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Clinical and Experimental Ophthalmology 2008; **36**: 437–442
doi: 10.1111/j.1442-9071.2008.01791.x

Original Article

Correlation between *CFH* Y402H and *HTRA1* rs11200638 genotype to typical exudative age-related macular degeneration and polypoidal choroidal vasculopathy phenotype in the Japanese population

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Abstract

Background: Typical exudative age-related macular degeneration (AMD) and polypoidal choroidal vasculopathy (PCV) are two of the major macular diseases found in Asians. Although genomic studies have shown a contribution by *CFH* and LOC387715/*HTRA1* polymorphisms to the development of these two diseases, the correlation of the clinical phenotypes to these genotypes has not been determined in Asian patients.

Methods: The prevalence of the *CFH* Y402H and *HTRA1* rs11200638 genotypes was determined in 116 patients with typical exudative AMD and in 204 patients with PCV. Potential correlations of these polymorphisms were tested retrospectively and cross-sectionally for bilaterality of the disease, final visual acuity and the greatest linear dimension of the choroidal neovascular (CNV) lesion.

Results: There was no significant difference in the incidence of *CFH* Y402H ($P=0.598$) and *HTRA1* rs11200638 ($P=0.290$) between eyes with typical exudative AMD and with PCV. There was a significant association between the lesion size and *HTRA1* rs11200638. For eyes with typical AMD, the size of the lesion ($6363 \pm 2837\,\mu m$) was significantly larger in the high-risk homozygous group (AA), than in the low-risk homozygous group (GG) ($3866 \pm 1947\,\mu m$; $P=0.0003$). The same tendency was observed for the size of the lesion in PCV cases (homozygous group: $6347 \pm 2673\,\mu m$, non-risk homozygous group: $4405 \pm 2066\,\mu m$, $P=1.3\times10^{-5}$).

Conclusions: A common genetic background may exist between typical exudative AMD and PCV patients. Among the patients with these two clinical entities, those with a homozygous *HTRA1* rs11200638 risk allele had larger CNV lesions.

Key words: age-related macular degeneration, choroidal vasculopathy, genetic analysis.

Introduction

Macular diseases are the most common cause of visual impairment in the elderly in developed countries.[1,2] The impact of macular diseases is similar for Caucasians and Asians, but the prevalence of the different types of macular disease differs between the two populations.[3,4] In Asians, typical exudative age-related macular degeneration (AMD) and polypoidal choroidal vasculopathy (PCV) account for around 90% of all cases of senile macular disease. Of these, the percentage of eyes with PCV is about 30–50%, and geographic atrophy (GA) is quite rare in Asians.[3,5] Although the pathophysiology of typical AMD and PCV has been believed to be different,[6] there is an overlap in the clinical findings for both entities, at least in type I choroidal neovascularization (CNV).[7]

Genetic studies of Caucasian patients with AMD have detected two disease-contributing polymorphisms.

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Received 14 November 2007; accepted 6 June 2008.

Complement factor H (*CFH*) on chromosome 1q32 has been identified by many studies as a major AMD-susceptible gene polymorphism in Caucasians.[8–12] In addition, biochemical and pathological studies have shown that a *CFH* Y402H polymorphism may be involved in AMD.[13,14]

Chromosome 10q26 has also been found to carry another important AMD susceptibility locus. Several studies have used refined linkage disequilibrium (LD) mapping and case–control association studies to probe the most susceptible alleles, LOC387715 rs10490924 and *HTRA1* rs11200638, for AMD.[15–18] In Asians, replication studies of cases of typical AMD have been reported for *CFH* Y402H.[19–24] In those with typical AMD, the contribution from *CFH* Y402H was found to be less strong than that for Caucasians. This is due to the much lower frequency of the risk allele, Y402H, that is seen in <6% of Asians compared with >35% of Caucasians.[19–25] The relationship of LOC387715 rs10490924 and *HTRA1*1200638 polymorphisms to typical AMD and PCV has been investigated in Asians.[15,26–30] Several studies have shown an almost complete LD in the two SNPs,[15,29,30] with both showing an identical and very strong positive association not only with typical AMD but also with PCV.[30]

Positive correlations have been reported between the phenotypes of Caucasian patients with exudative AMD and the polymorphic genotypes. Goverdhan *et al.* suggested that the risk allele *CFH* Y402H is significantly more prevalent in individuals with predominantly classic CNV, and that those who are homozygous for the *CFH* Y402H genotype tend to have the poorest visual acuity after the photodynamic therapy.[31] Chen *et al.* reported that the *HTRA1* rs11200638 homozygote odds ratios of wet AMD and GA are significantly greater in bilateral than in unilateral cases.[32] In Asians, we were unable to find any studies on the phenotype–genotype correlations.

Thus, the purpose of this study was to determine whether a significant correlation exists between the two major disease-associated polymorphisms, *CFH* Y402H and *HTRA1* rs11200638, and the clinical genotypes in cases of typical AMD and PCV in the Japanese population.

METHODS

Subjects

From April 2005 to December 2006, we studied 116 Japanese patients with typical AMD and 204 patients with PCV at the Center for Macular Diseases, Department of Ophthalmology, Kyoto University Hospital, and at the Department of Ophthalmology, Fukushima Medical University Hospital. All of the patients signed a written informed consent form (Table 1). Although the medical histories of the patients differed, all patients were being treated for exudative lesions. All patients had a complete ophthalmological examination including fluorescein and indocyanine green angiography with a scanning laser ophthalmoscope (HRA2, Heidelberg, Germany).

The inclusion criteria for PCV were the presence of reddish-orange, spheroidal, polypoidal structures detected by slit-lamp biomicroscopy of the macula with a contact lens, and the presence of a branching vascular network of choroidal vessels with terminal aneurysmal dilatations detected by indocyanine green angiography. The clinical presentation and angiographic findings were used to exclude secondary CNV diseases, for example, angioid streaks, degenerative myopia, idiopathic CNV and presumed ocular histoplasmosis syndrome.

Table 1. Characteristics of study population

Variable	tAMD	PCV
Number	116	204
Age (mean)	76.1	73.1
SD	8.27	7.70
Gender (% male)	74.1%	71.5%

PCV, polypoidal choroidal vasculopathy; SD, standard deviation; tAMD, typical exudative age-related macular degeneration.

The visual acuities of both eyes at the final examination were converted to logarithm of the minimum angle of resolution (logMAR) units. Whether the disease was monocular or binocular was also recorded. Before the photodynamic therapy, the greatest linear dimension (GLD) of the CNV lesion was measured on the angiographic images obtained by the HRA2 and the Heidelberg Eye Explorer software (Heidelberg).[33,34]

Genotyping

CFH Y402H rs1016670 (dbSNP build 126) was genotyped using the Taqman SNP assay (Applied Biosystems, Foster City, CA, USA). *HTRA1* rs11200638 was genotyped by direct sequencing with the following PCR primer sets: 5′-GACGTGTGAAGGATTCTATTCGAA-3′ and 5′-GCGTCCTTCAAACTAATGGAACTT-3′. The sequencing reactions were performed by the Dye Terminator method with an ABI PRISM 3730 DNA Analyzer (Applied Biosystems). The alignment of the sequences, SNP discovery and genotyping were performed with Genalys (http://www.software.cng.fr/docs/genalys.html).[35]

Statistical analyses

The deviations of the allelic distributions and bilaterality were assessed with the χ^2-test. The visual acuity of the poorer eye at the final visit and the lesion size were analysed with a one-way ANOVA. *P*-values obtained by these analyses were corrected for multiple testing using the Bonferroni correction by Statistica (StatSoft, Tulsa, OK, USA). The power calculation was made with the R public open software and its tools (http://www.r-project.org/).

