

1 Influence of *APOA5* locus on the treatment efficacy of
2 three statins: evidence from a randomized pilot study in
3 Chinese subjects

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18 Abbreviations: ApoA5, apolipoprotein A5; BMI, body mass index; FFA, free fatty acids; HDLc,
19 high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; Lp(a),
20 lipoprotein(a); SNP, single nucleotide polymorphism; T2D, type 2 diabetes; Tc, total cholesterol;
21 Tg, triglycerides.

22 **Abstract**

23 Pharmacogenetics or pharmacogenomics approaches are important for addressing the individual
24 variabilities of drug efficacy especially in the era of precision medicine. One particular interesting
25 gene to investigate is *APOA5* which has been repeatedly linked with the inter-individual
26 variations of serum triglycerides. Here, we explored *APOA5*-statin interactions in 195 Chinese
27 subjects randomized to rosuvastatin (5-10 mg/day), atorvastatin (10-20 mg/day), or simvastatin
28 (40 mg/day) for 12 weeks by performing a targeted genotyping analysis of the *APOA5* promoter
29 SNP rs662799 (-1131T>C). There were no significant differences between the treatment arms for
30 any of the statin-induced changes in clinical biomarkers. Reductions in LDL cholesterol were
31 influenced by the *APOA5* genotype in all three treatment groups. By contrast, changes in HDL
32 cholesterol and triglycerides were only affected by the *APOA5* genotype in the atorvastatin and
33 simvastatin groups and not in the rosuvastatin group. Our results support earlier findings
34 indicating that rosuvastatin is a better treatment option and that future studies should consider
35 stratifying subjects not only by genetic background but also by statin type.

36 **Keywords:** *APOA5* genotype, statins, triglycerides, dyslipidemia

37 Introduction

38 Although statins are the most prescribed class of drugs worldwide for prevention of various
39 cardiovascular diseases, about one third of patients do not respond well to this therapy with
40 respect to the lipid-lowering effect, suggesting that pharmacogenomics (Postmus et al., 2014) or
41 other environmental factors such as diet (Jenkins et al., 2005) or the gut microbiome (Kaddurah-
42 Daouk et al., 2011) may play substantial roles. To date, genome-wide association studies have
43 identified at least 39 genes that are associated with statin treatment efficacy (Gryn and Hegele,
44 2014). Most of these genes are involved in either the direct pharmacokinetic handling of statins or
45 in lipid metabolism pathways especially these involving cholesterol, the main target of statin
46 therapy (Mangravite et al., 2006). However, accumulating evidence indicates that statins can also
47 lower levels of triglycerides, potentially through altering degradation of apolipoprotein B (ApoB)
48 and related very low-density lipoprotein (VLDL) balance, although the precise mechanism
49 remains unclear (Ginsberg et al., 1987; Arad et al., 1992; Ginsberg, 1998).

50 One gene of particular interest within this context is *APOA5*, which has been repeatedly
51 associated with the high inter-individual variations of serum triglycerides in all reported
52 populations (Baum et al., 2003; Lai et al., 2004; Hubacek et al., 2008; Ouatou et al., 2014; Son et
53 al., 2015) since its identification in 2001 (Pennacchio et al., 2001; van der Vliet et al., 2001).
54 According to one estimation, the *APOA5* promoter SNP rs662799 (-1131T>C) alone can
55 contribute to 6.2% of the genetic component of hypertriglyceridemia (Hegele, 2009). Of note, the
56 minor C allele is much more common in the Asian population (26%-40%) than in Caucasians
57 (only ~8%) (Baum et al., 2003). In addition, accumulating evidence suggests that this gene also
58 confers risk for cardiovascular disease (Lai et al., 2004) and myocardial infarction (Do et al.,
59 2015). Although previous studies have suggested a link between this gene and statin treatment
60 (Brautbar et al., 2011; O'Brien et al., 2015), available statins differ in terms of their
61 pharmacodynamic and pharmacogenetic properties (Kivisto et al., 2004; Schachter, 2005) and
62 potency (Palmer et al., 2013; Arshad, 2014; Karlson et al., 2016). To the best of our knowledge,
63 no well-designed prospective study, has investigated whether *APOA5*-statin interactions
64 dependent on the statin type while controlling for differences in potency of the statins. One
65 retrospective study did not observe an effect of statin type when investigating the interaction
66 between the *APOA5* rs662799 variants and statins (Hubacek et al., 2009); however, this study did
67 not include rosuvastatin, which is often considered to be a better treatment choice (Scott et al.,
68 2004; McKenney, 2005; Schachter, 2005).

