2）調査方法

自記式の調査票により，過去1年間の転倒の有無とその発生状況を調べた．転倒の状況は，対象者が転倒をしたときの時刻，場所，転倒に関する活動，転倒の原因，転倒によるケガの程度を調べた．対象者が1年に2回以上転倒をしたとき，転倒の状況はもっとも重大なケガを引き起こした転倒，あるいはもっとも近い時期の転倒について質問した．

また，転倒に対する恐怖感はすべての対象者において，選択形式の質問「現在，あなたは転ぶことがこわいと感じますか」で回答を求め，選択肢は「とてもこわい」，「少しこわい」，「こわくない」の3分類を用い，前者2つを「こわい」，後者1つを「こわくない」とした．転倒の定義は既存の研究[10]を参考にして「自らの意志によらず，足底以外の部分が床，地面についた場合」とした．

転倒者の割合，転倒の恐怖を有する者の割合の性差についてはχ^2検定により分析した．他の発生状況について，ケガがもっとも重度であった転倒についてのみ記録されており，全転倒の状況が把握されているわけではない．そこで，本研究では，転倒の発生状況について調査結果の単純集計のみを報告し，統計学的な分析は実施しなかった．解析には統計パッケージSAS（ver.8.2）を使用した．

2. 結　果

転倒を経験した人数は150名で，転倒者の割合は13.3％であった．性別による転倒者の割合は女性が男性よりも有意に高かった（表1）．転倒回数についても調査をしたが，無回答が多かったため今回は分析を行なわなかった．

1日を深夜・早朝（0～6時），午前（6～12時），午後（12～18時），夜（18～24時）の4つの時間帯に分け，各時間帯に起きた転倒発生の割合を図1に示した．転倒は午前6時から午後6時までの時間帯に多く発生した．

転倒の場所に関して，屋内における転倒は28.0％，屋外における転倒は72.0％であり，転倒は屋内よりも屋外で頻繁に発生していた（図2）．

転倒に関係した活動は図3に示したように分類された．その他／不明を除いて，転倒は歩いている間にもっとも頻繁に発生し，階段を下りる際の転倒が次いで多かった．

転倒の主な原因は既存の研究[2,13]にしたがって，外因性の転倒（79.5％），内因性の転倒（2.1％），その他／不明の転倒（18.5％）に分けられた（表2）．転倒の大多数は外因性の要因によるものであった．外因性の要因の中でも，「つまずいた」，次いで「滑った」ことによる転倒が多かった．

転倒後のケガについて，図4に示した．ケガのない場合は最多で全転倒の43.5％であり，次いで，41.5％が打撲傷であった．骨折を伴う転倒は一例のみであったが（0.7％），高齢者の転倒で問題になる大腿骨頚部骨折ではなかった．

転倒の恐怖に関して，約30％が「こわい」と報告している（表3）．性別にみると，男性では20.0％，女性では47.9％であり，女性が男性よりも転倒恐怖感を有する人の割合が高かった．

表1　性別の転倒者数と割合

	転倒者数（%）
合計（n = 1,130）	150（13.3）
男性（n = 572）	57（10.0）
女性（n = 558）	93（16.7）
χ^2（男性 vs 女性）	11.02 **

**p < 0.01

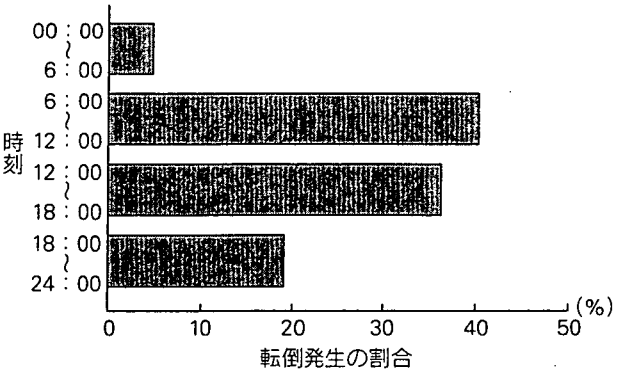

図1　転倒時間（n = 127）

3. 考　察

わが国の1年間の転倒発生率は在宅高齢者では20％弱と報告する研究が多い[2-4]．今回調査した地域在住の中年者における転倒者の割合は13.3％であり，高齢者に比べ，低い割合であった．高齢者では，視力や筋力，平衡能力の低下，歩行機能の低下など，さまざまな心身機能の低下により転倒が増えるとされている[1,11]．中年者ではこのような機能低下が少ないと考えられ，高齢者を対象者にした調査に比べ転倒発生率が低い今回の結果に特に矛盾はないといえるだろう．

本研究では，転倒者の割合は男性に比べ女性が有意に高かった．過去の高齢者に関する報告でも，転倒は女性に多いとされているが[1,4,5]，性差を認めないともいわれている[2,3]．女性は平衡維持機能が悪いとする報告もあるが[12]，安村ら[3]はさまざまな要因の関与が想定される転倒の発生は必ずしも女性に不利であると断定はできないと指摘している．

新野ら[13]は在宅高齢者における転倒の発生状況として，日中，屋外，歩行中の転倒が多い傾向にあるとしている．本研究でも，日中，屋外，歩行中の転倒が多く，転倒の発生状況に関して中年者は高齢者と同様の傾向が認められた．また新野[14]や安村ら[11]は転倒の発生状況について，高齢者では利用量，活動量の多い場所，時間帯を反映すると指摘している．このことは中年者の転倒にも当てはまると考えられる．転倒の原因は「つまずいた」，「滑った」の外因性の原因が多く，新野らの報告する高齢者の結果と一致していた[13]．比較的若くADLのよい高齢者では外因性の関与が強く[15]，高齢で病弱であるほど内因性の関与が大きい[11]と考えられている．今回の研究対象とした中年者は比較的元気な高齢者と同様，外因性の原因から転倒が発生することが認められた．

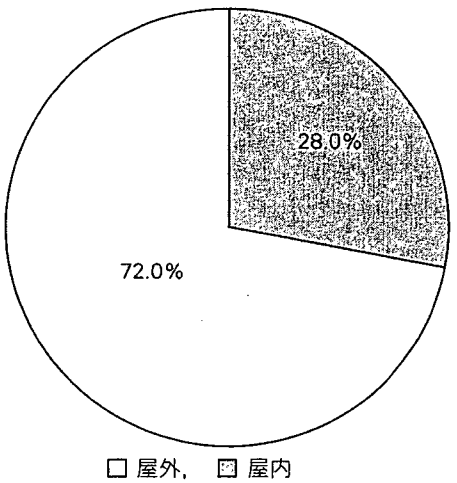

図2　転倒時の場所（n = 132）

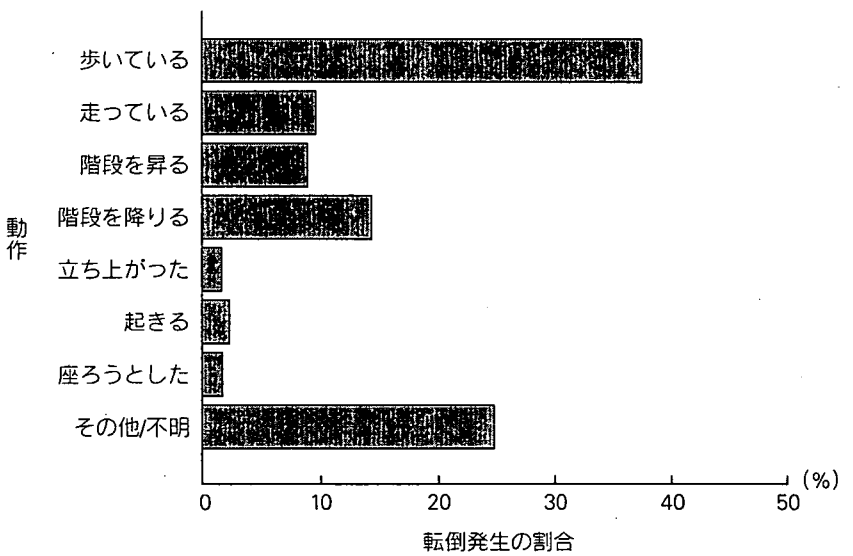

図3　転倒時の動作（n = 146）

表2　転倒の最大原因の分布

	転倒者数（%）
外因性	
つまずいた	66（45.2）
滑った	39（26.7）
何かにぶつかった	2（ 1.4）
足を踏み外した	9（ 6.2）
/小計	/116（79.5）
内因性	
めまい	0（ 0.0）
ふらついた	3（ 2.1）
/小計	/3（ 2.1）
その他/不明	27（18.5）
合計	146

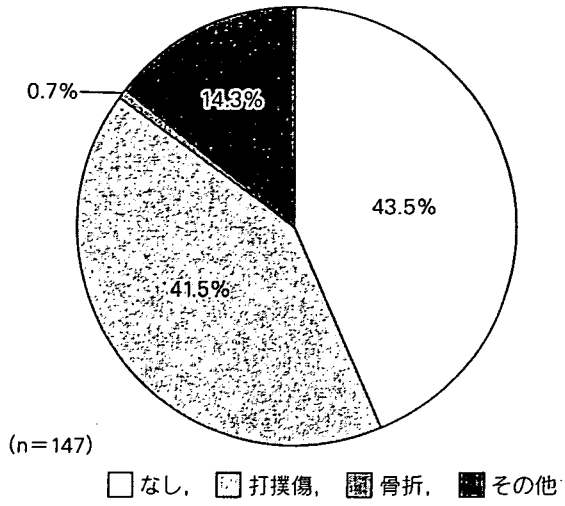

図4　転倒時のケガ

表3　転倒恐怖感を有する人の数と割合

	人数（%）
合計（n = 1,120）	337（33.7）
男性（n = 571）	114（20.0）
女性（n = 549）	263（47.9）
χ^2（男性 vs 女性）	97.85**

**p < 0.01

転倒に伴うケガについて調べた結果，ケガのない場合が最多で43.5%であり，他の高齢者の研究報告と同様の割合であった[2,4,13]．特に，注目すべき点は転倒に伴う骨折で，高齢者を対象にした研究では転倒による骨折は5～15%と報告されている[2,4,13]が本研究では0.7%とわずかであり，転倒による骨折は中年者において少ないことが示された．

転倒恐怖感を有する人の割合は中年者で約30%であった．性別に比較すると，転倒者の割合と同様，女性において転倒に対する恐怖感が有意に高かった．わが国では転倒恐怖感の研究が少なく単純に頻度を比較することはできないが，欧米の報告では地域在住高齢者において，全体の30～43%の人が転倒恐怖感を報告している[16-18]．転倒恐怖感により，活動の制限，身体機能の低下につながる可能性もあり，中年者についても転倒恐怖感のさらなる研究が必要である．Vellasら[16]は地域在住の高齢女性における転倒恐怖感の割合が高いことを報告し，虚弱な高齢女性が転倒恐怖感を表現するのではないかと指摘している．われわれの中年者の結果も，男性に比べ筋力など身体機能等が劣る女性に転倒恐怖感の表現が多く，そのため性差が生じたと考えられた．

なお，本研究では転倒回数について無回答が多かったため詳しい検討をしていないが，高齢者に比べ比較的機能が保持されている中年者では複数回転倒が少ないことが考えられる．今後この複数回転倒の問題も含め，さらに中年者の転倒について調査していきたいと考えている．

4. まとめ

中年期の地域住民を対象に，転倒をした人の割合，発生時刻，場所などの発生状況に関する調査を行なった．その結果，日中，屋外，歩行中の転倒が多いなど，地域在住高齢者と同様の傾向が認められた．ただし，転倒の予後に関しては中年者で骨折が少ない可能性が示された．

調査にご協力いただいた愛知県大府市および東浦町の方々，並びに調査に関係したスタッフに感謝いたします．本研究の一部は厚生労働科学研究費長寿科学総合研究事業，健康科学総合研究事業の補助を

受けて行なわれた.

