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RESEARCH ARTICLE

CD36 single nucleotide polymorphism is associated with variation in low-density lipoprotein-cholesterol in young Japanese men

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Abstract

Macrophages uptake oxidized low-density lipoprotein (LDL) via a scavenger receptor such as CD36 from plasma, and then become foam cells. We examined the association of CD36 gene single nucleotide polymorphisms (SNPs) with certain metabolic characteristics in a young male Japanese population ($n = 494$). The G allele in a SNP located at +30215 on the 3′-untranslated region (UTR) was significantly correlated with the plasma LDL-cholesterol concentrations ($r = 0.13$, $p < 0.01$). The difference in LDL-cholesterol concentrations was 10 mg dl^{-1} between GG- and AA-genotype carriers ($p < 0.05$). The CD36 gene SNP is a novel maker of the variation in the LDL-cholesterol levels in young Japanese men.

Keywords: *CD36; single nucleotide polymorphism; LDL-cholesterol*

Introduction

CD36 (or fatty acid translocase) is a transmembrane glycoprotein of the class B scavenger receptor family, which is expressed on macrophages, platelets, microvascular endothelium, adipocytes, skeletal muscle and smooth muscle cells (Greenwalt et al. 1992, Koonen et al. 2007, Podrez et al. 2007). Macrophages uptake oxidized low-density lipoprotein (LDL) via a scavenger receptor such as CD36 from plasma, and then become foam cells. Although regulation of lipid metabolism in rodents and humans is quite different, these findings suggest that CD36 may mediate oxidized LDL removal at a common essential step for both species.

Macrophages isolated from CD36-null mice are profoundly deficient in oxidized LDL uptake and foam cell formation (Febbraio et al. 2000). Breeding the CD36 deficiency onto a proatherogenic apoE-null background resulted in a phenotype that was significantly protected from developing atherosclerotic lesion (Zhao et al. 2005). Furthermore, CD36 is necessary for the transport of long-chain fatty acids in adipocytes, heart and skeletal muscle, where these fatty acids are important substrates for energy production. Transgenic mice overexpressing CD36 in skeletal muscle demonstrated enhanced fatty acid oxidation, decreased circulating fatty acids and triacylglycerols, and decreased fat deposition in atheroma (Ibrahimi et al. 1999).

In humans, the gene encoding CD36 lies on the chromosome 7q11.2 (Rac et al. 2007). CD36 deficiency is more prevalent in Asian (Ikeda et al. 1989) and African (~3%) than in North American populations (0.34%) (Furuhashi et al. 2003, Yamamoto et al. 1990). A biochemical study demonstrated that CD36

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deficiency in monocytes, was found in 5.7% of the population investigated and was associated with elevated LDL-cholesterol (LDL-C) levels in apparently healthy Japanese males (Yanai et al. 2000). A few recent studies have suggested an association of specific CD36 polymorphisms with cardiovascular risk factors in humans (Lepretre et al. 2004a, b). In a French diabetic population, the -178 A/C single nucleotide polymorphism (SNP) of the promoter region in the CD36 gene was associated with adiponectin levels, a marker for insulin sensitivity (Lepretre et al. 2004b). This SNP of CD36 was also associated with higher concentrations of apoB, total cholesterol and LDL-C in an obese French population (body mass index (BMI) >30 kg m^{-2}) (Lepretre et al. 2004a). However, it has not been fully elucidated whether or not the CD36 genotype is associated with LDL-C concentrations and insulin sensitivity, and whether or not a particular genotype contributes to cardiovascular disorders. Therefore, we decided to examine the relationship between CD36 gene polymorphisms and metabolic and cardiovascular characteristics in a young male Japanese population. We have focused exclusively on a young male population (median age 22 years, $n = 494$). Sampling data from a young male population is advantageous for genetic research, because their genetic factors predominantly affect metabolic constituents rather than acquired factors, such as stress, smoking, excessive salt or caloric intake, physical activity or menstrual cycle.