69 Here, we performed a pilot study to explore *APOA5*-statin interactions in 195 Chinese subjects
70 randomized to rosuvastatin, atorvastatin, or simvastatin therapy for 12 weeks. To address whether
71 the clinical responses of three types of statins differ between subjects with the same *APOA5*
72 genetic background, we genotyped *APOA5* rs662799 SNP and measured the fasting plasma
73 concentrations of triglycerides, cholesterols, free fatty acids, and four apolipoproteins both before
74 and after statin treatments.

75 Materials and Methods

76 Study subjects and study design

77

78 We recruited 195 patients at Shanghai Ruijin Hospital Luwan Branch (affiliated to Shanghai
79 Jiaotong University). In brief, the inclusion criteria were: (i) aged 18 years or older; (ii) newly
80 diagnosed with dyslipidemia and/or increased risk of atherosclerotic cardiovascular disease and
81 recommended to receive statins according to the 2013 American College of Cardiology (ACC)
82 and the American Heart Association (AHA) Blood Cholesterol Guidelines (Stone et al., 2014);
83 (iii) absence of major systematic diseases such as malignancy; and (iv) without medication
84 (especially antibiotics) in the previous three months except antihypertensive therapy.

85 The subjects were then randomly divided into three treatment arms to receive rosuvastatin (5-10
86 mg per day), atorvastatin (10-20 mg per day), or simvastatin (40 mg per day) for 12 weeks. To
87 achieve comparable clinical efficacies in response to the three statins, the different statin doses
88 were selected based on both clinical practice and evidence suggesting that each rosuvastatin dose
89 is equivalent to 3-3.5 times of atorvastatin and 7-8 times of simvastatin (at least in terms of
90 cholesterol reduction) (Hubacek et al., 2009). All treatments were tolerated with no side effects
91 reported.

92 Written informed consent was obtained from all the study participants. This study conforms to the
93 ethical guidelines of the 1975 Declaration of Helsinki and was approved by Ethics Committee of
94 Shanghai Ruijin Hospital Luwan Branch. Complete clinical trial registration is deposited at
95 chictr.org.cn (ChiCTR-RRC-16010131).

96 **Laboratory analyses**

97 Fasting plasma concentrations of triglycerides, total cholesterol, HDL cholesterol (HDLc), LDL
98 cholesterol (LDLc), free fatty acids (FFA), and three different apolipoproteins (ApoA1, ApoB-
99 100, ApoE) and lipoprotein(a) were measured by enzymatic methods using a Beckman Coulter
100 Chemistry Analyzer AU5800 Series (United States) at both baseline and 12 weeks after
101 treatments. ApoA5 was not measured since consistent evidence suggests rs662799
102 polymorphisms were not associated with the circulating levels of this apolipoprotein (Talmud et
103 al., 2006; Henneman et al., 2007)

104 DNA was isolated using the TIANamp Blood DNA kit (purchased from Tiangen, Beijing, China)
105 and individual *APOA5* variants (-1131T>C – rs662799) were genotyped using a base-quenched
106 probe method combined with polymerase chain reaction (PCR) as described before (Luo et al.,
107 2009). In brief, a 19-nt probe (5'-GGCAAATCTCACTTTCGCT-3') containing the targeted SNP
108 site was first conjugated with 6-carboxyfluorescein and then hybridized to its complementary
109 target sequence from PCR amplification. An analytical melting program that involves heating the
110 amplicon/probe heteroduplex will produce different fluorescence curves depending on the
111 genotypes of rs662799. Both the probe and primers (forward: 5'-
112 AGGAGTGTGGTAGAAAGACCTGTTG-3'; reverse: 5'-
113 AACTACCCAGAGTCACTGTGTCCC-3') used were synthesized by Sangon (Shanghai, China).