文　献

1) Tideiksaar R : Falling in old age : Its prevention and management (2nd ed). Springer, 1997.
2) 安村誠司ほか：地域の在宅高齢者における転倒発生率と転倒状況. 日公衛誌, **38** (9)：735—741, 1991.
3) 安村誠司ほか：農村部の在宅高齢者における転倒の発生要因. 日公衛誌, **41** (6)：528—537, 1994.
4) 新野直明，小坂井留美，江藤真紀：在宅高齢者における転倒の疫学. 日老医誌, **40** (5)：484—486, 2003.
5) Haga H, Shibata H, Shichita K et al. : Falls in the institutionalized elderly in Japan. Arch Gerontol Geriatr, **5** (1) : 1—9, 1986.
6) 鈴木みずえ，岡村カルロス竹男，嶋津祐子ほか：高齢者の転倒経験に関する調査研究—養護老人ホームの居住者を対象として—. 日公衛誌, **39** (12)：927—939, 1992.
7) 新野直明，中村健一：老人ホームにおける高齢者の転倒調査：転倒の発生状況と関連要因. 日老医誌, **33** (1)：12—16, 1996.
8) 厚生統計協会編：国民衛生の動向・厚生の指標. 臨時増刊, **50** (9)：396—397, 2003.
9) 下方浩史：長期縦断研究の目指すもの. Geriat Med, **36** (1)：21—26, 1998.
10) Gibson MJ : Falls in later life. Improving the health of older people ; A world view (eds by Kane et al). Oxford Univ Press, pp. 296—315, 1990.
11) 安村誠司，新野直明：高齢者の転倒因子. 理学療法, **14** (3)：199—205, 1997.
12) Overstall PW, Exton-Smith AN, Imms FJ et al. : Falls in the elderly related to postural imbalance. Br Med J, **1** (6056) : 261—264, 1977.
13) 新野直明：高齢者の転倒とその対応. 治療, **83** (10)：80—83, 2001.
14) 新野直明：運動障害1) 転倒. Geriat Med, **36** (6)：849—853, 1998.
15) Perry BC : Falls among the elderly : a review of the methods and conclusions of epidemiologic studies. J Am Geriatr Soc, **30** (6) : 367—371, 1982.
16) Vellas BJ, Wayne SJ, Romero LJ et al. : Fear of falling and restriction of mobility in elderly fallers. Age Aging, **26** (3) : 189—193, 1997.
17) Cumming RG, Salkeld G, Thomas M et al. : Prospective study of the impact of fear of falling on activities of daily living, SF-36 scores, and nursing home admission. J Gerontol A Biol Sci Med Sci, **55** (5) : M299—M305, 2000.
18) Murphy SL, Williams CS, Gill TM et al. : Characteristics associated with fear of falling and activity restriction in community-living older persons. J Am Geriatr Soc, **50** (3) : 516—520, 2002.

Inactive Aldehyde Dehydrogenase-2 Increased the Risk of Pancreatic Cancer Among Smokers in a Japanese Male Population

Kyoko Miyasaka, MD, PhD, Takako Kawanami, PhD,† Hiroshi Shimokata, MD, PhD,‡ Shigeo Ohta, MD, PhD,§ and Akihiro Funakoshi, MD, PhD†*

Objectives: Most of the acetaldehyde, a recognized animal carcinogen, generated during alcohol metabolism is eliminated by liver mitochondrial aldehyde dehydrogenase 2 (ALDH2). More than 40% of Japanese have the inactive form of ALDH2, and inactive ALDH2 is a risk factor for multiple cancers of the esophagus as well as head and neck cancer. Possible associations between pancreatic cancer and ALDH2 gene polymorphism, in conjunction with smoking and/or drinking habits, were examined in a Japanese population.

Methods: We investigated 114 patients (70 male and 44 female) with pancreatic cancer and compared them with 2070 control subjects (1050 male and 1020 female). The drinking (5 g ethanol consumption/d) and/or smoking habits as well as ALDH2 gene polymorphism were examined.

Results: In male subjects, the frequency of the active form of ALDH2 (2*1/2*1) was lower in pancreatic cancer patients than in control subjects ($P = 0.018$). The frequency of subjects with both smoking and drinking habits was significantly higher in pancreatic cancer patients than in control subjects having ALDH2*1/2*1 and ALDH2*1/2*2. The frequency of smoking habit alone was significantly higher in pancreatic cancer patients compared with control subjects having inactive ALDH2. Drinking habit had no relation to pancreatic cancer. In female subjects, neither habit had a relation to pancreatic cancer.

Conclusions: Smoking habit did increase the risk of pancreatic cancer, and this risk was further enhanced in subjects with inactive ALDH2 in a male population but not in a female population. There was no relationship between drinking habit and pancreatic cancer in either sex population.

Key Words: pancreatic cancer, alcohol, smoking, ALDH2, genotype

(*Pancreas* 2005;30:95–98)

Received for publication May 17, 2004; accepted August 30, 2004.

From the *Department of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan; †Department of Gastroenterology, National Kyushu Cancer Center, Fukuoka, Japan; ‡Department of Epidemiology, National Institute for Longevity Sciences, Ohbu, Japan; and §Department of Biochemistry and Cell Biology, Institute of Gerontology, Nippon Medical School, Kanagawa, Japan.

Reprints: Kyoko Miyasaka, Department of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakaecho Itabashiku, Tokyo 173-0015, Japan (e-mail: miyasaka@tmig.or.jp).

In Japan, pancreatic cancer ranks as the fifth most common cause of cancer death, and the 5-year survival rate of its victims is less than 10%.[1] Smoking is a well-documented risk factor for the development of pancreatic adenocarcinoma.[2] In contrast, alcohol intake has not been firmly established as causally related or unrelated to pancreatic cancer.[3,4] Heavy alcohol intake may cause chronic pancreatitis. Alcoholic pancreatitis, which accounts for 55.5% of pancreatitis cases, is the most common type in Japanese men (68.5%).[5] Chronic pancreatitis has been indicated as a risk factor for pancreatic cancer.[6–9]

In the body, alcohol is processed as follows. Orally ingested ethanol is metabolized by alcohol dehydrogenase, and the first metabolite is acetaldehyde. Most of the acetaldehyde generated during alcohol metabolism is eliminated by liver mitochondrial aldehyde dehydrogenase 2 (ALDH2) by converting the acetaldehyde into acetic acid. The Japanese population is deficient in ALDH2 because of the high frequency of a mutant allele in the ALDH2 gene (ALDH2*2). The ALDH2*2 allele encodes a Glu-to-Lys amino acid substitution at the 14th and last codon. More than 40% of Japanese have the inactive form of ALDH2, encoded as either heterozygous ALDH2*1/2*2 or homozygous ALDH2*2,[10] while the majority of whites possess the active form of ALDH2 (2*1/2*1).

Acetaldehyde is a recognized animal carcinogen.[11] A recent report[12] showed that inactive ALDH2 is a risk factor for multiple carcinomas of the esophagus in alcoholics and that acetaldehyde appears to play a critical role in field cancerization. More recently, there has been an association between ALDH2 gene polymorphisms and cancers of the head and neck.[13]

In this study, we investigated 114 Japanese patients with pancreatic cancer to determine whether it is associated with ALDH2 gene polymorphism, particularly in conjunction with smoking and/or drinking habits.

MATERIALS AND METHODS

Subjects

This study was approved by the Ethics Committees of the National Kyushu Cancer Center, of the National Institute of Longevity Sciences (NILS), and of the Tokyo Metropolitan Institute of Gerontology. Written informed consent was obtained from each subject.

The 70 male subjects (mean age 62 years; range, 41–80) and 44 female subjects (mean age, 66 years; range, 43–93) had been consecutively hospitalized at the National Kyushu Cancer Center. Pancreatic cancer was diagnosed clinically by imaging techniques including ultrasound, CT scanning, and magnetic resonance tomography and was proved by histologic examination.

The age-matched control subjects consisted of 1050 male participants (mean age, 59 years; range, 40–79) and 1020 female participants (mean age, 58 years; range, 40–79) in the NILS Longitudinal Study of Aging (LSA).[14]

Subjects who consumed more than 5 g of ethanol per day were judged as having a drinking habit. The smoking status classifications were current smoker, ex-smoker, and never smoked. Only current smokers were judged as having a smoking habit.

Genotyping Procedures

The genotype of the ALDH2 gene was determined by a mismatched PCR-restriction fragment length polymorphism (RFLP) method reported previously.[15]

Statistical Analysis

Statistical differences between pancreatic cancer subjects and control subjects were assessed using the χ^2 test or Fisher direct test. Probability differences of $P < 0.05$ were considered statistically significant.

RESULTS

Smoking and/or Drinking Habits

The frequency of male subjects who had both smoking and drinking habits was significantly higher in the pancreatic cancer patients than in control subjects (Table 1). The frequency of male subjects who had a smoking habit with or without a drinking habit was significantly higher in pancreatic cancer patients than in control subjects (70% for pancreatic cancer patients vs. 37.5% for controls), whereas a drinking habit with or without a smoking habit was not different between the 2 groups (64.5% for pancreatic cancer patients vs. 66.7% for controls) (Table 1).

In contrast, more than 70% of female subjects had neither habit (Table 1). Although the frequency of subjects who had a smoking habit alone tended to be higher and the frequency of subjects who had a drinking habit alone tended to be lower in pancreatic cancer patients than control subjects regardless of sex, but the differences were not statistically significant.

Distribution of ALDH2 Genotype Between Pancreatic Cancer Patients and Control Subjects

The distribution of the ALDH2 genotype in the control subjects of both sexes was similar to those in previous reports[10] (Table 2). In male subjects, the frequency of the active form of ALDH2 was significantly lower in pancreatic cancer patients than in control subjects ($P < 0.02$), whereas no difference was observed in female subjects in pancreatic cancer patients and control subjects (Table 2).

Smoking and Drinking Habits, and ALDH2 Gene Polymorphism Between Pancreatic Cancer Patients and Control Subjects

In male subjects, the frequency of subjects who had both smoking and drinking habits was significantly higher among the pancreatic cancer patients than among control subjects with either ALDH2*1/2*1 or ALDH2*1/2*2 (Table 3). The odds ratio was 3.13 for the subjects with ALDH2*1/2*1 and 3.12 for those with ALDH2*1/2*2. The frequency of subjects with ALDH2*1/2*2 who had a smoking habit alone was significantly higher in pancreatic cancer patients than in control subjects ($P = 0.048$) (Table 2). On the other hand, none of the 8 pancreatic cancer patients with ALDH2*2/2*2 had a drinking habit. Four of the ALDH2*2/2*2 subjects had a smoking habit (Table 3), but the difference between the pancreatic cancer patients and the controls was not significant ($P = 0.44$) because the absolute number of subjects with ALDH2*/2* was small. However, among the subjects with inactive ALDH2 (including ALDH2*1/2*2 and ALDH2*2/2*2), the frequency of the smoking habit alone was again significantly higher in pancreatic cancer patients than in control subjects ($P < 0.03$).

In contrast, in female subjects because few subjects had smoking and/or drinking habits, there were no significant differences between pancreatic cancer patients and control subjects in terms of habits and/or ALDH2 genotypes (Table 4).

