Appendix 2
Rouleau's protocol: the score is calculated by a sum of three components (I, II, III)

I Integrity of the clock face (maximum: 2 points)
- 2 Present without gross distortion
- 1 Incomplete or some distortion
- 0 Absent or totally inappropriate

II Presence and sequencing of the numbers (maximum: 4 points)
- 4 All present in the right order and at most minimal error in the spatial arrangement
- 3 All present but errors in spatial arrangement
- 2 Numbers missing or added but no gross distortions of the remaining numbers; numbers placed in counterclockwise direction or all present but gross distortion in spatial layout (i.e. hemineglect, numbers outside the clock)
- 1 Missing or added numbers and gross distortions
- 0 Absence or poor representation of numbers

III Presence and placement of the hands (maximum: 4 points)
- 4 Hands are in correct position and the size difference is respected
- 3 Slight errors in the placement of the hands or no representation of size difference between the hands
- 2 Major errors in the placement of the hands
- 1 Only one hand or poor representation of two hands
- 0 No hands or perseveration on hands

Appendix 3
Cahn's protocol: the global score is calculated by subtracting qualitative score (II) from quantitative score (I)

I Quantitative CDT score = maximum 10 points: assesses the presence and correctness of the clock; the clock face (0–2 points), the placement of the hands (0–4 points) and the placement of the numbers (0–4 points)

II Qualitative CDT score = maximum 8 points: summary of the following errors
- 1 Stimulus-bound response: the tendency of the drawing to be dominated or guided by a single stimulus
- 2 Conceptual deficit: this error type reflects a loss or deficit in accessing knowledge of the attributes, features and meaning of a clock
- 3 Perseveration: the continuation or the recurrence of activity without an appropriate stimulus
- 4 Neglect of left hemispace: all attributes of the clock are written on the right half of the clock face
- 5 Plannning deficit: this error type is represented by gaps before 12, 3, 6 or 9
- 6 Nonspecific spatial error: a deficit in the spatial layout of numbers, without any specific pattern in spatial disorganization
- 7 Numbers written on the outside of the clock: numbers written either around the perimeter of the circle or the circle itself
- 8 Numbers written counterclockwise: arrangement of the numbers with '12' at the top of the clock face and then continuing around in a counterclockwise fashion

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Alcohol dehydrogenase 2 variant is associated with cerebral infarction and lacunae

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Abstract—The authors examined the association of the alcohol dehydrogenase 2 (ADH2) genotype with vascular events in community-dwelling Japanese (1,102 men/1,093 women). The allele *ADH2*2* encodes an isozyme with a higher level of activity than ADH2*1. Here, the authors show that the *ADH2*1* carriage is associated with high prevalence of cerebral infarction and lacunae in men. Multiple regression analyses confirmed that the risk of lacunae and cerebral infarction was increased by the *ADH2*1* allele.

NEUROLOGY 2004;63:1711–1713

Alcohol dehydrogenase (ADH) is one of the key enzymes in alcohol metabolism. *ADH2* and *ADH3* have alleles that encode isoenzymes with distinct enzymatic properties.[1] Among Caucasians, a variant *ADH3* allele is found. On the other hand, among Mongoloids, especially the Japanese, about 85% of individuals are carriers of the β2-subunit encoded by the *ADH2*2* allele, compared to only 5% or less of European and white American populations. The β1 (encoded by *ADH2*1*) and β2 subunits (encoded by *ADH2*2*) differ by only one amino acid residue, Arg-47 in the β1 subunit substituted with His-47 in the β2 subunit. ADH2 functions as a dimer and the β2β2 dimer exhibits about 100 times more catalytic activity than the β1β1 dimer.[1]

We previously reported on the influence of the *ADH2* and aldehyde dehydrogenase 2 genotypes on diabetic vasculopathy in type 2 diabetes.[2] Here we examined whether the *ADH2* genotype would also be associated with vascular events in community-dwelling Japanese and show the association of the *ADH2*1* allele with cerebral infarction.

Materials and methods. A population-based prospective cohort study of aging and age-related diseases was begun in Japan in 1997. All participants (1,126 men and 1,106 women) were independent residents of Aichi prefecture. Residents aged 40 to 79 years old were randomly selected from the register in cooperation with the local government. A total of over 1,000 characteristics, including medication, food and nutrition, bone mineral density, blood and urine analysis, psychological examinations, visual and auditory examinations, physical function tests and physical activities, anthropometry and body composition, and head MRI, were examined (see http://www.nils.go.jp/index-j.html).[3] The study protocol was approved by the Committee on the Ethics of Human Research of National Chubu Hospital and the National Center for Geriatrics and Gerontology. Written informed consent for the entire procedure was obtained from each participant.

Samples of DNA were isolated from peripheral blood cells. Genotypes were determined with a fluorescence-based allele-specific DNA primer-probe assay system (Toyobo Gene Analysis, Tsuruga, Japan). Brain MRI was performed using a 1.5-tesla scanner (Toshiba Visart, Tokyo). The first scanning sequence consisted of a T1-weighted sagittal series centered in the midline to define the orbitomeatal line. The second series of T1-weighted axial images and T2-weighted axial images were oriented parallel to the orbitomeatal line. Fourteen slices were taken at each examination.

