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ASSOCIATION OF CHOLECYSTOKININ-A RECEPTOR GENE POLYMORPHISM WITH ALCOHOL DEPENDENCE IN A JAPANESE POPULATION

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Abstract—Aims: Cholecystokinin (CCK), one of the most abundant neurotransmitter peptides, interacts with dopamine. Dopaminergic neurotransmission between the ventral tegmental area and the limbic forebrain is a critical neurobiological component of alcohol and drug self-administration. CCK modulates dopamine release in the nucleus accumbens via the CCK-A receptor (R). We recently determined the transcriptional start site of the human CCK-AR gene, and detected two sequence changes (–81A/G and –128G/T) in the promoter region. The aims of the present study were to determine the prevalence of the –81A/G and –128G/T polymorphism of the CCK-AR gene between alcoholics and normal control subjects and the occurrences of the polymorphisms in subtypes of alcoholics. **Methods:** The above polymorphisms were examined in 435 alcoholics and 1490 control subjects. We excluded subjects with inactive ALDH2 and employed the subjects with ALDH2*1/2*1 (384 alcoholics and 792 controls). **Results:** The allelic frequency of –81G in the CCK-AR gene polymorphism (–81A/G) was significantly higher in alcoholics than in control subjects. However, there were no differences between the two groups with respect to the frequency of –128G/T. Alcoholic patients with antisocial personality disorder and with first-degree alcoholic relatives were significantly associated with a higher frequency of the –81G allele. In addition, the age of onset of alcohol dependence was significantly earlier in patients with this allele. **Conclusions:** The CCK-AR gene –81A/G polymorphism, especially in the –81G allele, may be associated with intractable alcoholism.

INTRODUCTION

Alcohol-related behaviours and/or alcohol sensitivities are associated with the actions of various neurotransmitters and neuropeptides such as dopamine (Kalivas, 1993; Self and Nestler, 1995). Cholecystokinin (CCK), one of the most abundant neurotransmitter peptides in the brain, is known to interact with dopamine (Crawley, 1991; Marshall *et al.*, 1991; Woodruff *et al.*, 1991; Ladurelle *et al.*, 1994; Hamilton and Freeman, 1995). Thus far, two types of CCK receptors (R) (types A and B) have been cloned (Wank, 1995). Although CCK-BR is widely distributed throughout the central nervous system, CCK-AR is found in specific regions, such as the amygdala, nucleus tractus solitarius, posterior nucleus accumbens, ventral tegmental area, substantia nigra, and raphe nucleus. CCK coexists in the mesolimbic dopamine neurons, and CCK-AR mediates the release of dopamine in the nucleus accumbens (Crawley, 1991; Marshall *et al.*, 1991; Woodruff *et al.*, 1991; Ladurelle *et al.*, 1994; Hamilton and Freeman, 1995; Wank, 1995). The dopaminergic neurotransmission between the ventral tegmental area and the limbic forebrain is a critical neurobiological component of self-administration of alcohol and drugs (Kalivas, 1993; Self and Nestler, 1995).

Recent reports (Blum *et al.*, 1990; Muramatsu *et al.*, 1996) in human subjects showed an association of polymorphisms of the dopamine D2 and/or D4 receptor gene with alcohol dependence, although results have been equivocal. In contrast, Okubo *et al.* (2000) reported that the CCK gene polymorphism does not play a major role in alcohol withdrawal symptoms. Based on our recent finding of two sequence changes in the promoter region (a G to T change in nucleotide –128 and an A to G change in nucleotide –81; GenBank database accession number D85606; Funakoshi *et al.*, 2000), in the present study, we examined the association between CCK-AR gene polymorphisms and alcohol dependence.

Liver mitochondrial aldehyde dehydrogenase-2 (ALDH2) is responsible for metabolizing the acetaldehyde produced from ethanol into acetate. More than 40% of Asians have the inactive form of ALDH2, encoded either as heterozygous ALDH2*1/2*2 or homozygous ALDH2*2 (Higuchi *et al.*, 1995), while the majority of Caucasians possess the active form of ALDH2 (2*1/2*1). A previous report (Murayama *et al.*, 1998) showed that the clinical characteristics of alcoholic patients having inactive ALDH2 differed from those of alcoholic patients with active ALDH2. In this study, we excluded subjects with inactive ALDH2 to avoid the influence of its overwhelming effect as a negative risk factor for alcoholism.

SUBJECTS AND METHODS

Subjects

This study was approved by the ethics committees of the National Alcoholism Center, Kurihama Hospital, of the National Institute of Longevity Sciences (NILS) and of the Tokyo Metropolitan Institute of Gerontology. Written informed consent was obtained from each subject.

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The subjects consisted of 435 (aged 32–74 years) Japanese male alcoholics who had been consecutively hospitalized at Kurihama Hospital. They were diagnosed as having DSM-III-R (American Psychiatric Association, 1987) alcohol dependence, based on the Structured Clinical Interview for DSM-III-R (SCID) assessment (Spitzer *et al.*, 1990).

The age-matched control subjects consisted of 1134 male participants in the NILS Longitudinal Study of Aging (LSA) (Shimokata *et al.*, 2000) and 356 males who were Institute employees. They were free of alcohol dependence, based on the results of the Kurihama Alcoholism Screening Test, the most widely used alcoholism screening test in Japan, which was administered to potential controls before entering into this study.

First, the genotype of the ALDH2 gene was determined by mismatched polymerase chain reaction restriction fragment length polymorphism (PCR–RFLP) method reported previously (Kamino *et al.*, 2000). Then the CCK-AR gene polymorphism was determined in the subjects with ALDH2*1/2*1 (384 alcoholics and 792 controls).

Genotyping procedures

The polymorphism in the promoter region of CCK-AR gene was examined using a mismatched PCR–RFLP method (Funakoshi *et al.*, 2000). In brief, a pair of primers (sense primer = 5′-CATATGTACACATGTGTGTAAAAAGCAGCC-AGAC-3′ and anti-sense primer = 5′-GCCCTTTCCTGGG-CCAGACT-3′), were designed to amplify the 103-bp product, which was subsequently digested with restriction enzyme *Hin*fI, and analysed by 12% polyacrylamide gel electrophoresis. Six genotypes were identified: a wild type (–81A/A, –128G/G); heterozygous mutant types (–81A/G, –128G/G), (–81A/G, –128G/T), (–81G/G, –128G/G), (–81G/G, –128G/T); and a homozygous mutant type (–81G/G, –128T/T).

Clinical data

We used a structured clinical interview for DSM-III-R to diagnose alcohol dependence and antisocial personality disorder (Spitzer *et al.*, 1990). We also used a structured interview to obtain responses to questions on social background as well as history of drinking and alcohol withdrawal. Family histories of alcohol dependence among all biological first-degree relatives were evaluated by using the Family History Research Diagnostic Criteria (Andreasen *et al.*, 1977). Age at onset of alcoholism was defined as the age at which the individual first met the DSM-III-R diagnostic criteria for alcohol dependence.

Statistical analyses

Statistical differences between alcohol-dependent and control subjects were assessed using the chi-squared test. A continuity correction was performed when the frequency of at least one cell was less than 5. An odds ratio (OR) with a 95% confidence interval (CI) was calculated to evaluate the genotype frequencies between groups. Probability differences of $P < 0.05$ were considered statistically significant. To assess the linkage disequilibrium between the two polymorphisms of the CCK-AR gene, we calculated the D value and its significance, using the ASSOCIAT program (downloaded from the website of J. Ott: ftp://linkage.rockefeller.edu/ software/utilities/). All statistical computations were carried out using the Statistical Analysis System package, version 6.12 (SAS Institute, 1998).

RESULTS

The frequency of a wild-type (–81A/A, –128G/G) genotype was lower in alcoholics than in controls, though the difference was not significant ($P = 0.053$) (Table 1). These polymorphisms were in linkage disequilibrium and in Hardy–Weinberg equilibrium. The genotypes of (–81A/A, –128G/T), (–81A/A, –128T/T), and (–81A/G, –128T/T) were not detected. These were not detected in our previous reports, either (Funakoshi *et al.*, 2000; Shimokata *et al.*, 2000).

When the allelic frequencies were estimated, significant differences in that of the –81A/G were detected between alcoholics and controls, as shown in Table 2 ($P = 0.023$). However, the frequencies of the –128 G to T change were not significantly different between the two groups.

Based on the finding that the allelic frequency of –81G was significantly higher in alcoholics than in controls (Table 2), the association between CCK-AR gene –81A/G polymorphism and the clinical features of alcoholics was assessed (Table 3). Comparison of the genotype distributions of the CCK-AR gene –81A/G polymorphism in alcoholics and control subjects revealed that the frequencies of –81A/A were quite similar among the subgroups of alcoholic patients with

Table 1. Distribution of CCK-AR gene –81A/G, –128G/T polymorphisms in alcoholics and control subjects (participants had ALDH2*1/2*1 genotype)

Genotype	Alcoholics ($n = 384$) n (%)	Control subjects ($n = 792$) n (%)
–81A/A, –128G/G	205 (53.3)	470 (59.3)
–81A/G, –128G/G	75 (18.8)	111 (14.0)
–81A/G, –128G/T	76 (19.8)	168 (21.2)
–81G/G, –128G/G	6 (1.6)	9 (1.1)
–81G/G, –128G/T	16 (4.2)	19 (2.4)
–81G/G, –128T/T	9 (2.3)	15 (1.9)

Percentages may not total 100 due to rounding up. The difference between the wild-type genotype and the mutations (the sum of the five different types) was tested by 2 × 2 chi-squared test. $\chi^2 = 3.75$, d.f. = 1, $P = 0.053$.

