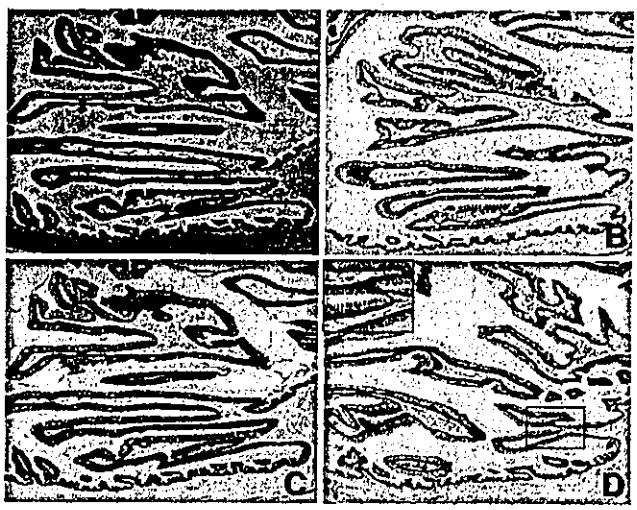

Figure 1. Intraductal papillary-mucinous tumor (adenoma). (A) H&E staining; (B) MUC5AC is positive in the cytoplasm; (C) MUC2 is evident in the cytoplasm; (D) Cdx2 nuclear staining is apparent. Inset, higher magnification of tumor cells. Original magnification, x50; the red square insets are magnified x400.

Postoperative survival analysis of the invasive pancreatic carcinoma patients with reference to phenotypic classification. Prognostic information after surgery for 30 of the 41 IDCs could be obtained. The 5-year survival evaluated by Kaplan-Meier analysis of the Cdx2-positive and -negative groups were 36.7% and 63.3%, respectively (Fig. 3), with the Cdx2-positive group having a better outcome (p=0.015).

Discussion

Our present data provide clear evidence that patients with Cdx2-positive IDCs demonstrate a better outcome than those with Cdx2-negative lesions, the Cdx2 expression correlating with intestinal expression in pancreatic tumors. Werling *et al* (12) earlier showed Cdx2 to be associated with the expression of villin as an intestinal phenotypic marker by immunohistochemical analysis in pancreatic adenocarcinomas, which is compatible with our data, although, they did not evaluate the relation with prognosis. To our knowledge, our present data provide the first evidence of any link between Cdx2 expression and outcome with pancreatic cancers. Several authors have pointed to a tumor-suppressor potential of Cdx2 in human colorectal tumorigenesis (24,27-29), and Aoki *et al* have shown that reduced expression of CDX2 is important in colon tumorigenesis through mTOR-mediated chromosomal instability (35). Bai *et al* have also demonstrated evidence that Cdx2 up-regulates transcription of p21/WAF1/CIP1, which plays a critical role in differentiation and tumor suppression (30). With stomach neoplasms, we and others have previously shown that Cdx2-positive tumors have a significantly better outcome than negative lesions, suggesting that this factor might suppress invasion by gastric cancer cells (14,31). Taking into account the previous reports and our present data, we consider that Cdx2 might suppress the expansion of cancer cells accompanied by their intestinalization in various organs.

Table II. The relation between Cdx2 and the intestinal phenotyic expression in pancreatic tumors.

Phenotypic classification[a]	Cdx2 expression[b]		
	Positive	Negative	Total
GI and I types	25	7	32
G and N types	15	30	45
Total	40	37	77

[a]GI and I types, The positive groups of the intestinal phenotypic expresssion (GI and I types); G and N types, The negative groups of the intestinal phenotypic expression (G and N types). [b]p=0.0002.

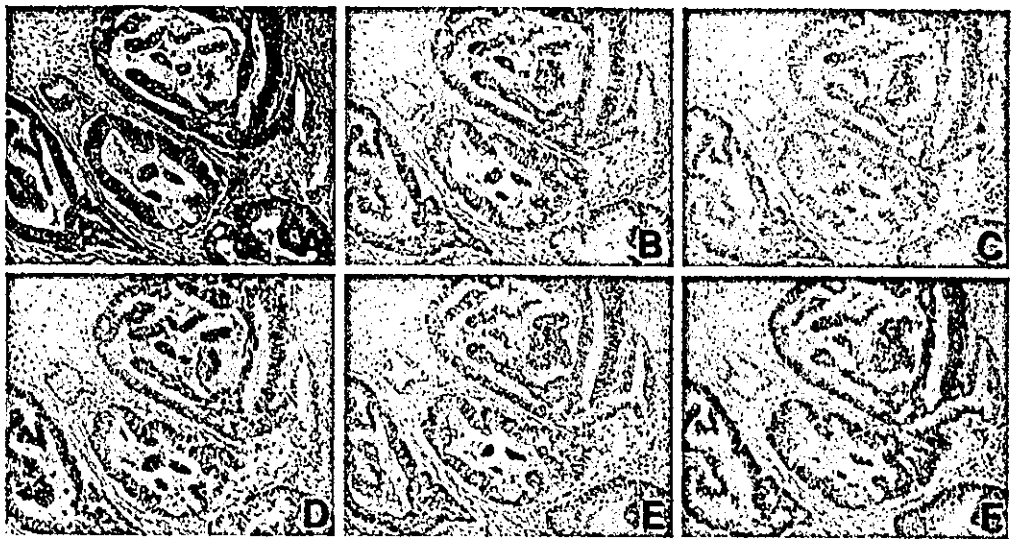

Figure 2. An invasive ductal carcinoma (moderately differentiated). (A) H&E staining; (B) MUC5AC is partially positive in the cytoplasm; (C) MUC6 is not detectable in the cytoplasm; (D) MUC2 is evident in the cytoplasm; (E) Villin is detected at the luminal surfaces of cancer cells; and (F) Cdx2 nuclear staining is apparent. Original magnification, x100.