A cerebral infarction was defined as a lesion more than 0.3 cm in diameter appearing as a low-signal-intensity area on T1-weighted images that was also visible as a hyperintense lesion on T2-weighted images as described.[3,4] Small lesions (<1.5 cm) were diagnosed as a lacunae. One of the authors (M.F.), a neurologist, who was blinded to the clinical status of the subjects, interpreted all MRI series.

Results. When the subjects were grouped into three according to the genotype of *ADH2*, *ADH2*2/ADH2*2 (ADH2*2/2)*, *ADH2*2/ADH2*1 (ADH2*2/1)*, and *ADH2*1/ADH2*1 (ADH2*1/1)*, the distribution of the *ADH2* genotypes was in Hardy-Weinberg equilibrium. There was no significant difference in characteristics among the three genotypic groups in women (data are not shown). In contrast, in men, the level of total cholesterol (TC) and LDL-cholesterol (LDL-C) significantly differed between the *ADH2*2/2* and *ADH2*1/2* genotypic groups by multiple comparisons (table 1). Although group *ADH2*1/1* did not significantly differ in the levels of TC and LDL-C from the other groups, probably due to an insufficient number in members of group *ADH2*1/1* (5.2%), the *ADH2*1* allele tended to increase the levels of TC and LDL-C. Additionally, alcohol consumption was higher in the *ADH2*1/1* group than the other groups, whereas there was no differ-

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Supported by a grant from the Ministry of Health, Labor and Welfare, Japan, to H.S. and S.O.

Received March 25, 2004. Accepted in final form June 24, 2004.

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*Table 1 Comparison of clinical characteristics in men among ADH2*2/2, ADH2*2/1, and ADH2*1/1 genotypic groups*

	*ADH2*2/2*	*ADH2*2/1*	*ADH2*1/1*	Genotype: *p* value
No. (%)	689 (61.2)	378 (33.6)	59 (5.2)	NS
Age, y	59.4 ± 0.4	58.8 ± 0.6	58.0 ± 1.4	NS
Alcohol, g/d	28.8 ± 1.4	29.5 ± 1.9	44.5 ± 4.5	2/2 vs 1/1: $p = 0.0049$*
				2/1 vs 1/1: $p = 0.0102$*
Nonsmoker & smoker, %†	21/40/39	22/40/37	24/39/37	NS
Systolic BP, mm Hg‡	120.1 ± 0.8	121.8 ± 1.0	126.1 ± 2.6	NS
Diastolic BP, mm Hg‡	74.9 ± 0.5	76.1 ± 0.6	77.3 ± 1.6	NS
Percent with hypertension§	32.6	37.0	40.7	NS
Height, cm	164.4 ± 0.2	164.7 ± 0.3	164.6 ± 0.8	NS
BMI	23.0 ± 0.1	22.8 ± 0.1	22.9 ± 0.4	NS
T-cho, mg/dL	210.1 ± 1.3	215.7 ± 1.7	217.6 ± 4.3	2/2 vs 2/1: $p = 0.0231$*
LDL, mg/dL	129.7 ± 1.2	135.8 ± 1.7	134.4 ± 4.2	2/2 vs 2/1: $p = 0.0115$*
HDL, mg/dL	57.3 ± 0.6	57.6 ± 0.8	57.4 ± 1.9	NS
TG, mg/dL	134.9 ± 3.7	130.8 ± 5.0	150.2 ± 12.4	NS
Glucose, mg/dL	105.7 ± 0.9	106.1 ± 1.2	103.9 ± 2.9	NS
HbA1c, %	5.32 ± 0.03	5.34 ± 0.04	5.33 ± 0.10	NS
Percent with diabetes	13.3	13.3	13.6	NS
Insulin, μU/mL	8.5 ± 0.2	7.8 ± 0.3	8.7 ± 0.7	NS
Estradiol, pg/mL	28.2 ± 0.4	27.1 ± 0.5	25.9 ± 1.4	NS
F-Testosterone, pg/mL	13.1 ± 0.2	13.3 ± 0.2	13.6 ± 0.5	NS
Brain examination, n (%)	n = 678	n = 367	n = 57	
Lacunal infarction	60 (8.9)	55 (15.0)	8 (14.0)	$p = 0.0085$¶
				2/2 vs 2/1: $p = 0.0025$‖
Cerebral infarction	68 (10.0)	59 (16.1)	9 (15.8)	$p = 0.0129$¶
				2/2 vs 2/1: $p = 0.0043$‖

Values are mean ± SD or n (%).

* *p* Value obtained by the Turkey-Kramer method for multiple comparisons.
† Nonsmoker & smoker = percentage of complete nonsmokers/percentage of past smokers who stopped smoking/percentage of current smokers.
‡ Blood pressure (BP) was analyzed only with subjects not taking oral antihypertension medications.
§ Hypertension was defined as either a systolic blood pressure of over 140 mm Hg or a diastolic blood pressure of over 90 mm Hg, or as receiving antihypertension medication.
¶ *p* Value obtained by the contingency table analysis.
‖ *p* Value by the chi-square analysis between groups *ADH2*2/2* and *ADH2*2/1*.

NS = not significant by multiple comparisons; BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

ence in amounts of alcohol consumption between groups *ADH2*2/2* and *ADH2*2/1*.