Table 2. Allele frequencies of CCK-AR gene –81A/G, –128G/T polymorphisms in alcoholics and control subjects (participants had ALDH2*1/2*1 genotype)

Allele	Alcoholics ($n = 768$) n (%)	Control subjects ($n = 1584$) n (%)
–81A	*558 (72.7)	1219 (77.0)
G	210 (27.3)	365 (23.0)
–128G	658 (85.7)	1367 (86.3)
T	110 (14.3)	213 (13.7)

Percentages may not total 100 due to rounding up. $\chi^2 = 5.12$, d.f. = 1, *$P < 0.023$ for the –81A/G polymorphism. Odds ratio = 1.26. There were no differences with respect to –128G/T.

Table 3. Clinical characteristics of alcoholics with CCK-AR gene −81A/G polymorphism (participants had ALDH2*1/2*1 genotype)

	Genotype of the CCK-AR Gene −81A/G Polymorphism		
Parameter	A/A *n* (%)	A/G + G/G *n* (%)	2 × 2 table χ^2 test
Antisocial personality disorder (ASP)			
Negative	204 (54.4)	171 (45.6)	$\chi^2 = 4.99$, d.f. = 1, $P = 0.025$ (continuity adjusted)
Positive	1 (11.1)	8 (88.9)	
Delirium tremens			
Negative	142 (56.8)	108 (43.2)	$\chi^2 = 3.36$, d.f. = 1, $P = 0.067$
Positive	63 (47.0)	71 (53)	
First-degree relatives			
Negative	191 (55.7)	152 (44.3)	$\chi^2 = 6.83$, d.f. = 1, $P = 0.009$
Positive	14 (34.2)	27 (65.9)	
Age of onset of alcohol dependence	41:8 ± 10.7	38.9 ± 10.7	$t = 2.54$, d.f. = 361, $P = 0.012$

Percentages may not total 100 due to rounding up.

negative ASP, with negative delirium tremens, and with negative first-degree relatives, and the control group (55.7, 57.5, and 56.9% for respective subgroups of alcoholics versus 59.3% for control subjects, as shown in Table 1). A comparison among alcoholic subgroups revealed that the frequency of genotype −81A/A was significantly lower in alcoholics with ASP and with first-degree relatives than in those without ASP and without family history (Table 3). The frequency of −81A/A tended to be lower in alcoholics with delirium tremens than in those without delirium tremens, though the difference was not significant ($P = 0.067$). The age at onset of alcohol dependence was significantly earlier in alcoholics with genotypes −81A/G and G/G than in those with wild-type (−81A/A).

DISCUSSION

Our results showed a higher frequency of the G allele of the CCK-AR gene −81A/G polymorphism in alcoholics than in control subjects. Moreover, the allelic frequency of −81G was significantly higher in alcoholic patients with ASP and with family history than in those without ASP and family history. Patients with delirium tremens tended to possess the −81G allele more frequently than did patients without delirium tremens, although the difference was not statistically significant ($P = 0.067$). Furthermore, the age at onset of alcohol dependence was earlier in patients with the −81G allele than in those without it. These findings suggest that the −81G allele of the CCK-AR gene may be associated with intractable alcohol dependence.

The comorbidity rate of antisocial personality disorder was only 2.3% and an average age at onset of alcohol dependence was around 40 years in our samples. These figures are substantially different from those of US alcoholic samples recruited from inpatient treatment settings (Hesselbrock *et al.*, 1986; Raimo *et al.*, 1999). Although reasons are not clear, we have observed a relatively low comorbidity rate of antisocial personality disorder in Japanese alcoholic samples (Yoshino and Kato, 1996; Murayama *et al.*, 1998). In addition, age of onset of our alcoholic samples is comparable to that of other Japanese alcoholic inpatients. (Murayama *et al.*, 1998). These comparisons suggest that our samples did not deviate from general Japanese alcoholic samples.

There have been several previous reports of CCK-AR gene polymorphisms (Inoue *et al.*, 1997; Tachikawa *et al.*, 2000; Okubo *et al.*, 2002). Okubo *et al.* (2002) determined five mutations, −388 $(GT)_8/(GT)_9$, −333G/T, −286A/G, −241G/A, and −85C/G in the promoter region of the CCK-AR gene, and reported a significant association between −85C to G change and alcoholic patients with hallucinations. However, once we had determined the transcriptional start site of the CCK-AR gene (Funakoshi *et al.*, 2000), we discovered that the −85 is not in the promoter region, but is in the 5′ untranslated region. Okubo *et al.* (2002) numbered not from the transcriptional start site but from the initial site of the coding region of exon 1. We examined CCK-AR gene polymorphisms in 50 patients with gallstone and 300 patients with diabetes mellitus before the establishment of the RFLP method (Funakoshi *et al.*, 2000). We found one case with G to A in intron 1, and another case with C to G in exon 3, without any change in amino acid (Thr). The polymorphisms of the promoter region (between −351 and +176) were also examined, and no polymorphisms other than −81A to G and −128G to T were detected. Those designated as −333G/T and −286A/G by Okubo *et al.* (2002) were identical to −128G/T and −81A/G in the present study, respectively. No association of these polymorphisms (−128G/T and −81A/G) with alcohol dependence was observed (Okubo *et al.*, 2002). One possible explanation for the differences between the study by Okubo *et al.* (2002) and our study is that Okubo *et al.* (2002) did not exclude subjects with inactive ALDH2. Inactive ALDH2 (2*1/2*2 and 2*2/2*2) is a strong negative risk factor for alcohol dependence (Higuchi *et al.* 1995). Tachikawa *et al.* (2000) reported an association of the 201A allele (201A/G is identical to −81A/G in the present study) of the CCK-AR gene with schizophrenia. Given the potential differences between alcohol dependence and other psychiatric disorders, our results do not completely contradict their findings.

We recently reported that functional comparison of the A and G variants of the −81 A/G polymorphism by luciferase assay demonstrated a slight decrease in the G variant, but no

significant difference (Takata *et al.*, 2002). However, we used STC-1 (Rindi *et al.*, 1990), established from a transgenic mouse expressing a viral oncogene under the control of the insulin promoter, because no human-derived cell line expressing CCK-AR was available. Further studies employing various experimental conditions are needed before conclusions can be drawn regarding the effect of this polymorphism on expression of the CCK-AR gene.

A recent report mapped the CCK-AR gene to chromosome 4 (4p15.2–15.1), in the vicinity of the dopamine D5 receptor gene (4p16.1–15.1) (Beischlag *et al.*, 1995). The dopamine D5 receptor binds dopamine with a 10-fold greater affinity than that of dopamine receptor 1. The dopamine D5 receptor protein is also localized in the prefrontal cortex. Thus, alterations in the CCK-AR gene may lead to some modification of dopamine release, and alteration of dopaminergic neurotransmission may be involved in alcohol misuse (Crawley, 1991; Marshall *et al.*, 1991; Woodruff *et al.*, 1991; Kalivas, 1993; Ladurelle *et al.*, 1994; Hamilton and Freeman, 1995; Self and Nestler, 1995; Wank, 1995).

In summary, the CCK-AR gene –81A/G polymorphism was found to be associated with alcohol dependence, and the –81G allele of the CCK-AR gene to be possibly associated with intractable alcohol dependence.

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Brief Research Communication

Association of Cholecystokinin-A Receptor Gene Polymorphisms and Panic Disorder in Japanese

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Several lines of evidence have suggested that naturally occurring alterations in cholecystokinin (CCK) systems could contribute to the development of panic disorder (PD). Among recent investigations, polymorphisms of the CCK and CCK-B receptor (R) genes were investigated, but the results were inconclusive. We recently cloned the genomic structures of human CCK-AR, and determined the transcriptional start site of the human CCK-AR gene. Two sequence changes were detected in the promoter region: a G to T change in nucleotide −128 and an A to G change in nucleotide −81 (GenBank database under accession number D85606). The frequencies of the genotypes and haplotypes of these two polymorphisms were compared in 109 Japanese patients with PD and 400 age- and gender-matched normal Japanese control subjects. The frequency of variant genotypes (−81A/G, −128G/T; G/G, G/T, and G/G, T/T) having variant haplotype (−81G/−128T) was significantly higher in PD than in controls ($P < 0.0001$, OR = 2.81, 95% CI = 1.74–4.39). The statistical differences between the haplotype distributions in the PD and control groups were highly significant: the frequency of variant haplotype (−81G/−128T) was higher in the former group than in the latter ($P < 0.0001$). This association was not affected by clinical characteristics such as age, gender, and age at onset of PD. In this study, the first to report the positive association of the CCK-AR polymorphisms and PD, haplotype analyses further strengthened the association based on our comparison of genotype distributions. The CCK-AR gene polymorphism may be involved in the neurobiology of PD.
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KEY WORDS: **panic disorder; cholecystokinin; CCK-A receptor; gene; polymorphism**

Panic disorder (PD) is a common anxiety condition, characterized by unprovoked anxiety attacks distinguished by such symptoms as palpitations, chest pain, dyspnea, choking, tremors, faintness, and sweating, in addition to fear of dying, losing control, or going crazy [American Psychiatric Association, 1987]. The carboxy-terminal tetrapeptide of cholecystokinin (CCK-4) induces panic-like attacks when administered as an intravenous bolus in healthy volunteers, and in patients with PD [De Montigny, 1989; Bradwejn et al., 1991].