Methods

Study population, physical examination and clinical characteristics

Young healthy Japanese men were consecutively recruited from an annual health maintenance program at Keio University between 1999 and 2004. Approximately 70% of the health checkup examinees agreed to enrol in the study in each year. There were a total of 515 enrolled subjects at the end of the study. They underwent (1) a physical examination, including measurements of BMI, systolic and diastolic blood pressure (BP) and heart rate (HR); (2) fasting blood chemistry, including glucose, immunoreactive insulin (IRI), total cholesterol (TC), LDL-C, high-density lipoprotein (HDL)-cholesterol (HDL-C), triglycerides, free fatty acids; and (3) a medical questionnaire for personal and familial medical histories. The rate–pressure product (RPP) was calculated by the equation, HR × systolic BP, as an index of cardiac energy expenditure. The insulin resistance index based on a homeostasis model assessment (HOMA-R) was calculated as [fasting blood glucose (mg dl^{-1}) × IRI (μU ml^{-1})/405]. At the end of sample collection, the data from 494 subjects were statistically analysed after exclusions due to genetic or acquired diseases, or failure either in DNA extraction or in biochemical analyses. The Institutional Ethic Committee of Keio University approved this study and all the participants gave written informed consent. Concentrations of fasting plasma glucose, IRI, TC, LDL-C, HDL-C, triglycerides, and free fatty acids were measured by the previously described methods (Morii et al. 2005).

SNP genotyping

Genomic DNA was extracted from peripheral blood lymphocytes by a standard protocol. To handle a large number of samples, the TaqMan polymerase chain reaction (PCR) method (Applied Biosystems, Foster City, CA, USA) was used to genotype SNPs. We examined three SNPs: +9794 located on intron 2, +10214 located on intron 3 and +30215 located on 3′-untranslated region (UTR) of the CD36 gene (Figure 1). The sequences of PCR primers for genotyping are shown in Table 1. All the SNPs are listed in the dbSNP Database from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/). PCR was performed with a GeneAmp System 9700 thermal cycler (Applied Biosystems) under the following conditions: the initial denaturation at 95°C for 10 min, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. The fluorescence level of PCR products was measured with an ABI PRISM 7900 Sequence Detector (Applied Biosystems), resulting in clear identification of the CD36 gene genotypes.

Statistical analysis

Genotype and allele frequencies between the genotype groups were assessed by the χ^2-test for Hardy–Weinberg equilibrium. Linkage disequilibrium between the three SNPs was calculated using SNPAlyze V3.1Pro (Dynacom Inc., Chiba, Japan). The analysis was conducted using log-transformed variables because the distributions of all the biomarkers except uric acid were right-skewed and the Kolmogorov–Smirnov statistic denied normal distributions. The relationship between the parameters was examined using Pearson's correlation. The results of clinical characteristics were compared by analysis of variance followed by contrast. *p*-Values were corrected with Bonferroni's correction by being multiplied by 3 in the analysis of variance followed by contrast, as multiple comparisons for three SNPs were carried out in this study. Stepwise multivariate linear regression analysis was performed to predict LDL-C concentrations from both genetic and biochemical parameters. Statistical analyses were carried out using the SPSS statistical program (SPSS V15, Chicago, IL, USA).

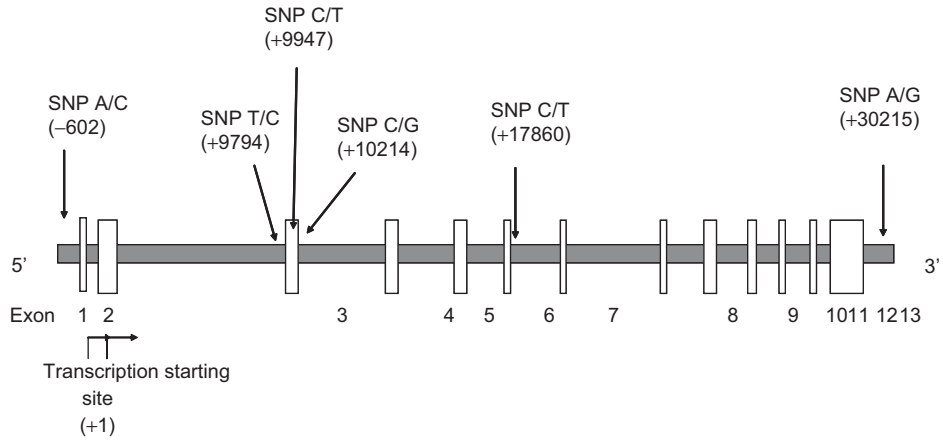

Figure 1. Schematic representation of the CD36 gene from the 5′ to the 3′-untranslated region. Three single nucleotide polymorphisms (SNPs) were analysed. Numbering indicates the transcription starting site of the CD36 gene.

Table 1. TaqMan PCR primers and probes used for genotyping (Nucleotide Reference Sequence NT_079595.1).