A total of 1,102 male and 1,093 female subjects were examined by MRI. More striking, in men, higher frequencies of lacunae and cerebral infarction were found in the *ADH2*2/1* group than the *ADH2*2/2* group (see table 1). The frequencies of other abnormal signs on MRI did not differ among the three groups (data are not shown). In women, there was no difference in prevalence of abnormal MRI signs among the three *ADH2* genotypic groups (data not shown).

To confirm the significant difference in the frequencies of lacunae and cerebral infarction according to the *ADH2* genotype, multiple logistic analyses were performed based on 1,102 subjects with an adjustment for aging (table 2). Aging is the most significant risk for lacunae and cerebral infarction. More interestingly, OR and *p* values clearly indicated that the *ADH2*1* allele is a distinct risk for lacunae and cerebral infarction. Even when the effect of alcohol consumption was included, the main conclusion was not altered (see table 2).

Discussion. An influence on lacunae and cerebral infarction by the *ADH* genotype was found only in Japanese men. This discrepancy between genders may be speculated to be due to a difference in alcohol consumption. However, even when the effect of alcohol consumption was included, the main conclusion was not altered. Therefore, the effect by alcohol consumption does not seem responsible for the discrepancy between genders. Instead, ADH2 activity modulated by several hormones may be responsible for the discrepancy. In fact, experiments with ani-

Table 2 Multiple logistic analyses (number of subjects = 1,102)

	OR (95% CI)	*p* Value
Lacunar state in men		
A: Multiple logistic analyses		
ADH2 (carriage of *ADH2*1* allele)	2.16 (1.44–3.25)	0.0002
Age - 10 y	3.46 (2.69–4.45)	<0.0001
B: Multiple logistic analyses including alcohol consumption		
ADH2 (carriage of *ADH2*1* allele)	2.18 (1.49–3.38)	0.0005
Age - 10 y	3.53 (2.68–4.65)	<0.0001
Cerebral infarction in men		
A: Multiple logistic analyses		
ADH2 (carriage of *ADH2*1* allele)	2.06 (1.39–3.06)	0.0003
Age - 10 y	3.44 (2.70–4.37)	<0.0001
B: Multiple logistic analyses including alcohol consumption		
ADH2 (carriage of *ADH2*1* allele)	2.05 (1.35–3.11)	0.0008
Age - 10 y	3.49 (2.70–4.52)	<0.0001

mals indicated that testosterone reduces enzymatic activity in the liver, and that estrogen increases the activity.[5]

ADH catalyzed the first step in the metabolism of ethanol, and in addition, has a wide substrate range, using both aliphatic and aromatic alcohols, aldehydes, sterols, and ω-hydroxy fatty acids. It is worth noting that ADH catalyzes the oxidation of 3,3-dimethylallyl alcohol, the intermediary alcohol of the shunt pathway of mevalonate metabolism, and the branching between the sterol and the shunt pathway could also occur at the level of geranyl pyrophosphate and farnesyl pyrophosphate.[6] Therefore, the genetic variant of *ADH2* may change the flow of the shunt pathway of cholesterol synthesis, thereby causing LDL-C levels to vary between the *ADH2*2/2* and *ADH2*2/1* groups. As for cardiovascular diseases, it was reported that an ADH3 polymorphism is associated with HDL-C levels and myocardial infarction in Caucasians.[7] Thus, our results may provide insight into ethnic differences in the incidence of cerebral or myocardial vascular disease between Mongoloids and Caucasians.

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Otol Jpn 14(2): 154-159, 2004

加齢及び全身性基礎疾患の歪成分耳音響放射に及ぼす影響

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The influence of aging and generalized diseases on distortion product otoacoustic emissions

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Objective : Investigations using otoacoustic emissions have great potential to detect cochlear impairment, especially nonlinear mechanical functions of the outer hair cells. Distortion product otoacoustic emissions (DPOAE) mostly reflect audiometric thresholds ; however, there could be an inconsistency between DPOAE response and audiometric thresholds depending upon the pathologic lesion. The objective of the present study is to assess the effects of aging and generalized diseases on auditory function using DPOAE after adjustment of confounding factors including audiometric thresholds.

Subjects and method : Of 1534 participants in a population-based study, 1265 subjects aged between 41 and 82 years who were administered DPOAE and other auditory tests were selected for the present analysis. Loss of DPOAE was defined as a signal-to-noise ratio of DPOAE amplitude equal or less than 0 dBSPL. Statistical analysis according to sex was performed in order to identify factors associated with loss of DPOAE using a multiple logistic regression model in which the independent variables were age, hypertension, hyperlipidemia, diabetes mellitus, ischemic heart disease, renal disease, liver disease, pure-tone average of 5 frequencies, resonant frequency of middle ear, ear disease, smoking habit, and occupational noise exposure.

Results : Age (odds ratio [OR] per 10 year = 1.36, 1.40, 1.53, at f2 = 5188, 5652, 6165 Hz, respectively, in male and OR = 1.32, 1.52, 1.42, 1.57, 1.46 at f2 = 1001, 1086, 4004, 4358, 6165 Hz, respectively, in female), presence of ischemic heart disease (OR = 2.25, 2.61 at f2 = 2002, 2185 Hz, respectively, in male), presence of hyperlipidemia (OR = 1.89 at f2 = 4358 Hz in male) and presence of liver disease (OR = 2.55 at f2 = 3662 Hz in female) showed a significant statistical association with loss of DPOAE.

Conclusion : Aging, ischemic heart disease, hyperlipidemia, and liver disease each may have an independent influence on auditory function from the effects on the pure-tone thresholds.

