANDER K, ISAACS A, SIJBRANDS EJ, UITTERLINDEN AG, WITTEMAN JC, OOSTRA BA, ELLIOTT P, RUOKONEN A, SABATTI C, GIEGER C, MEITINGER T, KRONENBERG F, DÖRING A, WICHMANN HE, SMIT JH, MCCARTHY MI, VAN DUIJN CM, PELTONEN L; ENGAGE CONSORTIUM. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. *Nat Genet* **41**: 47-55, 2009.
- BERGER J. The age of biomedicine: current trends in traditional subjects. *J Appl Biomed* **9**: 57-61, 2011.
- DAY IN, HUMPHRIES SE. Electrophoresis for genotyping: microtiter array diagonal gel electrophoresis on horizontal polyacrylamide gels, hydrolink, or agarose. *Anal Biochem* **222**: 389-395, 1994.
- HACHEM SB, MOORADIAN AD. Familial dyslipidaemias: an overview of genetics, pathophysiology and management. *Drugs* **66**: 1949-1969, 2006.

HAYMAN LL: Abnormal blood lipids: is it environment or is it genes? *J Cardiovasc Nurs* **14**: 39-49, 2000.

HUBACEK JA, ADAMKOVA V, PRUSIKOVA M, SNEJDRLOVA M, HIRSCHFELDOVA K, LANSKA V, CESKA R, VRABLIK M. Impact of apolipoprotein A5 variants on statin treatment efficacy. *Pharmacogenomics* **10**: 945-950, 2009.

HUBACEK JA, VRABLIK M. Effect of apolipoprotein E polymorphism on statin-induced decreases in plasma lipids and cardiovascular events. *Drug Metabol Drug Interact* **26**: 13-20, 2011.

HUBÁČEK JA, ADÁMKOVÁ V, LÁNSKÁ V, DLOUHÁ D, RYNEKROVÁ J, ZLATOHLÁVEK L, PRUSÍKOVÁ M, ČEŠKA R, VRABLÍK M. Variant within *CELSR2/PSRC1/SORT1*, but not within *CILP2/PBX4*, *PCSK9* and *APOB* genes, has a potential to influence statin treatment efficacy. *J Appl Biomed* **10**: 19–28, 2012.

KAJINAMI K, BROUSSEAU DE, ORDOVAS JM, SCHAEFER EJ. A promoter polymorphism in cholesterol 7 α -hydroxylase interacts with apolipoprotein E genotype in the LDL-lowering response to atorvastatin. *Atherosclerosis* **180**: 407-415, 2005.

KATHIRESAN S, MELANDER O, GUIDUCCI C, SURTI A, BURTT NP, RIEDER MJ, COOPER GM, ROOS C, VOIGHT BF, HAVULINNA AS, WAHLSTRAND B, HEDNER T, CORELLA D, TAI ES, ORDOVAS JM, BERGLUND G, VARTIAINEN E, JOUSILAHTI P, HEDBLAD B, TASKINEN MR, NEWTON-CHEH C, SALOMAA V, PELTONEN L, GROOP L, ALTSHULER DM, ORHOMELANDER M. Six new loci associated with blood low-density lipoprotein

- cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. *Nat Genet* **40**: 189-197, 2008. Erratum in: *Nat Genet* **40**: 1348, 2008.
- KJOLBY M, ANDERSEN OM, BREIDERHOFF T, FJORBACK AW, PEDERSEN KM, MADSEN P, JANSEN P, HEEREN J, WILLNOW TE, NYKJAER A. Sort1, encoded by the cardiovascular risk locus 1p13.3, is a regulator of hepatic lipoprotein export. *Cell Metab* **12**: 213-223, 2010.
- KOONER JS, CHAMBERS JC, AGUILAR-SALINAS CA, HINDS DA, HYDE CL, WARNES GR, GÓMEZ PÉREZ FJ, FRAZER KA, ELLIOTT P, SCOTT J, MILOS PM, COX DR, Thompson JF. Genome-wide scan identifies variation in MLXIPL associated with plasma triglycerides. *Nat Genet* **40**: 149-151, 2008.
- MAGGO SD, KENNEDY MA, CLARK DW. Clinical implications of pharmacogenetic variation on the effects of statins. *Drug Saf* **34**: 1-19, 2011.
- MANGRAVITE LM, KRAUSS RM. Pharmacogenetics of statin response, *Curr Opin Lipidol* **18**: 409-419, 2007.
- MANGRAVITE LM, MEDINA MW, CUI J, PRESSMAN S, SMITH JD, RIEDER MJ, GUO X, NICKERSON DA, ROTTER JI, KRAUSS RM. Combined influence of LDL and HMGCR sequence variation on lipid-lowering response to simvastatin. *Arterioscler Thromb Vasc Biol* **30**: 1485-1492, 2010.
- MILLER SA, DYKES DD, POLESKY HF. A simple salting out procedure for DNA extraction from human nucleated cells. *Nucleic Acid Res* **16**: 1215, 1988.
- MUNAFÒ MR. Reliability and replicability of genetic association studies. *Addiction* **104**: 1439-1440, 2009.
- ORDOVAS JM, MOOSER V. The APOE locus and the pharmacogenetics of the lipid response. *Curr Opin Lipidol* **13**: 113-117, 2002.

PITHA J, BOBKOVÁ D, KOVÁR J, HAVLÍCKOVÁ J, POLEDNE R. Antiatherogenic effect of simvastatin is not due to decrease of LDL cholesterol in ovariectomized golden Syrian hamster. *Physiol Res* 59: 401-406, 2010.

POLEDNE R, HUBÁČEK JA, STANĚK V, ASCHERMANN M, MATOUŠKOVÁ J, VESELKA J, WIDIMSKÝ P, CÍFKOVÁ R, LÁNSKÁ V, PITHA J. Why we are not able to find the coronary heart disease gene - apoE as an example. *Folia Biol (Praha)* 56: 218-22, 2010.

ROSENBERG NA, HUANG L, JEWETT EM, SZPIECH ZA, JANKOVIC I, BOEHNKE M. Genome-wide association studies in diverse populations. *Nat Rev Genet* 11: 356-366, 2010.

SADOWITZ B, MAIER KG, GATHAN V. Basic science review: Statin therapy - Part I: The pleiotropic effects of statins in cardiovascular disease. *Vasc Endovascular Surg* 44: 241-251, 2010.

SANDHU MS, WATERWORTH DM, DEBENHAM SL, WHEELER E, PAPADAKIS K, ZHAO JH, SONG K, YUAN X, JOHNSON T, ASHFORD S, INOUE M, LUBEN R, SIMS M, HADLEY D, MCARDLE W, BARTER P, KESÄNIEMI YA, MAHLEY RW, MCPHERSON R, GRUNDY SM; WELLCOME TRUST CASE CONTROL CONSORTIUM, BINGHAM SA, KHAW KT, LOOS RJ, WAEBER G, BARROSO I, STRACHAN DP, DELOUKAS P, VOLLENWEIDER P, WAREHAM NJ, MOOSER V. LDL-cholesterol concentrations: a genome-wide association study. *Lancet* 371: 483-491, 2008.

SEKI S, KAWAGUCHI Y, CHIBA K, MIKAMI Y, KIZAWA H, OYA T, MIO F, MORI M, MIYAMOTO Y, MASUDA I, TSUNODA T, KAMATA M, KUBO T, TOYAMA Y, KIMURA T, NAKAMURA Y, IKEGAWA S. A functional SNP in CILP, encoding

the cartilage intermediate layer protein, is associated with susceptibility to lumbar disc disease. *Nat Genet* **37**: 607-612, 2005.

SEVER PS, DAHLOF B, POULTER NR, WEDEL H, BEEVERS G, CAULFIELD M, COLLINS R, KJELDSSEN SE, KRISTINSSON A, MCINNES GT, MEHLSSEN J, NIEMINEN M, O'BRIEN E, OSTERGREN J; ASCOT INVESTIGATORS. Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial. *Lancet* **361**: 1149-1158, 2003.