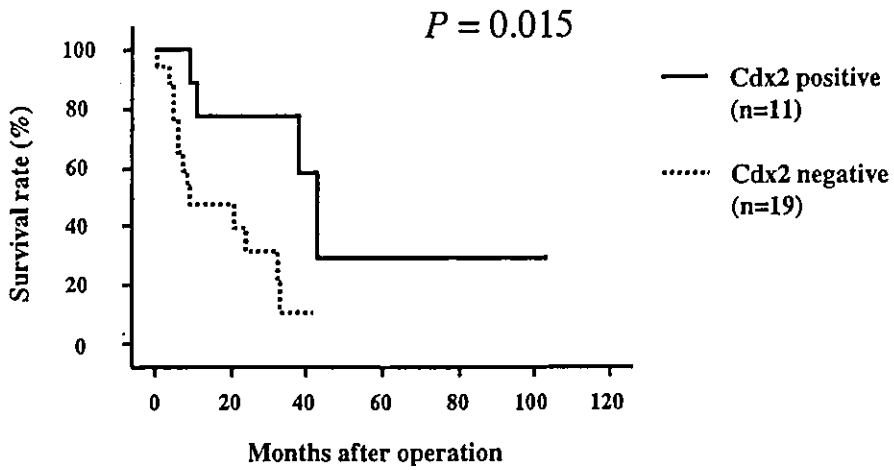

Figure 3. Postoperative survival curves of IDC patients with reference to the Cdx2 expression.

Cdx2 nuclear staining, however, was out of accord with other intestinal phenotypic markers in a few of the present series of cases. Regarding regulation of MUC2, Yamamoto *et al* (36) showed that Cdx2 induces its expression in goblet cells. Cdx2 nuclear staining was also strongly found to be associated with positivity for intestinal phenotypic markers such as MUC2 and villin in many gastric cancers (14,15,34). However, Siedow *et al* (21) demonstrated that *de novo* expression of the MUC2 gene in pancreas carcinoma cells is associated with promoter demethylation under epigenetic regulation. Therefore, we consider the possibility that several distinct systems may regulate the intestinal phenotypic expression.

Our present data demonstrated most pancreatic tumors to be positive for gastric phenotypic expression. In MCTs, columnar cells have been reported to exhibit strong cytoplasmic MUC5AC staining as a gastric phenotypic marker, irrespective of the grade of dysplasia (5). Several reports have documented MUC5AC gene expression in the majority of IPMT cases (4,18,19), and 35 of 41 our cases (85.4%) were judged to be positive for a gastric phenotype. We earlier proposed that gastric cancers at early stages, independent of the histological type, mainly consist of gastric phenotypic cells (37-42). Kim *et al* (3) demonstrated that MUC 6 and *de novo* expression of MUC5AC might occur early in the development of pancreatic adenocarcinoma. Taking into account the previous reports and our present data, we thus consider that gastric phenotypic expression might occur at an early stage in pancreatic tumorigenesis, independent of the histological types, similar to the gastric cancer case.

MUC2 was rarely expressed in invasive carcinomas of the pancreas (17-19), and Adsay *et al* (10) proposed it to be a marker of the 'indolent' pathway in pancreatic carcinogenesis. In gastric carcinomas, we and others have previously demonstrated that advanced gastric cancers with intestinal phenotypic expression have a relatively good prognosis compared to those harboring only gastric phenotypes (14,16). Similarly the lack of intestinal phenotypes and the emergence of gastric phenotypes might be associated with poor prognosis with pancreatic invasive ductal carcinomas.

In conclusion, our data suggest that Cdx2 is necessary for intestinal phenotypic expression even in pancreatic tumor cells, and provides a novel prognostic marker for patient survival in IDC cases. We further consider that a lack of intestinal phenotypic expression, including Cdx2, and increase of the gastric phenotypic expression may be associated with a poor outcome.

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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)

Parameter	Genotype of the CCK-AR Gene −81A/G Polymorphism		
	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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Histopathologic Difference Between Chronic Pancreatitis Animal Models and Human Chronic Pancreatitis

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Objectives: There are many experimental models for chronic pancreatitis. However, it remains unclear which animal models of pancreatic fibrosis can be categorized as chronic pancreatitis models. We compared the histologic features of some animal models of pancreatic fibrosis/chronic pancreatitis and chronic pancreatitis in humans.

Methods and Results: Human chronic pancreatitis due to chronic alcohol abuse and unknown etiology showed interlobular fibrosis and a cirrhosis-like appearance. Histopathologically, spontaneous pancreatitis models, WBN/Kob rats and OLETF rats, showed localized/nodular fibrotic lesions, which consisted of swollen, aggregated, atrophic islets of Langerhans; loss of the exocrine parenchyma and hemosiderin deposition that was seldom distributed in the interlobular area. On the other hand, fibrosis in the canine model, which was produced by combining alcohol administration with incomplete pancreatic duct obstruction, was characterized by interlobular fibrosis admixed with a cirrhosis-like appearance very similar to that in human chronic pancreatitis.

Conclusion: Most experimental models for chronic pancreatitis, except alcohol administration combined with other procedures such as incomplete pancreatic duct obstruction, are different from human chronic pancreatitis.

