An association of ABCG8: rs11887534 polymorphism and HDL-cholesterol response to statin treatment in the Polish population

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Abstract

**Background** Variation in lipid changes in response to statin treatment is associated with genetic polymorphism. Sterolin-1, encoded by ABCG5, and sterolin-2, encoded by ABCG8, together form a sterol transporter. There are some reports indicating association of rs11887534 (ABCG8:c.55G > C) polymorphism with lipid concentrations, both prior to and after statin treatment. The aim of this study was to analyze both baseline plasma lipids and their concentrations in response to statin treatment with regard to ABCG8: rs11887534 polymorphism in Caucasian patients of Polish origin.

**Methods** The study group consisted of 170 consecutive adult out-patients treated with atorvastatin or simvastatin for a minimum of 2 months. Concentrations of triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) were measured before and after statin treatment. The ABCG8 polymorphism was identified by mini-sequencing genomic DNA extracted from peripheral blood leukocytes.

**Results** There were no significant differences in regard to ABCG8 variants for baseline TG, TC, LDL-C and HDL-C as well as for TG, TC or LDL-C concentrations after statin treatment. However, patients carrying at least one C allele showed a decrease in post-statin HDL-C concentrations and the absolute and relative changes between post- and pre-statin HDL-C concentrations were negative in contrast to positive values in wild-type homozygotes.

**Conclusions** Our results suggest that the c.55C allele of the ABCG8: rs11887534 polymorphism might be associated with decrease in HDL-cholesterol in response to statin treatment in Polish patients.

Keywords Statin · Lipid response · ABCG8 · Gene polymorphism

Introduction

Statins are competitive inhibitors of the 3-hydroxy-3-methyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is a rate-limiting enzyme in cholesterol biosynthesis [1]. A decrease in cellular cholesterol concentrations caused by statins stimulates cell-surface expression of low-density lipoprotein (LDL) receptors on hepatocytes, which in turn increase the removal of circulating LDL cholesterol (LDL-C)[2]. Treatment with statins not only causes various degrees of LDL-C decrease but also a decrease in total cholesterol and triglyceride concentrations as well as a HDL-C increase [3, 4]. Hasvold et al. indicated that statin-induced changes in LDL-C and HDL-C are unrelated and many patients initiated on statins experience a paradoxical decrease in HDL-C [5]. There is also evidence that the inter-individual variability in lipid response to treatment with statins may be associated with genetic polymorphisms [1, 6–11].

The ABCG8 gene is located on chromosome 2p21 in a head-to-head orientation with the ABCG5 gene. ABCG5 encodes sterolin-1 and ABCG8 encodes sterolin-2. Both sterolins are non-functional half-transporters which have to form the heterodimer to gain sterol transport functionality [12]. Loss-of-function mutations in either ABCG5 or ABCG8 have been identified as a cause of sitosterolemia,
a rare autosomal recessive disorder characterized by elevated plasma levels of plant sterols due to increased intestinal absorption of dietary sitosterol and decreased biliary sterol secretion [13, 14]. The majority of patients with sitosterolemia are characterized by normal to moderately elevated plasma cholesterol concentrations [15, 16]. On the other hand, the common \(ABC\text{G8}: c.55G>C\) polymorphism (rs11887534) has been reported to account for variability in plasma concentrations of: triglycerides, total cholesterol concentrations, LDL-C concentrations and HDL-C concentrations [14, 17–20] as well as for the variability in plasma lipid parameters in response to treatment with statins [7, 14, 21]. However, other authors have not confirmed associations of \(ABC\text{G8}: rs11887534\) with plasma lipid levels [22–24]. In addition, till now only few studies on \(ABC\text{G8}: rs11887534\) have been conducted with Slavic populations [22, 25]. Therefore, we decided to analyze both baseline plasma lipids and changes in their concentrations in response to statin treatment in regard to the \(ABC\text{G8}: rs11887534\) polymorphism in Polish Caucasian patients.

