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ORIGINAL ARTICLE

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Hypertriglyceridemia associated with amino acid variation Asn985Tyr of the *RP1* gene

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Abstract Factors predisposing to the phenotypic features of hypertriglyceridemia have not been clearly defined. Here we report an association between a missense coding region polymorphism Asn985Tyr in the retinitis pigmentosa 1 gene (*RP1*) and plasma triglyceride (TG) levels in 332 adult individuals from an east-central area of Japan. Age and gender-adjusted levels of LDL-cholesterol, TG, and HDL-cholesterol were analyzed. When we separate the subjects into two genotypic groups regarding this amino acid variation, those who lack the 985-Asn allele (asparagine at residue 985) had significantly higher plasma TG levels than the others who had at least one 985-Asn allele (mean: 175.8 mg/dl vs 123.3 mg/dl; $P=0.0006$, Mann-Whitney test). Similarly, the former subjects had significantly lower HDL-cholesterol levels than the latter (mean: 48.0 mg/dl vs 53.8 mg/dl; $P=0.038$). Of the 280 individuals without a 985-Asn allele, approximately half of the individuals presented with hypertriglyceridemia, whereas only a quarter were hypertriglyceridemic among 52 individuals with the 985-Asn allele ($P=0.04$). Although this SNP marker may itself be in linkage disequilibrium with other unexamined functional variants within this locus, our data suggest that genetic variation at the *RP1* locus is one of the likely candidate determinants for plasma triglyceride and HDL-cholesterol metabolisms.

Keywords Retinitis pigmentosa 1 · Plasma triglyceride (TG) · HDL cholesterol (HDL-C) · Single nucleotide polymorphism (SNP) · Modifier gene

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Introduction

Accumulating evidence derived from clinical, epidemiological and experimental studies suggests that lipid and lipoprotein concentrations in plasma reflect the influence of complex genetic loci, at least in part (Zannis and Breslow 1985); defects are known only for some classical types of hyperlipoproteinemia that affect members of families, following Mendelian traits. However, the genetic mechanisms responsible for most types of familial dyslipoproteinemia appear to be complex, not monogenic.

Hyperlipoproteinemia is one of the most important predictors of cardiovascular diseases, determined by genetic risk factors as well as environmental factors (Hegele, 2001; Cullen et al. 1997). Clarification of the genetic risk factors is essential for diagnosis, prevention, and early effective treatment of hyperlipidemias. In addition, if the relevant genes were identified, the pathogenesis of these diseases would be explained by the variation of those genes or adjacent genes. Several gene polymorphisms have previously been associated with plasma lipoprotein abnormalities (reviewed in Goldstein et al. 1995). However, those are clearly only a part of the all determinants.

In the course of serial investigations of population genetics for hyperlipoproteinemia in a cohort of an area located in east-central Japan, we recognized a correlation between lipoprotein variations and polymorphism of the *RP1* locus. Here we focused on the analysis of the potential effect of genetic variation in a locus encoding

the retinitis pigmentosa 1 gene (*RP1*), investigating the correlation of the plasma lipoprotein profile with the genetic variation of this gene.

Subjects and methods

Subjects

Subjects were obtained from the participants of the cohort study of an area located in east-central Japan that was originally carried out concurrently with health check screening. The entire 22,228 participants for the health check were initially screened by distinctive criteria consisting of two issues that define individuals harboring hyperlipidemic risks (T-Chol ≥250 mg/dl, or HDL-C ≤ 35 mg/dl). From about 2,000 subjects sufficing these criteria, 332 individuals were randomly selected for the present study. All the selected participants were volunteers who gave their written informed consent prior to this study, which was approved by the Institutional Review Boards of the Research Consortium. Physical and clinical profiles of these subjects (ages, male to female ratios, body mass indices, and plasma lipoprotein and lipid levels) are indicated in Table 1. None of the selected participants had medical complications or was undergoing treatment for conditions known to affect plasma lipoprotein levels, such as pituitary disease, hypo- or hyperthyroidism, diabetes mellitus, liver disease, and renal disease. None was receiving anti-hyperlipidemic therapy. The selected participants visited lipid clinics for detailed examination of their lipoprotein profile and other clinical parameters. Blood was collected after 12–16 h of fasting in each participant. Genomic DNA was extracted as previously described (Shinohara et al. 2001).

