Association of the Functional Variant in the 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase Gene With Low-Density Lipoprotein-Cholesterol in Japanese

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**Background:** The association between single nucleotide polymorphisms (SNPs) at 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) and low-density lipoprotein-cholesterol (LDL-C) levels has been well replicated in genome-wide association studies (GWAS) of white populations. Recently, the common intronic SNP of HMGR (rs3846662) has been reported to be a functional variant, influencing the alternative splicing of exon 13. The aim of this study was to examine the association between rs3846662 of HMGR and the level of LDL-C in Japanese.

**Methods and Results:** Significant differences in LDL-C levels were observed among the genotypes of rs3846662 (P=0.0002 (n=2,686) and P=0.004 (n=2,110)) for the Suita and Ehime samples, respectively. The G allele of rs3846662 was associated with higher LDL-C levels (β 3.56; P=4.91x10^-9). Consistent with this observation, the risk G allele at rs3846662 was more prevalent in subjects with myocardial infarction (MI) (n=701) than in subjects without MI (n=3,118): 0.559 and 0.511 in MI cases and controls, respectively (nominal P=0.0038). The odds ratio adjusted for age, sex, diabetes, hypertension, and drinking and smoking habits was 1.15 (95% confidence interval 1.04–1.28; P=0.0075).

**Conclusions:** The previously reported association of rs3846662 with LDL-C levels was replicated in the present Suita and Ehime samples. The LDL-associated SNP, rs3846662, appears to confer susceptibility to MI in Japanese. (Circ J 2010; 74: 518–522)

**Key Words:** Genetics; Lipids; Myocardial infarction; Polymorphism

As outlined in the 2007 edition of the Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese,1 elevated levels of low-density lipoprotein-cholesterol (LDL-C) are an important risk factor. LDL-C is known to be determined by both genetic and environmental factors. Substantial progress has been made toward detecting genes influencing circulating levels of LDL-C. In a recently published genome-wide association study (GWAS, n=19,840) of white populations,1,2,7 previously reported loci (APOE/C1/C4/C2, APOB, HMGR, LDLR, PCSK9, CELSR2/PSRC1/SORT1, CILP2/PBX4) have shown genome-wide significant association with LDL-C levels. Although GWAS of lipid and lipoprotein levels have been predominantly conducted in populations of European ancestry, there have been only a few replication studies conducted in non-European populations.1,3-11

The association between single nucleotide polymorphisms (SNPs) of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) and LDL-C levels has been well replicated in GWAS of white populations.3,4,12 HMGR is the rate-limiting enzyme in cholesterol synthesis, and inhibitors of HMGR have been widely used as cholesterol-lowering drugs.13 Recently, the common SNP in intron 13 of HMGR (rs3846662) has been reported to be a functional variant, influencing the alternative splicing of exon 13.14 In that study, lymphoblastoid cells from subjects homozygous for the major A allele showed higher levels of an alternatively spliced isoform missing exon 13 compared with those from
Table 1. Clinical Characteristics of the Study Populations