SNP ID	refSNP ID	VIC probe sequence	FAM probe sequence	Forward primer sequence	Reverse primer sequence
9794[a]	rs3173798	TGCTATGGAAAAGAA	CTTGCTATGAAAAAGA	TCCAGAAGTGCCTGTACT-TACTACAAA	TTTAAAAGCAATTGT-ACCTTCTTCGA
10214	rs3212165	AGAAAAGATAAAGAGATTTT	AGAAAAGATAAACAGATTTT	GAAAAAAGTGGAAGATATA-TACATTATCCAAA	CATAGGGAAGTTATT-TGATACTGGTAAGTTT
30215	rs7755	TTTGCTATCATAATCG	TTTGCTGTCATAATC	AAGCGTAGACTATGCATTG-TTATTCATT	TTTAATGGTTGAAAC-CTGTCTTTATGAG

Single nucleotide polymorphism (SNP) ID indicates the position within the CD36 gene; [a]antisense direction.All sequences are in the 5′ to 3′ direction.

Results

Table 2 demonstrates anthropometric and biochemical data of the study subjects. For each CD36 SNP, +9794 located on intron 2, +10214 located on intron 3, +30215 located on 3′-UTR (Figure 1), the genotyping results were proved to satisfy the Hardy–Weinberg equilibrium because no statistically significant differences in the allele frequencies were detected between the observed and the expected values by χ^2-test. Also, the three SNPs did not show any linkage disequilibrium.

We examined the correlation between the parameters. Age demonstrated weak, but significant correlation with LDL-C and TC concentrations, respectively ($r=0.10$, $p<0.05$, and $r=0.10$, $p<0.05$ by Pearson's correlation, respectively). BMI showed substantial correlation with LDL-C and TC concentrations, respectively ($r=0.32$, $p<0.0001$ and $r=0.28$, $p<0.0001$ by Pearson's correlation, respectively). The G allele at the SNP located at +30215 on 3′-UTR was weakly but significantly correlated with LDL-C and TC concentrations, respectively ($r=0.13$, $p<0.01$ and $r=0.12$, $p<0.01$ by Pearson's correlation calculated as AA = 0, AG = 1 and GG = 2), whereas it was not correlated with HR, RPP, BMI, IRI and HOMA-R. Next, we examined the association between the genotypes and clinical parameters. LDL-C and TC concentrations were significantly higher in GG- than in AA-genotype carriers ($p<0.05$ by contrast). The differences in the median LDL-C and TC concentrations between GG- and AA-genotype carriers were 10 and 11 mg dl^{-1}, respectively. In addition, HR and RPP were significantly higher in GG- than in AA+AG-genotype carriers ($p<0.05$ and $p<0.05$ by contrast, respectively). IRI and HOMA-R tended to be higher in GG- than in AA+AG-genotype carriers ($p=0.09$ and $p=0.09$ by contrast, respectively).

In the stepwise multivariate linear regression analysis, LDL-C concentration was the dependent variable**,** whereas age, BMI and the 3′-UTR CD36 SNP were the independent variables. The most significant multivariate regression was determined to be: log LDL-C = 0.504 ($t=1.937$) + 0.313 × log BMI ($t=7.3$) + 0.10 × log Age (years) ($t=2.3$) + 0.09 × the G-allele of 3′-UTR SNP (AA : AG : GG = 0 : 1 : 2) ($t=2.1$) ($p<0.05$).

In contrast, for the SNPs at positions +9794 and +10214, neither significant correlation nor association was found between genotypes and clinical characteristics (data not shown).

Discussion

The present study demonstrates that the CD36 gene polymorphism is weakly, but significantly correlated with the LDL-C concentration in healthy young Japanese

Table 2. Clinical measures for each genotype carrier at single nucleotide polymorphism position +30215 in the whole study population.