RESULTS

CFH Y402H polymorphism and phenotypes

The genotype and allelic distributions for *CFH* Y402H are given in Table 2. The frequency of the risk allele C was 0.129

Table 2. Distribution of single nucleotide polymorphisms CFH Y402H and HTRA1 rs11200638 in Japanese patients with tAMD and PCV

	tAMD (116 cases)		PCV (204 cases)	
	CFH Y402H	HTRA1 rs11200638	CFH Y402H	HTRA1 rs11200638
Genotype	CC/CT/TT	AA/GA/GG	CC/CT/TT	AA/GA/GG
Count	4/22/90	48/47/21	2/43/159	73/88/43
Allele	C/T	A/G	C/T	A/G
Count	30/202	143/89	47/361	234/174
Bilaterality				
Monocular	CC/CT/TT	AA/GA/GG	CC/CT/TT	AA/GA/GG
	1/16/64	29/37/15	1/31/119	50/71/30
Binocular	CC/CT/TT	AA/GA/GG	CC/CT/TT	AA/GA/GG
	3/6/26	19/10/6	1/12/40	23/17/13

PCV, polypoidal choroidal vasculopathy; tAMD, typical exudative age-related macular degeneration.

in typical AMD patients and 0.115 in PCV patients. This difference in the frequency of the C allele was not significant ($P = 0.598$).

We next determined whether a correlation between the risk allele of *CFH* Y402H and the clinical presentation was significant. Six patients with the risk of homozygous CC were studied, and analyses were made between patients having at least one C allele and those having a non-risk TT genotype. Among the patients with typical AMD, those with the C allele showed a tendency to be binocularly affected in 34.6%, and those with the TT genotype in 28.9% (Table 2).

The visual acuity of patients with typical AMD with the C allele was 0.71 ± 0.50, and that for the TT genotype was 0.94 ± 0.55 (logMAR system). In patients with PCV, the correlation between the presence of the C allele and bilaterality (28.9%) and the TT genotype (25.2%) was not significant. The visual acuity for PCV patients with the C allele was 0.70 ± 0.57 and 0.64 ± 0.53 with the TT genotype. Although the differences for bilaterality and visual acuities between the groups was not statistically significant ($P > 0.055$), a power calculation indicated that the number of participants was too small to evaluate the negative findings for either bilaterality or visual acuity.

The GLD of the patients with typical AMD with the C allele was 4976 ± 2349 μm and that for the TT genotype was 5381 ± 2679 μm ($P = 0.491$). The GLD of patients with PCV with the C allele was 5376 ± 2323 μm and that for the TT genotype was 5219 ± 2598 μm ($P = 0.715$). Because the association between GLD and the *CFH* Y402H variant in our sample was not significant, we calculated that we would have to analyse almost 1000 patients in order to achieve a power of 0.8 ($\alpha = 0.05$).

HTRA1 rs11200638 polymorphism and phenotype

The genotype and allelic frequencies for *HTRA1* rs11200638 are given in Table 2. The frequency of the risk allele A of rs11200638 was 0.616 in typical AMD patients and 0.574 in PCV patients. This difference was not significant ($P = 0.290$).

We studied whether there was any significant correlation between the risk allele of rs11200638 and the clinical phenotype. In patients with typical AMD, there was a greater tendency for the AA genotype to be found in patients binocularly affected (39.6%) than in patients with the GG genotypes (28.6%). In patients with PCV, an association of the AA genotype with bilaterality (31.5%) or the GG genotype (30.2%) was not significant (Table 2). A power calculation showed that because of the small number of participants, a statistical evaluation of the genetic influence for both bilaterality and visual acuity could not be done.

In typical AMD, the average GLD size in the patients with GG (3866 ± 1947 μm) was significantly smaller than that in those with AA genotype (6363 ± 2837 μm; $P = 0.0003$; Fig. 1). In PCV, the GLD was significantly smaller for the GG group (4405 ± 2066 μm) than for the AA group (6347 ± 2673 μm; $P = 1.3 \times 10^{-5}$; Fig. 1).

Only one patient was found to have double-risk polymorphisms, namely, CC genotype in *CFH* Y402H and AA genotype in *HTRA1* rs11200638. This patient was diagnosed with typical AMD with a GLD size of 5250 μm in the one eye that was affected. No statistical analysis was possible.

Discussion

We investigated whether a significant correlation exists between two disease-associated polymorphisms with AMD and PCV that are present in both Caucasians and Asians. PCV was first described by Yannuzzi.[36] who called it, idiopathic PCV. After a decade of many studies, the detail characteristics of PCV were finally established.[6] These studies showed that PCV is a distinct clinical entity that is more common in individuals with pigmented skin including Asians. In addition, PCV is a different clinical entity than typical CNV that is found in patients with AMD, and indocyanine green angiograms are necessary to make a definitive diagnosis.[6] Recently, this condition has been referred to as polypoidal CNV, and classified as PCV with

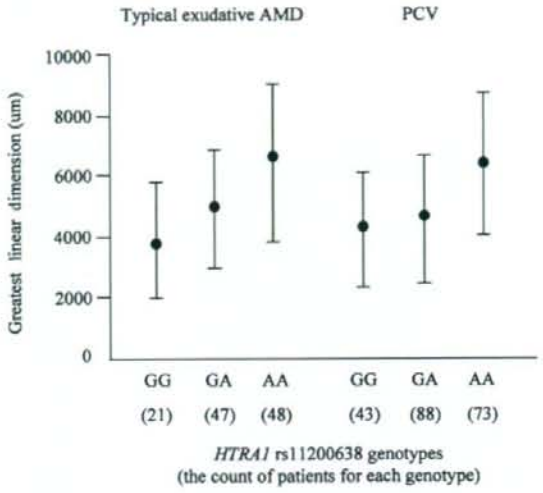

Figure 1. Correlations between the greatest linear dimension (GLD) in patients with typical exudative age-related macular degeneration (AMD) and patients with polypoidal choroidal vasculopathy (PCV) with the *HTRA1* rs11200638 genotypes. The circles represent mean lesion size and error bars represent standard deviation in patients carrying each genotype. Patients with typical AMD and the AA genotype had a significantly larger lesion size (6363 ± 2837 μm) than the other genotypes (GA, 4830 ± 2159 μm; GG, 3866 ± 1947 μm; $P = 0.0003$) as well as in the patients with PCV (AA 6347 ± 2673 μm, GA, 4762 ± 2316 μm and GG, 4405 ± 2066 μm; $P = 1.3 \times 10^{-5}$).

type I CNV.[7] Although many pathologic studies have been reported on whether PCV is abnormal choroidal vessels, intra-Bruch CNV, or a mixture of both is still undetermined.[37–42]

Our results showed that the frequency of the risk allele of *CFH* Y402H was not significantly different in patients with typical AMD and PCV. For *CFH* Y402H, Johnson *et al.* reported that individuals who are homozygous for the *CFH* Y402H risk allele have elevated levels of C-reactive protein in the choroid.[13] Lommatzsch *et al.* reported that a pathologic examination of classic CNV or occult CNV revealed that the subjects were either heterozygous or homozygous for the *CFH* Y402H risk allele.[14] In addition, in several studies in which no functional experiments were performed, more significant associations were reported in the haplotypes with other SNPs in both Caucasian and Asian populations.[12,20,21,24] In our study, we only reported data of *CFH* Y402H, and thus further studies are needed to be able to clearly define the contribution of other SNPs or haplotypes in *CFH*.