CCK is a classical gastrointestinal hormone and one of the most abundant neurotransmitter peptides in the brain. CCK receptor (R)s have been classified into two subtypes, CCK-A and CCK-B, on the basis of their affinities for a structurally and functionally related family of peptides that have identical COOH-terminal pentapeptide sequences but differences in sulfation at the sixth (gastrin) and seventh (CCK) tyrosyl residues [Wank, 1995]. Among recent investigations [Wang et al., 1998; Kennedy et al., 1999; Hamilton et al., 2001; Hattori et al., 2001a,b; Yamada et al., 2001] examined polymorphisms of the CCK and CCK-BR genes, but the results were inconclusive. There has been only one study to determine the CCK-AR gene polymorphism with no association [Kennedy et al., 1999], which was 5′ area of the 3′ untranslated region, and its functional role is unknown.

We recently cloned the genomic structures of human CCK-AR [Funakoshi et al., 2000], and determined the transcriptional start site of the human CCK-AR gene. Two sequence changes were detected in the promoter region: a G to T change in nucleotide −128 and an A to G change in nucleotide −81 [GenBank database under accession number D85606, Funakoshi et al., 2000]. Six genotypes, including a wild type (−81A/A, −128G/G) and five other variants, have been identified [Funakoshi et al., 2000; Shimokata et al., 2000]. The homozygote (−81G/G, −128T/T) showed a significantly higher percent body fat, although the real mechanism has not been clarified. In this study, we investigated a possible association between the CCK-AR gene and PD by evaluating the distribution of not only the genotypes but also the haplotypes of the two polymorphisms.

The subjects consisted of 109 Japanese patients with PD (64 males, 18–63 years old; 45 females, 21–71 years old), all of whom met DSM-III-R criteria for PD on the PD part of the Structured Clinical Interview for DSM-III-R (SCID) assessment. The age- and gender-matched control group consisted of 400 unrelated Japanese. The controls were employees and students in Kurihama National Hospital and in the Tokyo Metropolitan Institute of Gerontology. Nobody shows signs of

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psychiatric disorders (234 males, 20–62 years old; 166 females, 21–71 years old). The Ethics Committees of the National Alcoholism Center, Kurihama Hospital, and Tokyo Metropolitan Institute of Gerontology approved this study. Written informed consent was obtained from each subject. Genomic DNA was extracted from peripheral leucocytes.

Examination of the polymorphism in the promoter region of the CCK-AR gene was accomplished using a mismatch PCR-RFLP method [Funakoshi et al., 2000]. Briefly, a pair of primers (sense primer = 5′-GCATATGTACACATGTGTGT-AAAAAGCAGCCAGAC-3′, and anti-sense primer = 5′-GCC-CTTTCCTGGGCCAGACT-3′) were used to amplify the 103-bp product, which was subsequently digested with restriction enzyme *Hinf* I and fractionated by 12% polyacrylamide gel electrophoresis.

Statistical differences between PD and control subjects were assessed using Fisher's exact test. An odds ratio with 95% confidence intervals was calculated to evaluate the difference in genotype frequencies between the groups. Probability differences of $P < 0.05$ were considered statistically significant. To assess linkage disequilibrium between the two polymorphisms of the CCK-AR gene, we calculated the D value and its significance, using the ASSOCIAT program downloaded from the web site of Dr. J. Ott (ftp://linkage.rockefeller.edu/software/utilities/). All statistical computations were carried out using the Statistical Analysis System package, version 6.12 [SAS Institute Inc, 1988].

Comparison of the genotype and haplotype distributions of the CCK-AR gene −81A to G and −128G to T polymorphism in PD patients and control subjects (Table I) revealed frequencies among the controls that were quite similar to those reported in community-dwelling individuals. Three kinds of genotypes (−81A/A, −128T/T), (−81A/A, −128G/T), and (−81A/G, −128T/T) were not detected in the previous cohort studies [Funakoshi et al., 2000; Shimokata et al., 2000] and in the present study. Therefore, haplotype −81A/−128T was not present, either. These polymorphisms were in linkage disequilibrium (PD samples, $D = 0.1495$, $P < 0.0001$: controls, $D = 0.0865$, $P < 0.0001$). Both genotypic frequencies of distributions were in Hardy–Weinberg equilibrium.

TABLE I. Genotype and Haplotype Frequencies of the −81A to G and −128G to T Polymorphisms in Patients With Panic Disorder and Controls

	Polymorphisms		Panic disorder	Controls
	−81	−128	N (%)	N (%)
Genotype[a]			N = 109	N = 400
	A/A	G/G	48 (44.0%)	238 (59.5%)
	A/G	G/G	13 (11.9%)	71 (17.8%)
	A/G	G/T	36 (33.0%)	75 (18.8%)
	G/G	G/G	1 (0.9%)	6 (1.5%)
	G/G	G/T	9 (8.3%)	6 (1.5%)
	G/G	T/T	2 (1.8%)	4 (1.0%)
OR (95% CI)[b]			2.81 (1.74–4.39)	
Haplotype[c]			N = 218	N = 800
	A	G	145 (66.5%)	622 (77.8%)
	A	T	0 (0.0%)	0 (0.0%)
	G	G	24 (11.0%)	89 (11.1%)
	G	T	49 (22.5%)	89 (11.1%)

[a]Percentages may not total 100 due to rounding. Three genotypes (−81A/A, −128T/T), (−81A/A, −128G/T), and (−81A/G, −128T/T) were not present. $P < 0.0001$ (df = 5), $P < 0.0001$ (with −81G/−128T haplotype vs. without −81G/−128T haplotype, df = 1) when analyzed by Fisher's direct test.

[b]Ratio of odds (genotypes with −81G/−128T haplotype/genotypes without −81G/−128T haplotype) and 95% confidence interval.

[c]Haplotype (−81A/−128T) was not detected. $P < 0.0001$ when analyzed excluding −81A/−128T haplotype (df = 2), $P < 0.0001$ when compared between subjects with and without −81G/−128T haplotype (df = 1).

The frequency of variant genotypes (−81A/G, −128G/T; G/G, G/T, and G/G, T/T) having variant haplotype (−81G/−128T) was significantly higher in PD than in controls ($P < 0.0001$, OR = 2.81, 95% CI = 1.74–4.39). The statistical differences between the haplotype distributions in the PD and control groups were highly significant: The frequency of variant haplotype (−81G/−128T) was higher in the former group than in the latter ($P < 0.0001$; Table I).

Stratification of the PD samples and controls with respect to age and gender did not alter these relationships. Nor did the age at onset of PD affect the distributions of the CCK-AR gene polymorphisms (data not shown).

The frequencies of both the variant genotypes and haplotypes of the −81A to G and −128G to T polymorphisms of the CCK-AR gene were higher in our PD group than among our control subjects, suggesting that this gene is involved in the development of PD.

CCK-AR is expressed in specific brain regions such as the amygdala, nucleus tractus solitarius, posterior nucleus accumbens, ventral tegmental area, hypothalamus, substantia nigra, hippocampus, area postrema, and raphe nucleus, whereas CCK-BR is widely distributed throughout the central nervous system [Wank, 1995]. The expression patterns of these receptors overlap in the brain, and the cross-reactivity of each antagonist could not be excluded in pharmacological studies. Therefore, the functional differences of these two receptors remain unclear. Recently, we developed CCK-AR, BR, and ARBR gene knockout (−/−) mice and found that CCK-AR and BR may exert opposite influences on anxiety-related behaviors [Miyasaka et al., 2002a]. These evidences suggest that CCK-AR might be involved in induction of panic like attacks, although CCK-4 is a ligand of CCK-BR.

Our research has focused on two neighboring polymorphisms in the 5′ regulatory region of the CCK-AR gene, which shares the region involved in the regulation of the human CCK-AR promoter function [Takata et al., 2002]. We have examined CCK-AR gene polymorphisms in 50 patients with gallstone and 300 patients with diabetes mellitus before the establishment of RFLP method [Funakoshi et al., 2000]. We found one case with G to A in the intron 1, and another case C to G in the exon 3 without change in amino acid (Thr). The polymorphisms of promoter region (between −351 and +176) were also examined and no polymorphisms besides −81A to G and −128G to T were detected. Therefore, although various kinds of CCK-AR polymorphisms have been reported [Inoue et al., 1997; Tachikawa et al., 2001; Okubo et al., 2002], these may occur sporadically.

Although our recent investigation using the STC-1 murine neuroendocrine cell line showed that neither the −81A to G nor the −128G to T polymorphism affects luciferase activities [Takata et al., 2002], limitations in the experimental conditions suggest that those findings should be interpreted as inconclusive, because no human cell lines have been available. In a recent examination of the correlation of demethylation of the CCK-AR gene and its expression, we found significantly higher gene expression when the methylation level of the gene was low [Matsusue et al., 1999; Miyasaka et al., 2002b]. We observed many GC-rich segments in the CCK-AR promoter region, and the nucleotide position at −128 was methylated. Thus, a G to T replacement at the −128 position might be capable of altering CCK-AR gene expression.

In this study, the first to report the positive association of the CCK-AR polymorphisms and PD, haplotype analyses further strengthened the association based on our comparison of genotype distributions.