Key Words: chronic pancreatitis animal model, pancreatic fibrosis, interlobular fibrosis, cirrhosis-like appearance

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Chronic pancreatitis/pancreatic fibrosis have various causes, and chronic alcohol abuse is the most common cause of chronic pancreatitis.[1] Chronic alcoholic pancreatitis (CAP) is thought to be a progressive disease and is characterized by irregular sclerosis with destruction and loss of the exocrine parenchyma and finally complete replacement of this tissue by fibrotic tissue. In the progression of this disease, protein plugs and calculi and various complications such as diabetes mellitus, obstructive jaundice, duodenal stenosis, left-sided portal hypertension, pseudocyst, and mass formation mimicking pancreatic carcinoma, may occur.

WBN/Kob rats,[2,3] Otsuka Long Evans Tokushima fatty (OLETF) rats,[4] and Aly mice[5] are known to develop pancreatitis spontaneously, and experimental pancreatitis can be induced by ethanol feeding,[6] arginine injection,[7] and trinitrobenzene sulfonic acid (TNBS) infusion[8] in rats or mice. These conditions have thus been used as models for human pancreatitis.[9,10] However, pancreatic fibrosis in animal models is transient, decreasing noticeably sometime after development; thus, pancreatic fibrosis in animal models seems to be different from that in human chronic pancreatitis, which makes it difficult to extrapolate the mechanism of the development of pancreatic fibrosis in animal models to that in humans. Therefore, it remains unclear which animal models of pancreatic fibrosis can be categorized as chronic pancreatitis models.

In this study, we compared the histologic features of some animal models of pancreatic fibrosis/chronic pancreatitis and chronic pancreatitis in humans.

MATERIALS AND METHODS

Animal Models

WBN/Kob rats were purchased from Japan SLC (Shizuoka, Japan). The animals were maintained in an animal room with a controlled temperature (23 ± 2°C) and humidity (55 ± 15%) and a 12-hour light/12-hour dark cycle and were given a gamma ray-irradiated solid diet (MB-3; Funabashi Farm, Chiba, Japan) and water ad libitum during the experimental period. Animals aged 4, 8, 12, 16, 32, and 40 weeks (5 males at each of these ages) were used in this study. Each animal was killed by exsanguination.

OLETF rats (5 weeks old) were provided by Tokushima Research Institute, Otsuka Pharmaceutical Co. (Tokushima, Japan). The animal facilities were free of specific pathogens

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(SPF). The temperature (23°C), humidity (50%), and lighting (7:00–19:00) of the facilities were controlled. All rats were able to access a CRF-1 diet (Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum during the experimental period. Rats were killed at 5, 8, 15, 21, 23, 31, 32, 41, 52, and 60 weeks (5 males at each of these ages).

Nine adult mongrel dogs weighing 11–14 kg were used. The animals were fed standard dog chow (Oriental Yeast Co., Tokyo), and the food was withheld for 24 hours before the experiment. Surgical manipulations were performed aseptically under anesthesia with 20 mg/kg sodium pentobarbital (Nembutal). After creation of a gastric fistula, 2.0 $g \cdot kg^{-1} \cdot day^{-1}$, ethanol was administered via a gastric cannula, and after ligation of the minor pancreatic duct, the duodenum was incised to insert a polyethylene tube (0.4 mm ID, 1.7 mm OD, length of 7 mm) into the major duct, as described in detail in our previous study.[11] The laparotomy was repeated after 3 months. Resection of the pancreas and duodenum en masse was performed.

Human Materials

The human pancreatic specimens used for this study were from cases of the past 25 years as follows: 23 patients (mean age, 47.8 years) with clinically diagnosed CAP and 2 patients (16 and 18 years) with pancreatitis of unknown etiology, who were treated surgically at Juntendo University Hospital, Tokyo, and Yamanashi Prefectural Central Hospital, Yamanashi, Japan, for intractable back pain or significant suspicion of pancreatic carcinoma, were studied histopathologically. The 23 patients with CAP were considered heavy drinkers; all but 2 had consumed an amount of alcohol equal to >100 g of 100% ethanol daily for more than 20 years, and the 28-year-old man and the 29-year-old woman had done so for 9 years.

Histopathologic Study

The pancreatic tissue samples were collected from the splenic, duodenal, and colonic parts in rats, from both the left and right lobes in the dogs, and from 5–10 blocks taken from each patient with CAP or pancreatitis of unknown etiology and fixed in 10% phosphate-buffered formalin. The tissues were embedded in paraffin, sliced into 3- to 4-μm sections, and stained with hematoxylin and eosin, Masson trichrome, and elastica-van Gieson stains.

RESULTS

At 12 weeks of age, male WBN/Kob rats showed interlobular hemorrhage, edema, and inflammatory cell infiltration of the pancreas. The animals with severe inflammatory changes also showed degeneration and necrosis of acinar cells, proliferation of pancreatic ductules, and fibrosis to some degree. By 16 weeks of age, hemorrhage and edema had decreased, and the sites of these lesions had become fibrotic. Most fibrotic areas were nodular, located in the lobules, and included atrophic islets and proliferated ducts (Fig. 1). Interlobular fibrosis was partly seen in connection with the intralobular fibrosis. None of the animals showed protein plugs or calculi in the ducts.

At 31 weeks of age, OLETF rats showed focally localized/nodular lesions that consisted of swollen, aggregated, atrophic islets of Langerhans admixed with and without fibrosis, acinar vacuolation, loss of the exocrine parenchyma, and hemosiderin deposition that was found scattered or in clumps, also partly in the periductal area (Fig. 2). Fibrosis was seldom distributed in the interlobular area.