Characteristic	Entire study population (n=494)	AA genotype (n=167)	AG genotype(n=244)	GG genotype (n=83)
Age (years)	22 – **22** – 24	22 – **22** – 23	22 – **23** – 24	22 – **22** – 23
Systolic blood pressure (mmHg)	112 – **121** – 131	112 – **123** – 132	111 – **119** – 129	114 – **121** – 133
Diastolic blood pressure (mmHg)	64 – **69** – 75	65 – **70** – 75	63 – **69** – 75	66 – **71** – 76
Heart rate (bpm)	66 – **73** – 82	65 – **73** – 81	65 – **72** – 81	67 – **78** – 86$^{\sharp}$
Rate–pressure product(bpm × mmHg)	7506 – **8832** – 10455	7524 – **9087** – 10530	7278 – **8584** – 10062	7749 – **9580** – 11049$^{\sharp}$
Body mass index (kg m^{-2})	19.5 – **20.9** – 22.5	19.5 – **20.8** – 22.3	19.5 – **20.9** – 22.6	19.6 – **21.1** – 22.6
Immunoreactive insulin (μU ml^{-1})	4.2 – **5.7** – 7.9	4.5 – **5.6** – 7.2	4.0 – **5.5** – 8.0	4.7 – **6.5** – 8.6†
Fasting glucose (mg dl^{-1})	80 – **85** – 89	81 – **85** – 90	79 – **84** – 89	82 – **86** – 90
HOMA-R index	0.87 – **1.20** – 1.65	0.89 – **1.19** – 1.56	0.82 – **1.17** – 1.65	0.97 – **1.34** – 1.85†
Total cholesterol (mg dl^{-1})	155 – **174** – 196	153 – **168** – 193	159 – **175** – 195	154 – **179** – 204*
Triglyceride (mgdl^{-1})	47 – **64** – 92	47 – **65** – 91	47 – **63** – 91	49 – **66** – 98
LDL-cholesterol (mg dl^{-1})	83 – **99** – 117	81 – **97** – 114	87 – **99** – 117	80 – **107** – 128*
HDL-cholesterol (mg dl^{-1})	53 – **61** – 69	52 – **61** – 68	54 – **60** – 70	52 – **61** – 71
Free fatty acid (mEq l^{-1})	0.33 – **0.43** – 0.61	0.34 – **0.46** – 0.63	0.32 – **0.42** – 0.70	0.33 – **0.43** – 0.71

Values are expressed as 25 percentile, **median** and 75 percentile of each parameter. HOMA-R, homeostasis model assessment; LDL, low-density lipoprotein; HDL, high-density lipoprotein.*p<0.05 between AA vs. GG genotype carriers by contrast after Bonferroni's correction; $^{\sharp}p$<0.05 and $^{\dagger}p$=0.09 between AA + AG vs. GG genotype carriers by contrast after Bonferroni's correction.

men. This is, to our knowledge, the first evidence for the correlation between the CD36 gene polymorphism and LDL-C concentration**.** The CD36 SNP (GG vs AA) explains approximately 10 mg dl^{-1} variation of LDL-C and TC concentrations in early adulthood of Japanese men. Lepretre et al. reported a promoter SNP of CD36 is associated with adiponectin levels in a French type 2 diabetic population (Lepretre et al. 2004c). They also proposed that the promoter SNP in CD36 is associated with an atherogenic lipid profile in a French obese population (Lepretre et al. 2004a). Obese AA carriers (BMI >30 kg m^{-2}) of the promoter SNP in CD36 demonstrated significantly higher concentrations of apolipoprotein B, TC and LDL-C. In our preliminary experiment, we examined the association of the promoter SNP with lipid profiles in our population. However, the promoter SNP showed no association with lipid profile (data not shown). Therefore, we searched other SNPs in dbSNP Database from the National Center for Biotechnology Information and discovered that the 3′-UTR SNP at position +30215 affects lipid profile in young Japanese men. These findings suggest that the different SNPs of the two populations individually contribute to the variation in LDL-C levels.

Yanai et al. (2000) reported that serum TC and LDL-C levels were significantly elevated in type II CD36 deficiency (platelet CD36– and monocyte CD36+) using anti-CD36 antibody. Our study demonstrates that the GG genotype of the 3′-UTR SNP is associated with higher LDL-C concentrations. CD36 removes approximately 50% of oxidized LDL from plasma (Rac et al. 2007). CD36 is not specific to oxidized LDL, and CD36 also has a high affinity to native LDL (Calvo et al. 1997, 1998). CD36 deficiency may elevate free fatty acid and then may also increase hepatic very-low-density lipoprotein (VLDL) production and may induce an increase in serum LDL-C. These observations support the hypothesis that CD36 plays a key role in lipid metabolism. However, the prevalence rate of CD36 deficiency using anti-CD36 antibody was reported as ~3–6% in Asian populations (Ikeda et al. 1989, Yanai et al. 2000). Our study demonstrates the frequency of the GG genotype of the 3′-UTR SNP was 16.8% (83/494). Therefore, it is unlikely that all the GG carriers of 3′-UTR CD36 SNP are identical with the CD36 deficiency. Although we did not examine the biochemical CD36 function**,** a subtle change in expression or function of CD36 may be linked to the 3′-UTR CD36 SNP and contribute to the variation of LDL-C concentrations. SNPs in UTRs are involved in several post-transcriptional regulatory pathways that control the localization, stability and translation efficiency of mRNA (Pesole et al. 2001).