Key words : Distortion product otoacoustic emissions, Aging, Ischemic heart disease, Hyperlipidemia, Liver disease

はじめに

耳音響放射は外有毛細胞の能動的運動能と密接に関係するとされ、内耳機能の微少な変化を検出できる可能性がある。一般には、耳音響放射レベルの低下は純音聴力閾値上昇を反映する要素が大きいが、純音聴力閾値の変化と必ずしも一致するわけではない。

今回、歪成分耳音響放射（Distortion product otoacoustic emissions以下DPOAE）を用いることにより、加齢や全身性基礎疾患が聴覚に及ぼす影響を、純音聴力検査とは違った側面で捉えることを目的とした。解析にあたっては、可能性のある交絡因子について、調整するよう考慮した。

対象および方法

対象は、国立長寿医療センター疫学研究部において遂行中の『老化に関する長期縦断疫学研究』に、2000年4月から2001年9月までの間に参加した、41歳から82歳までの一般地域住民男女1534名のうち、解析に必要なすべての検査結果のある1265名である。

『老化に関する長期縦断疫学研究』は老化の過程の経時的観察を目的として、1997年10月に始動した、包括的調査研究で、地方自治体（愛知県大府市及び知多郡東浦町）の協力を得て、地域住民から年齢・性別に層化した無作為抽出を行い、選定された者の中から、自由意志により説明会、調査に参加した者を対象としている。各参加者の観察は、2年ごとに縦断的追跡を行う。参加者は、検査前に130以上の設問を含んだ自記式質問票を記入する。測定する検査項目は、血液検査、神経系検査、呼吸機能検査、循環機能検査、視覚、聴覚、骨、形態、体力、栄養、心理等、多岐にわたる。調査の詳細については過去の報告を参照されたい[1),2)]。全身性基礎疾患の有無については、この質問票のうち「現在または過去にかかった病気などがありますか。」という設問の項目から、高血圧、高脂血症、糖尿病、虚血性心疾患、腎疾患、肝疾患に対する回答を本解析に用いた。回答の選択肢のうち、「なし」と答えたものを「疾患なし」と取り扱い、「現在治療中」、「以前に治療した」、「治療していない」のいずれかを選んだものは、まとめて「疾患あり」として取り扱った。

DPOAEはOtodynamics社製Otodynamic Analyser IL092を用いて、静かな環境の室内（防音室前室）で測定した。2つの入力信号の周波数比はf2/f1≒1.2とし、入力音圧はf1、f2とも70dBSPLとした。F2の周波数領域を1001から6165Hzの範囲で変化させ、1オクターブあたり8測定点、計22点で、2f1−f2の周波数におけるDPOAEレベルを測定し、DP-gramを作成した。解析を行う際には、DP-gram上の22測定点それぞれについて、ノイズレベルを差引いたDPOAEレベルが0以下となる場合をDPレベル低下と定義した。

DPOAE測定と同日に、標準純音聴力検査、連続周波数ティンパノメトリを施行した。標準純音聴力検査はリオン社製AA-73Aを使用して防音室内で測定し、0.5、1、2、4、8kHzの5周波数の平均気導聴力レベルを連続変数として解析に用いた。連続周波数ティンパノメトリは、Grason-Stadler社製GSI33、Version2 Middle ear analyzerを用いた。GSI33ではプローブ音を250Hzから2000Hzまで50Hz毎に変化させ、各周波数毎に開始圧（+200daPa）とピーク圧との間のサセプタンスの差とアドミッタンスの位相の差を表示する。開始圧とピーク圧との間のサセプタンスの差が0になる周波数を、中耳共振周波数として解析に用いた[3)]。

統計学的解析には、Statistical Analysis System (SAS) ver.8.2を使用し、男女別で多重ロジスティック回帰分析を行った。目的変数は各f2周波数測定ポイントにおけるDPレベル低下の有無とした。説明変数として年齢及び、高血圧、高脂血症、糖尿病、虚血性心疾患、腎疾患、肝疾患それぞれの既往の有無の7変数をとった。他に5周波数平均気導聴力、中耳共振周波数、耳疾患の有無、喫煙習慣の有無、騒音職場での就労の有無の5変数を調製変数として用いた。耳疾患の有無、喫煙習慣の有無、騒音職場での就労の有無については、自記式質問票で得た回答をもとにした。ロジスティック回帰モデルは

$$\log \rho(x)/1-\rho(x)=\beta_0+\beta_1 x_1+\beta_2 x_2+\cdots+\beta_r x_r$$

である。ここで、年齢や全身性基礎疾患および調整の為に用いる因子を含んだ全12種の組み合わせである独立変数$x=(x_1, x_2, \cdots, x_r)$をもつ個体の、DPレベル低下が発生する条件付き確率を$\rho(x)$で表す。β_0は切片、β_1、β_2、…、β_rは回帰係数である。年齢に関しては、10歳増加毎のオッズ比で解析した。統計学的有意水準は5％とした。

結　果

表1に対象者の性年齢分布を示した。男性637名、女性628名とほぼ同数で、10歳ごとの男女別各年齢群の対象数は40歳台から70歳台までは120名以上であるが、80歳台は10名以下であった。