TABLE 1. Smoking and/or Drinking Habits in Pancreatic Cancer Patients and Control Subjects

	Both Smoking and Drinking Habits n (%)	Smoking Habit Alone n (%)	Drinking Habit Alone n (%)	Neither Habit n (%)	Total n (%)
Male					
Pancreatic cancer	35 (50.0)*	14 (20.0)	10 (14.3)	11 (15.7)	70 (100)
Control subjects	294 (27.5)	107 (10.0)	419 (39.2)	233 (21.8)	1050 (100)
Female					
Pancreatic cancer	1 (2.2)	5 (11.4)	3 (6.8)	35 (79.5)	44 (100)
Control subjects	25 (2.5)	46 (4.5)	227 (22.3)	722 (70.8)	1020 (100)

*The frequency was significantly lower compared with that in control subjects ($\chi^2 = 23.01$, df = 1, $P = 0.000$).
The difference between pancreatic cancer patients and control subjects was tested by 2 × 2 χ^2 test.

TABLE 2. Distribution of ALDH2 Gene Genotypes in Patients With Pancreatic Cancer and Control Subjects

Male	Pancreatic Cancer (n = 70) n (%)	Control Subjects (n = 1050) n (%)
Genotype		
ALDH2*1/2*1 (active ALDH)	26 (37.1)*	544 (51.8)
ALDH2*1/2*2 (inactive ALDH)	36 (51.4)	413 (39.3)
ALDH2*2/2*2 (inactive ALDH)	8 (11.4)	93 (8.9)
Female	**Pancreatic Cancer (n = 44) n (%)**	**Control Subjects (n = 1020) n (%)**
Genotype		
ALDH2*1/2*1 (active ALDH)	22 (50.0)	513 (50.3)
ALDH2*1/2*2 (inactive ALDH)	19 (43.2)	414 (40.6)
ALDH2*2/2*2 (inactive ALDH)	3 (6.8)	93 (9.1)

*The frequency was significantly lower compared with that in control subjects (χ^2 = 5.65, df = 1, P = 0.018).

The difference between the wild-type genotype and the mutations (the sum of the inactive form) was tested by 2 × 2 χ^2 test.

DISCUSSION

The present study showed that a smoking habit with or without a drinking habit is a risk factor for pancreatic cancer in male subjects as previously reported.[2] In contrast, alcohol drinking has no relation to pancreatic cancer. When the effect of drinking with or without smoking was investigated, the frequency of drinking habits did not differ between pancreatic cancer patients and control subjects, regardless of ALDH2 genotype (P = 0.53).

On the other hand, in spite of no relation between drinking and pancreatic cancer, the frequency of subjects with inactive ALDH2 was significantly higher in male pancreatic cancer patients than in control subjects. ALDH2 is responsible for metabolizing the acetaldehyde produced from ethanol into acetate. The inactive form of ALDH2 is considered to produce high levels of acetaldehyde to be accumulated in the blood, which has been known to be an animal carcinogen. We did not measure the blood concentration of acetaldehyde in the present study. In the previous report by Harada et al,[16] the acetaldehyde concentrations in the blood were significantly higher in subjects with inactive ALDH2 than those with active ALDH2 after 0.5 g/kg ethanol was administered orally (35.3 ± 12.8 μmol/L in 19 subjects with inactive ALDH2 vs. 2.1 ± 1.7 μmol/L in 25 subjects with active ALDH2), while the ethanol concentrations were comparable (10 mmol/L).

When the effect of smoking with or without drinking was investigated, the frequency of a smoking habit was significantly higher in pancreatic cancer patients than in control subjects. The frequency of male subjects who had both smoking and drinking habits was similar between subjects with ALDH2*1/2*1 and ALDH2*1/2*2 in both pancreatic cancer patients and controls (Table 3). In subjects with ALDH2*1/2*2, the frequency of smokers in pancreatic cancer (80%) was 2 times that of control (40%) (Table 3), and more subjects with pancreatic cancer had both smoking and drinking habits than control subjects. The odds ratio in the subjects with ALDH2*1/2*1 was 2.14 and 6.1 in the subjects with ALDH2*1/2*2. No subjects with ALDH2*1/2*1 had a smoking habit alone; however, 10 of the ALDH2*1/2*2 subjects had a smoking habit alone (Table 3). In contrast, only 2 patients (ALDH2*1/2*2) had a drinking habit alone. Therefore, a smoking habit would mask the contribution of acetaldehyde. Insofar as subjects with ALDH2*2/2*2 can hardly drink alcohol because of an inability to eliminate acetaldehyde, which causes an adverse reaction, known as the flushing response, after ethanol ingestion. Indeed, none of the 8 pancreatic cancer patients with ALDH2*2/2*2 had a drinking habit. Four of the ALDH2*2/2*2 subjects had a smoking habit (Table 3), although the difference between the pancreatic cancer patients and the controls was not significant (P = 0.44) (Table 3). It is suggested that the subjects with inactive ALDH2 might prefer to or be forced to smoke rather than drink during social intercourse. We did not determine how much ethanol or how much tobacco were consumed; thus, we could not further stratify these groups.

TABLE 3. Smoking and/or Drinking Habits and ALDH2 Gene Polymorphism in Male Pancreatic Cancer Patients and Control Subjects

Genotype	Both Smoking and Drinking Habits	Smoking Habit Alone	Drinking Habit Alone	Neither Habit	Total n (%)
Pancreatic cancer patients					
ALDH2*1/2*1	16 (61.5)*	0 (0)	8 (30.8)	2 (7.7)	26 (100)
ALDH2*1/2*2	19 (52.8)†	10 (27.8)‡	2 (5.6)	5 (13.9)	36 (100.1)
ALDH2*2/2*2	0 (0)	4 (50)	0 (0)	4 (50.0)	8 (100)
Control subjects					
ALDH2*1/2*1	184 (33.8)	18 (3.3)	291 (53.5)	54 (9.4)	544 (100)
ALDH2*1/2*2	109 (26.4)	58 (14.0)	125 (30.3)	121 (29.3)	413 (100)
ALDH2*2/2*2	1 (1.1)	31 (33.3)	3 (3.2)	58 (77.4)	93 (100)

*df = 1, P = 0.006, odds ratio = 3.13.
†df = 1, P = 0.002, odds ratio = 3.12.
‡df = 1, P = 0.048, odds ratio = 2.35.
The difference was tested by the Fisher direct test.

TABLE 4. Smoking and/or Drinking Habits, and ALDH2 Gene Polymorphism in Female Subjects

Genotype	Both Smoking and Drinking Habits	Smoking Habit Alone	Drinking Habit Alone	No Habit	Total n (%)
Pancreatic cancer patients					
ALDH2*1/2*1	1 (4.5)	3 (13.6)	2 (9.1)	16 (72.7)	22 (100)
ALDH2*1/2*2	0 (0)	1 (5.2)	1 (5.2)	17 (89.5)	19 (100)
ALDH2*2/2*2	0 (0)	1 (33.3)	0 (0)	2 (66.7)	3 (100)
Control subjects					
ALDH2*1/2*1	19 (3.7)	11 (2.1)	168 (32.7)	315 (61.4)	513 (100)
ALDH2*1/2*2	5 (1.2)	32 (7.7)	56 (13.5)	321 (77.5)	414 (100)
ALDH2*2/2*2	1 (1.1)	3 (3.2)	3 (3.2)	86 (92.5)	93 (100)

There were no significant differences.

Because few Japanese women have smoking and/or drinking habits, a relationship between smoking and pancreatic cancer was not significant. However, tendencies of higher frequency of subjects who had a smoking habit (13.6% for pancreatic cancer vs. 7% for controls) and of lower frequency of subjects who had a drinking habit (9% for pancreatic cancer vs. 24.8% for controls) in pancreatic cancer patients were observed in female subjects as well as in male subjects. The sex difference in habits might be one reason why the incidence of pancreatic cancer is higher in Japanese men than in women.

In conclusion, a smoking habit increased the risk of pancreatic cancer regardless of the presence or absence of a drinking habit, and smoking enhanced the risk of pancreatic cancer in male subjects having inactive ALDH2.

REFERENCES

1. Matsuno S, Egawa S, Shibuya K, et al. Pancreatic cancer: current status of treatment and survival of 16071 patients diagnosed from 1981–1996, using Japanese national pancreatic cancer database. *Int J Clin Oncol.* 2000;5:153–157.
2. Achuller HM. Mechanisms of smoking-related lung and pancreatic adenocarcinoma development. *Nature Rev Cancer.* 2002;2:255–463.
3. Karlson BM, Ekbom A, Josefsson S, et al. The risk of pancreatic cancer following pancreatitis: an association due to confounding? *Gastroenterology.* 1997;113:587–592.
4. Ye W, Lagergren J, Weiderpass E, et al. Alcohol abuse and the risk of pancreatic cancer. *Gut.* 2002;51:236–239.
5. Lin Y, Tamakoshi A, Matsuno S, et al. Nationwide epidemiological survey of CP in Japan. *J Gastroenterol.* 2000;35:135–141.
6. Lowenfels AB, Maisonneuve P, Cavallini G, et al. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med.* 1993;328:1433–1437.
7. Bansal P, Sonnenberg A. Pancreatitis is a risk factor for pancreatic cancer. *Gastroenterology.* 1995;109:247–252.
8. Howes N, Neoptolemos JP. Risk of pancreatic ductal adenocarcinoma in chronic pancreatitis. *Gut.* 2002;51:765–766.
9. Malka D, Hammel P, Maire F, et al. Risk of pancreatic adenocarcinoma in chronic pancreatitis. *Gut.* 2002;51:849–852.
10. Higuchi S, Matsushita S, Murayama M, et al. Alcohol and aldehyde dehydrogenase polymorphisms and the risk for alcoholism. *Am J Psychiatry.* 1995;152:1219–1221.
11. Woutersen RA, Appelman LM, Van Garderen-Hoetmer A, et al. Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. *Toxicology.* 1986;41:213–231.
12. Yokoyama A, Muramatsu T, Ohmori T, et al. Multiple primary esophageal and concurrent upper aerodigestive tract cancer and the aldehyde dehydrogenase-2 genotype of Japanese alcoholics. *Cancer.* 1996;77:1986–1990.
13. Muto M, Nakane M, Hitomi Y, et al. Association between aldehyde dehydrogenase gene polymorphisms and the phenomenon of field cancerization in patients with head and neck cancer. *Carcinogenesis.* 2002;23:1759–1765.
14. Shimokata H, Yamada Y, Nakagawa M, et al. Distribution of geriatric disease-related genotypes in the National Institute of Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). *J Epidemiol.* 2000;10(suppl): S46–S55.
15. Kamino K, Nagasaka K, Imagawa M, et al. Deficiency in mitochondrial aldehyde dehydrogenase increases the risk for late-onset alzheimer's disease in the Japanese population. *Biochem Biophys Res Commun.* 2000;273:192–196.
16. Harada S, Agarwal DP, Goedde HW. Aldehyde dehydrogenase deficiency as cause of facial flushing reaction to alcohol in Japanese. *Lancet.* 1981;ii:982.