114 **Statistical analysis**

115 Statistical differences between groups were estimated by Wilcox rank-sum test (between two
116 groups), Kruskal-Wallis test (among three groups) for continuous variables or by Chi-square test
117 for categorical variables. Different linear regression models were also built and compared using
118 Chi-square test to confirm the effect of *APOA5* genotype on different biomarkers and adjusted for
119 contributions from type 2 diabetes and sex. Associations between apolipoproteins and
120 concentrations of LDLc and HDLc were measured by Spearman's rank correlation analysis.
121 Hardy-Weinberg Equilibrium was accessed by exact test based on R package "HardyWeinberg"
122 (Graffelman, 2015). Raw P values were adjusted by the Benjamini-Hochberg method (Benjamini
123 and Hochberg, 1995) with a false discovery rate of 5%. A power of 99.98% was obtained using
124 pwr package (Champely, 2015) for this study based on 65 patients with paired design, 5%
125 significance, and an estimated effect size of 0.7 for statin in reducing LDL cholesterol
126 (Cholesterol Treatment Trialists et al., 2012; Ridker et al., 2016). All statistical tests and data

127 visualizations as well as the stratified randomization process by considering BMI as covariate
128 were performed under the R environment (Team, 2015).

129 Results and discussion

130 Baseline characteristics

131 The minor C allele frequency of *APOA5* rs662799 SNP in our cohort was 30%, consistent with
132 other reports based on larger Chinese cohorts (Baum et al., 2003; Jiang et al., 2010); the genotype
133 frequency of *APOA5* was in agreement with Hardy-Weinberg equilibrium ($n = 13, 91$ and 91 for
134 C/C, T/C, and T/T allele carriers, respectively; $P=0.171$). T/C and C/C subjects were pooled as
135 T(C)/C ($n = 104$) for further analyses to increase the power. With the exception of ApoE, there
136 were no significant baseline differences between the treatment arms, including the frequencies of
137 the T(C)/C and T/T genotypes ($P=0.342$) (**Table 1**). These data suggest that the treatment groups
138 are in general homogeneous and this study design is suitable for addressing the relationship
139 between *APOA5* variations and the clinical responses of three statins. When dividing the subjects
140 by genotype, the T(C)/C allele carriers had significantly higher plasma triglycerides than T/T
141 carriers at baseline (**Table 1**), in agreement with previous studies (Baum et al., 2003; Lai et al.,
142 2004; Jiang et al., 2010).

143 We also noted that subjects with the T(C)/C genotype had higher LDLc than T/T carriers at
144 baseline (**Table 1**); these findings were consistent with observations in a larger cohort (Lai et al.,
145 2004) but an earlier study in Chinese men did not observe significant *APOA5*-LDLc interactions
146 (Baum et al., 2003). It is not clear how *APOA5* variants affect LDLc as ApoA5 has only been
147 detected on HDL and VLDL and not on LDL particles (Ballantyne et al., 2006). However,
148 ApoA5 has been shown to directly interact with members of the LDL-receptor family (Nilsson et
149 al., 2007). In addition, an earlier study has shown a significant association between the *APOA5*
150 rs662799 SNP and increased risk of early-onset myocardial infarction even after adjusting for
151 triglycerides (De Caterina et al., 2011), providing further evidence that this SNP may
152 simultaneously affect other atherogenic lipids such as LDLc. It is also possible that this SNP is in
153 complete linkage disequilibrium with other polymorphism(s) that can explain the observed LDLc
154 levels.

155 **Rosuvastatin-induced changes in HDLc and triglycerides are not affected by**
156 **APOA5 genotype**

157 We next compared the clinical efficacies (in terms of cholesterol, triglyceride, and apolipoprotein
158 changes) of the statins. As expected, all three statins promoted significant reductions in total
159 cholesterol, ApoB, LDLc, ApoE and triglycerides and significant increases in ApoA1 and HDLc
160 (**Figure 1A**). However, there were no significant differences between the treatment arms for any
161 of the statin-induced changes in clinical biomarkers after adjusting for multiple testing (false
162 discovery rate 5%), confirming that the response to 5-10 mg of rosuvastatin is similar to that of
163 10-20 mg atorvastatin and 40 mg of simvastatin as suggested previously (Hubacek et al., 2009).
164 In agreement, results from a meta-analysis (Karlson et al., 2016), comparative pharmacology
165 (McTaggart, 2003) and the MERCURY II clinical trial (Ballantyne et al., 2006) have all shown
166 that rosuvastatin is more potent than the other statins and thus lower doses can be used to achieve
167 equivalent responses.