In a canine model, the fibrosis extended into the interlobular region, and parenchymal cell loss with chronic inflammatory cell infiltration was also observed, resulting in a cirrhosis-like appearance (Fig. 3). Three of 9 dogs had pancreatic calculi in the main pancreatic duct.

Fibrosis of CAP in humans was dense in the perilobular or interlobular areas, resulting in a cirrhosis-like appearance and not uniformly distributed in the representative tissues (Fig. 4); it was frequently marked in the peripheral or subcapsular regions and had extended into the intralobular area in advanced cases, in which protein plugs or pancreatic calculi were also found in 20 and 10 cases, respectively. Cases of advanced disease showed, in association with the degree of progression, increased interlobular fibrosis, disappearance of acinar cells, and complete replacement of pancreatic tissue by extensive fibrosis. In 2 patients with pancreatitis of unknown etiology, fibrosis was distributed in the interlobular area, showing the same pattern as that of CAP (data not shown).

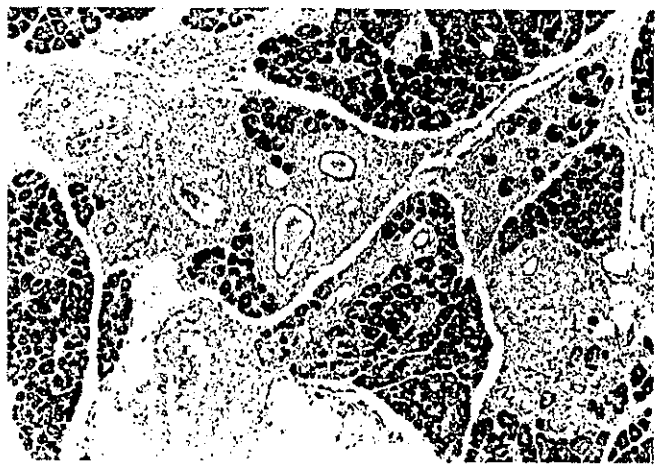

FIGURE 1. WBN/Kob rat at 16 weeks. Fibrotic lesions displayed a nodular formation that consisted of atrophic islets of Langerhans, proliferation of ducts, atrophy of the exocrine parenchyma, and hemosiderin deposition and were scattered in the intralobular/periductal areas. Hematoxylin and eosin stain, ×80.

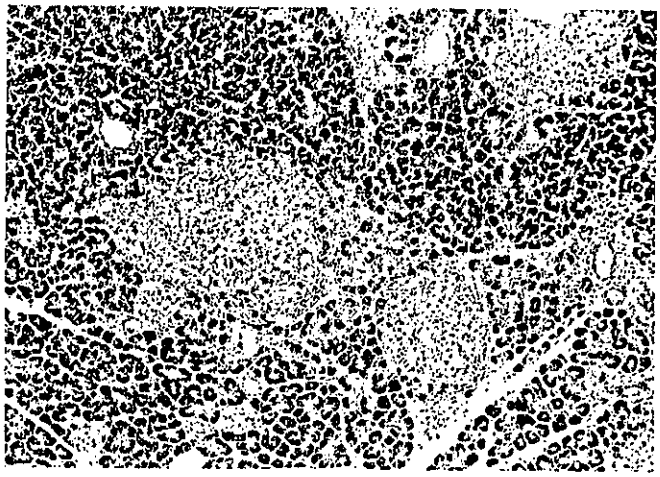

FIGURE 2. OLETF rat at 31 weeks. Fibrotic lesions consisted of aggregated/atrophic islets of Langerhans and parenchymal atrophy, distributed in a scattered pattern. Hematoxylin and eosin stain, ×100.

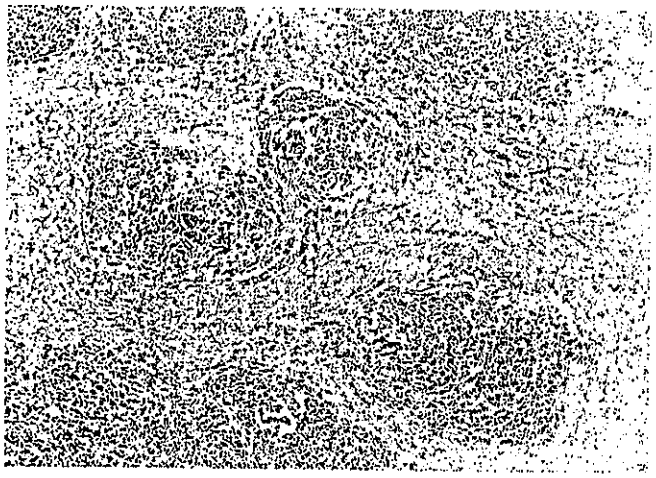

FIGURE 4. Human CAP. Fibrosis distributed in the interlobular area with a cirrhosis-like appearance. Hematoxylin and eosin stain, ×50.

DISCUSSION

Fibrosis in human CAP and pancreatitis of unknown etiology as seen in this study was dense in the interlobular or perilobular areas and not uniformly distributed in the representative tissue; the more extensive was interlobular fibrosis, and the smaller were lobules. Even in advanced cases, the cirrhosis-like appearance was well preserved. In most advanced cases, the pancreatic tissue had been completely replaced by extensive fibrosis.