ELSEVIER

Cholecystokinin A Receptor Gene Promoter Polymorphism and Intelligence

HIROSHI SHIMOKATA, MD, PHD, FUJIKO ANDO, MD, PHD, NAOAKIRA NIINO, MD, PHD, KYOKO MIYASAKA, MD, PHD, AND AKIHIRO FUNAKOSHI, MD, PHD

PURPOSE: To study the association between Cholecystokinin A receptor (CCKAR) genotypes and intelligence in community-living men and women.
METHOD: Subjects were 2251 community-dwelling Japanese men and women aged 40 to 79 years. The CCKAR gene promoter polymorphisms A-81G and G-128T were determined. Intelligence was assessed by Japanese Wechsler Adult Intelligence Scales – Revised Short Forms (JWAIS-R SF). The difference in intelligence between wild type and mutation was tested.
RESULTS: There were no subjects with *AA/GT*, *AA/TT*, or *AG/TT* genotypic combinations. Both A-81G and G-128T genotypes were related to intelligence quotient (IQ) estimated by JWAIS-R SF. The mean and SE of IQ levels of subjects with the wild-type allele and the mutation allele at nucleotide -128 were 103.4 ± 0.3 and 101.6 ± 0.6, respectively. There was a significant difference in IQ for G-128T ($p = 0.008$). The difference in IQ for A-81G was also significant ($p = 0.011$). The IQ level was 103.6 ± 0.4 in the subjects with the wild-type allele and 102.0 ± 0.5 in the subjects with the mutation. Differences in IQ levels by haplotypes for combinations of A-81G/G-128T were examined. IQ significantly decreased with an increasing number of mutation alleles ($p = 0.018$).
CONCLUSION: There were statistically significant differences in IQ for CCKAR gene promoter polymorphisms A-81G and G-128T in community-living Japanese.
Ann Epidemiol 2005;15:196–201.

KEY WORDS: Cholecystokinin, Intelligence, Genotype, Epidemiology.

INTRODUCTION

It is suspected that various genes influence intelligence, but the association between gene polymorphism and intelligence is still unclear. Cholecystokinin (CCK) is one of the major physiologic substances of gallbladder contraction and pancreatic enzyme secretion. CCK also plays an important role in the central nervous system (CNS) by interacting with dopamine and other neurotransmitters (1). CCK receptors have been classified into two subtypes, CCK type-A receptor (CCKAR) and type-B receptor (CCKBR). CCKAR has been found in the CNS (2). Associations with feeding disorders (3), anxiety (4), and schizophrenia (5) have been reported. It was also reported that learning and memory functions were impaired in CCKAR gene-knockout (OLETF) rats (6, 7). The CCKAR gene may be related to intelligence in humans. We examined the association between CCKAR gene promoter polymorphisms and intelligence in a group of 2251 community-dwelling Japanese men and women.

METHODS

Subject Selection

The subjects in this study were participants in the National Institute for Longevity Sciences – Longitudinal Study of Aging (NILS-LSA) (8). The NILS-LSA started in November 1997. The first phase of examinations was finished by the end of March 2000, and followed-up every 2 years. Participants in the NILS-LSA were independent residents in Obu city and Higashiura town in Aichi prefecture, central Japan. Data on all residents in the area are maintained in a Resident Registration System by local governments. Residents aged 40 to 79 years old were selected using Resident Registration. Samples of 7790 males and females were selected by age and gender stratified random sampling and invited to an explanatory meeting by mail. The number of replies was 3434. Of these, 881 refused to attend the meeting, 2553 agreed to attend, and 2513 actually attended. After the meeting, 2267 participated in the first phase examination. At the meeting, the procedures

From the Department of Epidemiology, National Institute for Longevity Sciences, Obu Aichi, Japan (H.S., F.A., N.N.); Department of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan (K.M.); and Department of Gastroenterology, National Kyushu Cancer Center, Fukuoka, Japan (A.F.).

Address correspondence to: Hiroshi Shimokata, M.D., Ph.D., Department of Epidemiology, National Institute for Longevity Sciences, 36-3 Gengo, Morioka-cho, Obu Aichi 474-8522, Japan. Tel.: +81-562-46-2311, ext. 815; Fax: +81-562-44-6593. E-mail: hiroshi@nils.go.jp

This work was supported in part by Research Grants for Longevity Sciences (12C-01) from the Ministry of Health and Welfare of Japan to Drs. H. Shimokata and A. Funakoshi.

Received June 6, 2004; accepted June 14, 2004.

1047-2797/05/$–see front matter
doi:10.1016/j.annepidem.2004.06.007

Selected Abbreviations and Acronyms

BMI = body mass index
CCK = cholecystokinin
CCKAR = cholecystokinin A receptor
CNS = central nervous system
DNA = deoxyribonucleic acid
GLM = general linear model
IQ = intelligence quotient
JWAIS-R-SF = Japanese Wechsler Adult Intelligence Scales – Revised Short Forms
NILS-LSA = National Institute for Longevity Sciences – Longitudinal Study of Aging
PCR-RFLP = polymerase chain reaction – restriction fragment length polymorphism
OLETF = Otsuka Long-Evans Tokushima Fatty
SE = standard error
WAIS-R = Wechsler Adult Intelligence Scales – Revised

for each examination and follow-up schedule were fully explained. Written informed consent to participate in all procedures was obtained from each subject. All persons in the Resident Registration list had Japanese nationality, and there were no persons who had a foreign name among the subjects. The subjects in this study were supposed to be ethnically homogenous Japanese.

Among the 2267 participants in the first phase examination, 2251 men and women were evaluated for CCKAR genotypes and intelligence. These subjects were analyzed for cross-sectional associations between genotype and intelligence. The number of the subjects by gender and age was almost equal (Table 1). The mean and standard deviation for age was 59.2 ± 10.9 years. Among the subjects, 26.7% had an educational background of college or greater. The Ethical Committee of Chubu National Hospital approved all procedures of the NILS-LSA.

Evaluation of Intelligence and Other Variables

The Wechsler Adult Intelligence Scales – Revised (WAIS-R) is one of the most popular tools used to assess intelligence (9). A Japanese version of the WAIS-R (JWAIS-R) has been developed and is widely used in Japan (10). In this study, intelligence was assessed by the Japanese Wechsler Adult Intelligence Scales - Revised - Short Forms (JWAIS-R-SF) (11). The JWAIS-R-SF consists of the following four subtests: Information, Similarities, Picture Completion, and Digit Symbol. Scaled scores of subtests were used in the analysis. The intelligence quotient (IQ) was estimated from the combination of these four subtests. Psychologists conducted the interviews and JWAIS-R-SF tests. Height and weight were measured while wearing lightweight clothes, and body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Life-style and medical history including annual income, education, and smoking status were checked by questionnaires. The questionnaires were checked by a physician at the medical examination. All drugs used during the previous 2 years were to be documented by participants; the physician confirmed them at an interview and coded the drugs used during the last 2 weeks. Among the 2251 subjects in the study, 213 had used drugs acting on the CNS, that is, hypnotic sedative agents, antianxiety agents, antiepileptic agents, stimulant drugs, antihypnotic drugs, anti-Parkinson drugs, and anti-psychotic drugs during the previous 2 weeks. The IQ was less than 70 in 11 subjects, and only one of them used drugs acting on the CNS.

TABLE 1. Distribution of the subjects by gender and age

	Age (years)				
Gender	40–49	50–59	60–69	70–79	Total
Males	291	282	281	280	1134
Females	278	278	283	278	1117
Total	569	560	564	558	2251

CCKAR Genotype Analysis

Genomic DNA was extracted from peripheral blood lymphocytes by a standard procedure. A mismatch PCR-RFLP method was used to analyze polymorphisms in the upstream region of the CCKAR gene [GenBank Accession No. U23427 (5)]. One pair of primers, sense primer = 5′-GCATATGTACACATGTGTGTAAAAAGCAGCCAGAC-3′, anti-sense primer = 5′-GCCCTTTCCTGGGCCAGACT-3) was designed to amplify a 103-base pair product, digested with restriction enzyme Hinf I, and analyzed by 3% agarose gel electrophoresis. Two sequence changes were detected: a G to T change at nucleotide -128, and an A to G change at nucleotide -81 (12).

Statistical Analysis

All values were expressed as the mean ± SE, if not specified. Both polymorphisms at nucleotides -128 and -81 were divided into two groups; as wild-type and mutation. Hetero groups were classified as mutation. The difference between wild-type and mutation groups was tested by the *t*-test for continuous variables and the 2 × 2 chi-square test for categorical variables. The difference in IQ and JWAIS-R subtests score by genotype was also tested by the *t*-test excluding subjects who had used drugs acting on the CNS or subjects with IQ less than 70. The trend among the three groups was tested by the general linear model (GLM) and the probability for trend (p for trend) was shown. Statistical analyses were performed using the SAS system (SAS Institute Inc., Cary, NC). All p-values were two-tailed.

AEP Vol. 15, No. 3
March 2005: 196–201

RESULTS

Distribution of CCKAR Promoter Genotypes

The distributions of CCKAR promoter single nucleotide polymorphisms A-81G and G-128T were both in Hardy-Weinberg equilibrium. The distribution of genotype combination was examined (Table 2). These polymorphisms were in linkage disequilibrium. There were no subjects with *AA/GT*, *AA/TT*, or *AG/TT* genotypic combinations. Thus, subjects with a mutation at -128 always had a mutation at -81.

Background Characteristics and CCKAR Genotype

Figure 1 shows the IQ distribution. The distribution was slightly skewed to the left (lower IQ) and close to a normal distribution. The mean value of the IQ of the all subjects was 103.0, and the median was also 103. The difference between the mean and median was very small. The lowest IQ was 43 and the highest IQ was 142 among the subjects. The number of subjects with IQ less than 70 was 11, and those with IQ 135 or over was 13. Background characteristics were compared by CCKAR G-128T and A-81G genotypes (Table 3). Age, body weight, body mass index, annual income, education, and smoking status did not differ between wild-type (GG) and mutation (*GT* or *TT*) for the CCKAR G-128T genotype. These variables also did not differ for the CCKAR A-81G genotype except for education status. Education status in the wild-type (*AA*) group was significantly higher than that in the mutation-type (*AG* or GG) group ($p = 0.009$). The IQ was significantly different by education status ($p < 0.001$). The IQ for the low education group was 100.3 ± 0.3 and that for the high education group was 110.6 ± 0.5.

Intelligence and CCKAR Genotype

The IQ levels in subjects with wild-type and mutation alleles at nucleotide -128 were 103.4 ± 0.3 and 101.6 ± 0.6, respectively. There was a significant difference in IQ for the G-128T genotype ($p = 0.008$). The score of Digit Symbol was lower in subjects with a mutation ($p = 0.003$). There

TABLE 2. Distribution of CCKAR G-81T and A-128G genotypes

CCKAR G-128T	CCKAR A-81G AA	AG	GG	Total
GG	1317 (58.5%)	307 (13.6%)	26 (1.2%)	1650 (73.3%)
GT	0 (0.0%)	491 (21.8%)	61 (2.7%)	552 (24.5%)
TT	0 (0.0%)	0 (0.0%)	49 (2.2%)	49 (2.2%)
Total	1317 (58.5%)	798 (35.5%)	136 (6.0%)	2251 (100.0%)

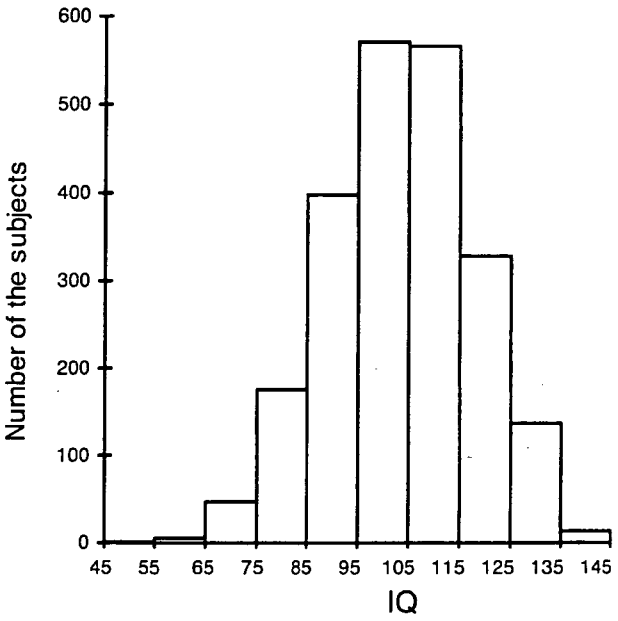

FIGURE 1. Distribution of IQ levels in the subjects.

was no difference in the scores of Information, Picture Completion, and Similarities subtests for polymorphism G-128T. The IQ level was 103.6 ± 0.4 in subjects with wild-type (AA) and 102.0 ± 0.5 in subjects with mutation (AG or GG) at nucleotide -81. The difference in IQ for the A-81G polymorphism was significant ($p = 0.011$). The Picture Completion and Digit Symbol subtest scores were significantly lower in subjects with the mutation ($p = 0.043$ and $p = 0.008$, respectively). The Similarities subtest score was marginally lower for a mutation at nucleotide -81 ($p = 0.051$).