次に解析の対象とした6種の全身性基礎疾患の、本研

究対象における有病率を表2に示した。男女ともに高血圧の有病率が最も高く、概ね4人に1人の割合であった。以下高脂血症、糖尿病と続くが、高脂血症は女性で多く、糖尿病は比較的男性で多かった。

年齢とDPレベル低下の関係について、ロジスティック回帰分析により検討した（表3）。10歳年齢が上昇する毎にDPレベル低下の危険性が、男性で5188、5652、6165Hzにおいてそれぞれ1.36、1.40、1.53倍、女性で1001、1086、4004、4358、6165Hzにおいてそれぞれ1.32、1.52、1.42、1.57、1.46倍高まることが示された。

6種の全身性基礎疾患の中で、DPレベル低下をきたす可能性のある要因として統計学的に有意であった疾患と、当該のf2周波数を表4に表した。男性で虚血性心疾患を有する群は、有しない群に比べて2002、2185Hzにおいてそれぞれ2.25、2.61倍、DPレベル低下をきたしやすかった。高脂血症を有する群は有しない群に比して4358Hzで1.89倍DPレベル低下をきたしやすかった。女性では3662Hzで肝疾患のある群で2.55倍DPレベル低下が起こりやすかった。

表1　対象者の性年齢分布

年齢群	40－49歳	50－59歳	60－69歳	70－79歳	80歳以上	計
男性	129	180	177	142	9	637
女性	137	174	162	147	8	628
計	266	354	339	289	17	1265

表2　本研究対象における有病率（%）

	高血圧	高脂血症	糖尿病	虚血性心疾患	腎疾患	肝疾患
男性	26.2	13.3	11.9	6.6	7.2	10.2
女性	27.1	20.4	6.7	5.9	5.3	5.9

考　察

DPOAEの成人における加齢変化については、多くの研究者が関心を寄せて検討してきた（表5）[4)~9)]。耳音響放射は一般的に純音聴力閾値で40dBを超えると検出されにくいため、高齢者では非検出例が多くなり、中高齢期の大規模な検討は難しい。過去の報告の中で常に議論されることは、加齢に伴って見られるDPOAEレベル

表3　年齢が有意にDPレベル低下に関与すると示されたf2周波数と10歳上昇毎のオッズ比

	DPレベル低下に関与する要因	対象数			オッズ比（括弧内は95%信頼区間）
		f2周波数	DPレベル低下あり	DPレベル低下なし	
男性	年　齢	5188 Hz	195	442	1.36（1.04－1.78）
		5652 Hz	244	393	1.40（1.08－1.82）
		6165 Hz	252	385	1.53（1.18－1.98）
女性	年　齢	1001 Hz	208	420	1.32（1.05－1.66）
		1086 Hz	161	467	1.52（1.18－1.97）
		4004 Hz	115	513	1.42（1.04－1.95）
		4358 Hz	107	521	1.57（1.13－2.18）
		6165 Hz	252	385	1.46（1.10－1.92）

ロジスティック回帰分析：目的変数を各f2周波数測定ポイントにおけるDPレベル低下の有無とし、説明変数として年齢および、高血圧、高脂血症、糖尿病、虚血性心疾患、腎疾患、肝疾患それぞれの既往の有無の7変数をとり、調整変数として5周波数平均純音聴力レベル、中耳共振周波数、耳疾患の有無、喫煙習慣の有無、騒音職場での就労の有無の5変数を用いた。

表4　有意にDPレベル低下に関与すると示された全身性基礎疾患

	DPレベル低下に関与する要因	対象数			オッズ比（括弧内は95%信頼区間）
		f2周波数	DPレベル低下あり	DPレベル低下なし	
男性	虚血性心疾患	2002 Hz	122	515	2.25（1.04－4.89）
	虚血性心疾患	2185 Hz	130	507	2.61（1.21－5.65）
	高脂血症	4358 Hz	204	433	1.89（1.04－3.43）
女性	肝疾患	3662 Hz	129	499	2.55（1.08－6.02）

ロジスティック回帰分析：目的変数を各f2周波数測定ポイントにおけるDPレベル低下の有無とし、説明変数として年齢および、高血圧、高脂血症、糖尿病、虚血性心疾患、腎疾患、肝疾患それぞれの既往の有無の7変数をとり、調整変数として5周波数平均純音聴力レベル、中耳共振周波数、耳疾患の有無、喫煙習慣の有無、騒音職場での就労の有無の5変数を用いた。

の変化が、純粋に年齢による効果なのか、純音聴力閾値の変化を反映しているに過ぎないのかという点である。DPOAEレベルとして表れる反応のうち、純音聴力検査で捉えられる変化の影響を分離するため、各研究デザインではそれぞれ独自の方法論を用いている。Strouseら[7]は対象に厳しい純音聴力閾値条件を設定し、Dornら[8]、Cilentoら[9]は重回帰分析を、Stoverら[5]は共分散分析を用いている。今回我々がロジスティック回帰分析を用いたのは、聴覚障害には多種の要因が関連する可能性が示唆されており、それら多くの要因を、互いに交絡する独立変数として採用できること、また耳疾患の既往や騒音職場での就労歴など、聴覚障害への影響が大きいと思われる要因も除外せず調整因子として用いて包括的に解析できることによる。性については、年代別純音聴力閾値[2]や、基礎疾患の有病率、騒音職場の就労歴に男女差が見られるため別々に取り扱った。調整因子として用いた変数の影響に関する詳細は、紙面の関係上別稿に譲るが、各々複数のポイントで、有効な調整因子と考えられる関与を示していた。中耳の状態を反映する指標としては、過去の報告で有用とされている中耳共振周波数を用いた[3]。