<table>
<thead>
<tr>
<th></th>
<th>Suita sample</th>
<th>Ehime sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>1,468</td>
<td>1,062</td>
</tr>
<tr>
<td></td>
<td>1,760</td>
<td>1,319</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66.0±10.7</td>
<td>58.6±15.3</td>
</tr>
<tr>
<td></td>
<td>63.8±10.5</td>
<td>62.1±13.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4±2.9</td>
<td>23.5±3.0</td>
</tr>
<tr>
<td></td>
<td>22.4±3.2</td>
<td>23.3±3.3</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)*</td>
<td>198.7±31.6</td>
<td>190.6±34.6</td>
</tr>
<tr>
<td></td>
<td>217.4±32.5</td>
<td>208.1±33.5</td>
</tr>
<tr>
<td>HDL-C (mg/dl)*</td>
<td>54.8±14.3</td>
<td>58.1±14.8</td>
</tr>
<tr>
<td></td>
<td>64.6±15.0</td>
<td>64.0±15.6</td>
</tr>
<tr>
<td>LDL-C (mg/dl)*</td>
<td>121.2±28.5</td>
<td>134.3±30.4</td>
</tr>
<tr>
<td></td>
<td>134.3±30.4</td>
<td>123.2±30.2</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)*</td>
<td>119.0±84.8</td>
<td>59.8±7.3</td>
</tr>
<tr>
<td></td>
<td>93.0±55.6</td>
<td>103.7±55.5</td>
</tr>
<tr>
<td>% Medication for dyslipidemia</td>
<td>11.0</td>
<td>18.5</td>
</tr>
<tr>
<td></td>
<td>18.5</td>
<td>6.7</td>
</tr>
<tr>
<td>% Smokers</td>
<td>28.8</td>
<td>38.2</td>
</tr>
<tr>
<td></td>
<td>5.3</td>
<td>2.1</td>
</tr>
<tr>
<td>% Drinkers</td>
<td>67.2</td>
<td>85.0</td>
</tr>
<tr>
<td></td>
<td>27.3</td>
<td>33.9</td>
</tr>
</tbody>
</table>

Continuous variables are mean±standard deviation. *Subjects with lipid-lowering medication were excluded. BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.

Methods

Study Populations

**Suite Sample**  The study design of the Suite Study has been described previously.17-24 In brief, the sample consisted of 14,200 men and women (30–79 years of age at enrollment), stratified by sex and 10-year age groups (10 groups and 1,420 subjects in each group) who had been randomly selected from the municipal population registry. They were all invited by letter to attend regular cycles of follow-up examination (every 2 years). Subjects were asked to estimate the amount and frequency of their alcohol intake per week, expressed as ethanol (g) per day.

To investigate the association of a genetic variation determining the LDL-C level with the risk of myocardial infarction (MI), genotyping of rs3846662 was carried out in 701 patients with MI randomly selected from in- and outpatient with documented MI and who were enrolled in the Division of Cardiology at the National Cardiovascular Center between May 2001 and April 2003. Those who were free from MI (n=3,118) served as controls.

Only those who gave written informed consent were included for the study. The study protocol was approved by the Institutional Ethics Committee and the Committee on Genetic Analysis and Gene Therapy of the National Cardiovascular Center.

**Ehime Sample**  Ehime sample comprised subjects from the Nomura study of Ehime University, which is a longitudinal epidemiological study based on the Nomura Town residents.25 Subjects were recruited through a community-based annual medical check-up process. Anthropometric and biochemical parameters were obtained from personal health records evaluated during the annual medical check-up. Information on smoking and drinking habits was obtained by interview. Subjects were asked to estimate average alcohol consumption per occasion expressed as ‘gou’, equivalent to 22.5 g of ethanol. All the study procedures were approved by the Ethics Committee of the Ehime University Graduate School of Medicine. Informed consent was given by each participating subject.

Genotyping Assays

Genotyping was performed by TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. Deviation from Hardy-Weinberg equilibrium and the degree of LD were analyzed using HaploView 4.0 (http://www.broad.mit.edu/mpg/haploview/).26 The exons 1–20 of HMGCR were sequenced in 48 subjects with low or high LDL-C levels using a 3730 DNA analyzer (Applied Biosystems) according to the manufacturer’s instructions.

Statistical Analysis

Data are expressed as mean±standard deviation. Continuous variables were tested for normality of distribution, and logarithmic transformation was applied to those with skewed distributions. Residuals, defined as the observed minus predicted values on the basis of confounding factors, were used for the genotype–phenotype association analysis by 1-way analysis of variance (ANOVA) tests. Covariates included in the model were derived from multiple logistic regression analysis and used to calculate a residual value for each variable. Genotype frequencies between control and MI cases were compared by chi-square test. Odds ratio (OR) and 95% confidence interval (CI) for the risk allele were estimated by logistic regression analysis with adjustment for covariates. Statistical analysis was performed using a JMP statistical package 7.0 (SAS Institute, Cary, NC, USA).