168 To determine how *APOA5* variations affected the clinical responses of the three statins, we
169 investigated how changes in the biomarker concentrations in response to each statin varied
170 between subjects with the T(C)/C or T/T genotype (**Figure 1B-E; Supplementary Table S1**).
171 Genotype did not affect the changes in total cholesterol (**Figure 1B**), apolipoproteins, FFA or
172 lipoprotein(a) (data not shown) in response to any of the three statins. However, patients
173 homozygous for the major T allele (T/T genotype) not only exhibited lower baseline LDLc levels
174 (**Table 1**) but also demonstrated significantly larger LDLc reductions compared with the C allele
175 carriers, independent of the type of statin used (**Figure 1C**). By contrast, rosuvastatin-induced
176 changes in HDLc and triglycerides showed little variation between patients with the T(C)/C and
177 T/T genotypes whereas changes in HDLc and triglycerides were more pronounced in T/T
178 compared with T(C)/T carriers upon atorvastatin or simvastatin treatment (**Figure 1D,E**). These
179 data suggest that rosuvastatin-induced responses may be less affected by *APOA5* variations than
180 the other two statins. The results were still valid after adjusting for type 2 diabetes and sex
181 (**Supplementary Table S2**). It has been suggested that the hydrophilic rosuvastatin is largely
182 excreted unchanged (Martin et al., 2003) whereas the other two lipophilic statins undergo
183 substantial metabolism by the CYP450 pathways and thus are more affected by gene
184 polymorphisms (Kivisto et al., 2004; Schachter, 2005), consistent with our findings. Additionally,
185 rosuvastatin differs from the other statins by its stronger binding to 3-hydroxy-3-methylglutaryl
186 coenzyme A (HMG-CoA) reductase, lower systemic bioavailability, longer elimination half-life
187 (McTaggart, 2003) and greater hepatoselectivity (Schachter, 2005). These physio-biochemical

188 differences may also potentially contribute to the different treatment responses according to
189 genotype. However, to fully understand how *APOA5* affects statin treatments, in-depth
190 characterizations of its functional role are needed.

191 **Statin-APOA5 interactions altered the correlations between apolipoproteins** 192 **and LDLc/HDLc**

193 Although most therapies to reduce cardiovascular disease risk currently focus on reduction of
194 LDLc and triglycerides, atherogenic proteins such as ApoB have also been suggested to have
195 great predictive value (Ballantyne et al., 2008). Accordingly, the American Diabetes Association
196 and the American College of Cardiology Foundation recommend that therapy for patients with
197 high cardiovascular disease risk should aim to lower ApoB concentrations to below 90 mg/dl in
198 addition to reducing LDLc levels (Brunzell et al., 2008). To address whether the well-known
199 strong associations between ApoB and LDLc both before and after statin treatments (Ballantyne et
200 al., 2008) differ among patients with different *APOA5* genotypes, we additionally analyzed
201 ApoB-LDLc correlations within each *APOA5* SNP subgroup. Before treatment, strong and
202 significant positive correlations were observed between ApoB and LDLc for both T(C)/C
203 (Spearman coefficient $\rho=0.55$; $P<0.001$) and T/T carriers ($\rho=0.78$; $P<0.001$; **Figure 2A**).
204 After treatment, a comparable strong correlation only existed for the C allele carriers ($\rho=0.50$;
205 $P<0.001$; **Figure 2B**). In contrast, the dramatic decrease in the ApoB-LDLc correlation among
206 T/T carriers (from 0.78 to 0.44) indicates the statin-induced reduction of ApoB in absolute values
207 was much smaller than reduction of LDLc. Thus, further treatment to reduce the levels of ApoB
208 even after achieving recommended LDLc reductions could be beneficial in T/T carriers. Similar
209 observations were found between ApoA1 and HDLc (**Supplementary Figure S1**).

210 **Conclusion**

211 In summary, our results show that low-dose rosuvastatin achieves improvements in clinical
212 responses that are comparable to those observed with higher doses of atorvastatin and simvastatin
213 but are less affected by *APOA5* genotype. These findings support the growing recognition that
214 rosuvastatin is a potentially better treatment option for patients with dyslipidemia and/or at high
215 risk of cardiovascular diseases. In addition, integrated efforts, such as the NIH Pharmacogenetics
216 Research Network (Giacomini et al., 2007), should be encouraged in the era of precision
217 medicine to accelerate pharmacogenetics or pharmacogenomics research. Future studies should
218 also consider stratifying populations by genetic background and by statin type.