In the low education group, IQ was 100.5 ± 0.4 in the -128 wild-type group and 99.5 ± 0.6 in the -128 mutation-type group. There was no significant difference in IQ between the wild- and mutation-type of G-128T genotype. However, the IQ for the -81 wild-type group was 100.8 ± 0.4, which was significantly higher than that for the mutation group (99.4 ± 0.4) ($p = 0.038$). In the high education group, the IQ was 111.5 ± 0.6 in the -128 wild-type group and 107.9 ± 1.1 in the -128 mutation-type group. There was a significant difference between the wild and mutation groups ($p = 0.004$). The IQ in the -81 wild-type group (111.1 ± 0.7) did not differ from that in the mutation group (109.8 ± 0.9).

Intelligence was compared excluding subjects who had used drugs acting on the CNS and subjects with IQ less than 70 (Table 4). The number of excluded subjects was 223. Differences in IQ between in the wild-type and mutation groups were still significant both for A-81G and G-128T

TABLE 3. Comparison of variables between wild-type and mutation alleles in CCKAR G-81T and A-128G genotypes

	CCKAR G-128T			CCKAR A-81 G		
	Wild type GG	Mutation GT or TT	p*	Wild type AA	Mutation AG or GG	p
n	1650	601		1317	333	
Age (years)	59.2 ± 0.3†	59.3 ± 0.4	NS‡	59.1 ± 0.3	59.5 ± 0.4	NS
Weight (kg)	57.5 ± 0.2	57.0 ± 0.4	NS	57.6 ± 0.3	57.0 ± 0.3	NS
BMI (kg/m^2)	22.9 ± 0.1	22.9 ± 0.1	NS	22.9 ± 0.1	22.9 ± 0.1	NS
Annual income (%; 54,000 US$ or over)	57.5	58.3	NS	58.3	57.0	NS
Education (%; college or over)	26.9	26.0	NS	27.4	25.6	0.009
Smoking (%; smoker)	22.8	22.8	NS	23.6	21.8	NS
JWAIS-R-SF						
IQ	103.4 ± 0.3	101.6 ± 0.6	0.008	103.6 ± 0.4	102.0 ± 0.5	0.011
Information	9.9 ± 0.1	9.7 ± 0.1	NS	9.9 ± 0.1	9.8 ± 0.1	NS
Picture Completion	10.2 ± 0.1	10.0 ± 0.1	NS	10.2 ± 0.1	10.0 ± 0.1	0.043
Similarities	10.3 ± 0.1	10.1 ± 0.1	NS	10.3 ± 0.1	10.1 ± 0.1	0.051
Digit Symbol	11.7 ± 0.1	11.3 ± 0.1	0.003	11.7 ± 0.1	11.4 ± 0.1	0.008

†Mean ± SE.
‡NS = not significant.
*p-value tested by the t-test or χ^2 test.

genotypes. The IQ levels of subjects with wild-type and mutation alleles at nucleotide -128 were 104.1 ± 0.4 and 102.0 ± 0.6, respectively. There was a significant difference in IQ ($p = 0.002$). The scores of Information and Digit Symbol were significantly lower in subjects with a mutation ($p = 0.012$ and $p = 0.003$, respectively). There were no differences in the scores of Picture Completion and Similarities subtests for polymorphism G-128T. The IQ level was 104.2 ± 0.4 in the subjects with wild-type and 102.6 ± 0.5 in the subjects with mutation at nucleotide -81. Difference in IQ by A-81G polymorphism was significant ($p = 0.008$). Similarities and Digit Symbol subtest scores were significantly lower in subjects with the mutation ($p = 0.033$ and $p = 0.013$, respectively). The Information subtest score was marginally lower with mutation of nucleotide -81 ($p = 0.078$). However, there was no significant difference in the score of Picture Completion subtest.

Haplotype Analysis

Possible haplotypes in the combinations of polymorphism A-81G/G-128T were GA, GG, TG, and TA. However, there were no subjects with AA/GT, AA/TT, or AG/TT genotypic combinations (Table 2). The common haplotype of AA/GT, AA/TT, or AG/TT genotypic combinations was TA. It was considered that no subject had a TA haplotype. The distribution of haplotypes GA, GG, and TG is shown in Table 5. The number of GA haplotypes was 3432; GG was 420; and TG was 650. There was a significant difference in IQ among haplotypes GA, GG, and TG. The IQ for haplotype GA was the highest and the IQ for haplotype TG was the lowest. With an increase in the number of mutation alleles, the IQ level decreased ($p = 0.018$). Digit Symbol scores also significantly decreased with an increasing number of mutation alleles ($p = 0.012$).

TABLE 4. Comparison of intelligences between wild-type and mutation alleles in CCKAR G-81T and A-128G genotypes. Subjects who had used drugs acting on the CNS or subjects with IQ less than 70 were excluded

	CCKAR G-128T			CCKAR A-81G		
	Wild type GG	Mutation GT or TT	p*	Wild type AA	Mutation AG or GG	p
n	1489	539		1178	850	
JWAIS-R-SF						
IQ	104.1 ± 0.4†	102.0 ± 0.6	0.002	104.2 ± 0.4	102.6 ± 0.5	0.008
Information	10.0 ± 0.1	9.6 ± 0.1	0.012	10.0 ± 0.1	9.8 ± 0.1	0.078
Picture Completion	10.2 ± 0.1	10.1 ± 0.1	NS‡	10.3 ± 0.1	10.1 ± 0.1	NS
Similarities	10.4 ± 0.1	10.2 ± 0.1	NS	10.4 ± 0.1	10.2 ± 0.1	0.033
Digit Symbol	11.8 ± 0.1	11.4 ± 0.1	0.003	11.8 ± 0.1	11.5 ± 0.1	0.013

†Mean ± SE.
‡NS = not significant.
*p-value tested by the t-test.

AEP Vol. 15, No. 3
March 2005: 196–201

TABLE 5. Comparison of intelligences between wild-type and mutation alleles in CCKAR G-81T and A-128G genotypes

	Haplotype			
	GA	GG	TG	p for trend*
n	3432	420	650	
JWAIS-R-SF				
IQ	103.2 ± 0.2†	103.0 ± 0.7	101.7 ± 0.6	0.018
Information	10.0 ± 0.1	9.8 ± 0.1	9.7 ± 0.1	NS‡
Picture Completion	10.2 ± 0.1	10.1 ± 0.1	10.0 ± 0.1	NS
Similarities	10.3 ± 0.1	10.1 ± 0.1	10.1 ± 0.1	NS
Digit symbol	11.6 ± 0.1	11.6 ± 0.1	11.3 ± 0.1	0.012

†Mean ± SE.
‡NS = not significant.
*Trend of the three groups was tested by the general linear model.

DISCUSSION

Accumulating data support the involvement of the dopaminergic system in cognitive processing. It is known that CCKAR modulates CCK-stimulated dopamine release in the brain, and mutations in the CCKAR gene may influence the dopaminergic system (5). Considerable preclinical and clinical evidence indicate that inhibitory effects on dopaminergic systems by antipsychotic medications may account for cognitive impairment. A report showed sustained activation of the human mesolimbic dopaminergic system during the performance of cognitive tasks (13). It was also reported that systemic administration of the CCKAR selective antagonist, devazepide, impaired the development of conditioned incentive learning in rats (14). From these data, it is suspected that mutation in the CCKAR gene may influence intelligence.

The CCKAR promoter genotypes were significantly related to IQ. The IQ levels of subjects with the mutant allele were significantly lower than those of subjects with the wild-type allele both for G-128T and A-81G genotypes. A difference in IQ by CCKAR promoter gene polymorphisms was seen in both middle-aged and elderly people. In analyses excluding the subjects who had used drugs acting on the CNS and subjects with IQ less than 70, there was also a significant difference in IQ between the wild and mutation genotypes. We carried out association studies of quantitative traits with haplotypes, and found that the IQ became lower with an increase in the number of mutation alleles.

The CCKAR gene polymorphisms of G-128T and A-81G are located in the promoter region of the gene. It is suspected that mutation of these genotypes is related to the amount of CCKAR production. However, it is still unclear whether these CCKAR polymorphisms are functional or if they are in linkage disequilibrium with other as yet unknown polymorphisms in the CCKAR gene or in a neighboring gene.

In the studies on intelligence in the general population, investigation of genetic factors is an important issue (15). However, at the present time, gene polymorphism has infrequently been reported to be associated with cognition (16). It is suspected that there are many genes associated with individual differences in intelligence, and intelligence is determined from interactions of these gene polymorphisms. However, the contribution of each gene to intelligence may be small as indicated by the results of this study. Testing of thousands of subjects is required to detect small but significant differences. A detailed assessment of IQ requires interviews by psychologists. Assessment of IQ in a large-scale community-dwelling population is generally difficult. It is also difficult to obtain DNA specimens from community-dwelling populations. Because of this, studies on the association between genotype and intelligence have not progressed. In the present study, we showed the relationship between intelligence and CCKAR promoter mutations G-128T and A-81G in community-living middle-aged and elderly Japanese. CCKAR-promoter genotyping may provide useful information for assessing intelligence and preventing cognitive impairment.

REFERENCES

1. Hansen TV. Cholecystokinin gene transcription: Promoter elements, transcription factors, and signaling pathways. Peptides. 2001;22:1201–1211.
2. Hill DR, Shaw TM, Graham W, Woodruff GN. Autoradiographical detection of cholecystokinin-A receptors in primate brain using 125I-Bolton Hunter CCK-8 and 3H-MK-329. J Neurosci. 1990;10:1070–1081.
3. Brenner LA, Ritter RC. Type A CCK receptors mediate satiety effects of intestinal nutrients. Pharmacol Biochem Behav. 1996;54:625–631.
4. Lydiard RB. Neuropeptides and anxiety: Focus on cholecystokinin. Clin Chem. 1994;40:315–318.
5. Tachikawa H, Harada S, Kawanishi Y, Okubo T, Shiraishi H. Novel polymorphisms of the human cholecystokinin A receptor gene: An association analysis with schizophrenia. Am J Med Genet. 2000;96:141–145.
6. Nomoto S, Miyake M, Ohta M, Funakoshi A, Miyasaka K. Impaired learning and memory in OLETF rats without cholecystokinin (CCK)-A receptor. Physiol Behav. 1999;66:869–872.
7. Li XL, Aou S, Hori T, Oomura Y. Spatial memory deficit and emotional abnormality in OLETF rats. Physiol Behav. 2002;75:15–23.
8. Shimokata H, Ando F, Niino N. A new comprehensive study on aging—The National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). J Epidemiol. 2000;10:S1–S9.
9. Wechsler D. Wechsler Adult Intelligence Scale – Revised manual. New York: Psychological Corporation; 1981.
10. Shinagawa F, Kobayashi S, Fujita K, Maekawa HH. Japanese Wechsler Adult Intelligence Scale–Revised [in Japanese]. Tokyo: Nihon-Bunka-Kagaku-Sha (Japan Culture and Science Company); 1990.
11. Kobayashi S, Fujita K, Maekawa H, Dairoku H. Short form method in Japan [in Japanese]. In: Misawa G, ed. Japanese Wechsler Adult Intelligence Scale-Revised Short Forms. Tokyo: Nihon-Bunka-Kagaku-Sha (Japan Culture and Science Company); 1993:23–55.
12. Funakoshi A, Miyasaka K, Matsumoto H, Yamamori S, Takiguchi S, Kataoka K, et al. Gene structure of human cholecystokinin (CCK) type-A receptor: Body fat content is related to CCK type-A receptor gene promoter polymorphism. FEBS Lett. 2000;466:264–266.