The frequency of the risk allele of *HTRA1* rs11200638 was not significantly different in typical AMD and PCV. We genotyped rs10490924, but a complete LD led us to analyse the clinical association only with rs11200638 (data not shown). In a previous study on Japanese patients, it was concluded that rs11200638 made a greater contribution to typical AMD than to PCV.[30] However, we recalculated the reported genotype and allele data using the same method as the original article and found that no significant difference existed between typical AMD and PCV in the two *HTRA1* polymorphisms.[30]

For *CFH* Y402H and *HTRA1* rs11200638, it may well be that patients with typical AMD and PCV have the same genetic background. It has been reported that PCV can co-exist with typical AMD.[3,43]

We examined the potential contribution of genetic factors to clinical presentation. However, because the number of participants was too small to establish a high-enough power to determine the statistical significance of the findings, only positive associations could be considered. We obtained a statistically significant correlation between the frequency of the polymorphisms and the lesion size, particularly for the GLD and the *HTRA1* rs11200638 polymorphisms. To measure the size of the CNV lesion, we chose the GLD as this was clearly defined in the treatment of age-related macular degeneration with photodynamic therapy (TAP) study,[33] and therefore the measurements for typical AMD and PCV was less biased, especially in Asians who tend to have haemorrhagic or fibrovascular pigment epithelial detachments.

There were two limitations of this study. The border of the lesion tended to be not well demarcated in some Japanese patients, and we did not measure the lesion size in these patients. Additionally, the number of subjects was limited because this was a retrospective and cross-sectional examination, and a patient's consent to participate was required for the genomic analyses.

In summary, our results indicate a possible common genetic background of patients with typical AMD and PCV. We also noted a potential association between the disease-risk allele *HTRA1* rs11200638 polymorphism and larger lesion size, which was observed in both typical AMD and PCV patients.

ACKNOWLEDGEMENTS

This study was supported in part by the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Japan National Society for the Prevention of Blindness.

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nature genetics

Lung cancer susceptibility locus at 5p15.33

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We carried out a genome-wide association study of lung cancer (3,259 cases and 4,159 controls), followed by replication in 2,899 cases and 5,573 controls. Two uncorrelated disease markers at 5p15.33, rs402710 and rs2736100 were detected by the genome-wide data ($P = 2 \times 10^{-7}$ and $P = 4 \times 10^{-6}$) and replicated by the independent study series ($P = 7 \times 10^{-5}$ and $P = 0.016$). The susceptibility region contains two genes, *TERT* and *CLPTM1L*, suggesting that one or both may have a role in lung cancer etiology.

We and others have recently reported a susceptibility locus for lung cancer in gene region 15q25, an area that includes a cluster of nicotinic acetylcholine receptor genes[1–3]. In order to identify further susceptibility gene loci, we genotyped an additional 1,291 cases and 1,561 controls from three further studies (Toronto case-control study, HUNT2/Tromsø cohort study and CARET cohort study) for a total of 3,259 cases of lung cancer and 4,159 controls with genome-wide data (**Table 1** and **Supplementary Methods** online). After exclusion of subjects because of genotyping quality or evidence of non-European ancestry (**Supplementary Methods** and **Supplementary Fig. 1** online), we analyzed under a log-additive model 315,194 SNPs for 2,971 lung cancer cases and 3,746 controls, adjusting for age, sex and country (**Supplementary Fig. 2** online). Using principal-component analysis (**Supplementary Methods**) to adjust for population stratification, we found only minor differences in the estimates of risk and significance (**Supplementary Table 1** online).

Eight SNPs exceeded the genome-wide significance level of 5×10^{-7} (**Supplementary Fig. 2b** and **Supplementary Table 1**). Seven of these are located at 15q25.1, the locus previously reported as being associated with lung cancer[1–3], with the most prominent association with rs1051730 ($P = 1 \times 10^{-15}$). The eighth SNP, rs402710, is located at 5p15.33 ($P = 2 \times 10^{-7}$), indicating a potentially new susceptibility locus for lung cancer. Three additional SNPs in the 5p15.33 region showed evidence of association $P < 5 \times 10^{-6}$ (**Supplementary Table 1**). Two of these, rs31489 and rs401681, were in strong linkage disequilibrium (LD) with rs402710 ($r^2 > 0.680$) in the 3,746 controls genotyped on the Illumina platform. In contrast, rs2736100 showed relatively little LD with rs402710 ($r^2 = 0.026$) (**Supplementary Fig. 3** online).

We subsequently genotyped rs402710 and rs2736100 using Taqman in an additional 2,899 lung cancer cases and 5,573 controls from four separate studies (**Table 1** and **Supplementary Methods**). These included the EPIC cohort study, the Liverpool case-control study, the Szczecin lung cancer study and, uniquely for rs402710 because of limited DNA availability, additional cases and controls from the CARET cohort study. This independent sample provided evidence for replication of the initial finding for both variants ($P = 7 \times 10^{-5}$ for rs402710 and $P = 0.016$ for rs2736100). A combined association using all 5,870 cases and 9,319 controls with correction for the 315,194 comparisons in the genome-wide analysis yielded P values of 4×10^{-6} for rs402710 and 0.02 for rs2736100. The estimated allelic odds ratio (OR) in the replication series was more modest than that of the initial GWA series, subject to the 'winner's curse'. The more conservative OR in replication series is the preferred estimate.

More detailed information on the association between lung cancer and the SNPs rs402710 and rs2736100 is presented in **Figure 1**. The

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Received 21 May; accepted 10 September; published online 2 November 2008; doi:10.1038/ng.254

Table 1 Description of the seven studies contributing to the genome-wide and replication analysis

	No. of subjects on ILLUMINA		No. of subjects passing QC			
Study	Cases	Controls	Cases	Controls	Location	Study design
Genome-wide association studies						
Central Europe	1,968	2,598	1,841	2,441	Romania, Hungary, Poland, Russia, Slovakia, Czech Republic	Case control
Toronto	438	709	330	500	Greater Toronto area (Canada)	Case control
HUNT2/Tromsø	433	433	403	412	North Trondelag County (Norway) and Tromsø city in Tromsø County (Norway)	Cohort
CARET	420	419	397	393	United States	Cohort
Total	3,259	4,159	2,971	3,746		
Replication studies						
EPIC	–	–	1,213	2,591	Sweden, Netherlands, UK, France, Germany, Spain, Italy, Norway, Denmark, Greece	Cohort
Szczecin	–	–	908	1,037	Poland	Case control
CARET2	–	–	363	1,128	United States	Cohort
Liverpool	–	–	415	817	UK	Case control
Total			2,899	5,573		
Total overall			5,870	9,319		

For quality control (**Supplementary Methods**), we excluded samples with call rate <95%, sex discrepancy or non-European ancestry. We also excluded non-expected duplicates and first-degree relatives from the final analysis.

risk-associated allele was the more common allele of rs402710 and the less common allele of rs2736100. The association with rs402710 was prominent in never-smokers ($P = 0.01$), ex-smokers ($P = 0.0007$) and current smokers ($P = 0.0001$), and there was no evidence of any heterogeneity by study, histology, age or sex. There was no apparent geographical heterogeneity in the allele frequencies of rs402710. Adjustment for smoking exposure (pack years) had no effect on the observed association with a smoking-adjusted OR per allele of 1.19 (1.12–1.26). We also investigated rs402710 in the context of smoking intensity among controls and did not observe any association between number of cigarettes consumed per day and rs402710 ($P = 0.74$). The effects observed with rs2736100 were similar, with the associations for the less common (risk) allele being largely comparable to those for rs402710.