表5に見られるように、過去の報告で、純音聴力検査結果を、DPOAEの交絡因子に用いて調整した後に、年齢とDPOAEの有意な関連が示された研究は少ない。その意味において、本研究では、男性で5000-6000Hz周辺の比較的高音域で、女性では1000Hz-6000Hzにわたり、交絡の可能性のある他の因子の影響とは独立して、年齢がDPレベル低下に対し有意な影響を及ぼす可能性が示されたことは非常に意義深い。耳音響放射は、外有毛細胞の伸縮によって能動的に増幅された基底板振動が逆向きに放射され検出される反応で、この増幅機能には相当な予備能力の存在が推定されており、純音聴力検査閾値に影響が出ない程度の微少な蝸牛増幅機能の低下が、耳音響放射で捉え得るのではないかと考えられている[8),9)]。

全身性基礎疾患と耳音響放射は、一部の疾患を除いて、その関連について未だ十分な検討がなされていない。本研究では、感音難聴の危険因子として、過去の報告より

表5　DPOAEと成人の加齢に関する報告例と本研究

報告者	報告年	対象数	対象の年齢	対象の純音聴力閾値条件	DPOAEレベルと年齢	純音聴力検査結果を、交絡する連続変数に用いて調整したか
Lonsbury-Martinら[4]（米）	1991	30名	31－60歳	20dBHL≧	高齢になるほどレベルが有意に低下	調整なし
Stover and Norton[5]（米）	1993	42名	20－80歳	25dBHL＞	有意な関連なし	調整あり
Castorら[6]（仏）	1994	75名	20－88歳	ISOの年齢基準聴力	高齢群で有意にレベル低下、ただし高齢群と同等の聴力障害がある若年群とは有意差なし	調整なし
Strouseら[7]（米）	1996	20名	20－79歳	15dBHL≧	年齢群間に有意差を認めず	調整なし
Dornら[8]（米）	1998	800名以上（単一周波数で聴力条件を満たす耳も採用）	5－79歳	20dBHL≧	複数の周波数で聴力条件を満たす群では、年齢の効果見られず	調整あり
Cilentoら[9]（米）	2003	486名	31－82歳	4－6kHzにnotchのある例は除外	女性でのみ加齢に伴うレベル低下が有意に見られた	調整あり
本研究（日本）	2003	1265名	41－82歳	なし	男女とも年齢が10歳上がる毎に、レベル低下の危険性が高まる	調整あり

可能性のある疾患を取り上げて検討した。高脂血症に関しては、難聴との因果関係が1960年代より[10]疫学的または実験的に研究されてきた。病因としては、高脂血症に続発する血液の粘稠性増加及び動脈硬化によってもたらされる蝸牛血流減少が論じられてきた。別の病理メカニズムとしてNguyenら[11]が、コレステロールを取り込んだ外有毛細胞が硬化することをin vitroで示し、それを受けてPreyerら[12]が、DPOAEの入出力特性が病的な群では正常群に比べて有意に血清コレステロール値やLDLコレステロール値が高かったと報告して、高コレステロール血症患者では蝸牛の非線形性が障害されている可能性を示唆した。またErdemら[13]は、純音聴力正常の高トリグリセリド血症群と糖尿病群両群において、4 kHzでのDPOAEレベルが有意に減少していたことを報告した。その考察の中で、過粘稠度は2－4 kHzの比較的高周波数領域の感音難聴をもたらす可能性があるとしたGatehouseらの報告と[14]、高コレステロール食が騒音性難聴の易障害性を高めることを示したSikoraら[15]の実験結果を紹介して、根拠としている。今回我々の研究においても、男性ｆ2周波数4.4kHz周辺において、高脂血症がDPレベル低下に有意に関連していたことは、これらの研究結果と一致する。

心循環器系疾患と難聴は、血管条をはじめとする蝸牛血流への影響が論じられるが[16]、今回、男性のｆ2周波数2000Hz周辺において、虚血性心疾患の既往をもつ群では、DPレベル低下を有意にきたしやすいと示されたことより、血流を介した間接的な関与であるにせよ、外有毛細胞機能障害に影響を及ぼしている可能性が示唆された。

肝疾患と難聴の関連については、渉猟しえた範囲において、詳細な議論がなされていないが、耳毒性薬剤の血中濃度を高める可能性があることや、肝炎治療のために用いられるインターフェロンによって、可逆性の感音難聴をきたすという報告が見られた[17]。肝疾患が内耳に及ぼす病理学的影響については、今後の研究が待たれる。

今回の結果では、DPレベル低下に影響を示した全身性基礎疾患は、ｆ2周波数によっても、男女間でも違っていたが、有病率が高くない疾患については今後さらに対象数を増やして検討することが課題である。

ま と め

1．41歳から82歳の男女1265名について、年齢及び全身性基礎疾患と歪成分耳音響放射との関連を、純音聴力検査による聴力レベルをはじめとした交絡因子を考慮に入れて検討した。