Pancreatic fibrosis/chronic pancreatitis is attributable to various causes, such as alcohol abuse, pancreatic duct obstruction, biliary disease, autoimmune-related disease, and unknown causes. Chronic alcohol abuse is the most common cause of pancreatic fibrosis and chronic pancreatitis. According to Martin,[12] there are at least 3 types of fibroatrophic states found in the pancreas, ie, predominantly intralobular sclerosis (IS), which is always homogeneous and diffuse; predominantly perilobular sclerosis (PS), which presents a cirrhosis-like appearance but is irregular and sometimes patchy; and mixed IS and PS (MS), which is often homogeneously distributed in the gland. According to our previous study,[13] the pancreatic fibrosis associated with alcohol abuse can show any of Martin's classification patterns. We have emphasized that CAP can be identified by the presence of PS. Moreover, we consider that MS should be included in the IS category because fibrosis of the MS type is seen mainly in the intralobular, or periacinar, areas and is uniformly distributed. Based on the distribution of fibrosis, perilobular or interlobular, versus intralobular, differences in various components and accompanying diseases, such as protein plugs/calculi, liver cirrhosis, peripancreatic fibrosis, splenic vein involvement, choledochus involvement, pseudocyst, ductal hyperplasia, and duodenal stenosis, pancreatic fibrosis can be classified into 2 distinct pathogenic entities, which occur by different mechanisms.[14] Hence, intralobular or periacinar fibrosis is different from chronic pancreatitis, and the periacinar pattern of experimental fibrosis described by Haber et al[9] and that produced in the TNBS model[8] are not similar to that seen in human chronic pancreatitis at all.

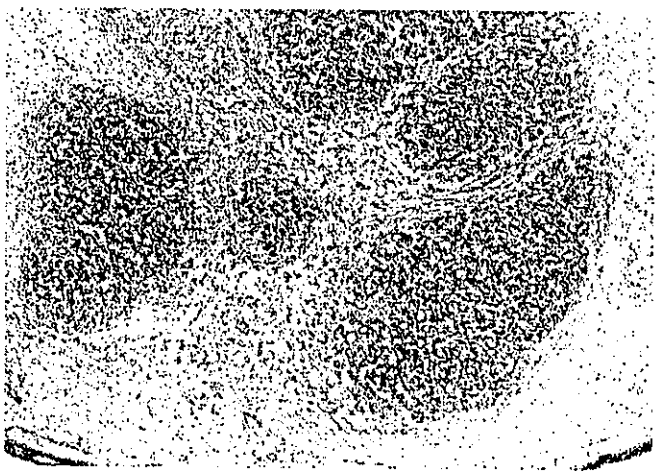

FIGURE 3. Canine model produced by alcohol administration combined with incomplete obstruction of the pancreatic duct at 3 months. There were interlobular fibrosis distribution and a cirrhosis-like appearance. Hematoxylin and eosin stain, ×50.

Despite decades of research, the pathophysiology underlying CAP remains unknown. Accordingly many experimental models, such as spontaneous, ethanol feeding, and drug induced, have been considered and studied. In both of the spontaneous models, male WBN/Kob rats and male OLETF rats in

this study, localized, nodular fibrotic lesions were found at 16 and at 31 weeks of age, which were considered the peak times of fibrosis and lesions, respectively. These fibrotic lesions consisted of swollen, aggregated, atrophic islets of Langerhans with and without fibrosis and loss of the exocrine parenchyma and hemosiderin deposition and were seldom distributed in the interlobular area. Fibrosis in these animal models was potentially reversible, and the morphology in the chronic stage showed features different from those of chronic pancreatitis in humans.[15]

On the other hand, the fibrosis in the canine model, which was produced by combining alcohol administration with incomplete pancreatic duct obstruction, was mainly characterized by interlobular fibrosis admixed with a cirrhosis-like appearance very similar to that in human CAP. According to our previous study,[16] cases of mild changes in patients with ampullary carcinomas that showed diffuse mild interlobular fibrosis and expansive lobular patterns had histologic findings similar to those of patients with CAP and pancreatitis of unknown etiology, except for excessive fibrosis with patchy distribution. Moreover, as mentioned by Schneider et al,[17] models of ethanol feeding in combination with other procedures such as duct obstruction[11] have revealed several mechanisms that play a role in ethanol-induced pancreatic injury. These animal models have provided insights into several factors that predispose the pancreas to the development of pancreatic injury and contribute to alcoholic pancreatitis.

In conclusion, most experimental models of chronic pancreatitis that cause nodular/localized or intralobular/periacinar fibrosis are different from human chronic pancreatitis, especially cases with intractable back pain and significant suspicion of pancreatic carcinoma.

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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.

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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Brief Genetic Analysis

Association of Cholecystokinin 1 Receptor and β_3-Adrenergic Receptor Polymorphisms with Midlife Weight Gain

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Abstract

KODA, MICHIKO, FUJIKO ANDO, NAOKIRA NIINO, HIROSHI SHIMOKATA, KYOKO MIYASAKA, AND AKIHIRO FUNAKOSHI. Association of cholecystokinin 1 receptor and β_3-adrenergic receptor polymorphisms with midlife weight gain. *Obes Res.* 2004;8:1212–1216.