Results

Clinical characteristics of the study populations are shown in
Table 1 and Figure summarizes the association of rs3846662 genotypes with LDL-C levels in the Suita and Ehime samples. Significant differences in residual values of LDL-C were observed among the genotypes of rs3846662 (P=0.0002 (n=2,686) and P=0.004 (n=2,110)) for the Suita and Ehime samples, respectively. In accordance with the previous report, the G allele of rs3846662 was associated with higher LDL-C levels in the Suita sample ($\beta$, 3.56, $P=4.91 \times 10^{-5}$ with adjustment for sex, body mass index (BMI) and ethanol consumption). Although the association was in the same direction in both the Ehime and Suita samples, the frequency of the risk G allele was more common in the Suita than in the Ehime sample (0.511 among the Suita sample, 0.495 among the Ehime sample). In the Ehime sample, homozygotes for the A allele had significantly lower levels of LDL-C ($\beta$, −3.22, $P=0.001$ with adjustment for age, sex, BMI and ethanol consumption).

To examine the association between rs3846662 and the risk of MI, genotype frequencies were compared between patients with MI (n=701) and those free from MI (Table 2). The risk G allele of rs3846662 was more prevalent in subjects with MI than in subjects without MI (0.559 and 0.511 in MI cases and controls, respectively; nominal $P=0.0038$). The OR adjusted for age, sex, diabetes, hypertension, and smoking and drinking habits was 1.15 (95% CI 1.04–1.28; $P=0.0075$).

In order to assess whether a functional rare variant of HMGCR with a large effect is involved in influencing the variation in LDL-C levels in Japanese, we sequenced the exon regions of HMGCR in 48 subjects with low (n=18; residual LDL-C adjusted for sex, BMI and daily ethanol consumption: −71.76 to −4.25 mg/dl) or high (n=30; residual LDL-C adjusted for sex, BMI and daily ethanol consumption: 54.05–135.87 mg/dl) LDL-C levels. The sequencing analysis revealed 1 synonymous mutation on exon 17 (Thr758Thr) and 2 non-synonymous mutations on exon 9 (Tyr311Ser) and 19 (Gln824Lys). The minor allele frequency (MAF) for Thr758Thr, Tyr311Ser and Gln824Lys were 0.01, 0.03 and 0.01, respectively. Exons 11–20 are known to encode a catalytic domain. Because only 1 subject with low LDL-C (uncorrected LDL-C: 46 mg/dl; residual LDL-C adjusted for sex, BMI and daily ethanol consumption: −71.8 mg/dl) had Gln824Lys, further genotyping of Gln824Lys on exon 19 was carried out in 192 subjects. However, we did not find any other subject with this mutation. Overall MAF (n=240) for Gln824Lys was 0.002.

**Table 2. Logistic Regression Analysis of MI**

<table>
<thead>
<tr>
<th>rs3846662</th>
<th>Risk allele frequency</th>
<th>Genotype frequency</th>
<th>P value*</th>
<th>HWE</th>
<th>OR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=3,118)</td>
<td>0.511</td>
<td>0.232</td>
<td>0.514</td>
<td>0.254</td>
<td>0.0038</td>
<td>0.119</td>
</tr>
<tr>
<td>MI cases (n=701)</td>
<td>0.559</td>
<td>0.193</td>
<td>0.496</td>
<td>0.311</td>
<td>0.905</td>
<td></td>
</tr>
</tbody>
</table>

*Genotype frequencies between control and MI cases were compared by chi-square test. 
†Deviation from HWE was analyzed by an exact test and P values are presented. 
‡OR and 95%CI for the risk allele were estimated by logistic regression analysis with adjustment for age, sex, diabetes, hypertension, and drinking and smoking habits. BMI and the presence of hyperlipidemia were not significant predictors for MI and not included in the model.