Several lines of evidence suggest that the associations observed with rs402710 and rs2736100 are independent. We found little LD between rs402710 and rs2736100 using all available controls. After incorporation of either one of these SNPs into the logistic regression, the association with the other remained significant, and there was no change in the risk estimate (OR per allele for rs402710 = 1.17 ($P = 2 \times 10^{-8}$) with adjustment for rs2736100 and OR per allele for rs2736100 = 1.11 ($P = 0.0004$) with adjustment for rs402710). Second, when cases and controls were compared for the number of risk alleles for rs402710 and rs2736100, there was an increasing trend with increasing number of risk alleles ($P = 2 \times 10^{-13}$) reaching an OR of 1.65 (1.34–2.02) for those who were homozygous for both risk variants (**Supplementary Table 2** online). Finally, when we imputed genotypes (**Supplementary Methods**) at 5p15.33 in the 2,971 cases of lung cancer and 3,746 controls with genome-wide data, we did not identify any SNPs more strongly associated with risk than rs402710 (**Supplementary Table 3** online). The top 11 imputed SNPs ($P < 0.0001$) were genotyped subsequently in the cases and controls of central European ancestry (**Supplementary Methods**) and comparison of haplotype frequencies from this direct genotyping indicated that the prevalence of two distinct haplotypes differed between cases and controls (**Supplementary Table 4** online). One haplotype carried the minor allele of rs402710 and eight additional SNPs in high LD ($r^2 > 0.644$) with rs402710, and the second haplotype tagged the minor allele of both rs2736100 and a second SNP rs2736098. Nevertheless, the possibility remains that rs402710 and rs2736100, although only weakly associated with each other, are in LD with one or more causal variants in this region.

The 5p15.33 locus contains two known genes: the *TERT* (human telomerase reverse transcriptase) gene and the *CLPTM1L* (alias *CRR9*; cleft lip and palate transmembrane 1 like) gene. There is no clear evidence to suggest that rs2736100 or rs402710 are themselves causative alleles. The rs2736100 variant is located in intron 1 of *TERT*, and rs402710 is located in a region of high LD that includes the proximal and putative promoter regions of *TERT*, as well as the entire coding region of the *CLPTM1L* gene (**Supplementary Fig. 3**). Current knowledge of the role of these genes would seem to implicate *TERT* as the more plausible candidate. *TERT* is the reverse transcriptase component of telomerase[4], making it essential for telomerase enzyme production and maintenance of

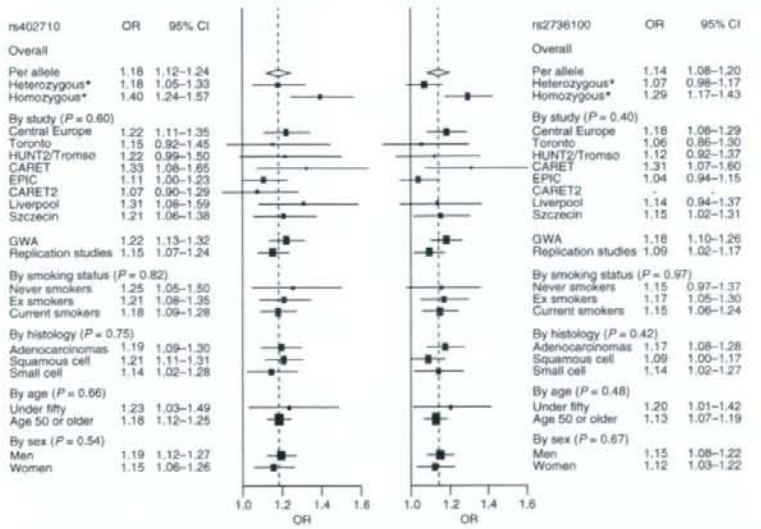

Figure 1 Forest plot representing lung cancer risk and the two variants in the 5p region (rs402710 and rs2736100). Apart from the odds ratios for heterozygous and homozygous effect (*), odds ratios and 95% confidence intervals are derived from the per-allele model. All models are adjusted for age, sex and country. The overall OR is shown by the broken vertical line. *P* values are from heterogeneity tests.

telomeres[5]. The telomerase enzyme is responsible for telomere regeneration, and up to 90% of human tumor samples, including lung cancer[6], show telomerase activity, indicating that regeneration of telomeres is a vital step for most forms of carcinogenesis[7]. *TERT* expression is actively present in germ cells, although is found in very low levels for most types of normal cells[8]. Activation of the *TERT* promoter seems to be a key step in synthesis of the TERT protein and resulting telomerase activity[9]. Such activity may be measured with the telomeric repeat amplification protocol (TRAP) and has been associated with both lung cancer progression and prognosis[6,10,11]. Inhibitors of TERT are clearly of much interest for potential chemoprevention and treatment of cancer, although their development has so far been unsuccessful[6]. DNA resequencing has shown that there is little common genetic variation in the *TERT* coding region which, along with its high conservation between species, implies that the gene itself is under strong evolutionary restraint[12]. Rare mutations in the *TERT* coding sequence have been implicated in dyskeratosis congenita[13], an autosomal dominant syndrome characterized by bone marrow abnormalities, but also pulmonary fibrosis and increased risk of some cancers[14].

The other gene in this region, *CLPTM1L*, named for its similarity to a gene implicated in susceptibility to cleft lip palate, was identified through screening for cisplatin (CDDP) resistance–related genes and was found to be upregulated in CDDP-resistant ovarian tumor cell lines and to induce apoptosis in CDDP-sensitive cells[15]. The *CLPTM1L* gene is well conserved and expressed in various tissues, including lung tissue. On the basis of these properties, it could be hypothesized that *CLPTM1L* induces apoptosis of lung cells under genotoxic exposures such as tobacco carcinogen–related stress.

In summary, we have identified a new susceptibility locus for lung cancer that comprises two potential candidate genes: *TERT*, an essential component of telomerase production and of carcinogenesis, and *CLPTM1L*, which may induce apoptosis. The nature of the causative alleles remains unclear. Further studies to identify the causal genetic variants and elucidate their function will aid our understanding of the etiology of lung cancer.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

P. Brennan and M. Lathrop designed the study. J.D.M., R.J.H., V.G., M.B.E., A.B. and H. Blanche coordinated the preparation and inclusion of all biological samples. J.D.M., S.H. and V.G. undertook the statistical analysis. Bioinformatics analysis was undertaken by F.M., M.F. and S.H. D.Z., D.L. and I.G. coordinated the genotyping of the central Europe samples. A.M. and R.J.H. coordinated the genotyping of the Toronto samples. J.D.M., D.Z., M.D., A.C., T.A. and H.E.K. coordinated the genotyping of the other studies. All other coauthors coordinated the initial recruitment and management of the studies. M. Lathrop obtained financial support for genotyping of the central Europe study; P. Brennan, R.J.H. and H.E.K. obtained financial support for genotyping of the other studies. P. Brennan and J.D.M. drafted the manuscript with substantial contributions from R.J.H. and M. Lathrop. All authors contributed to the final paper.

The authors thank all of the participants who took part in this research and the funders and support and technical staff who made this study possible. Support for the central Europe, HUNT2/Tromsø and CARET genome-wide studies and follow-up genotyping was provided by Institut National du Cancer, France. Support for the HUNT2/Tromsø genome-wide study was also provided by the European Community (Integrated Project DNA repair, grant no. LSHG-CT-2005-512113), the Norwegian Cancer Association and the Functional Genomics Programme of Research Council of Norway. Funding for the Toronto genome-wide study was provided by the Ontario Institute of Cancer Research. Funding for the Szczecin/Poland replication study was provided by European Community program "Marie-Curie Host Fellowships for the Transfer of Knowledge," grant no. MTKD-CT-2004-510114. Additional funding for study coordination, genotyping of replication studies and statistical analysis was provided by the US National Cancer Institute (R01 CA092039).