We investigated the relationship of polymorphisms in the *cholecystokinin 1 receptor* [*CCK1R*; *G* to *T* (n-128), *A* to *G* (n-81)] and the *β_3-adrenergic receptor* (*β_3-AR*; Trp64Arg) with midlife weight gain. The participants were 1012 Japanese men and women (40 to 59 years of age). Their weight at 18 years old was obtained from a questionnaire. Weight change was defined as the current weight minus the weight at 18 years old. Subjects were grouped into four categories by these genotypes: W/W = noncarriers, W/H = Arg^{64} carriers of the *β_3-AR*, H/W = *T* (n-128) or *G* (n-81) carriers of the *CCK1R*, H/H = *T* (n-128) or *G* (n-81) and Arg^{64} carriers. In men, the interaction between the *CCK1R* and *β_3-AR* polymorphisms was significant (two-way ANOVA, $p < 0.05$), but neither the *CCK1R* nor the *β_3-AR* was individually associated with weight gain. The H/H group showed a higher possibility of weight gain of 10 kg or more compared with the W/W group in men. The odds ratio for weight gain (≥10 kg) of H/H was 2.54 (95% confidence interval: 1.50 to 4.30) compared with W/W. In women, neither main effect nor interaction was significant. These results suggest that the combination of *CCK1R* and the *β_3-AR* polymorphisms is a contributing factor for midlife weight gain in men.

Key words: combination of polymorphism, body weight gain, middle-aged men

Age-related increases in body weight in young adult men and postmenopausal women have been reported. Weight gain is as harmful to the health as being overweight. In a previous study, weight gain from 20 years of age was closely associated with cardiovascular risk factors in middle-aged men (1), and weight gain from 18 years of age was associated with coronary heart disease risk in women (2). According to a Japanese national cross-sectional survey in 1999 (3), although the rate of excess weight (BMI ≥ 25 kg/m^2) was 19.2% in those 20 to 29 years old, it increased to 29.6% in those 50 to 59 years old for men. In women, it was 7.3% in those 20 to 29 years old and 27.5% in those 50 to 59 years old.

There are several causes associated with weight gain, such as smoking, physical activity during leisure, alcohol consumption, and genetic factors (4–6). Regarding obesity, we reported the possibility that the polymorphism of the *cholecystokinin 1 receptor* (*CCK1R*)[1] gene may be related to an increase in body fat content in middle-aged and elderly people (7). Cholecystokinin (CCK) is a peptide hormone found in the central nervous system and gastrointestinal tract. *CCK1R* has been shown to mediate the CCK-induced suppression of food intake (8), and the peripheral administration of *CCK1R* antagonists increased food intake (9). However, Hamann et al. (10) found no evidence for its association with early-onset obesity in children and adolescents.

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[1] Nonstandard abbreviations: CCK1R, cholecystokinin 1 receptor; CCK, cholecystokinin; β_3-AR, β_3-adrenergic receptor; NILS-LSA, National Institute for Longevity Sciences-Longitudinal Study of Aging.

The *β_3-adrenergic receptor* (*β_3-AR*) genotype has also been cited as a gene candidate related to obesity (6,11,12), and it is involved in the regulation of lipolysis and thermogenesis. Japanese (12), Pima Indians (6), and Alaskan Eskimos (13) have higher frequencies of the *β_3-AR* gene polymorphism than whites. However, some studies have suggested that the *β_3-AR* gene is not associated with obesity (13,14). Therefore, we investigated the relationship between *CCK1R* and *β_3-AR* gene polymorphisms and weight gain from 18 years of age to middle age.

The means and SD of current weight, weight at 18 years, and weight change from 18 years by genotype are shown in Table 1. The means of weight change were 8.2 kg in men and 5.1 kg in women.

Genotype and polymorphism allele frequency distributions for *CCK1R* and *β_3-AR* are shown by gender in Table 2. These genotype frequencies were found to be in Hardy-Weinberg equilibrium in men and women. Gender differences in those frequency distributions were not significant. The frequency of the *T* (n-128) allele in *CCK1R* was 26% and that of the *G* (n-81) allele was ~40%. Funakoshi et al. (7) has found that there are two sequence changes in human *CCK1R*, a *G* to *T* change in n-128 and an *A* to *G* change in n-81. Six genotypes were identified as wild-type (G/G, A/A), heterozygote type (G/T, A/G), (G/G, A/G), (G/T, G/G), (G/G, G/G), and homozygote type (T/T, G/G). The genotype combinations G/T, A/A; T/T, A/G; and T/T, A/A were not found. On the other hand, the genotype frequency of the *β_3-AR* gene polymorphism is ~33%, similar to previous studies in other Japanese (12).

Two-way ANOVA was carried out in which weight gain was taken as the dependent variable and the *CCK1R* and *β_3-AR* polymorphisms were independent variables. Neither *CCK1R* nor *β_3-AR* was individually associated with weight gain in men. However, the interaction between *CCK1R* and *β_3-AR* polymorphisms was significant ($p < 0.05$; Table 3). The main effects and the interaction were not significant in women.

Comparisons of the distributions of weight change from 18 years by genotype are shown in Table 4. Of the 564 men, 227 (40%) were noncarriers (W/W), 110 (20%) were *Arg^{64}* carriers of the *β_3-AR* (W/H), 149 (26%) were *T* (n-128) or *G* (n-81) carriers of the *CCK1R* (H/W), and 78 (14%) were *T* (n-128) or *G* (n-81) and *Arg^{64}* carriers (H/H). Of the 548 women, 211 (38%) were W/W, 113 (21%) were W/H, 158 (29%) were H/W, and 66 (12%) were H/H. The frequency of weight gain (≥10 kg) was 40% for men and 24% for women. The distribution of weight change in men was different among the genotypes ($p < 0.01$). The frequency of a weight gain of ≥10 kg was higher in the H/H group than in the other three groups. The distribution in women was not different.

Finally, the risk of weight gain (≥10 kg) was estimated using multiple logistic regression analysis in men (Table 5).

Table 1. Characteristics of participants by gender

	Men (n = 564)	Women (n = 548)
Height	164.1 ± 5.9	154.1 ± 4.9
Current weight	65.0 ± 8.7	54.1 ± 8.0
Weight at 18 years	56.8 ± 6.7	48.9 ± 6.0
Weight change	8.2 ± 7.4	5.1 ± 7.7

Mean ± SD.