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The Impact of Health Problems on Depression and Activities in Middle-Aged and Older Adults: Age and Social Interactions as Moderators

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In this study, we compared the impact of health problems (HPs) on everyday activities and depressive symptoms between middle-aged and older adults. We also examined what type and source of social interactions moderate the noxious effects of HPs. Longitudinal analyses of data with 1,802 Japanese community-dwelling adults indicated that HPs were significantly related to (a) an increase in depressive symptoms among middle-aged adults and (b) a decline in everyday activities among older adults. The former was buffered by emotional family support, whereas the latter (b) was buffered by instrumental family support and, surprisingly, by negative interactions with family. In contrast, social interactions with other friends and acquaintances did not show any moderating effect.

MANY researchers have reported the relationship between health problems (HPs), such as disease or injury, and lower activities among older adults (Dargent-Molina, Hays, & Breart, 1996; Guccione et al., 1994; Mor et al., 1989). Yet, as aging per se is a normal phenomenon that leads to a limitation of activities (Dickerson & Fisher, 1993), the pathologic effects of disease on disability are sometimes confused with the results of normal aging (Pearson, 2000). However, the decline of physical function that is due to normal aging is relatively small and should not interfere with the ability of older adults to live an autonomous and high-quality life (Ferrucci et al., 2002). In fact, a noticeable percentage of persons who reach extreme longevity are still able to perform activities of daily living (ADLs; Franceschi et al., 2000). Consequently, we could expect older adults to live primarily free of disability if they do not suffer from HPs.

This assumption does not mean that the role age plays in the disablement process is negligible. For instance, Pohjasvaara, Erkinjuntti, Vataja, and Kaste (1997) examined whether ischemic strokes have an equivalent negative impact on the daily activities of younger versus older patients. They found that ADL functions deteriorated more significantly among the older patients than the younger patients after the stroke. The results suggest that older adults are more likely than younger adults to decrease their activities because of HPs. However, although most prior studies have statistically controlled for age when estimating the impact of HPs, the question of how age and HPs interact and influence everyday activities has rarely been addressed.

Psychosocial stress theory also considers HPs to be influential life events leading to a decline in psychological well-being such as depression (Holms & Rahe, 1967; Murrel, Norris, & Hutchins, 1984). Although little is known about the role of age in the stress process (Folkman, Lazarus, Pimley, & Novacek, 1987; Martin, Gruendahl, & Martin, 2001), theoretical and empirical findings suggest that HPs exert less impact on well-being in older adults than in younger adults. For example, although HPs affect middle-aged adults' well-being by producing social role strain regarding work or parenting (Karasz & Ouellette, 1995), the number of such social roles decreases as people age (Aldwin, Sutton, Chiara, & Spiro, 1996). As Krause (1994) argued, stressors will be more strongly associated with well-being when they arise in important social roles. Thus, the fewer social roles of older adults may make dealing with HPs psychologically less challenging for them than it is for middle-aged adults. Indeed, Aldwin and colleagues (1996) found that older adults were not more likely to appraise HPs in terms of loss or threat, despite the fact that they were actually dealing with HPs.

Furthermore, according to the normative life events theory, events that are not expected for a particular period in life will have a greater impact on the well-being of individuals during that particular period than on individuals during other periods (Pearlin & Lieberman, 1979). Following the logic, the higher morbidity in older adults (Bowling & Grundy, 1997; Kriegsman, van Eijk, Penninx, Deeg, & Boeke, 1997) may attenuate the psychological impact of HPs on the age group, compared with younger groups. In fact, Hurwicz, Durham, Boyd-Davis, Gatz, and Bengtson (1992) found that, although over 30% of the older adults tended to report "ill health" as the event that had had the greatest impact on them, compared with 21% of the middle-aged and 16% of the younger adults, a significant association between the ill health and depressive symptoms was revealed only in the younger adults.

Taken as a whole, the primary purpose of the present study was to examine age differences in the impact of HPs on everyday activities and depressive symptoms among middle-aged and older adults. However, disability and depression are not merely dependent on a person's HPs but are also subject to psychosocial factors. This study also focused on one such factor, social interactions, and its role in the relationships between HPs and outcomes.

An extensive body of research has demonstrated that positive social interactions, such as social support, contribute to the improvement of a person's psychological and physical well-being (Cohen & Willis, 1985). However, there is a growing consensus that social support is a multidimensional concept, and the type and source of support should be considered when its effect is estimated. Some studies have found that social support from friends is more effective for psychological well-being than that from family (Dean, Kolody, & Wood, 1990; Lee & Ishii-Kuntz, 1987). Others assert that family support is more effective than support from friends (Chi & Chou, 2001; Yanagisawa et al., 2002). The findings from Felton and Berry (1992) are more complicated: Emotional support was more beneficial for psychological well-being when provided by nonkin, whereas instrumental support was more beneficial when provided by kin. Regarding physical health outcomes, some researchers have found support to be beneficial in improving physical function and activities (Duke, Leventhal, Brownlee, & Leventhal, 2002; Seeman et al., 1995), whereas Mendes de Leon, Gold, Glass, Kaplan, and George (2001) have indicated that instrumental support was associated with an *increase* of disability risk among older adults.

Although the previous findings are not altogether consistent, a careful review of the literature by Crohan and Antonucci (1989) revealed that, whereas friends tend to play their largest role in the arenas of socialization and the provision of day-to-day companionship, support provided by family becomes important when long-term sick care or daily living help is needed. This suggests that support from family would be more beneficial than support from other relationships, at least for people suffering from HPs.

The mixed findings on the effect of support by type may be partly derived from the analytical designs. Most previous studies have focused, on one hand, on the *direct* effect of social support: the effect of improving well-being regardless of whether stressors, such as HPs, are present or not. The *buffering* effect, on the other hand, posits that support is related to well-being for persons under stress, as it protects persons from the potentially pathologic influence of stress events (Cohen & Willis, 1985). Indeed, Penninx and associates (Kriegsman et al., 1997; Penninx et al., 1997, 1998) have reported that instrumental support buffered the incidence of mobility difficulties for older people with chronic lung disease, whereas emotional support buffered the increase of depressive symptoms for older people with cardiac disease and arthritis. As Cohen and Willis (1985) argued, these findings suggest that there must be a reasonable match between the type of available support and the consequences of HPs in order for buffering to occur.

The present study also investigated how negative interactions, that is, interference and criticism dimensions of social interactions, may moderate the relationship between HPs and outcomes. Although negative interactions are theoretically assumed to exacerbate the noxious association of stressors with well-being (Shinn, Lehman, & Wong, 1984), this negative buffering, or stress-amplification hypothesis (Okun, Melichar, & Hill, 1990), has not been well established. For example, Finch, Okun, Barrera, Zautra, and Reich (1989) failed to confirm the effects of negative interactions to aggravate the associations between HPs and psychological distress in older adults. However, because their study had a cross-sectional analytical design using a relatively small sample ($N = 246$) and lacked attention to disability outcome, the results still have opened the door for further examination of the moderating effect of negative interactions on HPs and their consequences.

Hypotheses and Analytic Strategy

On the basis of the aforementioned grounds, this study examined the moderating effects of age and social interactions in the impact of HPs on everyday activities and depressive symptoms, using longitudinal data collected from Japanese middle-aged and older adults. In Japan, as in other Western countries, the population is aging rapidly, and estimating the influence of HPs on older adults' lives has become an important issue (Health and Welfare Statistics Association, 2002). This study would contribute to the available data for developing a cross-cultural view on the experience of HPs in later life.

The analysis was twofold. First, we addressed the question of whether the impact of HPs differed between middle-aged and older adults. We expected that the impact of HPs in decreasing everyday activities would be greater in older adults, compared with middle-aged adults. In contrast, compared with older adults, middle-aged adults would be more likely to increase their depressive symptoms because of HPs. To test the hypotheses, we conducted repeated measures analyses with a mixed procedure (SAS Institute Inc., 1996) to examine pattern differences of changes in activities and depression scores according to age group and HP experience.

Second, we examined what type and source of social interactions would moderate the negative consequences of HPs. To avoid problems associated with multicollinearity, we examined in separate analyses whether the effect of HPs is moderated by the level (high vs. low) of three types of social interactions (emotional support, instrumental support, and negative interactions), provided by the two interaction sources of family and others (friends or acquaintances). We expected that social interactions with family would more effectively buffer the negative consequences of HPs than interactions with others. Furthermore, we expected that emotional support would buffer the increase of depressive symptoms, whereas instrumental support would buffer the decline of everyday activities caused by HPs. We also predicted that negative interactions would amplify the noxious effects of HPs on activities and depressive symptoms.

METHODS

Participants

The data for this study are taken from the baseline (Wave 1, from November 1997 to April 2000) and the follow-up (Wave 2, from April 2000 to May 2002) surveys of the National Institute for Longevity Sciences–Longitudinal Study of Aging (NILS–LSA). The average follow-up interval was 2.1 years. The NILS–LSA participants were Japanese community-dwelling adults between 40 and 79 years of age, randomly recruited from areas around the institute. Details of the NILS–LSA have been described elsewhere (Shimokata, Ando, & Niino, 2000). The study sample consisted of 1,802 men and women who had completed examinations at both Wave 1 and Wave 2. The average age for the entire sample was 58.3 ± 10.6 years. For

the analyses in this article, we divided the sample into two groups according to their age at Wave 1 (older adults: 60–79 years old, $n = 833$; middle-aged adults: 40–59 years old, $n = 969$).