Published online at http://www.nature.com/naturegenetics/
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Endocrine Journal 2008, **55** (2), 429–432

NOTE

Subclinical Hypercortisolism in Hospitalized Patients with Type 2 Diabetes Mellitus

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Abstract. Pre(sub)clinical Cushing's disease is a recently described entity defined by the autonomous secretion of ACTH and the absence of a cushingoid appearance. We screened 77 hospitalized patients with diabetes mellitus for subclinical hypercortisolism and detected pre(sub)clinical Cushing's disease in 2 (2.6%) of them. In both patients, transsphenoidal surgery was performed and a microadenoma was removed. Their metabolic clearance rate of glucose measured by a glucose clamp study, an index of insulin sensitivity, significantly improved after surgery. Our results indicate that screening for subclinical hypercortisolism in diabetic patients might be useful, as surgery improves glucose tolerance and insulin sensitivity.

Key words: Subclinical hypercortisolism, Pre(sub)clinical Cushing's disease, Diabetes mellitus, Insulin sensitivity, Glucose clamp study

(*Endocrine Journal* **55**: 429–432, 2008)

THE prevalence of subclinical hypercortisolism in patients with diabetes mellitus in Western countries is higher than previously believed [1–3], but it is not known whether this is the case in Japan. Screening and detection of subclinical hypercortisolism in diabetic patients might be useful because surgery may improve glucose tolerance.

Pre(sub)clinical Cushing's disease is a recently described entity defined by the autonomous secretion of ACTH and the absence of a cushingoid appearance [4], and the diagnostic criteria were recently published in Japan. We screened hospitalized patients with diabetes mellitus for subclinical hypercortisolism. In patients in whom pre(sub)clinical Cushing's disease was detected, transsphenoidal surgery was performed. Insulin sensitivity was assessed with the glucose clamp technique before and after surgery.

Received: September 6, 2007
Accepted: October 20, 2007
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Methods

The subjects were recruited from 135 consecutive patients admitted to Ohtsu Red Cross Hospital for control of diabetes mellitus from September 2005 to September 2006. Exclusion criteria were cushingoid appearance, type 1 diabetes, diabetes secondary to other causes, alcoholism, renal failure, depression, and acute illness. A total of 77 patients (47 males and 30 females, age 61 ± 13 years, BMI 25.0 ± 4.2, HbA1c 10.4 ± 2.1%) were studied. None of the patients displayed moon face, buffalo hump, central obesity, striae cutis, skin atrophy, ecchymosis, or weakness associated with proximal muscle wasting. The study protocol was approved by the hospital ethics committee, and informed consent was obtained from all patients.

After at least 36 h after admission , the serum cortisol concentration was measured between 2300 and 2400 h (midnight cortisol). Although the cut-off value of midnight cortisol for screening is 2.5 μg/dl according to the diagnostic criteria for pre(sub)clinical Cushing's disease (Guidelines of Diagnosis and Treatment of Pre(sub)clinical Cushing's Disease by the Research Committee of the Ministry of Health, Labor, and Wel-

fare of Japan, 2006), we set the cut-off value as 5 μg/dl because the diurnal rhythm of cortisol is insufficient in diabetic patients [5]. Patients with a midnight cortisol level of more than 5.0 μg/dl underwent a 0.5 mg overnight dexamethasone suppression test (DST). Patients in whom the plasma cortisol levels were greater than 3 μg/dl after the 0.5 mg overnight DST were regarded as positive according to the diagnostic criteria, and basal ACTH and cortisol levels were measured. Patients with ACTH levels above 10 pg/ml underwent dynamic magnetic resonance imaging (MRI) of the pituitary. Abdominal computed tomography would have been performed in patients with ACTH levels of less than 10 pg/ml, however none of the patients had such low levels.

After improvement of glycemic control by medical treatment and stabilization for 4 to 6 months, transsphenoidal surgery was performed and a microadenoma was identified and removed in patients diagnosed with pre(sub)clinical Cushing's disease.

A euglycemic hyperinsulinemic glucose clamp study was performed 1 week before and 4 to 6 weeks after surgery as described earlier [6] with slight modification [7]. Serum C-peptide values were measured 6 min after intravenous administration of 1 mg glucagon before and after surgery.

Results

Of the 77 patients, 27 patients had a midnight cortisol level of more than 5.0 μg/dl. Among these 27, there were 7 in whom plasma cortisol levels were greater than 3 μg/dl after the 0.5 mg overnight DST. One patient declined further study. Basal ACTH and cortisol levels were measured in the other 6 patients, all of whom had ACTH levels above 10 pg/ml. Dynamic MRI of the pituitary revealed cystic lesions in two of the patients, and the findings in the other 4 patients were normal.

The endocrinologic data of the two patients with positive MRI findings are shown in Table 1. Basal ACTH and cortisol levels were normal, and the peak/basal ratio of plasma ACTH after a CRH stimulation test was above 1.5. Serum cortisol was not suppressed by an overnight low-dose (0.5 mg) DST, but was suppressed by an overnight high-dose (8 mg) DST. The central-peripheral ratio of ACTH measured by bilateral simultaneous sampling of the cavernous sinuses after stimulation with 100 μg CRH was above 3.0. Both patients were diagnosed with pre(sub)clinical Cushing's disease based on the diagnostic criteria. Transsphenoidal surgery was performed and a microadenoma was identified and removed in both patients. The pathologic diagnosis based on immunohistochemistry was an

Table 1. Endocrinologic data of the patients with positive MRI findings

	Patient 1	Patient 2
Age, sex	72, M	47, M
Midnight F (μg/dl)	5.8	7.5
Basal ACTH (pg/ml)	32.6	22.1
Basal F (μg/dl)	17.3	10.6
0.5 mg DST-F (μg/dl)	10.1	5.7
8 mg DST-F (μg/dl)	2.4	1.4
peak/basal ACTH ratio post-CRH	4.1	3.9
c/p ACTH ratio post-CRH	7.3	418

peak/basal ACTH ratio post-CRH, peak/basal ratio of plasma ACTH after a CRH stimulation test; c/p ACTH ratio post-CRH, central-peripheral ratio of ACTH measured by bilateral simultaneous sampling of the cavernous sinuses after stimulation with 100 μg CRH

Table 2. Diabetologic data of the two patients with pre(sub)clinical Cushing's disease.

	Patient 1	Patient 2
BMI		
pre op	26.6	25.4
post op	25.1	23.5
HbA1c (%)		
on 1.st admission	9.6	12.9
pre op	7.0	5.2
post op	6.4	4.7
MCR-G (ml/kg/min)		
pre op	2.9	5.1
post op	4.0	7.0
CPR 6 min. after glucagon test (ng/ml)		
pre op	5.4	5.3
post op	5.2	4.8
treatment		
pre op	Insulin 20 U/day	OHA
post op	Insulin 6 U/day →OHA	Diet only

After improvement of glycemic control by medical treatment and stabilization for 4 to 6 months, transsphenoidal surgery was performed. A euglycemic hyperinsulinemic glucose clamp study was performed 1 week before and 4 to 6 weeks after surgery. Hydrocortisone replacement therapy was stopped 2.5 weeks after surgery. Post-op, post-operation; MCR-G, metabolic clearance rate of glucose measured by a euglycemic hyperinsulinemic glucose clamp study; CPR; serum C-peptide

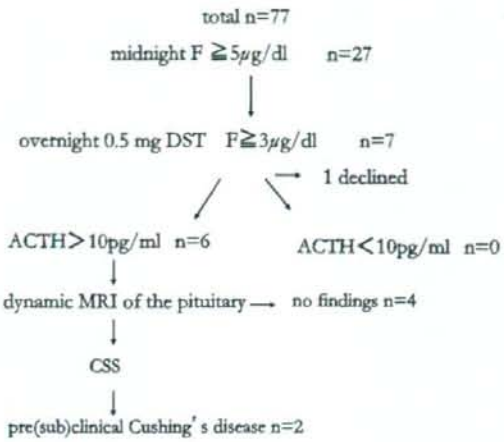

Fig. 1. Study design.
F; serum cortisol, DST; dexamethasone suppression test, MRI; magnetic resonance imaging

ACTH-secreting adenoma.