THUNSDALL-PEDOE H, KUULASMAA K, TOLONEN H, DAVIDSON M, MENDIS S. MONICA monograph and multimedia sourcebook. Thunsdal-Pedoe H, editor. Geneva: World Health Organisation, 2003.

VRABLIK M, CESKA R, ADAMKOVA V, PEASEY A, PIKHART H, KUBINOVA R, MARMOT M, BOBAK M, HUBACEK JA. MLXIPL variant in individuals with low and high triglyceridemia in white population in Central Europe. *Hum Genet* **124**: 553-555, 2008.

WANG K, LI M, HAKONARSON H. Analysing biological pathways in genome-wide association studies. *Nat Rev Genet* **11**: 843-854, 2010.

WATERWORTH DM, RICKETTS SL, SONG K, CHEN L, ZHAO JH, RIPATTI S, AULCHENKO YS, ZHANG W, YUAN X, LIM N, LUAN J, ASHFORD S, WHEELER E, YOUNG EH, HADLEY D, THOMPSON JR, BRAUND PS, JOHNSON T, STRUCHALIN M, SURAKKA I, LUBEN R, KHAW KT, RODWELL SA, LOOS RJ, BOEKHOLDT SM, INOUE M, DELOUKAS P, ELLIOTT P, SCHLESSINGER D, SANNA S, SCUTERI A, JACKSON A, MOHLKE KL, TUOMILEHTO J, ROBERTS R, STEWART A, KESÄNIEMI YA, MAHLEY RW,

GRUNDY SM; WELLCOME TRUST CASE CONTROL CONSORTIUM, MCARDLE W, CARDON L, WAEBER G, VOLLENWEIDER P, CHAMBERS JC, BOEHNKE M, ABECASIS GR, SALOMAA V, JÄRVELIN MR, RUOKONEN A, BARROSO I, EPSTEIN SE, HAKONARSON HH, RADER DJ, REILLY MP, WITTEMAN JC, HALL AS, SAMANI NJ, STRACHAN DP, BARTER P, VAN DUIJN CM, KOONER JS, PELTONEN L, WAREHAM NJ, MCPHERSON R, MOOSER V, SANDHU MS. Genetic variants influencing circulating lipid levels and risk of coronary artery disease. *Arterioscler Thromb Vasc Biol* **30**: 2264-2276, 2010.

Table 1

Basic characteristics of the analyzed groups. P is given for the differences before and after treatment in the patients group.

Character	Patients		Controls	P
Number	895		672	
Age	60.3 ± 13.1		42.5 ± 10.2	
% of males	46		40	
	Before	After		
Total cholesterol	7.00 ± 1.53	5.15 ± 1.17	4.35 ± 0.42	0.0001
HDL cholesterol	1.43 ± 0.48	1.41 ± 0.40	1.38 ± 0.35	0.08
Triglycerides	2.03 ± 1.01	1.65 ± 1.23	1.03 ± 0.37	0.0001

Table 2

Primer sequences, restriction enzymes and size of the restriction fragments used for detection of polymorphisms of interest.