Controversy remains over the relationship between CD36 gene and lipid metabolism. A study with mouse adenovirally overexpressing CD36 indicated that CD36 did not play a significant role for either HDL-C or LDL-C concentration (de Villiers et al. 2001). These findings are inconsistent with the earlier French and the present Japanese genetic epidemiological studies. The discrepancy can be explained by the following points: (1) species diversity in lipid metabolism between mice and humans, and (2) differences between a model based on

the overexpression of a single gene and human beings in whom multiple environmental and genetic factors interact in the expression of phenotypes. Spontaneously hypertensive rats (SHR) derived from a NIH colony provides a spontaneous CD36 deficiency model, which displays several characteristics of metabolic syndrome such as hypertension and defects in glucose and fatty acid metabolism (Aitman et al. 1999, Aitman 2001, Pravenec et al. 2001). In the SHR, transgenic expression of Cd36 ameliorates insulin resistance and lowers serum fatty acids levels (Pravenec et al. 2001). Yanai et al. reported that human CD36 deficiency demonstrates higher LDL-C levels (Yanai et al. 2000), although they did not report SNPs of the CD36 gene. We discovered that GG-genotype carriers of the 3′-UTR CD36 SNP are associated with increased HR, PPR, TC and LDL-C levels and also tend to be insulin resistant. These findings support the hypothesis that genetic CD36 deficiency causes cardiometabolic abnormalities through dyslipidemia. The link between abnormal lipid metabolism and insulin resistance has been well established *in vivo*. The higher mean HR in the GG-genotype carriers may be partially explained by the heightened sympathetic nervous system activity due to compensatory hyperinsulinemia (Watanabe et al. 1999, Esler et al. 2001, Lindmark et al. 2003). In healthy individuals who are insulin sensitive, experimentally induced hyperinsulinemia decreases the level of circulating free fatty acids, hence reducing VLDL production (Lewis et al. 1995, Lewis et al. 1997). In contrast, insulin resistance in adipose tissue increases the free fatty acid flux toward insulin-sensitive tissues, such as muscle and liver, resulting in increased lipoprotein production (Lewis et al. 2002). Significant association between the CD36 G-allele and LDL-C concentrations became obscure after adjusting confounding factors such as BMI and IRI by analysis of covariance (data not shown). This implies that the effect of the CD36 gene on LDL-C should be partially dependent on body fat or insulin resistance. Therefore, the higher HR and LDL-C levels in the GG-carriers of 3′-UTR SNP should be partially attributable to insulin resistance.

High concentrations of LDL-C are a major risk factor for coronary atherosclerosis which produces fatty streaks in adolescence (Berenson et al. 1992). Results of the Pathobiological Determinants of Atherosclerosis in Youth emphasized the importance of risk factor modifications in the young to delay development of the clinically significant atherosclerotic lesions (Zieske et al. 2002), which would promote future development of hypertension and premature atherosclerosis and could increase the risk of cardiovascular diseases later in life (Kohen-Avramoglu et al. 2003). The 3′-UTR CD36 SNP could be a useful tool for preventing a series of derangements of cardiovascular and metabolic disorders in young Japanese men.

The study has certain limitations. Because the simultaneous analysis of genetic and environmental factors is extremely difficult, we chose to perform the study exclusively with young Japanese men, as younger subjects are less influenced by environmental factors. Multivariate stepwise regression analysis clarified that LDL-C levels were primarily predicted by BMI rather than the CD36 genotypes even in the relatively lean young population (median age 22 years old and BMI 20.9 kg m^{-2}). Moreover, genetic and environmental interactions may be different in young versus older individuals. However, CD36 SNP can discriminate a 10 mg dl^{-1} difference in LDL-C levels between GG- and AA-genotype carriers in young Japanese men. As the difference in LDL-C levels between the 25th and 75th percentile of this age group was from 117 to 83 (34 mg dl^{-1}), the impact of CD36 SNP on LDL-C is substantial. Besides, we chose to exclude women from this study because their profiles would be profoundly affected by ovarian hormones. Therefore, our findings are limited to a young male population.

In conclusion, we discovered that the CD36 3′-UTR SNP is a genetic marker for LDL-C levels and a potential marker of pleiotropic cardiovascular and metabolic profiles in young Japanese men. Further genetic studies in various different populations should be carried out to confirm this observation.

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