Patient 2 had transient hypoglycemia postoperatively. In both patients, however, the ACTH and cortisol response after stimulation with CRH 2 weeks after surgery was normal, consistent with preoperative pre(sub)clinical Cushing's disease without severe suppression of the residual pituitary gland, and hydrocortisone replacement therapy was stopped. Serum cortisol was suppressed by an overnight low-dose (0.5 mg) DST.

The diabetologic data of the two patients are shown in Table 2. Neither patient had a family history of diabetes. Glucose tolerance improved after surgery in both patients.

Notably, the metabolic clearance rate of glucose measured by a glucose clamp study, an index of insulin sensitivity, significantly improved after surgery. There was no significant difference between the serum C-peptide values 6 min after intravenous administration of 1 mg of glucagon before and after surgery. Patient 1 required mixed insulin at 20 U/day to control blood glucose levels before surgery; the dose was reduced to 6 U/day after surgery, and the patient is currently under good control on oral hypoglycemic agents. Patient 2 was able to stop taking an oral hypoglycemic agent after surgery.

The blood pressure of Patients 1 and 2 was 140/75 mmHg and 132/74 mmHg preoperatively, and 125/70 and 110/70 mmHg postoperatively, respectively. Patient 1 was taking antihypertensive drugs, the dosages of which were not changed either before or after surgery. Although bone mineral density was not measured, osteoporosis was observed in the thoracic spine in a lateral view chest X-ray in Patient 2, which improved 8 months after surgery.

Discussion

The prevalence of subclinical hypercortisolism in diabetic patients in Western countries is reported to be 2% to 9.4%, depending on the population studied and the diagnostic criteria [1–3]. In the present study, we detected 2 patients with pre(sub)clinical Cushing's disease (2.6%) out of 77 hospitalized diabetic patients. Our findings suggest that the prevalence of subclinical hypercortisolism in diabetic patients in Japan might be higher than expected, although further studies with larger numbers of patients are required to determine the actual prevalence.

Because glucose tolerance and insulin sensitivity improved after surgery in both patients, screening for subclinical hypercortisolism in diabetic patients might be useful. This is the first report of pre(sub)clinical Cushing's disease with the insulin sensitivity and secretion evaluated by a euglycemic hyperinsulinemic glucose clamp study and glucagon test before and after surgery. It is unlikely that the improved insulin sensitivity was the result of ameliorated glucose toxicity, because a preoperative glucose clamp test was performed after glycemic control had been stabilized for 4 to 6 months.

The mechanism of insulin resistance in pre(sub)clinical Cushing's disease without clinical features of Cushing's disease remains speculative. One possible explanation is that sensitivity to cortisol may differ among tissues. Another possibility is that differences in the activity of 11βHSD-1, an enzyme that generates active cortisol from inactive cortisone, may cause differences in glucocorticoid actions among various tissues [8].

There are some limitations in this study. The possibility that midnight cortisol was increased due to venipuncture cannot be excluded. In this sense, screening by midnight salivary cortisol is promising [9], although this is currently not clinically available in Japan. Although various approaches have been studied, there is no single screening test that can detect all cases of

subclinical hypercortisolism. Combination of a 0.5-mg overnight DST and midnight cortisol might be desirable [10], although the risk of causing hyperglycemia in uncontrolled diabetic patients is not negligible. We performed DST in patients screened by midnight cortisol after fair glycemic control was achieved.

The detection rate of a pituitary adenoma in Cushing's disease by dynamic MRI is approximately 60%. Indeed, in the present two cases of pre(sub)clinical Cushing's disease, microadenoma was found apart from the cysts detected by preoperative MRI. In 4 cases with negative MRI findings, the plasma cortisol levels after the 0.5-mg overnight DST were between 3 and 5 μg/dl, and basal ACTH levels were between 44.5 and 65.5 pg/ml. Thus, ectopic ACTH syndrome or adrenogenic preclinical Cushing's syndrome is unlikely. All four cases had hypertension. Pre(sub)clinical Cushing's disease cannot be excluded, but the patients were reluctant to undergo further studies including sampling of the cavernous sinuses. Therefore, we might have underestimated the prevalence of pre(sub)clinical Cushing's disease in the present study.

In conclusion, screening for subclinical hypercortisolism in diabetic patients might be useful , as glucose tolerance and insulin sensitivity improve after surgery.

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Original Article

Effects of Pitavastatin on Lipid Profiles and High-Sensitivity CRP in Japanese Subjects with Hypercholesterolemia: Kansai Investigation of Statin for Hyperlipidemic Intervention in Metabolism and Endocrinology (KISHIMEN) Investigators

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Aim: The effect of pitavastatin on high-sensitivity C-reactive protein (hs-CRP) has not been reported, yet, in humans. We, therefore, investigated the effects of pitavastatin on lipid profiles and hs-CRP in Japanese subjects with hypercholesterolemia.

Methods: The subjects were 178 Japanese with hypercholesterolemia, including 103 (58%) with type 2 diabetes. Pitavastatin (1–2 mg/day) was administered for 12 months. Serum low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), remnant-like particle cholesterol (RLP-C), triglycerides (TG) and hs-CRP levels were measured for 12 months.

Results: Serum LDL-C and RLP-C levels were significantly decreased by 30.3% and 22.8%, respectively. Serum TG levels were decreased by 15.9% in subjects with basal TG levels above 150 mg/dl. Serum HDL-C levels were significantly increased. The administration of pitavastatin reduced serum hs-CRP levels by 34.8%. No serious adverse events were observed, including changes in glycosylated hemoglobin levels of diabetic patients.

Conclusion: These results suggest that pitavastatin significantly improves lipid profiles and reduces proinflammatory responses, without adverse effects, in Japanese subjects with hypercholesterolemia, including those with diabetes mellitus.

J Atheroscler Thromb, 2008; 15:345-350.

Key words; Inflammation, Diabetes, LDL-C, HDL-C

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Received: March 7, 2008
Accepted for publication: July 22, 2008

Introduction

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are well known to reduce circulating low-density lipoprotein cholesterol (LDL-C) levels by inhibiting *de novo* cholesterol synthesis in the liver and thereby inducing the

bexpression of hepatic LDL receptors[1, 2]. However, in clinical trials, the overall reductions in cardiovascular events following statins appear to occur much earlier and to a greater extent than expected from the levels of LDL-C lowering alone[3, 4]; therefore, it has been considered that statins have pleiotropic effects independent of the reduction in circulating LDL-C levels[3, 4], including anti-inflammatory effects[5-8].