13. Fried I, Wilson CL, Morrow JW, Cameron KA, Behnke ED, Ackerson LC, et al. Increased dopamine release in the human amygdala during performance of cognitive tasks. Nat Neurosci. 2001;4:201–206.
14. Josselyn SA, Franco VP, Vaccarino FJ. Devazepide, a CCKA receptor antagonist, impairs the acquisition of conditioned reward and conditioned activity. Psychopharmacology. 1996;123:131–143.
15. Bouchard TJ Jr, McGue M. Familial studies of intelligence: A review. Science. 1981;212:1055–1059.
16. Payton A, Holland F, Diggle P, Rabbitt P, Horan M, Davidson Y, et al. Cathepsin D exon 2 polymorphism associated with general intelligence in a healthy older population. Mol Psychiatry. 2003;8:14–18.

Taylor & Francis
Taylor & Francis Group

Original Article

International Journal of Audiology 2005; 44:86–91

*Yasue Uchida**
Tsutomu Nakashima†
Fujiko Ando‡
Naoakira Niino‡
Hiroshi Shimokata‡

*Department of Otorhinolaryngology, Chubu National Hospital, National Institute for Longevity Sciences, Japan
†Department of Otorhinolaryngology, Nagoya, Japan University School of Medicine
‡Department of Epidemiology, National Institute for Longevity Sciences, Japan

Key Words
Noise
Smoking
Hearing
Age

Is there a relevant effect of noise and smoking on hearing? A population-based aging study

¿Existe un efecto relevante del ruido y el tabaquismo en la audición? Un estudio de envejecimiento de base poblacional

Abstract

The objectives of the present study were to evaluate both the respective and combined effects of occupational noise exposure and smoking on hearing, taking age into consideration. The evaluation was conducted using 1478 subjects without a history of ear disease out of a population-based sample of 2267 adults, aged 40–79 years. Pure-tone audiometry and a questionnaire were administered. A deleterious effect of noise exposure on hearing was significantly observed in both genders at many frequencies after adjustment for age, income, and education. The smoking habit alone significantly affected hearing deterioration at 4000 Hz in noise-unexposed males. The combined effect of noise and smoking was not interactive but additive. A dose-response effect of smoking on hearing loss was observed in middle-aged males without noise exposure. Smoking and noise exposure were associated with hearing loss respectively. This result is noteworthy for the preservation of good hearing especially at the beginning of aging.

Sumario

El propósito de este estudio fue evaluar los efectos aislados o combinados en la audición de la exposición a ruido ocupacional y el tabaquismo, tomando en cuenta la edad. La evaluación se efectuó en 1478 sujetos sin historia de enfermedad otológica, a partir de una muestra de población de 2267 adultos con edades entre 40 y 79 años. Se realizó una audiometría de tonos puros y se administró un cuestionario. Se observó un efecto significativo de deterioro en la audición por la exposición a ruido en ambos géneros y en muchas frecuencias, después de hacer ajustes por la edad, ingresos y educación. El hábito de tabaquismo aislado afectó significativamente el deterioro auditivo en 4000 Hz, en varones no expuestos a ruido. El efecto combinado de ruido y tabaquismo no fue interactivo sino de sumación. Se observó un efecto de respuesta de acuerdo con la magnitud del tabaquismo sobre la pérdida auditiva, en varones de edades medias sin exposición a ruido. Se asoció el tabaquismo y la exposición al ruido, con la hipoacusia. El resultado es de mucho interés para la preservación de una buena audición, especialmente al principio del período de envejecimiento.

Whether it is possible to modulate presbycusis has been debated from various aspects. Noise exposure is one of the major etiologies of sensorineural hearing loss. Cigarette smoking has also been investigated as a factor associated with hearing loss. Other contributing variables such as ototoxic drugs,and medical conditions (e.g. cardiovascular disease, hypercholesterolemia, and diabetes mellitus) have been researched. Each of these potential risk factors, might contribute to age-related changes in hearing. It has not been clarified whether, excluding all extraneous causes, presbycusis will still occur, and there are numerous hurdles to discovering all extraneous causes which contribute to this type of hearing loss. Conducting a population-based study which compares subjects, matched for age and gender in the presence and absence of given factors, would help identify which factors contribute to hearing loss.

Cigarette smoking is known to be injurious to health. Over 2000 chemical compounds have been identified in tobacco leaves, some of which are released through smoking. Tobacco products contain more than 50 established or identified carcinogens (Kuper et al, 2002). Tobacco smoking is considered to increase the risk of many diseases, such as heart disease, chronic obstructive pulmonary disease, stroke, peripheral vascular disease, peptic ulcers, and osteoporosis. Many epidemiological studies have been performed to investigate the effect of smoking on hearing. Several studies have suggested a positive association between smoking and hearing loss (Siegelaub et al, 1974; Rosenhall et al, 1993; Cruickshanks et al, 1998; Itoh et al, 2001). Cruickshanks et al (1998) reported on a study of 3753 subjects aged 48 to 92 years seen in Beaver Dam, Wisconsin. After adjustment for other factors (age, history of cardiovascular disease, alcohol consumption, occupational noise exposure, and education), current smokers had an increased risk of hearing loss compared with nonsmokers (odds ratio [OR], 1.69; 95% confidence interval [CI], 1.31–2.17). Itoh et al (2001) found in an epidemiological study of health screening participants in Japan, that current smokers had a significantly increased risk of hearing loss compared with nonsmokers (OR, 2.10; 95% CI, 1.53–2.89) after adjustment for sex, age, and eight other potential confounders, such as body mass index and% vital capacity. On the other hand, some studies have not found a significant relationship between cigarette smoking and hearing loss (Gates et al, 1993; Brant et al, 1996). Gates et al (1993)

ISSN 1499-2027 print/ISSN 1708-8186 online
DOI: 10.1080/14992020500031256

Received:
December 12, 2003
Accepted:
June 28, 2004

Yasue Uchida
Department of Otorhinolaryngology, Chubu National Hospital, National Institute for Longevity Sciences, 36-3 Gengo, Morioka, Obu, Aichi, 474-8511, Japan
E-mail:yasueu@nils.go.jp

reported that there was no association between cigarette smoking and hearing loss in 1662 elderly men and women in the Framingham Study. There continues to be debate on the dose-response relationship between smoking and hearing loss, active or passive smoke exposure, and reciprocal action with the other confounders in sensorineural hearing loss.

The objectives of the present study were to evaluate both the respective and combined effects of occupational noise exposure and smoking on hearing, taking age into consideration; and to find out whether there is a dose-response relationship between the amount of smoking and the hearing threshold in subjects with and without a history of occupational noise exposure.

Methods

The present study was conducted as part of the baseline examination in the 'Longitudinal Study of Aging (NILS-LSA)' conducted by the National Institute for Longevity Sciences; which consists of measurements of visual and auditory function, blood chemical analysis, body composition and anthropometry, physical function, nutritional analysis, and psychological tests. This cohort study looked at 2267 subjects aged 40 to 79 years old with a 2-year follow-up. Individuals in resident registrations of Ohbu-city (population 70,000) and Higashiura-town (population 40,000) in Aichi prefecture were stratified by both age and gender in cooperation with their local governments, and then randomly selected for participation. The residents selected were invited to an explanatory meeting at which the study design and detailed procedures of examinations of the NILS-LSA were fully described. Only those persons who understood and accepted this project participated. All procedures for the study were reviewed by the Ethical Committee of the Chubu National Hospital, and written informed consent was obtained from all participants. The baseline examinations were performed between November, 1997, and April, 2000. In the present study, 579 subjects who reported suffering from any ear disease at any time in their life, and 105 subjects who were uncertain whether they were suffering or not were excluded. Participants who refused to or did not complete the hearing test and those who responded invalidly to the necessary questions for the present analysis were excluded. Data on 1478 subjects with no missing data out of 1583 subjects without a history of ear disease were analyzed.

Participants filled out detailed questionnaires in advance of the examination visit. The questionnaires consisted of over 130 question items designed to obtain demographic characteristics, personal history, family history, lifestyle habits, and various medical problems. Occupational noise exposure and smoking history were obtained. Occupational noise was defined in our questionnaires as background noise in a work environment over which the worker could not hold a conversation in a normal voice. Former and current noise exposure were put together into a Noise (+) group. In addition, ex-smokers and current smokers were put together into a Smoking (+) group. The subjects were divided into four groups by gender to analyze the effects of noise exposure and smoking habits: 1. Noise (−) Smoking (−), 2. Noise (−) Smoking (+), 3. Noise (+) Smoking (−), 4. Noise (+) Smoking (+). Regarding the amount of smoking, the number of pack-years was calculated. Pack-years of smoking were defined as the number of packs (one pack is equal to 20 cigarettes) smoked per day, multiplied by the duration of smoking in years.

Air-conduction pure-tone thresholds at octave intervals from 500 to 8000 Hz for the right and left ears were obtained employing a test method recommended by the Japan Audiological Society (Audiology Japan, 1990), which uses a diagnostic audiometer (AA-73A, RION, Tokyo) calibrated according to JIS (Japanese Industrial Standards T 1201). The thresholds over the maximum output level of this audiometer were treated as a level plus an additional 5 dB; that is to say, 105 dB at 500 to 4000 kHz and 100 dB at 8000 Hz. The threshold of both the better and the worse ear were analyzed in order not to overlook subjects with at least one affected ear. For each subject, the ears were classified as the better ear (BE) and the worse ear (WE), respectively, depending on a pure-tone average of thresholds at 500, 1000, 2000, and 4000 Hz.

Statistical analyses were conducted using the Statistical Analysis System (SAS) version 6.12 software (SAS Institute Inc., 1997). Differences in the mean pure-tone thresholds at each frequency between groups of Noise (+) and Noise (−) by gender and smoking habits were compared using the GLM Procedure. Adjustments were made for age, income, and education. Age was adjusted to the control of 60 years of age. Income level was defined as family income and was divided into two categories: yearly income <6.5 million yen and >6.5 million yen. Educational level was divided into two categories: less than or equal to high-school graduates, and more than high school. Differences in the mean pure-tone thresholds at each frequency between groups of Smoking (+) and Smoking (−) by gender and the existence of noise exposure were also compared using the GLM Procedure, with adjustment for age with the control of 60 years of age, income and education. The interaction of noise and smoking was analyzed by a two-way analysis of variance (ANOVA) using the GLM Procedure with adjustment for age, income, and education. Correlation analysis between the pack-years and pure-tone thresholds by age groups and the existence of noise exposure was performed using the Pearson correlation analysis with adjustment for income and education. The level of significance was set at $p < 0.05$. Since there were not enough female smokers for the correlation analysis, it was investigated only in males.