Measures

Health problems.—We identified HPs by using a life event checklist at Wave 2. The trained interviewer presented participants with the checklist of 43 items and asked them to report the occurrence of each event between Wave 1 and Wave 2 periods. Participants who reported the occurrence of "major injury or disease," one of the listed items, were classified into the HP-present (HPP) group, whereas all others were classified into the HP-absent (HPA) group. Although information about the type of HP was collected from the HPP participants, no type-specific analyses were conducted in the study.

Everyday activities.—We measured everyday activities both at Wave 1 and Wave 2 by using the Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG-IC; Koyano, Shibata, Nakazato, Haga, & Suyama, 1987). This scale includes 13 items conceptually grouped into three categories: instrumental ADLs (using transportation; shopping, preparing meals, paying bills, and making bank deposits and withdrawals), intellectual or cultural activities (filling out forms, reading papers, reading books and magazines, and watching television), and social engagement activities (visiting a friend's home, helping others, going to see someone in the hospital, and talking to others). Acceptable reliability ($\alpha = .86$) and validity (association with mortality) of the index have been reported (Koyano, Shibata, Nakazato, Haga, & Suyama, 1991). Participants indicate whether or not they can perform each activity with a "yes" (= 1) or a "no" (= 0), and then the responses are totaled and used as an index of everyday activities (a higher score indicates better performance). In the study, the internal reliability of the scale was .72.

Depressive symptoms.—We measured depressive symptoms at both Wave 1 and Wave 2 by using a Japanese version of the Center for Epidemiologic Studies Depression (CES-D) scale (Radloff, 1977; Shima, Shikano, Kitamura, & Asai, 1985). Participants indicated how often during the previous week they had experienced any of the 20 depressive symptoms described in the scale. Each item was rated on a 4-point scale ranging from 0 (rarely or none of the time) to 3 (most or all the time). Four positively worded items were reverse scored. The points were added together so that a higher score represented a higher level of depressive symptoms. For our sample, the internal reliability of the scale was .91.

Social interactions.—We measured social interactions at Wave 2, using a scale developed by Noguchi (1991). This scale comprises three subscales: emotional support, instrumental support, and negative interactions, each of which consists of four items. Participants indicated whether there was someone to provide specific interactions such as "listening to them when they have worries or problems" (emotional support), "caring for them when they are confined to bed for several weeks" (instrumental support), or "being critical of them" (negative interactions). We rated the responses on a 4-point scale, ranging from 1 (none) to 4 (many), with higher scores indicating more social interactions. Each item was duplicated to refer to the two interaction sources of family and others (friends or acquaintances). Summing up item scores by type and source, we generated six social interaction measures (emotional support from family, emotional support from others, instrumental support from family, instrumental support from others, negative interactions with family, and negative interactions with others). The internal reliability of the measures ranged from .71 to .86. For analyses, participants with scores 1 *SD* below the age-specific mean of emotional support from family were classified into the low-emotional family support group and others were classified into the high-emotional family support group. We conducted the same operations for the other social interaction measures. However, for negative interaction measures, participants with scores 1 *SD above* the mean were classified into the high-negative interactions group and others were classified into the low-negative interactions group.

Control variables.—We used gender and annual family income at Wave 1 as control variables because of their significant associations with the outcome variables (data not shown). We measured annual family income by using a scale with 11 options (from 1 = income less than ¥1,500,000 to 11 = income greater than ¥20,000,000). In addition, in the analyses we considered the following information regarding participants' health status at Wave 1. Subjective health was assessed by a single self-reported question ("How would you rate your health?"), with five options (from 1 = very bad to 5 = very good). The presence and history of seven diseases (stroke, hypertension, cardiovascular disease, diabetes, bronchitis, arthritis, and cancer) were totaled as an index of participants' existing chronic conditions.

Results

Sample Characteristics

Table 1 presents the results of bivariate tests of age differences in the study variables. Male participants represented just over half of the individuals in both age groups. The older group had a significantly lower income than the middle-aged group. The older group also felt less healthy and was in worse condition than the middle-aged group, although the differences were not very large. Those who reported experiencing HPs between the surveys made up 13.9% of the older group and 11.6% of the middle-aged group, which was not significantly different. Regarding the HP subtypes, participants who reported disease outstripped those who reported injury and those who reported both disease and injury or malfunctions from an unknown cause such as indefinite complaint (categorized as "other" in Table 1) in both age groups. The outcome variables did not differ between the age groups, except for the TMIG-IC score at Wave 2, which was slightly lower in the older group. Regarding social interactions, older adults reported fewer negative interactions with both family and others. Furthermore, older adults reported slightly less emotional and instrumental support from others than did middle-aged adults.

Table 1. Descriptive Information for Study Variables and Age Differences

Variable	Older	Middle-Aged
Gender, male (%)	53.4	51.0
Income (*M*)	5.1 (2.4)	7.3 (2.0)***
Subjective health (*M*)	3.1 (0.6)	3.3 (0.7)***
Chronic conditions (*M*)	0.8 (0.8)	0.3 (0.5)***
HPP (%)	13.9	11.6
Disease	7.7	5.6
Injury	5.0	4.6
Other	1.2	1.3
TMIG-IC (*M*)		
Wave 1	12.3 (1.1)	12.4 (1.0)
Wave 2	12.2 (1.2)	12.3 (1.1)*
CES-D (*M*)		
Wave 1	7.0 (6.7)	6.9 (6.2)
Wave 2	7.4 (6.7)	7.3 (6.7)
Social interactions, source–type (*M*)		
Family–emotional	12.5 (2.3)	12.4 (2.1)
Family–instrumental	12.1 (2.2)	12.0 (2.0)
Family–negative	8.6 (2.4)	9.6 (2.2)***
Other–emotional	11.2 (2.7)	11.6 (2.5)***
Other–instrumental	9.0 (2.8)	9.3 (2.7)*
Other–negative	7.0 (2.3)	8.3 (2.3)***

Notes: Entries in parentheses are standard deviations. HPP = health problem present; TMIG-IC = Tokyo Metropolitan Institute of Gerontology Index of Competence; CES-D = Center for Epidemiologic Studies Depression (scale).

*$p < .05$; ***$p < .001$.

Age and the Impact of HPs

We conducted repeated measures analyses to examine pattern differences of changes in the TMIG-IC and CES-D scores according to age (middle-aged vs. older) and HP (HPP vs. HPA) groups. The design was a split plot, involving the main effects of age, HP, and time (Wave 1 vs. Wave 2), and their two-way and three-way interaction terms. Gender, income, subjective health, chronic conditions, and the baseline outcome (TMIG-IC or CES-D) were used as covariates.

The analysis indicated that, among between-subject factors, gender (female), $F(1, 1741) = 10.88$, $p < .001$, subjective health, $F(1, 1741) = 9.45$, $p < .01$, and the baseline outcome, $F(1, 1741) = 5751.82$, $p < .001$, had significant positive associations with the TMIG-IC score. No significant main effects were found for age, HP, and Age × HP interaction. Regarding within-subject factors, time, $F(1, 1743) = 13.32$, $p < .001$, and Age × Time, $F(1, 1743) = 6.73$, $p < .01$, were revealed to be significant, suggesting participants decreased their activities during the surveys, regardless of age or HP effects, and the decline was more rapid in the older group. However, a significant three-way interaction among Age × HP × Time, $F(1, 1743) = 5.34$, $p < .05$, was also revealed, suggesting that the change in the TMIG-IC score depended on the participant's age and HP experience.

We performed the same analysis with the CES-D outcome. The results indicated that, among between-subject factors, chronic conditions, $F(1, 1737) = 8.02$, $p < .01$, and the baseline outcome, $F(1, 1737) = 5271.15$, $p < .001$, had significant positive associations with the CES-D score, whereas income, $F(1, 1737) = 4.23$, $p < .05$, and subjective health, $F(1, 1737) = 18.44$, $p < .001$, had significant inverse associations with the CES-D score. Furthermore, significant main effects with regard to age, $F(1, 1737) = 12.53$, $p < .001$, and HP, $F(1, 1737) = 3.93$, $p < .05$, emerged, suggesting that those in the middle-aged group and those in the HPP group had more depressive symptoms compared with those in the older group and those in the HPA group, respectively. However, these effects are qualified by a between-subject factor, Age × HP interaction, $F(1, 1737) = 6.18$, $p < .05$, and within-subject factors, time, $F(1, 1701) = 8.90$, $p < .01$, and Age × HP × Time, $F(1, 1701) = 4.39$, $p < .05$. These results suggest that changes in depressive symptoms relating to HPs were not equivalent between the age groups.

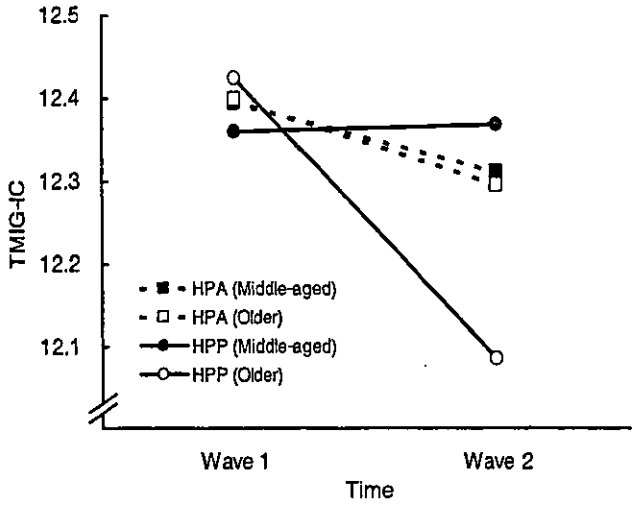

Figure 1. Changes in everyday activities (Tokyo Metropolitan Institute of Gerontology Index of Competence, or TMIG-IC) over time relating to age and health problem (HP) experience. HPA = HP absent; HPP = HP present.