219 Conflict of Interest

220 The authors declare no conflict of interest.

221 Author contributions

222 S.H., G.L., N.X. and J.Z. designed the study; S.H. performed the randomization process and
223 clinical intervention; J.L. and W.W. enrolled participants and measured the lipids and
224 apolipoproteins; J.Z. and G.L. performed the genotyping analysis; S.H., C.M., J.L. and W.W.
225 collected and analyzed the data; S.H., C.M, G.L. and J.Z. wrote the manuscript.

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380

381 Table 1. Baseline characteristics summarized by statin treatment and *APOA5* genotypes,
382 respectively.

Characteristics	Statin treatment ^a			<i>P</i> ^b	<i>APOA5</i> genotype ^a		<i>P</i> ^c
	Atorvastatin	Rosuvastatin	Simvastatin		T(C)/C	T/T	
n	36/29 ^d	30/35 ^d	38/27 ^d	0.342 ^c	104	91	-
Male (%)	53.8	44.6	49.2	0.575 ^c	45.2	53.8	0.288 ^c
Age (years)	74.9±10.5	75.0±11.8	69.8±17.4	0.344	72.8±12.8	73.7±14.8	0.420
BMI (kg/m ²)	23.5±3.2	23.5±3.3	23.4±3.2	0.938	23.5±3.5	23.3±2.8	0.662
Tc (mg/dl)	193.1±49.5	180.2±40.6	186.7±49.0	0.306	191.9±45.3	180.7±47.6	0.093
Tg (mg/dl)	180.1±110.5	162.6±102.6	181.9±138.0	0.482	193.7±124.8	153.4±105.8	0.004
HDLc (mg/dl)	42.1±9.9	44.1±10.4	44.6±11.5	0.458	43.1±10.7	44.1±10.6	0.513
LDLc (mg/dl)	132.2±42.9	125.8±34.8	125.7±41.1	0.780	134.7±40.6	120.1±37.3	0.015
ApoA1 (mg/dl)	114.4±20.6	119.1±23.3	116.9±20.1	0.515	116.3±22.0	117.3±20.7	0.708
ApoB-100 (mg/dl)	86.3±31.2	86.5±25.0	94.1±29.2	0.181	92.1±28.6	85.4±28.5	0.145
ApoE (mg/dl)	4.4±1.5	3.8±1.0	4.5±1.4	0.019	4.4±1.3	4.2±1.3	0.382
FFA (mmol/l)	0.5±0.2	0.5±0.3	0.5±0.2	0.780	0.5±0.2	0.4±0.3	0.052
Lp(a) (mg/dl)	17.5±17.0	18.0±15.4	19.8±17.9	0.916	18.4±14.8	18.4±18.8	0.575
Type 2 diabetes (%)	43.1	40.0	32.3	0.430 ^c	40.4	36.3	0.658 ^c

383 ^aContinuous variables are expressed as mean ± standard deviations (sd);

384 ^b*P* values were estimated by Krukskal-Wallis test for continuous variables;

385 ^c*P* values were estimated by Chi-square test for categorical variables;

386 ^dSample sizes for individuals genotyped as T(C)/C and T/T, respectively;

387 ^e*P* values were estimated by Wilcox rank-sum test for continuous variables;

388 **Figure legends**

389 **Figure 1.** *APOA5*-statin interactions. (A) Box plots (with median) showing percentage changes in
390 the indicated biomarkers after treatment with rosuvastatin (5-10 mg per day), atorvastatin (10-20
391 mg per day) or simvastatin (40 mg per day). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ versus before
392 treatment. (Wilcoxon signed-rank test) (B-E) Box plots (with median) showing percentage
393 changes in total cholesterol (Tc) (B), LDLc (C), HDLc (D), and triglycerides (Tg) (E) in response
394 to each statin in subjects divided by genotype (*APOA5* rs662799 T(C)/C and T/T). Sample sizes
395 for each subgroup are given on top of panels B-E. *, $P < 0.05$; **, $P < 0.01$ (Wilcoxon rank-sum
396 test).

397 **Figure 2.** Both *APOA5* and statin alter the ApoB-LDLc correlations. Correlations between ApoB
398 and LDLc before (A) and after (B) statin treatment in subjects with *APOA5* rs662799 T(C)/C or
399 T/T allele.