Results

The gender and age distribution of the subjects by smoking habits and occupational noise exposure are shown in Table 1. The subjects were 758 males and 720 females, and the number of subjects in each decade (40s, 50s, 60s, 70s) was approximately the same. There were 588 male smokers (77.6% of the male subjects) and 68 females (9.4% of the female subjects). There were 201 male occupationally noise-exposed subjects (26.5% of the male subjects) and 104 females (14.4% of the female subjects).

The mean air conduction thresholds were compared between the Noise (−) and Noise (+) groups by gender and smoking habits. In the male Smoking (+) group, statistically significant deterioration in hearing was found in the Noise (+) group compared with the Noise (−) group at all frequencies in both BE and WE. In the female Smoking (+) group, no significant

Table 1. The distribution of respondents for the questions about smoking habit and occupational noise exposure by age groups and gender

	Male				*Female*				
Age Group	*40–49yr*	*50–59yr*	*60–69yr*	*70–79yr*	*40–49yr*	*50–59yr*	*60–69yr*	*70–79yr*	*Total*
Smoking (−), Noise (−)	24 (13)	34 (18)	41 (21)	27 (14)	112 (69)	143 (78)	156 (84)	148 (78)	685 (46)
Smoking (−), Noise (+)	10 (6)	11 (6)	14 (7)	9 (5)	18 (11)	25 (14)	19 (10)	31 (17)	137 (9)
Smoking (+), Noise (−)	112 (62)	103 (55)	104 (53)	112 (58)	28 (17)	12 (6)	9 (5)	8 (4)	488 (33)
Smoking (+), Noise (+)	35 (19)	38 (20)	39 (19)	45 (23)	5 (3)	3 (2)	1 (1)	2 (1)	168 (12)
	181 (100)	186 (100)	198 (100)	193 (100)	163 (100)	183 (100)	185 (100)	189 (100)	1478 (100)

The column percentages are indicated in parenthesis.

difference was observed between the Noise (−) and Noise (+) groups at any frequency in both ears. In the male Smoking (−) group, the deleterious effect of noise exposure was found significantly excepting at 500 and 8000 Hz in BE and at 500 Hz in WE. In the female Smoking (−) group, the significant adverse effect of noise exposure was shown excepting at 2000 Hz in BE and at 500, 4000 and 8000 Hz in WE. A comparative display of the effect of smoking, classified by gender and noise exposure with regard to frequencies, is presented in Table 2. A statistically significant deterioration in hearing was found in the Smoking (+) group compared to the Smoking (−) group at 4000 Hz in noise-unexposed males. No statistically significant difference was observed at the other frequencies in noise-unexposed males, and at any frequency in noise-exposed males and in any females. Focusing on BE at 4000 Hz in the male group, the joint effects of smoking habits and noise exposure are graphically presented in Figure 1. As mentioned above, statistically significant deterioration in hearing was found in the Smoking (+) group compared to the Smoking (−) group in noise-unexposed group and statistically significant deterioration in hearing was found in the Noise (+) group compared with the Noise (−) group regardless of smoking habits. The results for WE show a similar tendency to those for BE.

In the analysis by two-way ANOVA after adjustment for age, income, and education, a significant main effect of noise was observed at all frequencies in males and females. A significant main effect of smoking was found at 4000 Hz in males. No interaction between noise and smoking was observed in either gender.

Correlation analysis between the amount of smoking and hearing thresholds by age groups and the existence of noise exposure was performed to evaluate the further effect of smoking on hearing in each age population. The results of the analysis are shown in Table 3. There was weak but significant positive correlation between pack-years and air conduction pure-tone thresholds for the better ear in males in their forties and fifties without noise exposure at several frequencies. As smoking increased, the pure-tone thresholds significantly elevated. For the worse ear, significant positive correlation between pack-years and air conduction pure-tone thresholds was observed in their forties without noise exposure at some frequencies. No significant correlation between pack-years and hearing thresholds was found in male subjects with noise exposure.

Discussion

Whether there is an interrelated effect on sensorineural hearing loss between noise and smoking has been argued, especially in investigations for workers exposed to occupational noise (Barone et al, 1987; Virokannas & Anttonen, 1995; Toppila et al, 2001; Mizoue et al, 2003). Groups of workers exposed to occupational noise are likely to consist of younger males with a mean age between 30 and 49. The participants of the current study were sampled equally from middle-aged to elderly populations. The additional geographical feature of the district investigated in the present study was its location in the midsection of the main island of Japan, where employment by secondary industries such as construction and manufacturing is relatively higher than in other parts of Japan. According to a Labour Force Survey conducted in 1999, the ratio of secondary industries in the employment structure was 56.4% in the district included in this study, while it was 31.1% in the entire country, 40.8% in Aichi prefecture, and 76.8% in Toyota city in Aichi prefecture, where the Toyota car company is located. It was considered advantageous in the investigation of whether noise and smoking affect hearing to use a population-based stratification of age and gender of subjects from this particular district (Statistical Handbook of Japan 2001).

Barone et al (1987) have demonstrated, on the basis of a study of 2348 male employees of a large aerospace company, who were between the ages of 18 and 59 years (mean age: 35.3 in hearing loss group, 35.5 in control) that smoking contributes to noise-induced hearing loss. Statistically significant trends in risk were also reported for the number of pack-years of smoking, and the current packs per day consumption in present smokers. Mizoue et al (2003) assessed the interaction between smoking and noise exposure on hearing loss, using data from worker health examinations in a Japanese steel company. A dose-response relation was observed between smoking and high frequency hearing loss. The combined effect of smoking and occupational exposure to noise on hearing loss was additive. In other words, smoking did not enhance the effect of noise on hearing. In the present study smoking and noise exposure were associated with hearing loss respectively. The deleterious effect of noise

Table 2. Adjusted mean air conduction pure tone thresholds (dB) and standard error (SE) for age (with the control of 60 years of age), income and education in BE and WE by occupational noise exposure and smoking habits. Parenthesized data indicate 95% confidence interval. Asterisk shows statistically significant difference ($p < 0.05$)

BE			*0.5 kHz*		*1 kHz*		*2 kHz*		*4 kHz*		*8 kHz*	
			Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Male	Noise (−)	Smoking (−)	11.9 (10.5–13.2)	0.7	11.5 (10.0–13.1)	0.8	16.6 (14.6–18.5)	1.0	24.5 (21.8–27.1)	1.3 *	37.0 (34.2–39.8)	1.4
		Smoking (+)	12.9 (12.1–13.7)	0.4	12.2 (11.3–13.2)	0.5	17.5 (16.4–18.7)	0.6	27.5 (25.9–29.0)	0.8	37.9 (36.3–39.6)	0.8
	Noise (+)	Smoking (−)	15.1 (11.7–18.5)	1.7	16.2 (12.0–20.4)	2.1	22.3 (17.4–27.2)	2.5	34.7 (28.7–40.8)	3.1	41.7 (35.6–47.7)	3.1
		Smoking(+)	15.9 (13.8–18.1)	1.1	17.1 (14.4–19.7)	1.3	24.4 (21.3–27.5)	1.6	37.8 (33.9–41.6)	1.9	46.2 (42.4–50.00)	1.9
Female	Noise (−)	Smoking (−)	13.8 (13.0–14.7)	0.4	10.5 (9.7–11.4)	0.4	15.3 (14.3–16.3)	0.5	17.1 (15.9–18.3)	0.6	34.2 (32.6–35.8)	0.8
		Smoking (+)	14.3 (12.0–16.6)	1.2	12.1 (9.7–14.5)	1.2	16.4 (13.8–19.1)	1.3	19.6 (16.3–22.8)	1.7	34.9 (30.6–39.2)	2.2
	Noise (+)	Smoking (−)	18.0 (15.7–20.4)	1.2	13.9 (11.4–16.4)	1.3	17.9 (15.2–20.5)	1.3	21.3 (17.6–24.9)	1.8	38.1 (33.5–42.7)	2.3
		Smoking (+)	16.7 (10.9–22.6)	2.9	15.1 (8.8–21.4)	3.2	17.4 (10.7–24.0)	3.3	24.5 (15.3–33.8)	4.6	44.3 (32.7–55.8)	5.8
WE			*0.5 kHz*		*1 kHz*		*2 kHz*		*4 kHz*		*8 kHz*	
			Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Male	Noise (−)	Smoking (−)	15.3 (13.7–17.0)	0.8	15.1 (13.2–17.0)	1.0	21.9 (19.8–24.1)	1.1	30.5 (27.8–33.2)	1.4 *	39.9 (36.9–43.0)	1.6
		Smoking (+)	15.5 (14.5–16.4)	0.5	15.8 (14.7–16.9)	0.6	21.7 (20.4–23.0)	0.6	33.9 (32.3–35.5)	0.8	42.0 (40.3–43.8)	0.9
	Noise (+)	Smoking (−)	19.4 (15.4–23.4)	2.0	20.1 (15.8–24.5)	2.2	29.8 (24.9–34.6)	2.5	41.3 (35.7–46.8)	2.8	47.7 (41.4–54.1)	3.2
		Smoking (+)	19.6 (17.1–22.1)	1.3	21.0 (18.2–23.7)	1.4	30.4 (27.3–33.4)	1.6	42.9 (39.4–46.8)	1.8	50.4 (46.4–54.4)	2.0
Female	Noise (−)	Smoking (−)	17.5 (16.4–18.6)	0.6	14.5 (13.5–15.6)	0.5	20.3 (19.2–21.5)	0.6	23.3 (21.9–24.7)	0.7	38.5 (36.8–40.3)	0.9
		Smoking (+)	19.7 (16.7–22.6)	1.5	16.5 (13.6–19.3)	1.4	19.8 (16.7–22.9)	1.6	24.8 (21.0–28.5)	1.9	39.8 (35.1–44.5)	2.4
	Noise (+)	Smoking (−)	20.0 (17.1–23.0)	1.5	17.5 (14.5–20.5)	1.5	24.9 (21.7–28.1)	1.6	27.9 (23.8–32.0)	2.1	42.8 (38.2–47.5)	2.4
		Smoking (+)	18.7 (11.2–26.1)	3.8	16.6 (9.1–24.1)	3.8	21.7 (13.7–29.8)	4.1	34.5 (24.2–44.8)	5.2	49.0 (37.3–60.8)	5.9

exposure on hearing was significantly observed without regard to smoking habits, excepting the female Smoking (+) group after adjustment for age, income, and education. The female Smoking (+) group was in need of further data accumulation for effectual assessment. Smoking alone significantly affected hearing deterioration at 4000 Hz in male subjects without noise exposure. Since smoking habits were likely to be influenced by socio-economic status (Green & Potvin, 2002), the present analysis was performed with adjustment for income and education. An interactive effect of noise exposure and smoking on hearing was not statistically demonstrated as shown in Figure 1. The joint effects of noise exposure and smoking on hearing were additive as found in the study of Mizoue et al (2003). In their study, however, smoking was associated with increased odds of having hearing loss at 4000 Hz in both noise-unexposed and noise-exposed males. The trend association that odds ratio increased with higher numbers of cigarettes smoked per day was more evident among those who had worked in noisy environments. In the present study, there was no statistically significant difference between groups of Smoking (+) and Smoking (−) in noise-exposed subjects and a dose-response relation was not observed between smoking and hearing thresholds in noise-exposed subjects. The great effect of noise may control the development of hearing loss, regardless of smoking