Measurement of lipoproteins

Lipid and lipoprotein concentrations were measured by procedures described previously (Hattori et al. 2002); i.e., plasma cholesterol and triglyceride concentrations were assayed enzymatically, and concentrations of HDL-cholesterol were determined by the $MgCl_2$-dextran precipitation method. LDL-cholesterol concentration was calculated by subtracting HDL-cholesterol level from the fraction of both LDL-cholesterol and HDL-cholesterol as described elsewhere (Ishii et al. 2002).

Genotyping for single nucleotide polymorphism (SNP) in the *RP1* gene

PCR amplification of the polymorphism at the *RP1* locus was performed using conditions described previously (Nakazawa et al. 2001; Harada et al. 2001); primer sequences used are as follows: forward primer, 5′-CCTGAGGCTATTGCTCATCATTC-3′; reverse primer, 5′-TAGGCAAAGGCCACAGGAG-3′. The surrounding sequence of the amino acid-substituting SNP in the *RP1* gene, i.e. polymorphic nucleotides A or T in codons 985 (Aat/Tat), as well as primer sequences and experimental conditions, were obtained from published reports (Haga et al. 2002). SNP genotyping was performed by Invader assay (Third Wave Technologies. Madison, WI) using PCR products of the flanking sequence and probes of the Invader assay designed and synthesized by the supplier (Ohnishi et al. 2001).

Table 1 Physical and clinical profiles of the subjects. The*P* value was calculated by Mann-Whitney test, except for gender; χ^2 test was conducted for distribution analysis of gender. Values are expressed in mean ± SD;*NS* not significant

	Asn (+)	Asn (−)	*P* value
Number	52	280	–
Gender (M/F)	16/36	125/155	NS
Ages (years)	60.3 ± 8.4	60.6 ± 10.2	NS
BMI (kg/m^2)	24.7 ± 6.5	23.3 ± 3.5	NS
TG (mg/dl)	123.3 ± 89.8	175.7 ± 127.9	0.0006
TC (mg/dl)	234.2 ± 38.8	237.6 ± 36.9	NS
HDL-C (mg/dl)	53.8 ± 18.6	48.0 ± 15.6	0.04
LDL-C (mg/dl)	145.2 ± 82.1	184.6 ± 163.8	NS

Statistical analysis

Plasma levels of lipoproteins were adjusted by gender and ages of the subjects, using standard data obtained from 11,994 individuals of a 2001 cohort study of the general Japanese population. Coefficients of skewness and kurtosis were calculated to test deviation from a normal distribution. Because the clinical and biochemical traits in each genotypic group did not always distribute normally, we applied a non-parametric Mann-Whitney test or an analysis of variance (ANOVA) with linear regression analysis as a post hoc test ($P < 0.05$) to compare those traits among groups divided by a single SNP (Ota et al. 2001). Fisher's exact test was used to compare differences in the prevalence of hypertriglyceridemia among the population. Chi-square tests were invoked to detect Hardy-Weinberg equilibrium.

Results

To carry out a correlation analysis of the potential effect of the amino acid substituting SNP, Asn985Tyr, in the *RP1* gene, 332 individuals were genotyped. The analyzed subjects were selected from participants for a cohort of an area initially screened by criteria that define individuals harboring hyperlipidemic risks. Because of initial screening for the health check assessment, the basal level of each value was a little higher than that of data from the general population of Japanese (Table 1). However, the average differences were less than 0.5×SD in general. When the subjects were genotypically categorized into three groups (two homozygous minor 985-Asn allele carriers, 50 heterozygous carriers and 280 homozygous 985-Tyr allele carriers), no deviation of genotype frequencies from Hardy-Weinberg equilibrium was observed ($P = 0.99$, χ^2-test).