**Materials and methods**

The study group consisted of 170 consecutive adults (52 males and 118 females, aged from 38- to 84-years old) recruited in an outpatient clinic in Szczecin according to the protocol described previously [26]. All recruited participants were Caucasian patients of Polish origin living in Szczecin, the largest city in West Pomerania. Inclusion criteria were as follows: age > 18 years old, the presence of a lipid disorder and treatment either with atorvastatin (10–20 mg per day) or with simvastatin (20–40 mg per day) for a minimum of 2 months. Exclusion criteria were: smoking, thyroid disease (hyperthyroidism or hypothyroidism), or if, after extensive interview, patients had not complied fully with instructions, including a diet low in fat. Clinical data from patients’ records included: age, gender, body mass index (BMI) calculated as (body mass, kg)/(height, m\(^2\)), duration of statin treatment, the daily dose of statin, and the presence of arterial hypertension, diabetes mellitus or coronary artery disease (Table 1). Laboratory data from patients’ records included: serum concentrations of triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C). Lipid concentrations were measured before (time 1) and after (time 2) the statin treatments as described previously [26]. In addition, absolute (\(\Delta_{2-1} = \text{time 2} - \text{time 1}\)) or relative (\(\Delta\% = 100*(\text{time 2} - \text{time 1})/\text{time 1}\)) differences were calculated for TG, TC, LDL-C and HDL-C (Table 2). Peripheral blood samples (5 ml) were drawn before statin treatment and stored at −20 °C until DNA isolation. All patients gave informed, written consent to participate in the study, which was approved by the bioethics committee at the Pomeranian Medical University, Szczecin, Poland. Genomic DNA was extracted from peripheral blood leukocytes using a commercially available DNA isolation kit (QIAamp Blood DNA Mini Kit, QIAGEN, Germany). Each DNA sample was used as a template for PCR to amplify a 130-bp \(ABC\text{G8}\) sequence, including rs11887534. PCR was performed using: 5′–GCT GGG TCT AAG AGA GCT GC–3′ as the forward primer and 5′–CTT CCC ATT GCT CAC TCA CC–3′ as the reverse primer. Subsequently, the PCR amplification products were purified using Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (ThermoFisher Scientific Inc., Waltham, MA USA) according to manufacturer procedures. The purified amplicons were subjected to a mini-sequencing reaction using an ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems) with extension primer 5′–GAC TGA CTG ACT GAC TGA CTG ACT TGC TCA TGC TCA CTC ACC GAG GTTAC–3′. Capillary electrophoresis of

| Table 1 | Basic characteristics of patients in regard to their \(ABC\text{G8}\) genotype |
| --- | --- | --- | --- |
| Variable | All (n = 170) | \(ABC\text{G8}\) genotype | p |
| | | GG (n = 147) | GC + CC (n = 22 + 1) | GC + CC vs GG |
| Males, n (%) | 52 (30.6) | 47 (32.0) | 5 (21.7) | 0.455 |
| Age (years) | 66 (57.73) | 66 (56.73) | 68 (59.73) | 0.408 |
| BMI (kg/m\(^2\)) | 27.3 (24.4, 30.4) | 27.2 (24.4, 30.4) | 28.0 (24.2, 31.9) | 0.684 |
| Arterial hypertension, n (%) | 134 (78.8) | 118 (80.3) | 16 (69.6) | 0.372 |
| Diabetes mellitus, n (%) | 49 (28.8) | 41 (27.9) | 8 (34.8) | 0.584 |
| Coronary artery disease, n (%) | 37 (21.8) | 33 (22.4) | 4 (17.4) | 0.667 |
| Duration of treatment with statin (months) | 9 (4, 20) | 9 (4, 19) | 12 (5, 24) | 0.630 |
| Simvastatin equivalent dose (mg/day) | 40 (20–40) | 40 (20–40) | 40 (20–40) | 0.849 |
| Patients using higher (≥ 40 mg/day) simvastatin equivalent dose, n (%) | 85 (50.0) | 73 (50.0) | 12 (52.2) | 1.000 |

Quantitative data are presented as median (lower quartile, upper quartile)
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The mini-sequencing products was performed on an ABI PRISM 3100-Avant genetic analyzer (Applied Biosystems). The mini-sequencing products were visualized and analyzed with GeneMapper™ v4.1 Software (Applied Biosystems). All DNA samples were genotyped using a blind method, i.e. the samples were anonymously labeled by one person and then genotyped by the second person.

Normal distribution of quantitative data was tested using Shapiro–Wilk tests. Since the majority of quantitative variables were not normally distributed, we presented all of them as median values with lower (Q1) and upper quartiles (Q3). Quantitative data were compared between genotype groups using Mann–Whitney tests. Categorical data and the divergence of ABG8 genotype frequencies from Hardy–Weinberg equilibrium were assessed using chi-squared tests. Statistical significance was defined as $p < 0.05$. We calculated the statistical power of the study to detect significant differences of relative changes (Δ%) in lipid parameters during statin treatment between genotype groups, assuming that standard deviations of the changes were equal to 15% for TC, 20% for LDL-C, 25% for HDL-C and 50% for TG. The power with 170 subjects and a minor allele frequency of 7% was sufficient to detect with 80% probability true differences equivalent to 10% for TC, 13% for LDL-C, 16% for HDL-C and 32% for TG, between genotype groups using a dominant model. All data were analyzed using a data analysis software system (Dell Statistica, version 13. Dell Inc. 2016, software.dell.com).