Figure 1 and 2 illustrate the nature of the interactions by displaying the changes in the outcome scores over time by age and HP groups. We carried out further tests to examine simple main effects of time on each group by using the Tukey–Kramer adjusting method. After we controlled for gender, income, subjective health, chronic conditions, and the baseline outcome (TMIG-IC or CES-D), results revealed that the TMIG-IC score in older adults who had experienced HPs significantly decreased, $t(1743) = 3.64$, $p < .01$ (Figure 1), whereas the CES-D score in middle-aged adults who had experienced HPs significantly increased, $t(1701) = -3.14$, $p < .05$ (Figure 2), between the surveys. The HPA groups, in contrast, did not show any significant changes in their outcome scores, indicating stability of activities or mental health status over time irrespective of age group.

Moderating Effect of Social Interactions

Our second research goal was to determine the type and source of social interactions that moderate the noxious effects of HPs. On the basis of the results mentioned herein, we performed the analyses only on HPP participants. That is, for older adults with HPs, we carried out repeated measures analyses to determine whether the level (high vs. low) of each

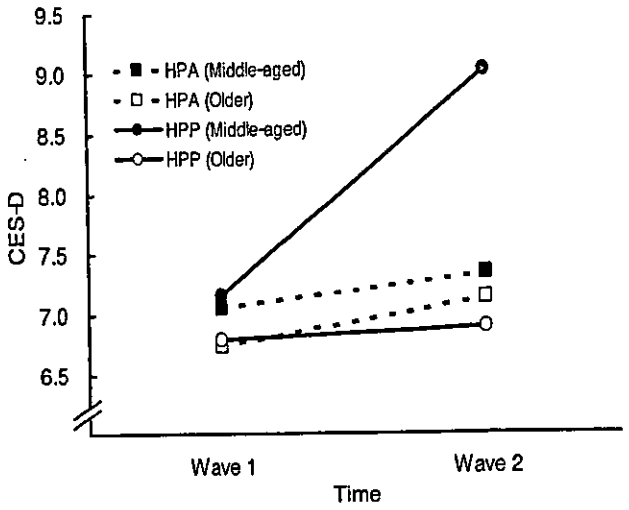

Figure 2. Changes in depressive symptoms (Center for Epidemiologic Studies Depression, or CES-D, scale) over time relating to age and health problem (HP) experience. HPA = HP absent; HPP = HP present.

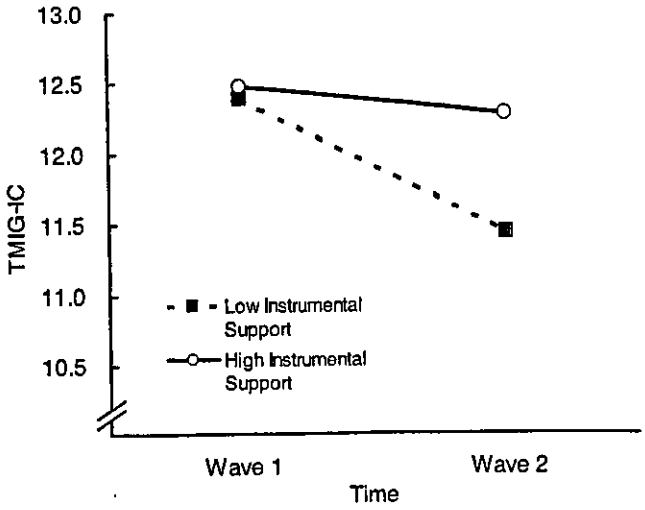

Figure 3. Changes in everyday activities (Tokyo Metropolitan Institute of Gerontology Index of Competence, or TMIG-IC) over time among older adults with health problems by the level of instrumental family support.

social interaction moderates (buffered or amplified) the change in TMIG-IC score over time. Similarly, for middle-aged adults with HPs, we examined whether the level of each social interaction moderates the change in CES-D score over time. The analyses tested the statistical significance of the main effect of each social interaction level and its interaction with time, controlling for gender, income, subjective health, chronic conditions, and the baseline outcome (TMIG-IC or CES-D).

The results were as follows. For older adults with HPs, significant main effects for levels of instrumental family support, $F(1, 104) = 11.09$, $p < .01$, emotional support from others, $F(1, 105) = 4.77$, $p < .05$, and negative interactions with family, $F(1, 104) = 4.45$, $p < .05$, emerged, with higher levels of these interactions increasing the TMIG-IC score. For middle-aged adults with HPs, significant main effects for levels of emotional family support, $F(1, 101) = 6.67$, $p < .05$, and instrumental family support, $F(1, 101) = 10.10$, $p < .01$, emerged, with higher levels of these interactions decreasing the CES-D score. No significant main effects were found for levels of other social interaction measures on both age groups, regardless of the outcome variable.

In addition to the main effects, three significant interactions emerged: Instrumental family support × Time, $F(1, 109) = 7.65$, $p < .01$, and Negative interactions with family × Time, $F(1, 109) = 5.55$, $p < .05$, in older adults, and Emotional family support × Time in middle-aged adults, $F(1, 104) = 3.97$, $p < .05$. The effects of these interactions are illustrated in Figures 3, 4, and 5. Among older adults with HPs, there was a significant decrease in the TMIG-IC score over time only when instrumental family support level was low, $t(109) = 3.89$, $p < .01$ (Figure 3). Surprisingly, there was also a significant decrease of the TMIG-IC score over time when the level of negative interactions with family was low, $t(109) = 3.95$, $p < .001$ (Figure 4). Among middle-aged adults with HPs, there was a significant increase of the CES-D score over time only when the emotional family support level was low, $t(104) = -2.72$, $p < .05$ (Figure 5).

Discussion

Age and the Impact of HPs

The three-way interaction of Age × HP × Time and the post hoc analyses indicated that the decline of everyday activities over time was statistically significant only for older adults who had experienced HPs between the surveys. The findings support our prediction that the impact of HPs in decreasing everyday activities would be greater in older adults, compared with middle-aged adults. As we noted earlier, aging per se is not so influential in limiting everyday activities of older adults (Dickerson & Fisher, 1993). However, although it is not inherently impaired, the reserve capacity to compensate for stress, metabolic derangement, and drug metabolism is increasingly limited with advancing age (Oskvig, 1999). Perhaps the age-related decline of reserve capacity in the human body allows HPs to have a greater impact on older adults, thus leading to limitations in the performance of everyday activities.

Another explanation for the difference is that the nature of HPs varies across age groups. Valderrama-Gama, Damian, Ruigomez, and Martin-Moreno (2002) argued that, whereas acute disease represents the main cause of HPs in young people, chronic conditions are more prevalent in older persons. Focusing on the type and, possibly, the severity of the HP would be the next step to developing and refining our findings.

In contrast, as we also expected, the increase of depressive symptoms over time was statistically significant only for middle-aged adults who had experienced HPs between the surveys. This is consistent with other studies (Aldwin et al., 1996; Hurwicz et al., 1992), including a Japanese one (Matsunaka, 2002), suggesting older adults are less emotionally reactive to HPs than younger adults. Thus, our results confirm the cross-cultural consistency in the psychological resilience of older adults with HPs. As we discussed earlier, because HPs threaten the salient social roles of middle-aged adults as parents or income earners (Karasz & Ouellette, 1995), they could become more critical for them and could lead to severe

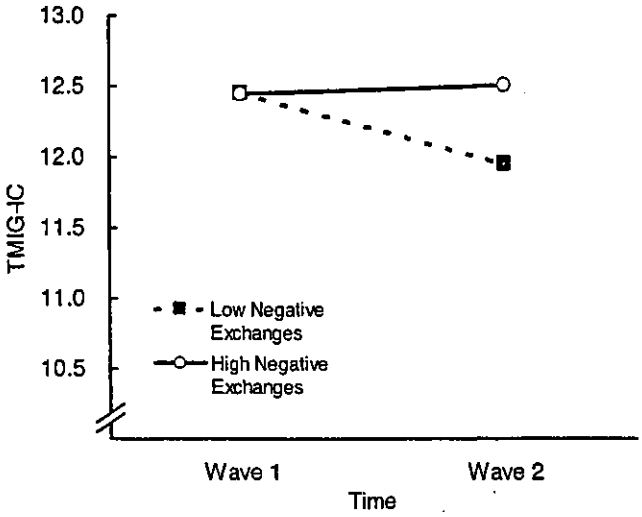

Figure 4. Changes in everyday activities (Tokyo Metropolitan Institute of Gerontology Index of Competence, or TMIG-IC) over time among older adults with health problems by the level of negative interactions with family.