The distribution and mean values of TG, LDL-cholesterol and HDL-cholesterol were analyzed among these groups. Although the LDL-cholesterol levels among these three groups were almost the same, TG and HDL-cholesterol levels were significantly different. Plasma TG levels of homozygous minor 985-Asn allele carriers ($n = 2$), heterozygous carriers ($n = 50$) and homozygous 985-Tyr allele carriers ($n = 280$) were 114.4 ± 51.7 mg/dl, 123.7 ± 91.3 mg/dl, and 175.7 ± 127.9 mg/dl, respectively, indicating a co-dominant TG lowering effect of minor 985-Asn allele ($r = 0.15$, $P = 0.0056$). Similarly, plasma HDL-cholesterol levels were 41.8 ± 3.2 mg/dl, 54.4 ± 18.8 mg/dl, and 48.0 ± 15.6 mg/dl, where a dominant effect was assumed. Because the subjects carrying a minor 985-Tyr allele was rare ($n = 2$), suitable categorization of the subjects was

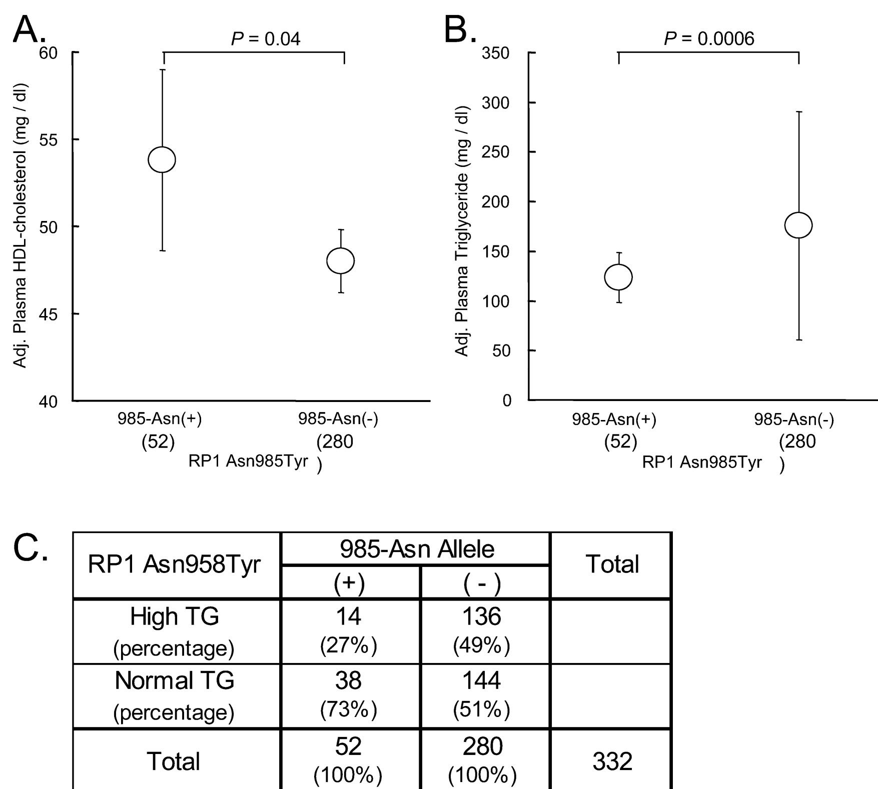
re-considered. We thus separated the subjects into two groups, those who lack the 985-Asn allele (asparagine at residue 985) and those who bear at least one 985-Asn allele, the former subjects had significantly higher plasma TG levels than the latter (mean ± SD: 175.8 ± 127.9 mg/dl vs 123.3 ± 89.8 mg/dl; $P = 0.0006$). Similarly, the former subjects had significantly lower HDL-cholesterol levels than the latter (mean ± SD: 48.0 ± 15.6 mg/dl vs 53.8 ± 18.6 mg/dl; $P = 0.038$) (Table 1).

Since the data presented here suggested involvement of the *RP1* locus in expression of the hypertriglyceridemic phenotype in this population, we correlated the manifestation of hypertriglyceridemia with respect to presence or absence of 985-Asn or 985-Tyr alleles of *RP1*. Hypertriglyceridemia was defined as plasma triglyceride level above the reference values after age and sex adjustment (TG > 150 mg/dl). This criterion is based on a recommended pre-clinical level of hyperlipidemic individuals who have to be alerted for high risk for ischemic heart disease. It classified 150 subjects as having hypertriglyceridemia among the study population consisting of 332 individuals. Of the 280 individuals without the 985-Asn allele, 136 presented with hypertriglyceridemia (49%), whereas only 14 did so among 52 individuals with 985-Asn allele (27%) The difference of the distribution was significant when tested by Fisher's exact test ($P = 0.04$; relative risk = 0.45; 95% confidence interval = 0.25–0.79) (Fig. 1).