Numerous studies have suggested that low-grade inflammation has a pivotal role in atherosclerosis[5, 9, 10]. Prospective studies have indicated that high-sensitivity C-reactive protein (hs-CRP) is an important risk factor for atherosclerotic cardiovascular disease[5, 9]. Ridker *et al.* demonstrated that slight elevation in hs-CRP could lead to the evolution of atherosclerosis in humans[5, 9]. We previously demonstrated that hs-CRP is associated with insulin resistance and fibrinogen levels in non-obese Japanese type 2 diabetic patients[11]. Athough few studies have investigatied the effect of statins on hs-CRP in humans, some conflicting reports exist. Several studies indicated that statins reduce hs-CRP levels[5, 10, 12, 13]; however, one study failed to demonstrate the inhibitory effect of a statin on hs-CRP levels in diabetic subjects[14].

Pitavastain is a synthetic strong statin, whose molecular structure is similar to atorvastain and rosuvastatin[15]. There has been a single report about the effect of pitavastatin on lipid profiles in humans[16]. Although several studies demonstrated that pitavastatin, as well as other lipophilic statins, has anti-inflammatory effects *in vitro*[6-8, 17], the effects of pitavastatin on inflammatory markers, including hs-CRP, have not been reported in humans *in vivo* to the best of our knowledge.

In the present study, therefore, we explored the effects of pitavastatin on lipid profiles as well as hs-CRP levels in Japanese subjects with hypercholesterolemia, including those with diabetes mellitus.

Patients and Methods

This study was a 12-month, multi-center, prospective, open-label study. Japanese patients, who met the following inclusion criteria: serum total-cholesterol (TC) ≥ 220 mg/dL, and triglycerides (TG) < 400 mg/dL, were recruited. A total of 209 Japanese subjects were enrolled, of which 31 were excluded because they did not follow the protocol. As a result, 178 cases were investigated. Pitavastatin in a dose of 1–2 mg/day was administered [1 mg/day in 44 cases (25%) and 2 mg/day in 134 cases (75%)]. Before the administration of pitavastatin, no lipid-lowering medications had been administered in 111 cases (62 %), whereas other lipid-lowering drugs had been prescribed in 67 cases (38 %; 27 pravastatin, 18 atorvastatin, 10 simvastatin, 8 fluvastatin, 1 rosuvastatin, 2 bezafibrate and 1 fenofibrate cases), all of which were withdrawn at least one week before the administration of pitavastatin.

Blood samples were obtained at the beginning and 3, 6 and 12months after the administration of pitavastatin. Serum TC, LDL-C, TG, HDL-C, glucose, glycosylated hemoglobin (A1C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and creatinine phosphokinase (CK) were measured by standard techniques. Remnant-like particle-cholesterol (RLP-C) and hs-CRP were measured by immunoaffinity gel (JIMRO, Japan) and N Latex CRP II (Dade Behring Marburg GmbH, Marburg, Germany), respectively.

Continuous variables are shown as the mean ± S.E.M. when the distribution was normal. Statistically significant differences among groups were analyzed by paired *t* test. When the distribution was skewed, statistically significant differences among groups were analyzed by Wilcoxon signed-rank test. The JMP Software computer program (Version 5.0 for Windows; SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. *P* values 0.05 were considered significant.

This study was approved by the local ethics committee, and informed consent was obtained from all participants before the study.

Results

Basal characteristics of the 178 patients are shown in **Table 1** [age: 62.0 ± 0.9 years, 83 men (47%) and 95 women (53%)]. The mean body mass index (BMI) and fasting glucose levels of the subjects were 24.5 kg/m^2 and 126.0 mg/dL, respectively. The participants in this study included 103 cases (58%) of type 2 diabetes, 62 cases (35%) of hypertension, 7 cases (4%) of peripheral arterial disease, 12 cases (7%) of cerebral infarction, and 32 cases (18%) of coronary heart disease. Serum LDL-C levels were significantly decreased by 32.6%, 31.0% and 30.3% after 3, 6 and 12 months, respectively (**Fig. 1A**). Serum TG levels were significantly decreased by 17.7% and 15.9% after 3 and 12 months, respectively, in subjects whose basal TG levels were more than 150 mg/dL, although serum TG levels were not significantly changed in overall subjects (**Fig. 1B**). Serum HDL-C levels were significantly increased by 3.1%, 5.9% and 2.6% after 3, 6 and 12 months, respectively (**Fig. 1C**). In subjects whose basal HDL-C levels were below 40 mg/dL, HDL-C levels were increased by 16.2%, 22.4% and 19.0% after 3, 6

Table 1. Basal characteristics of subjects.

Age	62.0±0.9 (178)
Male %	47% (83)
BMI (kg/m²)	24.5±0.3 (174)
LDL-C (mg/dL)	153.4±3.3 (174)
HDL-C (mg/dL)	57.8±1.4 (134)
TG (mg/dL)	154.7±5.5 (175)
TC (mg/dL)	242.2±3.6 (174)
RLP-C (mg/dL)	7.9±0.5 (63)
A1C (%)	7.2±0.2 (139)
FPG (mg/dL)	126.0±3.9 (116)
hs-CRP (mg/L)	0.69 (0.33-1.36) (31)
Diabetes mellitus %	58% (103)
Hypertension %	35% (62)
Peripheral arterial disease %	4% (7)
Cerebral infarction %	7% (12)
Coronary heart disease %	18% (32)

Data are presented as the mean±S.E.M. Numbers of subjects are shown in parentheses. Abbreviations: TC: total cholesterol, FPG: fasting plasma glucose.

and 12 months, respectively (**Fig. 1C**). Furthermore, serum RLP-C levels were significantly decreased by 14.0%, 20.2% and 22.8% after 3, 6 and 12 months, respectively (**Fig. 1D**).

Serum hs-CRP levels were significantly decreased in 31 subjects after 12 months (median: 0.69 mg/L to 0.45 mg/L, −34.8%, $p<0.01$, **Fig. 2A**). Pitavastatin similarly decreased hs-CRP levels in subjects with diabetes mellitus (median: 0.59 mg/L to 0.36 mg/L, −39.0%, $p<0.05$, **Fig. 2B**).

There was no significant correlation between changes in LDL-C and hs-CRP levels even after transformation of hs-CRP values into a logarithm after 12 months ($r=0.124$, $p=0.39$). We also found no significant correlation between changes in hs-CRP and changes in serum TG or serum RLP-C levels during the study (data not shown) and there were no serious adverse events. In a subgroup of diabetic patients, there was a slight and statistically insignificant decrease in A1C after pitavastatin treatment for 12 months (7.0% to 6.9%, $n=80$, $p=0.07$).

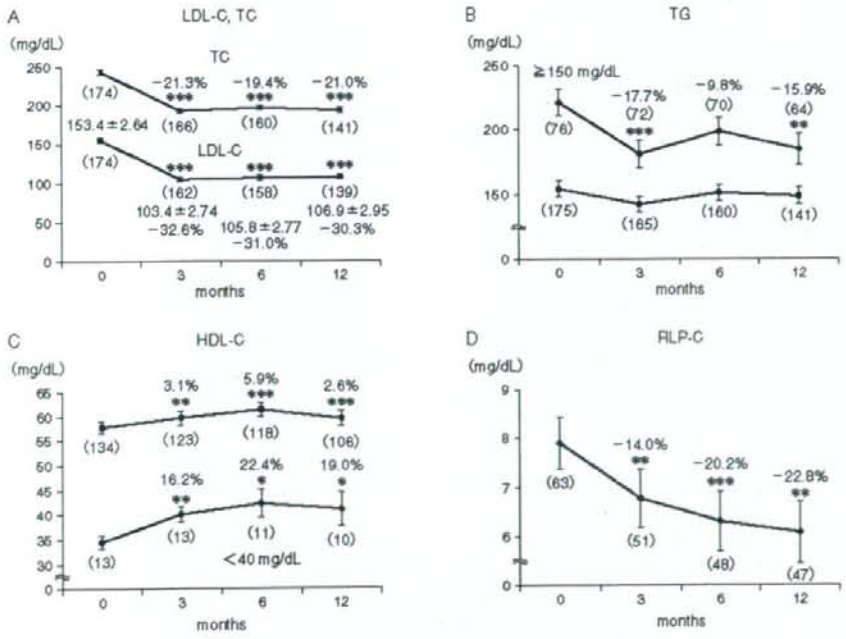

Fig. 1. Effect of pitavastatin on lipid levels in all subjects.