### Results

There were 147 GG homozygotes (86.5%), 22 GC heterozygotes (12.7%) and one CC homozygote (0.6%) in the studied group, and ABG8: rs11887534 genotype distribution conformed to expected Hardy–Weinberg equilibrium ($p = 0.582$). The frequency of the minor ABG8: c.55C allele was 7.1%. No significant differences in gender composition, age, BMI, prevalence of arterial hypertension, frequency of diabetes mellitus, prevalence of coronary artery disease, duration of treatment with statin and frequency of patients treated with a higher ($≥ 40$ mg/day) simvastatin equivalent dose were found between subjects homozygous for the wild-type ABG8 allele (c.55G) and individuals having at least one mutated allele (GC or CC genotype) (Table 1).

There were also no significant differences in regard to ABG8 variants for baseline TG, TC, LDL-C and HDL-C concentrations, for TG, TC or LDL-C concentrations after treatment with statin as well as for absolute and relative differences in TG, TC or LDL-C levels. The only significant differences between both genotype groups of patients concerned HDL-C concentrations after statin use as well as absolute and relative changes in HDL-C concentrations. In contrast to GG homozygotes, patients with GC + CC genotypes showed a decrease in post-statins HDL-C concentrations, and negative absolute and relative differences in HDL-C concentrations (Table 2).

### Table 2 Lipid parameters of the patients in regard to their ABG8 genotype

| Variable | Time code | All $(n = 170)$ | GGG $(n = 147)$ | GC + CC $(n = 22 + 1)$ | p |
|----------|-----------|----------------|----------------|------------------------|---|
| TG       | 1         | 1.57 (1.26, 2.18) | 1.59 (1.28, 2.29) | 1.46 (1.11, 1.81) | 0.089 |
|          | 2         | 1.26 (0.97, 1.77) | 1.25 (0.97, 1.84) | 1.28 (0.87, 1.55) | 0.571 |
|          | Δ2–1      | −0.34 (−0.82, 0.02) | −0.36 (−0.94, 0.01) | −0.02 (−0.65, 0.15) | 0.183 |
|          | ΔG       | −24.5 (−46.3, 2.1) | −25.4 (−47.4, 0.6) | −2.0 (−39.7, 9.6) | 0.234 |
| TC       | 1         | 6.66 (6.01, 7.38) | 6.73 (6.06, 7.36) | 6.37 (5.26, 7.59) | 0.238 |
|          | 2         | 4.51 (4.01, 5.13) | 4.53 (4.04, 5.15) | 4.27 (3.76, 4.92) | 0.294 |
|          | Δ2–1      | −2.10 (−2.88, −1.37) | −2.12 (−2.77, −1.37) | −1.94 (−2.64, −1.45) | 0.680 |
|          | ΔG       | −31.0 (−40.2, −22.1) | −31.1 (−40.2, −22.0) | −31.0 (−41.1, −23.6) | 0.973 |
| LDL-C    | 1         | 4.36 (3.66, 5.04) | 4.37 (3.68, 5.04) | 4.08 (3.43, 4.65) | 0.330 |
|          | 2         | 2.42 (2.00, 2.95) | 2.41 (1.97, 2.98) | 2.55 (2.01, 2.92) | 0.911 |
|          | Δ2–1      | −1.86 (−2.59, −1.09) | −1.92 (−2.59, −1.21) | −1.47 (−2.59, −0.94) | 0.272 |
|          | ΔG       | −44.2 (−53.4, −29.4) | −44.3 (−53.6, −30.3) | −34.9 (−51.3, −25.0) | 0.432 |
| HDL-C    | 1         | 1.45 (1.42, 1.66) | 1.45 (1.22, 1.63) | 1.45 (1.24, 1.92) | 0.466 |
|          | 2         | 1.42 (1.22, 1.66) | 1.45 (1.22, 1.71) | 1.27 (1.11, 1.53) | 0.028 |
|          | Δ2–1      | 0.00 (−0.23, 0.21) | 2.00 (−7.0, 0.21) | −0.13 (−0.44, −0.02) | 0.003 |
|          | ΔG       | 0.0 (−14.6, 14.3) | 3.1 (−12.9, 15.9) | −9.8 (−25.3, −1.8) | 0.002 |

Lipids concentrations and absolute differences in lipid concentrations are measured in millimols per liter (mmol/l). The relative differences in lipid concentrations are expressed as a percentage. Data are presented as median (lower quartile, upper quartile).
Discussion

The ABCG8 locus is one of many loci identified to be associated with blood lipid levels [27–29]. The c.55G > C transversion (rs11887534) in the ABCG8 gene causes the substitution of aspartic acid (Asp, D) by histidine (His, H) at amino acid position 19 (p.Asp19His) of the sterolin-2. An aspartic acid at amino acid position 19 is highly conserved from plants to vertebrates and its substitution by histidine results in the loss of negative charge [14]. Therefore, it has been speculated that this conformational change might increase the function of the ABCG5/ABCG8 transporter [19]. However, there are no experimental reports confirming the influence of rs11887534 on the expression or activity of this transporter so far [21, 30].