**Figure.** Association of rs3846662 with low-density lipoprotein (LDL)-cholesterol levels in the Suita and Ehime samples. Covariates used for the calculation of residual values for the Suita sample were sex, body mass index (BMI) and daily ethanol consumption. Covariates for the Ehime sample included age, sex, BMI and ethanol consumption per occasion.

**Discussion**

We have replicated the previously reported association of rs3846662 within intron 13 of HMGCR with LDL-C level in 2 independent Japanese populations: the Suita and Ehime samples. Furthermore, rs3846662 was found to be associated with the risk of MI. The risk allele frequency for rs3846662 was more common in patients with MI than in those without MI. The OR adjusted for age, sex, diabetes, hypertension,
and smoking and drinking habits was 1.15 (95% CI 1.04–1.28, P=0.0075).

Results of our GWAS conducted in 900 Japanese men and women using the Illumina Sentrix HumanHap550 BeadChip (Illumina Inc, San Diego, CA, USA) are also in line with our current observation (see Supplement for more details). Among the 368,274 SNPs with a call rate >90% and MAF >0.1, rs3846662 of HMGCR was 1 of the top 38 SNPs exceeding the arbitrary threshold of –log10P>4.0. Of 38 top-ranked SNPs, 20 were genotyped in the remaining Suita sample (n=1,000–1,500) for validation of the associations detected in the initial subgroup (n=900). Although the strength of the association for the 20 SNPs genotyped in the additional Suita sample was weakened by increasing the sample size, the strongest association for LDL-C was observed for rs3846662, indicating this SNP as a good candidate for replication. Although it is possible that unrecognized genes or loci influencing LDL-C levels could be newly identified by increasing the sample size of the initial screening, the observation that none of the markers (n=368,274) achieved genome-wide significance after Bonferroni correction suggests that there is no master gene involved in determining LDL-C levels.

Because it can be speculated that multiple rare alleles with a much greater effect may contribute to variations in LDL-C levels in Japanese, we sequenced the 20 exons of HMGCR in 48 subjects with high or low LDL-C levels. Despite our anticipation, we failed to identify any unrecognized SNP with a larger effect.

One of the limitations of the current study is the use of the Friedewald formula to estimate LDL-C levels. However, a recent study conducted in 27,331 women demonstrated a significant correlation between the fasting LDL-C concentration by Friedewald equation and the direct method. Nearly identical results were obtained for fasting LDL-C levels by the 2 methods in terms of the ability to predict cardiovascular disease, questioning the advantage of the direct method over the Friedewald formula. Therefore, it is unlikely that the use of the Friedewald formula altered the outcome of the results significantly.

We have replicated the association of rs3846662 with LDL-C in 2 independent Japanese populations. In contrast to the remarkable effect of HMGCR inhibitors as a cholesterol-lowering drug, the effect of rs3846662 on LDL-C is rather small, explaining only a fraction. The physiological consequence of genetic polymorphisms on MI using a candidate gene approach.

Although our findings need to be tested in a larger sample, the LDL-associated functional SNP, rs3846662, identified through GWAS appears to confer susceptibility to MI in Japanese. The GWAS approach is a powerful tool for identifying genes involved in pathogenic pathways and will provide new clues to fundamental strategies for disease prevention and therapy. The possible candidate for future validation may be found in the GWAS data included in the Supplement.

In conclusion, the previously reported association of rs3846662 with LDL-C levels was replicated in Japanese populations.

Acknowledgments

We thank all those who participated in the study. In addition, we gratefully acknowledge all the members of Suita City Health Center and the Suita Medical Association. The technical assistance of Ms Hiromi Sawamurara was much appreciated.

The present study was supported by a research grant from the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation, Japan.

References