Polymorphism	Primer sequence	Annealing temperature	PCR product	Enzyme	Size (bp)	Allele
CILP2/...	5' tgg ctc ttg tcc act ggc cac atc ccc	70°C	135 bp	Hin1II	137	G
rs16996148	5' ttc tcc cat gcc tcc agg ccc cca ag				82+54	T
Apo B	5'aga gga aac caa ggc cac agt tgc	57.5°C	163 bp	XhoI	136	C
rs693	5' tac att cgg tct cgt gta tct tct				110+26	T
Apo B	5'cct agt taa tcc tca gaa tga cac tg	59°C	369 bp	BglII	369	G
rs515135	5' att ggg gtg gca ata ggc gca aat tg				261+108	A
CELSR2/...	5' atc cag cta ttt ggg agc agt gtc ctg g	66°C	137 bp	Hin1II	139	A
rs646776	5'aag gtc tgg tct ctg gaa aac aga ag				107+32	G
<i>PCSK9</i>	5' ccc agc att gcc agc ttc tct gtc tc	68.9°C	130 bp	Hin6I	130	T
rs11206510	5' agc caa aga cgg cca cca cag aca gc				104+26	C
<i>PCSK9</i>	5' atg agt tgg agg agg gag ccc ctt cc	57.5°C	133 bp	PstI	133	G
rs6235	5'aag ttt tca taa ggg atg ttg agc tct gc				103+30	C
<i>HMG-CoA r</i>	5' atg tct ccc cct ctc cag gtg ttc ac	56.5°C	138 bp	Hin1II	138	T
rs12654264	5' cca tta aaa atg cac agt ctc tat atc t				110+28	A
<i>LDL-receptor</i>	5' acc ggg gat gat gat gat tgc	62°C	132 bp	DpnI	132	G
rs6511720	5' ttg cct aag act tca tta aca ttt g				106+26	T
<i>APOE/C1/C4</i>	5' tta act cca tgg tct caa aag agt ctt cc	59.2°C	340 bp	Mae I	340	G
rs4420638	5' tga ggc agg ata atc tct tga acc tgg g				185+155	A

Table 3 Genotype distributions within the analyzed groups

CILP2/PBX4							
rs16996148	GG		GT		TT		P
	N	%	N	%	N	%	
Patients	764	85.6	123	13.8	6	0.7	0.003
Controls	534	80.9	110	16.7	16	2.4	
Apolipoprotein B							
rs693	CC		CT		TT		P
	N	%	N	%	N	%	
Patients	194	22.2	451	51.7	227	26.0	0.001
Controls	190	29.6	327	50.9	125	19.5	
Apolipoprotein B							
rs515135	GG		GA		AA		P
	N	%	N	%	N	%	
Patients	581	68.4	242	28.5	26	3.1	0.79
Controls	433	66.9	195	30.1	19	2.9	
CELSR2/PSRC1/SORT1							
rs646776	AA		AG		GG		P
	N	%	N	%	N	%	
Patients	551	64.8	275	32.4	24	2.9	0.0001
Controls	357	55.8	248	38.8	35	5.4	
PCSK9							
rs11206510	TT		TC		CC		P
	N	%	N	%	N	%	
Patients	561	64.5	260	29.9	50	5.8	0.06
Controls	435	65.7	206	31.1	21	3.2	
PCSK9							
rs6235	CC		CG		GG		P
	N	%	N	%	N	%	
Patients	469	54.8	335	39.1	52	6.1	0.92
Controls	362	55.4	250	38.2	42	6.4	
HMG-CoA r							
rs12654264	AA		AT		TT		P
	N	%	N	%	N	%	
Patients	301	33.6	443	49.5	151	16.8	0.08
Controls	252	38.1	320	48.4	89	13.4	
LDL-receptor							
rs6511720	GG		GT		TT		P
	N	%	N	%	N	%	
Patients	740	87.7	100	11.8	4	0.5	0.0001
Controls	513	76.9	143	21.4	11	1.7	
APOE/C1/C3							
rs4420638	AA		AG		GG		P
	N	%	N	%	N	%	
Patients	523	62.4	284	33.9	31	3.7	0.0007
Controls	476	73.3	159	24.5	14	2.2	

Table 4

Changes of lipid parameters according to individual genotypes (expressed as percentage of the baseline value).

APOE/C1/C4	+G	AA	+G	AA	
rs4420638	Males		Females		P*
Δ T-C	19.4 ± 18.0	24.5 ± 10.9	27.8 ± 10.8	27.6 ± 11.6	0.14
Δ TG	20.3 ± 30.6	12.5 ± 35.1	24.1 ± 23.3	13.4 ± 31.1	0.01
Δ HDL- C	20.0 ± 29.6	11.2 ± 27.0	4.9 ± 28.1	4.5 ± 20.1	0.007

* uncorrected P values, adjusted for sex, age and baseline plasma lipid levels. After Bonferroni correction Δ for HDL- C are not significant at P = 0.063.