Discussion

Multiple environmental and genetic factors appear to influence the phenotypic variation of the plasma lipoprotein profile. Life-style variations among individuals in physical exercise, control of food-calorie intake, proper understanding and awareness to the disease and compliance with treatments, including medications, should influence lipoprotein variations among individuals. In addition, unidentified genetic modifiers may cause variability among the individuals.

Hypertriglyceridemia is one of the important risk factors of ischemic heart disease (Matsuzawa 1995; Norioka 2000). In the present study, we showed a tendency that hypertriglyceridemic patients lacked a minor variant allele of *RP1* gene (985-Asn). An association study revealed an elevating effect of plasma total triglyceride and a lowering effect of HDL-cholesterol by the lack of this *RP1* 985-Asn allele. The high prevalence (49%) of hypertriglyceridemia (mean ± SD: 175.7 ± 127.9 mg/dl) among the individuals with this genotype (985-Tyr/Tyr) is in contrast to the scarceness (27%) of hypertriglyceridemia (mean ± SD: 123.7 ± 91.3 mg/dl and 114.4 ± 51.7 mg/dl) among the rest of the study subjects (985-Tyr/Asn and 985-Asn/Asn, respectively). It might be interesting to test if distribution of familial combined hyperlipidemia patients among the subject groups of our cohort is different, although insufficient information of the subjects did not allowed us to analyze it at this point. Nevertheless, our results indicated



RP1 Asn958Tyr	985-Asn Allele		Total
	(+)	(-)	
High TG (percentage)	14 (27%)	136 (49%)	
Normal TG (percentage)	38 (73%)	144 (51%)	
Total	52 (100%)	280 (100%)	332

Fig. 1A–C Comparisons of plasma lipoprotein levels among genotypically determined groups according to the RP1 Asn985Tyr variation. **A** Adjusted plasma HDL-cholesterol levels were compared. **B** Adjusted plasma triglyceride levels were compared by Mann-Whitney test ($P < 0.01$). *Open circles* represent mean values. *Error bars* represent the 95% confidence interval. **C** Distribution analysis of hypertriglyceridemic patients among *RP1* Asn985Tyr classified-genotypes: *985-Asn*(+)/(−). The 2×2 table was analyzed by Fisher's exact test ($P < 0.05$). Clinical phenotypes were defined by adjusted plasma total triglyceride (*TG*) levels above 150 mg/dl

that *RP1* variation might modify the lipoprotein phenotype of plasma triglyceride and HDL-cholesterol.

The suspected link between functional changes of RP1 protein and plasma triglyceride metabolism was unexpected. *RP1* was originally identified as a gene response to in vivo retinal oxygen levels in mouse model, and a causative mutation gene for a human autosomal dominant type of retinitis pigmentosa (Pierce et al. 1999; Sullivan et al. 1999). RP1 protein is a cytosolic protein that mainly exists in connecting cilia of retina photoreceptor cells, and was suggested to participate in transport of proteins or maintenance of cilial structure. Although the exact tissue distribution of the *RP1* gene product has not been clarified yet, Western blot analysis of previous study in mouse implied its existence in several types of tissues other than in retina, including skeletal muscle (Liu et al. 2002). If it affects the regulation of triglyceride metabolism in plasma, the Asn985-Tyr variation might bring about conformational changes of RP1 protein, resulting in considerable differences in metabolic function. The possibility cannot be ruled out, however, that this SNP marker may itself be in linkage disequilibrium with other unexamined functional variants localized in close proximity to Asn985Tyr. The contribution of another four non-synonymous coding SNPs in *RP1* gene that are archived in the dbSNP database may have to be tested, although a statistically significant contribution of rare SNPs is less likely. A true mechanistic basis for the associations needs to be clarified (Shastry 2002). Functional studies would be required for ruling out the other possibilities. In addition, it would be important to confirm the association at geographically distinct populations and other ethnic groups, including domestic different cohorts as well as those from other countries. Those studies will be conducted in the future.

In summary, we noted an association between amino acid variation of the retinitis pigmentosa 1 (*RP1*) gene and hypertriglyceridemia in 332 subjects from an area of the east-central region of Japan. Given our genetic results, we expect that the effects of multiple genes, both additive and interactive (Lalouel and Rohrwasser 2001; Takada et al. 2002), will eventually prove to be responsible for many cases of common, inherited, mixed dislipidemias.

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