The odds ratio of the H/H group was significantly higher [2.54 (95% confidence interval: 1.50 to 4.30)] compared with that of the W/W group. However, in men with W/H or H/W, the odds ratios were not significant.

These results showed that the combination of *CCK1R* and *β_3-AR* polymorphisms was associated with a weight gain of ≥10 kg from 18 years of age in men. Hamann et al. (10) did not find that the *CCK1R* polymorphism was associated with early-onset obesity in children and adolescents. Although excess energy from increased food intake may be used for growth in a child, it is not usually used for growth in adults. After maturing, the polymorphism of the *CCK1R* gene may have an important role as a regulator of food intake. *β_3-AR* is involved in the regulation of lipolysis and thermogenesis. The resting metabolic rate in *Arg64* homozygotes is significantly lower than in Trp64 homozygotes (15). Moreover, *β_3-AR* is expressed in visceral fat in humans (16), and visceral fat increases with advancing age (17). Therefore, in men carrying the *T* or *G* allele of the *CCK1R* and *Arg^{64}* allele in *β_3-AR*, food intake may increase, but extra energy may not burn, leading to weight gain.

However, neither *CCK1R* nor *β_3-AR* was individually associated with weight gain. *CCK1R* or *β_3-AR* alone was not likely to be a strong independent contributing factor of weight gain. Therefore, the results of the association between a single gene and weight gain in many previous studies have been contradictory. A combination of polymorphisms in two or more candidate genes may contribute to weight gain (e.g., the *β_3-AR* and uncoupling protein gene) (18,19). The simultaneous existence of two polymorphisms was associated with weight gain.

It remains unclear why these results were revealed only in men. For women, the physiological and environmental factors are relatively strong (e.g., pregnancy, parity, and menopause involve hormonal changes) (20). Furthermore, women may try more frequently to lose weight, and these factors may be stronger than genetic factors.

There are some limitations in this study. First, there may be other factors related to body weight. Smoking influences weight and weight change (4), and we, therefore, performed an analysis excluding smokers. The results were similar to

Table 2. Genotype and allele frequencies for *CCK1R* and β_3-*AR* polymorphisms by gender

		Men (n = 564)		Women (n = 548)	
		Count	**Percentage**	**Count**	**Percentage**
CCK1R (n-128)	Genotype				
	G/G	415	73.6	403	73.5
	G/T	134	23.8	133	24.3
	T/T	15	2.7	12	2.2
	Allele				
	G	964	85.5	939	85.7
	T	164	14.5	157	14.3
CCK1R (n-81)	Genotype				
	A/A	337	59.8	324	59.1
	A/G	190	33.7	185	33.8
	G/G	37	6.6	39	7.1
	Allele				
	A	864	76.6	833	76.0
	G	264	23.4	263	24.0
β_3-*AR*	Genotype				
	Trp/Trp	376	66.7	369	67.3
	Trp/Arg	161	28.5	158	28.8
	Arg/Arg	27	4.8	21	3.8
	Allele				
	Trp	913	80.9	896	81.8
	Arg	215	19.1	200	18.2

the original results. Second, the weight estimate at 18 years of age might not be accurate, because this was assessed only by a questionnaire. Third, weight changes, either up or down, were not ascertained for the period between 18 years of age and the time of this study. We need to research this in the future.

Research Methods and Procedures

Subjects

The subjects were 564 Japanese men and 548 women, 40 to 59 years of age, who participated in the National Institute for Longevity Sciences–Longitudinal Study of Aging

Table 3. Relationship between weight gain and the polymorphisms in *CCK1R* and *β3-AR* (two-way ANOVA)

	Covariable		**Sum of squares**	*df*	*F*	*p*
Men	Main effects	*CCK1R*	173.73	1	3.18	0.075
		β3-AR	70.45	1	1.29	0.257
	Interactions	*CCK1R* × *β3-AR*	228.40	1	4.18	0.042
	Model		476.62	3	2.90	0.034
Women	Main effects	*CCK1R*	62.09	1	1.06	0.304
		β3-AR	2.08	1	0.04	0.851
	Interactions	*CCK1R* × *β3-AR*	78.5	1	1.34	0.248
	Model		141.58	3	0.80	0.492

Table 4. Comparison of the distributions of body weight change from 18 years by genotype

			<0 kg		≥0 to <10 kg		≥10 kg		*p* for genotype frequencies†
		Total	Number	Percentage	Number	Percentage	Number	Percentage	
Men	W/W*	227	29	12.8	116	51.1	82	36.1	
	W/H*	110	20	18.2	51	46.4	39	35.4	
	H/W*	149	19	12.8	73	48.9	57	38.3	
	H/H*	78	6	7.7	26	33.3	46	59.0	0.005
	Total	564	74	13.1	266	47.2	224	39.7	
Women	W/W*	211	50	23.7	112	53.1	49	23.2	
	W/H*	113	28	24.8	58	51.3	27	23.9	
	H/W*	158	40	25.3	78	49.4	40	25.3	
	H/H*	66	14	21.2	36	54.6	16	24.2	0.985
	Total	548	132	24.1	284	51.8	132	24.1	

* W/W, (*CCK1R*/β_3-*AR*) = (G/G, A/A)/(Trp/Trp); W/H, (G/G, A/A)/(Trp/Arg) or (Arg/Arg); H/W, (G/T, A/G), (G/G, A/G), (G/T, G/G) or (G/G, G/G)/(Trp/Trp); H/H, (G/T, A/G), (G/G, A/G), (G/T, G/G) or (G/G, G/G)/(Trp/Arg) or (Arg/Arg).
† Cochran-Mantel-Haenszel statistics.