The frequency of the minor ABCG8: c.55C allele of 7.1% in our patients was very similar to its frequency previously reported by Krawczyk et al. in Poles (7.5%) [25] or by Hubacek in Czechs, who are also of Slavic origin (6.7%) [22]. The prevalence of rs11887534 in Polish subjects was also close to its frequencies in other European populations, which ranged from 5.4 to 10.6% [10, 24, 31].

There was no significant association between ABCG8: rs11887534 polymorphism and plasma concentrations of triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol in our studied patients. The lack of such associations has also been reported not only in Czech patients [22], but also in white subjects of non-Hispanic origin in the Dallas metropolitan area [23], in a cohort of Indian patients with coronary artery disease [21] and in a large cohort of Dutch patients with heterozygous familial hypercholesterolemia [24]. However, the results of a study by Kajinami et al. carried out in 338 multi-ethnic patients in the USA revealed that plasma cholesterol concentrations in subjects carrying at least one minor ABCG8: c.55C allele were significantly lower than in wild-type homozygotes [14]. Gylling et al. reported that in mildly hypercholesterolemic Finns the minor ABCG8: c.55C variant was associated not only with lower total cholesterol but also with lower LDL cholesterol [18]. In addition, Acalovschi et al. found that both plasma cholesterol and plasma triglycerides were lower in Romanian patients carrying at least one mutated rs11887534 allele as compared to wild-type homozygous subjects [17]. In turn, Junyent et al. revealed that the participants of the Boston Puerto Rican Health Study carrying the minor rs11887534 allele displayed lower concentrations of HDL-C only if they were smokers [20]. In contrast to these aforementioned results, Chen et al. showed that the ABCG8: c.55C allele in Taiwanese subjects consuming an ordinary Chinese diet (a diet with lower cholesterol and higher phytosterol content compared to a Western diet) was associated with both higher total cholesterol and higher LDL-cholesterol [19].

Reports concerning the efficacy of statin treatment in regard to ABCG8: rs11887534 polymorphism are scarce [7, 14, 21]. In 2004 Kajinami et al. revealed that post-atorvastatin TC and post-atorvastatin LDL-C were significantly lower and adjusted percent reductions of LDL-C concentrations were significantly greater in subjects carrying at least one minor ABCG8: rs11887534 allele (c.55C) as compared to GG homozygotes [14]. Srivastava et al. reported that post-treatment TC was significantly lower, and percent reduction of LDL-C was significantly greater, in Indian patients with coronary artery disease having at least one ABCG8: c.55C allele than in subjects with GG homozygous genotype. However, the ABCG8: rs11887534 polymorphism in these patients was not independently associated with both absolute or a percent reduction in LDL-C in stepwise multiple regression analysis including: age, gender, pretreatment lipid levels and ABCG8 genotype as independent variables [21]. On the other hand, Chien et al. revealed a significant association of an ABCG8 haplotype including wild-type rs11887534 with reduction in LDL-C after statin treatment in a Chinese population [7].

In contrast to above reports, we have found no association of ABCG8: rs11887534 polymorphism with response of LDL-C, total cholesterol and triglyceride levels to statin treatment. However, we have revealed a significant decrease in HDL cholesterol after statin use in our patients carrying at least one minor c.55C allele as compared with wild-type ABCG8 homozygotes. Ethnic-dependent differences, both in the frequency of ABCG8 polymorphism and in the prevalence of environmental factors (e.g. dietary habits), should be taken into consideration as major reasons for the inconsistency of results among studies.

Treatment with statin usually moderately increases the serum concentration of HDL cholesterol in a majority of patients but some patients experience a paradoxical decrease in HDL-C levels after such pharmacotherapy [32]. In addition, Ota et al. suggested that a paradoxical decrease in HDL cholesterol after statin treatment might be an independent predictor for long-term adverse cardiovascular events in patients with acute myocardial infarction [32]. Hasvold et al. noted a decrease in HDL-C of > 0.1 mmol/l in 20% of patients treated with statins (96% of the cohort were initiated on simvastatin with a mean dose of 20 mg/day), and the group of patients with reduction in HDL-C comprised more women, had a higher HDL-C at baseline (1.69 mmol/L) and less diabetes compared with the unchanged HDL-C group [5]. In our study more than 34% of patients (58 out of 170 subjects) experienced an HDL-C lowering of > 0.1 mmol/l, but this phenomenon was not associated with gender, baseline HDL-C or the prevalence of diabetes. As our study was based on data from patients’ records in a primary care
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