2．男女ともに複数の周波数領域において、有意に、年齢が上昇するほどDPレベル低下をきたしやすいことが示された。全身性基礎疾患の既往のうち、有意にDPレベル低下を起こしやすいことが示されたのは、男性で虚血性心疾患、高脂血症、女性で肝疾患であった。加齢、虚血性心疾患、高脂血症、肝疾患は、純音聴力検査で捉えられる機能に依存しない影響を、聴覚に及ぼす可能性が示唆された。

本論文の要旨は、第13回日本耳科学会学術講演会（千葉）において口演した。

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論文受付　15年12月25日
論文受理　16年4月7日

別刷請求先：〒474-8511　大府市森岡町源吾36－3
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Psychology and Aging
2004, Vol. 19, No. 2, 346–351

0882-7974/04/$12.00 DOI: 10.1037/0882-7974.19.2.346

Age Differences in the Effect of Physical Activity on Depressive Symptoms

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This study examined associations between physical activity and depressive symptoms in 1,151 community-dwelling adults in Japan. Physical activity was measured using a pedometer, whereas depressive symptoms were assessed with the Center for Epidemiological Studies—Depression Scale. A structural equation modeling with a cross-lagged panel design revealed that for the older adults (65–79 years of age), daily walking at baseline predicted fewer depressive symptoms at the 2-year follow-up, even after adjusting for confounders. In contrast, the association was not confirmed for the middle-aged adults (40–64 years of age). Findings suggest that age should be considered when estimating the effect of physical activity on psychological well-being.

People aged 65 years or older constitute the fastest growing segment of many populations, especially in industrialized countries, and a significant percentage of the older population experiences psychological distress such as depression. In fact, with major depression affecting approximately 1% of older adults within a community, and another 8%–15% showing depressive symptoms (Blazer, 1994), promoting mental health is a top priority among professionals working with the aged.

The antidepressant effect of physical activity has been examined in recent years. Evidence indicates that the benefits of exercise are not restricted to experimental studies for moderately or clinically depressed persons (McNeil, LeBlanc, & Joyner, 1991; Singh, Clements, & Fiatarone, 1997) but extend to epidemiological studies of nonclinical community populations as well. Indeed, although cross-sectional analyses have consistently shown that active individuals report fewer depressive symptoms than those who are less active (Hassmen, Koivula, & Uutela, 2000; Herzog, Franks, Markus, & Holmberg, 1998; Ross & Hayes, 1988), longitudinal studies have also demonstrated that physical activity reduces subsequent depressive symptoms (Camacho, Roberts, Lazarus, Kaplan, & Cohen, 1991; Lampinen, Heikkinen, & Ruoppila, 2000). Camacho et al. (1991) found that regular physical exercise by individuals at baseline reduced their risk for depression at the 9-year follow-up, even after adjusting for confounding variables. Lampinen et al. (2000) reported that those who had reduced their intensity of physical exercise during the intervening 8 years were more depressed at the follow-up than those who had remained active or who had increased their physical activity.

Although previous findings are valuable, most of these studies have focused on younger or middle-aged Caucasian adults (Brosse, Sheets, Lett, & Blumenthal, 2002; Brown, 1992). Because it has been established that body size and composition differ by age and ethnicity and that the differences affect physical performance (Shephard, 2002), age should be considered when estimating the effects of physical activity on psychological well-being.

To our knowledge, two empirical studies directly addressed the question of whether the relationship between exercise and psychological well-being varies across age groups. Stephens (1988) conducted a secondary analysis of four surveys among household populations of the United States and Canada and found that the relationship between physical activity and mental health was stronger for persons 40 years and older than for those ranging in age from 20 to 39 years. Ruuskanen and Ruoppila (1995) found that intensive and regular physical exercise was significantly associated with a lower prevalence of depressive symptoms in two of the study's older age groups (65–69 and 70–75 years) but not in the oldest age groups (75–79 and 80–84 years). Although these findings suggest that there is an age difference in the effect that physical activity has on depressive symptoms, both studies have some methodological concerns: The analyses were cross-sectional, and physical activity was assessed by self-report measures.

Self-reporting is the most feasible approach to large population surveys for assessing physical activity, primarily because of its low cost, ease of administration, and potential for nonreactivity (Tudor-Locke, Williams, Reis, & Pluto, 2002). However, when using self-report measures, respondents and investigators must have a shared understanding of ambiguous terms such as *leisure, physical activity, moderate,* and *vigorous* (Sallis & Saelens, 2000). Furthermore, self-report measures can lead to information bias due to inaccurate recall or intentional misreporting, especially for older adults (Stone, 1995). In the present study, to avoid the issues for self-report measures, we used pedometers for a more objective

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An earlier version of this article was presented at the annual meeting of the Japanese Epidemiological Association, Fukuoka, Japan, January 2003. This study was supported by a Grant-in-Aid (H14-choju-004) for Comprehensive Research on Aging and Health from the Ministry of Health, Labor, and Welfare of Japan.

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monitoring of daily physical activity. Objective quantification of ambulatory activity via simple and inexpensive pedometers allows researchers and practitioners to easily assess activity levels along a continuum (Tudor-Locke, Bell, et al., 2002). In addition, because this portable monitoring device directly counts the number of steps walked, the data obtained are independent of a person's recall or misreporting and are substantially free of errors (Tsubono et al., 2002).

In the present study, we used longitudinal data from community-dwelling adults in Japan to expand on previous research regarding the relationship between physical activity and psychological well-being. More specifically, we addressed the question of whether physical activity affects depressive symptoms differently among middle-aged and older adults by incorporating the widely used Center for Epidemiological Studies—Depression Scale (CES–D; Radloff, 1977) and measuring individuals' regular walking activities.