(NILS-LSA) from November 1997 to April 2000. The NILS-LSA is a comprehensive longitudinal study on aging, which started in November 1997. The design of the NILS-LSA has been described elsewhere (21). Informed consent was obtained from all subjects. The study protocol was approved by the Ethical Committee of Chubu National Hospital.

Measurements

Body weight of subjects dressed in underwear only was measured with a digital scale. Weight at 18 years of age was collected by questionnaire. Weight change was defined as the current weight minus the weight at 18 years of age.

Venous blood was collected into tubes containing EDTA (disodium salt; 50 mM), and genomic DNA was isolated with an automated genomic DNA isolation system (NA1000; Kurabo, Osaka, Japan).

The polymorphism of the upstream region of the *CCK1R* gene was determined with a mismatch polymerase chain reaction-restriction fragment length polymorphism method (7). Genotyping of the β_3-*AR* Trp64Arg polymorphism was determined using polymerase chain reaction-restriction fragment length polymorphism analysis (11). These methods have already been described in detail elsewhere (22).

Data Analysis

There were two sequence changes in the *CCK1R*, a *G* to *T* transversion at nucleotide −128 (n-128) and an *A* to *G* change in nucleotide −81 (n-81) (GenBank accession no. D85606) (7). The β_3-*AR* genotype leads to the replacement of tryptophan by arginine at position 64 ($Trp^{64}Arg$). The genotype distributions were tested for Hardy-Weinberg equilibrium with χ^2 statistics. Gender differences in the genotypic distribution were analyzed using χ^2 statistics. Two-way ANOVA was used to evaluate the effect of the genotype and the interaction between that independent variable and weight gain.

Subjects were grouped into four categories by genotype: W/W, W/H, H/W, and H/H. Values for weight change were also grouped into three categories: <0, 0 to 9.9, and ≥10 kg. The distribution of weight change was tested by Coch-

Table 5. Odds ratios (ORs) and 95% confidence intervals (95% CIs) for body weight gain (≥10 kg) in men

	Case number	Referents number	OR	95% CI
W/W*	82	145	1.00	
W/H*	39	71	0.97	0.60–1.56
H/W*	57	92	1.10	0.72–1.68
H/H*	46	32	2.54	1.50–4.30

* W/W, (*CCK1R*/β_3-*AR*) = (G/G, A/A)/(Trp/Trp); W/H, (G/G, A/A)/(Trp/Arg) or (Arg/Arg); H/W, (G/T, A/G), (G/G, A/G), (G/T, G/G) or (G/G, G/G)/(Trp/Trp); H/H, (G/T, A/G), (G/G, A/G), (G/T, G/G) or (G/G, G/G)/(Trp/Arg) or (Arg/Arg).
† Cochran-Mantel-Haenszel statistics.

ran-Mantel-Haenszel statistics. The odds ratio for weight gain (≥10 kg) and its 95% confidence interval were estimated using a logistic regression model. The data were analyzed using the SAS statistical software package (release 8.2; SAS Institute, Cary, NC) (23). Probability values below 0.05 were regarded as significant.

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A randomized multicenter trial comparing resection and radiochemotherapy for resectable locally invasive pancreatic cancer

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Background. *Though the outcome of resection for locally invasive pancreatic cancer is still poor, it has gradually improved in Japan, and the 5-year survival is now about 10%. However, the advantage of resection over radiochemotherapy has not yet been confirmed by a randomized trial. We conducted this study to compare surgical resection alone versus radiochemotherapy without resection for locally invasive pancreatic cancer using a multicenter randomized design.*

Methods. *Patients with pancreatic cancer who met our preoperative criteria for inclusion (pancreatic cancer invading the pancreatic capsule without involvement of the superior mesenteric artery or the common hepatic artery, or without distant metastasis) underwent laparotomy. Patients with operative findings consistent with our criteria were randomized into a radical resection group and a radiochemotherapy group (200 mg/m^2/day of intravenous 5-fluorouracil and 5040 cGy of radiotherapy) without resection. The 2 groups were compared for mean survival, hazard ratio, 1-year survival, quality of life scores, and hematologic and blood chemical data.*

Results. *Twenty patients were assigned to the resection group and 22 to the radiochemotherapy group. There was 1 operative death. The surgical resection group had better results than the radiochemotherapy group as measured by 1-year survival (62% vs 32 %, P = .05), mean survival time (>17 vs 11 months, P < .03), and hazard ratio (0.46, P = .04). There were no differences in the quality of life score or laboratory data apart from increased diarrhea after surgical resection.*

Conclusions. *Locally invasive pancreatic cancer without distant metastases and major arterial invasion appears to be best treated by surgical resection. (Surgery 2004;136:1003-11.)*

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PANCREATIC CANCER remains one of the most difficult diseases to cure by resection. The results of surgical treatments including super-radical resections are poor.[1-3] Pancreatic cancer currently kills more than 17,000 persons per year in Japan and is the fifth leading cause of cancer death.[4,5] The overall 5-year survival in patients undergoing margin-negative resection varies from 6.8% to 25%.[6-10] However, most surgeons maintain that resection is the only way to provide long-term survival to pancreatic cancer patients, especially since improvements in operative and perioperative management have reduced operative mortality and decreased hospital stay.[9-11]

Pancreatic cancer is one of the most chemoresistant human malignancies.[12-14] The results

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