A: Effect of pitavastatin on total cholesterol (TC) and LDL cholesterol (LDL-C) levels. B: Effect of pitavastatin on triglyceride (TG) levels in all subjects and in subjects with basal TG levels of more than 150 mg/dL. C: Effect of pitavastatin on HDL cholesterol (HDL-C) levels. Data in subjects with basal HDL-C levels less than 40 mg/dL are indicated separately. D: Effect of pitavastatin on remnant-like particle cholesterol (RLP-C) levels.

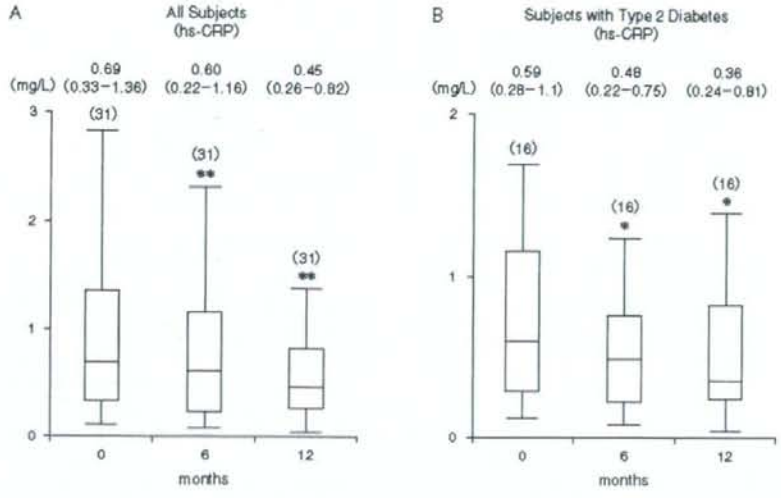

Fig. 2. Effect of pitavastatin on hs-CRP levels in all patients (panel A) and patients with type 2 diabetes (panel B) after 12 months.

Data are presented as the mean ± S.E.M. Numbers of subjects are shown in parentheses. $^{*}p<0.05$ vs. basal values, $^{**}p<0.01$ vs. basal values.

Discussion

The present study demonstrated that pitavastatin improved serum lipid profiles in Japanese subjects with hypercholesterolemia: a decrease in LDL-C, an increase in HDL-C, a decrease in RLP-C, and a decrease in TG among those with higher basal TG levels. The improvement in lipid profiles was compatible with a recent report on pitavastatin in Japanese subjects[16], although, in the present study, LDL-C levels before the administration of pitavastatin were lower (153 ± 3 mg/dL) than in the previous report (183 ± 21 mg/dL)[16].

Like other statins, pitavastatin has been indicated to have pleiotropic effects on vascular cells *in vitro*. In cell culture experiments, for example, pitavastatin is reported to reduce osteopontin expression in rat vascular smooth muscle cells[17], suppress interleukin-8 (IL-8), monocyte chemoattractant protein-1 and endothelin-1 expression, enhance endothelial nitric oxide synthase expression[7], and inhibit CRP-induced IL-8 production[8], thus showing its direct ant-inflammatory and anti-atherogenic effect. However, there have been no reports about anti-inflammatory effects of pitavastatin *in vivo* in humans.

The present study showed that pitavastatin lowered hs-CRP in patients with hypercholesterolemia. A recent study in Korea has showen that pitavastatin reduced mean hs-CRP levels from 24.6 to 16.5 mg/L by 8-week treatment[18]. In the present study, pitavastatin, at similar doses, significantly reduced hs-CRP levels in hypercholesterolemic subjects, including type 2 diabetes, with lower basal hs-CRP levels. These findings have been supported by previous reports which indicated anti-inflammatory effects of pitavastatin *in vitro*[6, 8]. There have been conflicting reports about the effects of statins on hs-CRP *in vivo* in diabetic subjects; one report did not show a significantly decrease in hs-CRP after atorvastatin[14]. Therefore, it is possible that diabetic patients are resistant to the inhibitory effects of statins on hs-CRP, although diabetic patients showed a significant decrease in hs-CRP after atorvastatin in a recent paper[19]. The present as well as the previous study[18], also demonstrated that pitavastatin, a new synthetic lipophilic strong statin, lowered hs-CRP levels in hypercholesterolemic subjects, including type 2 diabetes. There was no significant correlation between pitavastatin-induced decreases in hs-CRP and LDL-C, in the present study with pitavastatin, as previously reported with other statins[9, 10, 12, 19]. We also found no significant correlation between pitavastatin-induced decrement in hs-CRP and those in RLP-C in this study cohort. Thus, it is suggested that pitavastatin may have direct anti-inflammatory effects which are independent of improved lipid profiles. A previous study showed thst non-responders,

whose LDL-C levels were above 100 mg/dL after atorvastatin treatment, tender to be resistant to hs-CRP reduction by statin treatment[19]; however, the present study did not show such a tendency but hs-CRP reduction to be independent of LDL-C levels after pitavastatin treatment (data not shown).

There were no serious adverse events in the present study, including A1C changes in subjects with diabetes mellitus. In contrast, atorvastatin has been reported to result in a slight but significant increase of A1C in Caucasian diabetic subjects, as shown by the Collaborative Atorvastatin Diabetes Study (CARDS)[20]. It has been shown that Japanese subjects with type 2 diabetes are mainly insulin-deficient rather than insulin-resistant[21]. In addition, Asians have higher plasma levels of statins than Caucasians[22]. Therefore, it is possible to speculate that Japanese subjects with type 2 diabetes may be more prone to worsening glycemic control due to, if any, adverse effects of statins on insulin secretion; however, the persent study indicated that pitavastatin did not significantly worsen glycemic control in Japanese diabetic subjects.

This study is limited by the fact that it was an uncontrolled study with a moderate number of subjects, especially a relatively small number with hs-CRP measurement. It is to be noted, however, that the subjects showed lower basal hs-CRP levels before pitavastatin treatment (median: 0.69 mg/L) than those in previous studies of Caucasians (median: about 3 mg/L)[5, 9]. In the Pravastatin Inflammation/CRP Evaluation (PRINCE) study, baseline hs-CRP levels were the major determinants of the change in hs-CRP levels after pravastatin[12], indicating that anti-inflammatory effects of statins would be more pronounced in subjects with higher hs-CRP levels. It is intriguing that pitvastatin was able to reduce hs-CRP even in subjects with lower basal hs-CRP levels in the present study. Previous large scale cardiovascular event outcome studies with other statins have been conducted in Caucasians with high basal hs-CRP levels; therefore, it is of clinical importance to investigate whether pitavastatin can similarly reduce cardiovascular events among Japanese subjects with lower basal hs-CRP values than Caucasians[10, 23, 24].

Conclusions

It is concluded that pitavastatin improves lipid profiles, including the reduction of RLP-C levels, and that it decreases hs-CRP levels independently of improved lipid profiles, without any adverse effects, in Japanese subjects with hypercholesterolemia, including type 2 diabetes.

Acknowledgements

The authors are very grateful to Mr. Kazunobu Kasai for his assistance with data analyses.

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