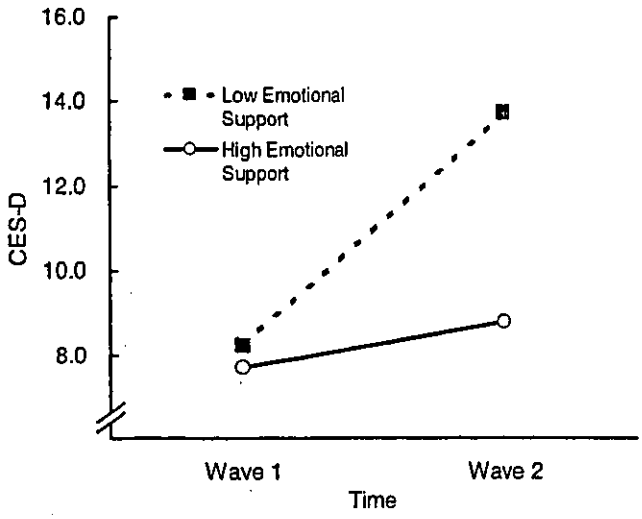

Figure 5. Changes in depressive symptoms (Center for Epidemiologic Studies Depression, or CES-D, scale) over time among middle-aged adults with health problems by the level of emotional family support.

psychological distress. In contrast, as disengagement theory (Cumming & Henry, 1961) implies, aging is a process whereby people gradually withdraw from society and are relieved of annoying obligations and social roles. The decreased demands from society may allow older adults to feel at ease and less stressed even when they are suffering from HPs.

Our findings would also be compatible with the normative life events theory, which posits that events that are expected for a particular period in the life span will have less impact on well-being (Pearlin & Lieberman, 1979). We found that the older group felt less healthy and was in worse condition even at the study entry, compared with the middle-aged group (see Table 1). As Norris and Murrell (1988) pointed out, the prior experience of a stressor provides "inoculation" against strong emotional reaction to a similar stressor experienced in later days. Thus, relatively worse health status at baseline in older adults might make provision against newly experienced HPs and attenuate the influence on psychological well-being.

Moderating Effect of Social Interactions

We found that instrumental family support buffered the decline of everyday activities in older adults, whereas emotional family support buffered the increase of depressive symptoms in middle-aged adults. In contrast, social support provided by other relationships (friends or acquaintances) did not show any buffering effect in either age group. One of the implications of the findings is that, as we expected, social support is more effective at preventing negative consequences of HPs when it comes from family rather than from other relationships. According to a hierarchical–compensatory model of social support (Cantor, 1979), people select supportive ties from a hierarchy of relationships, with family members always selected before nonfamily members. Additionally, as in other Asian countries such as China (Chi & Chou, 2001) and Korea (Mo, 1999), Japanese society maintains cultural values and traditional norms of caregiving in which the family provides care for its older members (Asahara, Momose, & Murashima, 2002). These values and norms may also strengthen the central roles of family as the support provider, especially for people suffering from HPs. However, because we aggregated friends and acquaintances into one support source category of "other relationships," the effects of key network nonkin members might be masked (Barrera, Chassin, & Rogosch, 1993). Distinguishing "other relationships" across levels of closeness would then be needed to further assess the beneficial effect of nonkin support.

Regarding the type of social support, the results also supported our predictions: Emotional support buffered the increase of depressive symptoms, whereas instrumental support buffered the decline of everyday activities. The results are in line with those of other cross-sectional findings (Kriegsman et al., 1997; Penninx et al., 1997, 1998) and the theoretical suggestion that posits that support closely linked to the specific need elicited by a stressor will exert the buffering effect (Cohen & Willis, 1985). For example, emotional support might reduce depressive symptoms caused by HPs because this type of support enhances the person's self-esteem, whereas instrumental support attenuates limitations of activities because it helps the person seek medical care for the HPs. However, contrary to our findings, Mendes de Leon and colleagues (2001) found that older adults with more instrumental support showed a decline of ADLs over time. They examined how baseline instrumental support predicts lower ADL functions at the follow-up, with no regard to the presence or absence of HPs in the study population, whereas we examined whether older adults with HPs maintain their activity level by instrumental support measured at the follow-up. The inconsistency between the studies would then be due to the differences in analytical designs.

An unexpected finding in the study is that negative interactions with family did not amplify but rather buffered the noxious effect of HPs on older adults' everyday activities. A possible explanation is that social network members may, by being critical and demanding of an older person, induce him or

her to engage in beneficial health behaviors (Lewis & Rook, 1999). For example, a wife may be critical of her diabetic husband for not following an appropriate diet (Krause, 2001). Indeed, Krause, Goldenhar, Liang, Jay, and Maeda (1993) found that negative interactions predicted more frequent exercise among older Japanese adults. Perhaps negative interactions would potentially contribute to discouraging health-compromising behavior and promoting health-enhancing behavior.

Future Directions

Some limitations should be kept in mind when our findings are interpreted. First, Lawrence and Jette (1996) described the multistep pathway in the disablement process: Presence of disease leads to anatomic and structural abnormalities, which in turn lead to restrictions in basic physical and mental actions, which then lead to difficulty performing ADLs. Analyses using a 2-point assessment method, as in our study, are too simple to examine such a multistep disablement process. Second, we did not pursue gender differences in the effects of HPs to avoid the complicated interpretation of a four-way interaction (HP × Age × Gender × Time). However, compared with men, women tend to report poorer physical health associated with chronic illness (O'Neill & Morrow, 2001) and more depressive symptoms (Nolen-Hoeksema, Larson, & Grayson, 1999). An elaborate analysis concerning these issues would modify and extend our findings.

Despite these limitations, the findings garnered from the present study add several specific points to the literature examining relationships between HPs and their negative consequences. Whereas most studies have restricted their sample to older adults or analyzed data by using age just as one of the confounders, this study made clear that the impact of HPs differs across age groups. Moreover, we highlight the importance of taking into account the type and source of social interactions when we are investigating how they moderate the impact of HPs. Although the results suggest that the relationships among HPs, everyday activities, and mental health are more complex than generally conceived, that is what makes it so challenging for researchers and professionals who are interested in stress and the development process in adulthood.

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The relationship between age and intraocular pressure in a Japanese population: The influence of central corneal thickness

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Abstract

Purpose. Previous cross-sectional Japanese studies have shown that intraocular pressure (IOP) decreases with age. However, central corneal thickness (CCT) variation should also be considered when examining the relationship between age and IOP, since tonometry has an inherent measurement error due to CCT variations. This study investigates the influence of CCT variation on the age-IOP relationship in a Japanese population.

Methods. The right eyes of 1317 subjects from 40 to 80 years old selected from a general population using a random sampling method were assessed in cross-section. The IOP was measured with a non-contact tonometer, and CCT was measured with a specular microscope. The relationships between age, IOP, and CCT were assessed using correlation analyses, while the relationship between age and IOP controlled for CCT, blood pressure, and body mass index was investigated using multivariate regression analyses.

Results. The mean (± SD) IOP value was 13.6 (± 2.6) mmHg in men and 13.3 (± 2.6) mmHg in women. The IOP correlated inversely with age in men ($r = -0.14$, $p < 0.001$), but showed only a marginal inverse correlation in women ($r = -0.07$, $p = 0.066$). The mean (± SD) CCT value was 518.3 (± 33.2) μm in men and 511.1 (± 33.0) μm in women. Only in men was an inverse correlation seen between CCT and age ($r = -0.10$, $p = 0.009$), but both genders had positive correlations between CCT and IOP (man: $r = 0.44$, $p < 0.001$; woman: $r = 0.48$, $p < 0.001$). In multivariate analyses, CCT was shown to have an effect on IOP measurement, however, it was shown that IOP still decreases with age in both sexes even when adjusted for CCT ($p = 0.001$).

Conclusions. The IOP decreases with age in the Japanese, and CCT variation has practically no effect on the unique age-IOP relationship.

Keywords: age; corneal thickness; intraocular pressure; population-based study

Introduction

In cross-sectional analyses, a positive correlation between age and intraocular pressure (IOP) has been reported in black and white populations,[1–6] while an inverse association was found in Japanese population studies.[7–9] The reason for this contrast in the age-IOP relationship between Japanese and other populations seemed to be related to blood pressure and obesity: the Japanese population tended to be less hypertensive and less obese with age than the other populations, and blood pressure and obesity have been demonstrated as having a positive association with IOP.[7] Our previous study in a Japanese population also showed a similar inverse association between age and IOP in a cross-sectional setting, although following the individual subjects over a longer time showed IOP did increase with advancing age.[10]

Variation in central corneal thickness (CCT) was assumed to be one of the reasons for this difference in IOP between populations for several reasons: 1) CCT has an important influence on IOP measurement, with thin corneas producing

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falsely low readings,[11-13] 2) there was a significant decrease in CCT with age in a Japanese population,[14] and 3) a significant positive correlation was identified between IOP and CCT.[15-18] In the present study, therefore, we measured IOP and CCT in a general Japanese population and investigated the influence of CCT variation on the relationship between age and IOP to examine this assumption.

Materials and methods

We measured the IOP and CCT of 1317 subjects (680 men and 637 women) as part of the National Institute for Longevity Sciences – the Longitudinal Study of Aging program (NILS-LSA) from April 1999 to November 2000. The subjects were randomly selected from the general population stratified by age and gender. Their ages ranged from 40 to 80 years. A detailed description of the sampling scheme has been reported elsewhere.[19] The NILS-LSA consists of clinical evaluations, body composition and anthropometry, physical function, nutritional analysis, and psychological tests. All procedures for the study were reviewed by the Ethical Committee of the Chubu National Hospital, and written informed consent was obtained from all subjects. The NILS-LSA participants who had a history of eye surgery or current users of contact lenses were excluded from the present study.