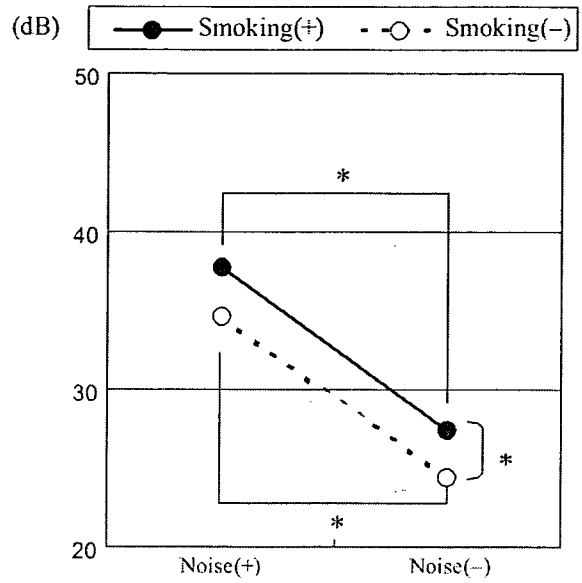

Figure 1. Mean air conduction pure tone thresholds (dB) for BE at 4 kHz in the male group by smoking habits and noise exposure. Asterisk shows statistically significant difference ($p < 0.05$).

habits. Previous studies have suggested that the effects of noise are so large as to overwhelm the effects of smoking (Siegelaub et al, 1974; Drettner et al, 1975). In addition, there is a limitation in classification based on self-reported examination regarding intended variables. Although there were various characteristics of the noise that workers were exposed to, depending on their occupation or working environments, noise levels at the workplaces were unknown in the present study. Toppila et al (2001) have found in their investigation of paper mill workers, forest workers, and shipyard workers, that for subjects with less than two confounders, the extent of lifetime noise exposure determined the development of noise-induced hearing loss. They have classified 706 male workers (mean age: 40 ± 9) by lifetime occupational noise exposure with the confounders in noise-induced hearing loss, such as smoking habits, serum cholesterol, systolic or diastolic blood pressure, and the use of analgesics. Virokannas and Anttonen (1995) found a dose-response relationship between smoking and the impairment of hearing acuity in workers exposed to occupational noise when the exposure time to noise was used as a covariance. Since the current study was a population-based sample, the target occupation was not specified and we were therefore unable to classify or adjust for an absolute quantity of noise exposure. Imbalance in the noise levels that respective groups were exposed to may possibly make the target effects obtuse.

A weak but statistically significant positive correlation between the amount of smoking and hearing threshold was observed only in middle-aged subjects without noise exposure in the present study. A dose-response effect of smoking on hearing loss has been indicated in previous studies (Rosenhall et al, 1993; Virokannas & Anttonen, 1995; Cruickshanks et al, 1998; Mizoue et al, 2003). A marked increase in hearing impairment prevalence in the subjects who smoked more than 20 pack-years was found in a previous study (Noorhassim & Rampal, 1998). Regarding the likely affected frequencies, one study reported 3000 and 4000 Hz were mostly affected rather than 6000 Hz (Virokannas & Anttonen, 1995). Another study demonstrated the prevalence of hearing impairment was highest at 6000 Hz among 500, 1000, 2000, 3000, 4000, and 6000 Hz

Table 3. Correlation coefficients between pack year and air conduction pure tone thresholds for BE and WE in male subjects after adjustment for income and education. Asterisk shows statistically significant correlation ($p < 0.05$)

BE			*0.5 kHz*	*1 kHz*	*2 kHz*	*4 kHz*	*8 kHz*
	40–49	yr	0.07	0.28 *	0.22 *	0.16	0.01
Noise (−)	50–59	yr	0.19*	0.07	0.05	−0.07	0.03
$n = 557$	60–69	yr	0.10	0.05	0.09	0.06	0.10
	70–79	yr	0.01	0.03	−0.02	0.09	0.03
	40–49	yr	0.03	−0.22	−0.17	−0.08	−0.15
Noise (+)	50–59	yr	0.10	0.19	0.03	0.04	0.07
$n = 201$	60–69	yr	0.04	0.01	−0.08	0.13	0.05
	70–79	yr	−0.03	−0.20	−0.11	−0.01	0.01
WE			*0.5 kHz*	*1 kHz*	*2 kHz*	*4 kHz*	*8 kHz*
	40–49	yr	0.06	0.30 *	0.23*	0.16	0.11
Noise (−)	50–59	yr	0.02	0.00	−0.06	−0.02	−0.13
$n = 557$	60–69	yr	−0.05	0.06	0.01	0.12	0.17
	70–79	yr	0.01	−0.01	−0.03	−0.01	0.00
	40–49	yr	−0.14	−0.08	−0.17	−0.03	−0.19
Noise (+)	50–59	yr	0.11	0.10	0.10	0.03	0.11
$n = 201$	60–69	yr	0.06	0.00	−0.10	−0.03	−0.07
	70–79	yr	−0.09	−0.22	−0.22	−0.13	−0.05

(Noorhassim & Rampal, 1998). A third study (Rosenhall et al, 1993) indicated significant correlation at any of 250, 500, 1000, 2000, 4000, and 8000 Hz in different age cohorts. In the current study, a dose-response relationship between smoking and hearing deterioration was observed at 500, 1000, and 2000 Hz, while a statistically significant difference between smokers and non-smokers was found at 4000 Hz.

Toppila et al (2001) have reported in the above-mentioned investigation, that as the number of confounders increased, the noise exposure was overruled by the other confounding factors in the development of hearing loss. Since many of influential confounders in the development of hearing loss were age-related in statistical analysis, elderly subjects could have more numbers of confounders unadjusted here than young subjects. The reason that a dose-response effect of smoking on hearing loss was observed only in middle-aged subjects in the present study was speculative. Further research using many potential variables not discussed here, should be our next assignment for modulation of presbycusis. However, even in middle-aged subjects without noise exposure, it is surely worthwhile to note for the preservation of good hearing, that a dose-response effect of smoking on hearing loss was found in the current study.

The interaction between smoking and noise on hearing has remained obscure. An experimental study has demonstrated that smokers evidenced significantly less temporary threshold shift following noise exposure than did non-smokers (Dengerink et al, 1992). Carbon monoxide, which is one of the major ingredients in cigarette smoke, appeared to play a large role in the long-term effects of smoking on the reduction of the temporary threshold shift. Smoking may have some beneficial effect on the temporary threshold shift. It appears, however, that the longer term effects of smoking on permanent hearing loss shown by the research mentioned previously (Rosenhall et al, 1993; Virokannas & Anttonen, 1995; Cruickshanks et al, 1998) is harmful rather than beneficial. The carbon monoxide in the smoke forms carboxyhemoglobin, which in turn, reduces the blood's oxygen-carrying capacity (Glantz & Parmley, 1991). Nicotine increases platelet aggregation (Saba & Mason, 1975), which plays an important role in the development of atherosclerosis. Although the vasoconstrictory effects of nicotine in the blood vessels are typical, vessels of the central nervous system and coronary arteries are known to dilate in response to nicotine (Matschke, 1991). It remains to be investigated whether the longer term effects of smoking are associated with the effect of noise exposure on permanent hearing loss.

Conclusions

In the present study, smoking and noise exposure were associated with hearing loss respectively. The deleterious effect of noise exposure on hearing was significantly observed in both genders at many frequencies after adjustment for age, income, and education. The smoking habit alone significantly affected hearing deterioration at 4000 Hz in male subjects without noise exposure. The combined effect of noise exposure and smoking on hearing was not interactive but additive. A dose-response effect of smoking on hearing loss was observed at 500, 1000, and 2000 Hz in middle-aged males without noise exposure. This result is noteworthy for the preservation of good hearing especially at the beginning of aging.

Acknowledgements

This study was supported by a Grant-in-Aid for Research on Eye and Ear Sciences, Immunology, Allergy and Organ Transplantation from the Ministry of Health and Welfare of Japan.

References

Anonymous 1990. *Audiology Japan*, 33, 792–806. (in Japanese).

Barone, J.A., Peters, J.M., Garabrant, D.H., Bernstein, L. & Krebsbach, R. 1987. Smoking as a risk factor in noise-induced hearing loss. *J Occup Med*, 29, 741–745.

Brant, L.J., Gordon-Salant, S., Pearson, J.D., Klein, L.L., Morrell, C.H., et al. 1996. Risk factors related to age-associated hearing loss in the speech frequencies. *J Am Acad Audiol*, 7, 152–160.

Cruickshanks, K.J., Klein, R., Klein, B.E.K., Wiley, T.L., Nondahl, D.M., et al. 1998. Cigarette smoking and hearing loss: the epidemiology of hearing loss study. *JAMA*, 279, 1715–1719.

Dengerink, H.A., Lindgren, F.L. & Axelsson, A. 1992. The interaction of smoking and noise on temporary threshold shifts. *Acta Otolaryngol (Stockh)*, 112, 932–938.

Drettner, B., Hedstrand, H., Klockhoff, I. & Svedberg, A. 1975. Cardiovascular risk factors and hearing loss. *Acta Otolaryngol*, 79, 366–371.

Gates, G.A., Cobb, J.L., D'Agostino, R.B. & Wolf, P.A. 1993. The relation of hearing in the elderly to the presence of cardiovascular disease and cardiovascular risk factors. *Arch Otolaryngol Head Neck Surg*, 119, 156–161.

Glantz, S.A. & Parmley, W.W. 1991. Passive smoking and heart disease. Epidemiology, Physiology, and Biochemistry. *Circulation*, 83, 1–12.

Green, L.W. & Potvin, L. 2002. Education, health promotion, and social and lifestyle determinants of health and disease. In R. Detels, J. McEwen, R. Beaglehole & H. Tanaka (eds.) *Oxford Textbook of Public Health. The Scope of Public Health*. (Fourth Edition) Oxford: Oxford University Press, pp. 113–130.

Itoh, A., Nakashima, T., Arao, H., Wakai, K., Tamakoshi, A., et al. 2001. Smoking and drinking habits as risk factors for hearing loss in the elderly: epidemiological study of subjects undergoing routine health checks in Aichi, Japan. *Public Health*, 115, 192–196.

Kuper, H., Adami, H.O. & Boffetta, P. 2002. Tobacco use, cancer causation and public health impact. *J Intern Med*, 251, 455–466.

Matschke, R.G. 1991. Smoking habits in patients with sudden hearing loss. *Acta Otolaryngol (Stockh), 476 (Suppl)*, 69–73.

Mizoue, T., Miyamoto, T. & Shimizu, T. 2003. Combined effect of smoking and occupational exposure to noise on hearing loss in steel factory workers. *Occup Environ Med*, 60, 56–59.

Noorhassim, I. & Rampal, K.G. 1998. Multiplicative effect of smoking and age on hearing impairment. *Am J Otolaryngol*, 19, 240–243.

Rosenhall, U., Sixt, E., Sundh, V. & Svanborg, A. 1993. Correlations between presbyacusis and extrinsic noxious factors. *Audiology*, 32, 234–243.

Saba, S. & Mason, R. 1975. Some effects of nicotine on platelets. *Thromb Res*, 7, 819–824.

SAS Procedures Guide, Release 6.12 Edition. 1997. SAS Institute Inc., Cary, NC.

Siegelaub, A.B., Friedman, G.D., Adour, K. & Seltzer, C.C. 1974. Hearing loss in adults: relation to age, sex, exposure to loud noise, and cigarette smoking. *Arch Environ Health*, 29, 107–109.

Statistical Handbook of JAPAN 2001, Statistics Bureau, Ministry of Public Management, Home Affairs, Posts and Telecommunications. http://www.stat.go.jp/

Toppila, E., Pyykko, I. & Starck, J. 2001. Age and noise–induced hearing loss. *Scand Audiol*, 30, 236–244.

Virokannas, H. & Anttonen, H. 1995. Dose-response relationship between smoking and impairment of hearing acuity in workers exposed to noise. *Scand Audiol*, 24, 211–216.