IOP was measured three times with a non-contact tonometer (NT-3000; Nidek, Gamagori, Japan) between 9:00 a.m. and 12:00 a.m, and the mean value of the three measurements was used for analysis. The tonometer was periodically calibrated. Following the IOP measurements, CCT was measured with a Topcon specular microscope (SP-2000P; Topcon, Tokyo, Japan) which uses non-contact optical pachymetry to focus automatically on the epithelial and endothelial surfaces along with the optical axis of the eye. The device has been reported to be accurate and reproducible,[20] including when it was used in our previous study for the NILS-LSA.[21] Because there was a high correlation between left and right eyes for both IOP ($r = 0.82$, $p < 0.0001$) and CCT ($r = 0.92$, $p < 0.0001$) in our subjects, the values of IOP and CCT from the right eye only were used for analyses in the present study.

Height and weight were measured according to a standardized protocol, and body mass index was calculated by dividing weight (in kilograms) by height squared (in meters). Systolic and diastolic blood pressures were measured with a standard mercury sphygmomanometer on the right arm between 9:00 a.m. and 12:00 a.m.

All statistical analyses were performed by gender using the Statistical Analysis System (SAS) release 6.12,[22] with the study population data presented as the mean and SD. Comparisons of mean IOP and CCT by gender were performed by the Student's *t* test. The correlations among age, IOP, CCT, blood pressure, and body mass index were calculated as Pearson's correlation coefficients. Subsequently, the correlates of IOP were estimated by multiple linear regression analyses.

Results

Mean values for age, IOP, CCT, systolic blood pressure, diastolic blood pressure, and body mass index are shown by gender in Table 1. Men in this sample, on average, had significantly higher IOP ($p = 0.037$) and CCT ($p < 0.001$) than women.

Table 2 shows the age and gender specific IOPs and CCTs. In men, both IOP and CCT significantly decreased with age ($r = -0.14$, 95% confidence interval (CI): −0.21, −0.07; $r = -0.10$, 95% CI: −0.17, −0.03, respectively). In women, there was also a marginal inverse correlation between age and IOP ($r = -0.07$, 95% CI: −0.15, 0.01), though no significant association between age and CCT was detectable.

In Table 3, we summarize the results of correlation analyses for age, IOP, CCT, and the other variables considered. There was a strong positive association between IOP and CCT in both men and women ($r = 0.44$, 95% CI: 0.38, 0.50; $r = 0.48$, 95% CI: 0.42, 0.54, respectively). There was also a significant positive association between IOP and systolic

Table 1. Characteristics of the subjects.

	Men (n = 680)		Women (n = 637)		Total (n = 1317)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	59.8	10.5	59.6	10.5	59.7	10.5
Intraocular pressure (mmHg)	13.6	2.6	13.3	2.6	13.4	2.6
Central corneal thickness (μm)	518.3	33.2	511.1	32.5	514.8	33.0
Systolic blood pressure (mmHg)	122.8	18.3	123.2	20.5	123.0	19.4
Diastolic blood pressure (mmHg)	76.0	10.9	74.4	11.6	75.2	11.3
Body mass index (Kg/m^2)	23.1	2.7	22.9	3.1	23.0	2.9

SD: standard deviation.

Table 2. Age and gender specific intraocular pressure and central corneal thickness.

Age group (years)	Men					Women				
	n	IOP (mmHg)		CCT (μm)		n	IOP (mmHg)		CCT (μm)	
		mean	SD	mean	SD		mean	SD	mean	SD
40–49	157	14.18	2.62	521.3	33.0	133	13.53	2.82	512.3	36.
50–59	162	13.62	2.66	522.4	31.1	183	13.29	2.64	509.3	37.
60–69	209	13.29	2.40	515.1	31.5	176	13.20	2.52	515.0	32.
70–80	152	13.32	2.84	515.2	37.0	145	13.07	2.50	507.5	27.

IOP: intraocular pressure.
CCT: central corneal thickness.
SD: standard deviation.

Table 3. Simple Pearson's correlation coefficients for intraocular pressure and covariates.

	Men (n = 680)			Women (n = 637)		
	Age	Intraocular pressure	Central corneal thickness	Age	Intraocular pressure	Central cornea thickness
Age	1.000			1.000		
Intraocular pressure	−0.140***	1.000		−0.073†	1.000	
Central corneal thickness	−0.100**	0.441***	1.000	−0.019	0.480***	1.000
Systolic blood pressure	0.137***	0.178***	0.034	0.263***	0.228***	0.060
Diastolic blood pressure	0.011	0.201***	0.055	0.160***	0.210***	0.062
Body mass index	−0.135***	0.140***	0.033	0.023	0.088*	0.036

†: $p < 0.10$.
*: $p < 0.05$.
**: $p < 0.01$.
***: $p < 0.001$.

blood pressure, diastolic blood pressure, and body mass index, whereas no association could be found between CCT and systolic or diastolic blood pressures, or body mass index in either men or women.

In Table 4, we present results from the multiple regression analyses using IOP as the dependent variable. In both genders, CCT and systolic blood pressure were positively associated, age was inversely associated, and body mass index had no significant association with IOP. Diastolic blood pressure was excluded from these analyses because there was a high correlation between the systolic and diastolic blood pressures in both men (r = 0.89) and women (r = 0.90). These multivariate analyses showed that a change in corneal thickness by 10 μm would alter the measured IOP by 0.34 mmHg (95% CI: 0.28, 0.39) and 0.37 mmHg (95% CI: 0.32, 0.42) in men and women, respectively. It also showed that a change in age by a decade would alter the measured IOP by −0.28 mmHg (95% CI: −0.45, −0.11) and −0.30 mmHg (95% CI: −0.48, −0.13) in men and women, respectively.

Discussion

This study was based on a population-based sample of adu Japanese and found that there are significant inverse assoc ations between age and IOP in both men and women aft adjusting for CCT, blood pressure, and obesity. The IOP i an essential physiological parameter for diagnosis and mar agement of glaucoma. Goldmann applanation tonometry : one of the most common methods used for measuring IO worldwide, even though many factors such as tear film, shap of the anterior cornea, corneal thickness, or scleral rigidit can influence its accuracy in healthy corneas. It has been su gested that CCT is a major source of error in applanatio tonometry:[13,23] a thick cornea leading to an overestimation IOP, and a thin one leading to an underestimation.[11–13,18] Pr vious studies suggested a range of error for Goldmann appl nation tonometry between 0.11 and 0.71 mmHg for eve 10 μm of deviation from a normal CCT measurement.[23] non-contact tonometer uses the fundamental principles

Table 4. Multiple linear regression coefficients for intraocular pressure in men and women.

	Men (n = 680)			Women (n = 637)		
	Parameter estimate	Standard error	p value	Parameter estimate	Standard error	p value
Age (years)	−0.028	0.009	0.001	−0.030	0.009	0.001
Central corneal thickness (μm)	0.034	0.003	<0.001	0.037	0.003	<0.001
Systolic blood pressure (mmHg)	0.023	0.005	<0.001	0.029	0.005	<0.001
Body mass index (Kg/m²)	0.064	0.034	0.062	0.005	0.030	0.862
Adjusted R^2	0.235		<0.001	0.280		<0.001

applanation tonometry with a wider applanation area. Therefore, it has been suggested that non-contact tonometry is influenced even more than Goldmann applanation tonometry by the corneal thickness.[24,25] Our results also showed that an increase in CCT of 10 μm was associated with an increase in IOP when measured with a non-contact tonometer (0.34 mmHg in men and 0.37 mmHg in women). Thus, it seems that the true relationship between age and IOP can be fully investigated when taking into account the error in IOP measurements due to CCT variation.

Several cross-sectional studies showed that IOP decreased with advancing age in Japanese populations.[7-9] In contrast, IOP was sustained or increased with advancing age in black and white populations.[1-6] Similarly, there was a significant decrease in CCT with advancing age in Japanese populations,[14,21] although the association was rarely significant in white populations.[16,26] Therefore, it would not seem appropriate to compare the effect of aging on IOP among different races without also considering the effect that CCT variation has on IOP readings. As shown in Table 4, since the inverse association between age and IOP was detected even after controlling for CCT, the difference in the age-specific CCT between Japanese and other populations is an unlikely explanation for the contrasting age-IOP relationship between these populations.

Previous studies have shown that blood pressure and obesity have independent, positive associations with IOP[27-29] and age,[30,31] and the positive association between age and IOP disappeared in a white population when cardiovascular risk variables were taken into account.[27,32] Therefore, the effects of blood pressure and obesity should be considered when investigating the relationship between age and IOP. Because the present study also showed that IOP correlated positively to systolic blood pressure, diastolic blood pressure, and body mass index as an indicator of obesity, these variables were adjusted in our multiple linear regression analyses.

In the present study, we measured IOP and CCT in middle-aged and elderly Japanese. Consistent with previous Japanese studies, IOP and CCT decreased with advancing age, especially in men, and there is a strong positive association between IOP and CCT in both men and women. Our multivariable analysis shows that CCT does have an effect on IOP measurements, however, even when controlled for CCT, IOP still decreases with advancing age. We conclude that IOP does decrease with advancing age in this Japanese population and that CCT variation has practically no effect on this unique age-IOP relationship.

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