Method

Participants

The data for the present study were collected as part of the National Institute for Longevity Sciences–Longitudinal Study of Aging (NILS-LSA). The population of the NILS-LSA was a sex- and age-stratified random sample of Japanese community-dwelling adults, who were between 40 and 79 years of age at baseline. We recruited the participants from the neighborhood of the Institute (Obu City and Higashiura Town), in cooperation with the local governments. Informed consent was obtained from each participant at the study entry. Details of the NILS-LSA have been described elsewhere (Shimokata, Ando, & Niino, 2000).

Because physical disability can preclude walking activity, 4 persons with any of six functional disabilities (bathing, dressing, toileting, transfer, continence, or feeding) were excluded from the analyses. The baseline assessment of Katz's Index of Activities of Daily Living (Katz, Ford, Moskowitz, Jackson, & Jaffe, 1963) was used for the exclusion procedure. The study sample was then 1,151 men and women who had completed both the baseline (Wave 1: from 1997 to 2000) and the 2-year follow-up (Wave 2: from 2000 to 2002) surveys, with no missing data in the study variables. The average age for the entire sample was 57.4 years (SD = 10.2 years). For the analyses presented in this article, the sample was divided into two groups according to their age upon entering the study (middle-aged adults: 40–64 years old, n = 837; and older adults: 65–79 years old, n = 314).

Measures

Physical activity. Daily walking steps were counted by an electronic digital pedometer (Select II, Suzuken Co., Nagoya, Japan) at both Wave 1 and Wave 2. The reproducibility and validity of the tool were fully evaluated (Niimi, 1999). We supplied a pedometer to each participant on the examination day (the NILS-LSA requires participants to visit the Institute and spend 1 day for extensive examinations regarding medical, psychological, and other health-related domains). Participants were given instructions for wearing the device firmly at the belt line over 7 consecutive days, from waking up to falling asleep. After completing the assessment, participants returned the device by mail. To estimate the participants' usual walking activity, we discarded the maximum and minimum daily records from the entire data. The data for the remaining 5 days were summed up and divided by 5, generating average daily walking steps for use in the analyses.

Depressive symptoms. We measured depressive symptoms at both Wave 1 and Wave 2 by means of a Japanese version of the CES-D Scale (Shima, Shikano, Kitamura, & Asai, 1985). The scale was mailed to participants to complete and bring to the Institute on the examination day. Participants indicated how often during the previous week they had experienced any of the 20 symptoms included in the scale. Each item was rated on a 4-point scale ranging from 0 (*rarely or none of the time*) to 3 (*most or all the time*). Four positively worded items were reverse scored. The points were added together so that a higher score represented a higher level of depressive symptoms. Cronbach's alphas were .86 and .86 for the middle-aged group and .89 and .85 for the older group at Waves 1 and 2, respectively.

Control variables. We controlled for the following characteristics in the statistical analyses. Gender was coded as a contrast effect (men were assigned a score of 0 and women a score of 1). Annual family incomes at Wave 1 were rated on an 11-point scale (1 = *income less than ¥1,500,000,* 11 = *income greater than ¥20,000,000*). The presence and history of seven diseases (stroke, hypertension, cardiovascular disease, diabetes, bronchitis, arthritis, and cancer) at Wave 1 were also totaled and were used as an index of participants' chronic conditions.

Results

Sample Characteristics

Table 1 presents the sample characteristics by age group. Compared with the middle-aged group, the older group consisted of more men, $\chi^2(1, N = 1{,}151) = 4.38, p < .05$, and had participants with lower incomes, $t(492) = 15.39, p < .01$. The older group also reported more chronic conditions, $t(426) = -9.70, p < .01$, than the middle-aged group. Daily walking steps in the middle-aged group were significantly greater than in the older group at both Waves 1 and 2, $ts(1149) = 7.08$ and 7.98, respectively, $ps < .01$. In contrast, the CES–D scores did not differ between the age groups at either baseline or follow-up.

Longitudinal Analyses

We used a structural equation modeling (SEM) procedure with a cross-lagged panel design to test the relationships between walking activity and depressive symptoms. All analyses were conducted using the AMOS 4.0 computer program (Arbuckle & Wothke, 1999). Figure 1 illustrates the possible relationships between four study variables: walking steps at Waves 1 and 2 and depressive symptoms at Waves 1 and 2. Walking steps at Waves 1 and 2 are enclosed in boxes as observed variables. Depressive

Table 1
Descriptive Information for Study Variables by Age Group

	Middle-aged (n = 837)		Older (n = 314)	
Variable	*M*	*SD*	*M*	*SD*
Gender (% male)	51.4		58.3	
Income	7.1	2.1	4.7	2.4
Chronic conditions	0.3	0.6	0.8	0.8
Steps per day (Wave 1)	6,395	2,438	5,281	2,214
Steps per day (Wave 2)	8,436	3,087	6,764	3,375
CES-D (Wave 1)	6.5	6.0	6.7	6.9
CES-D (Wave 2)	7.1	6.3	7.2	6.3

Note. Score ranges of income, chronic conditions, and the CES–D scores are 1–11, 0–7, and 0–60, respectively. CES-D = Center for Epidemiological Studies—Depression Scale.

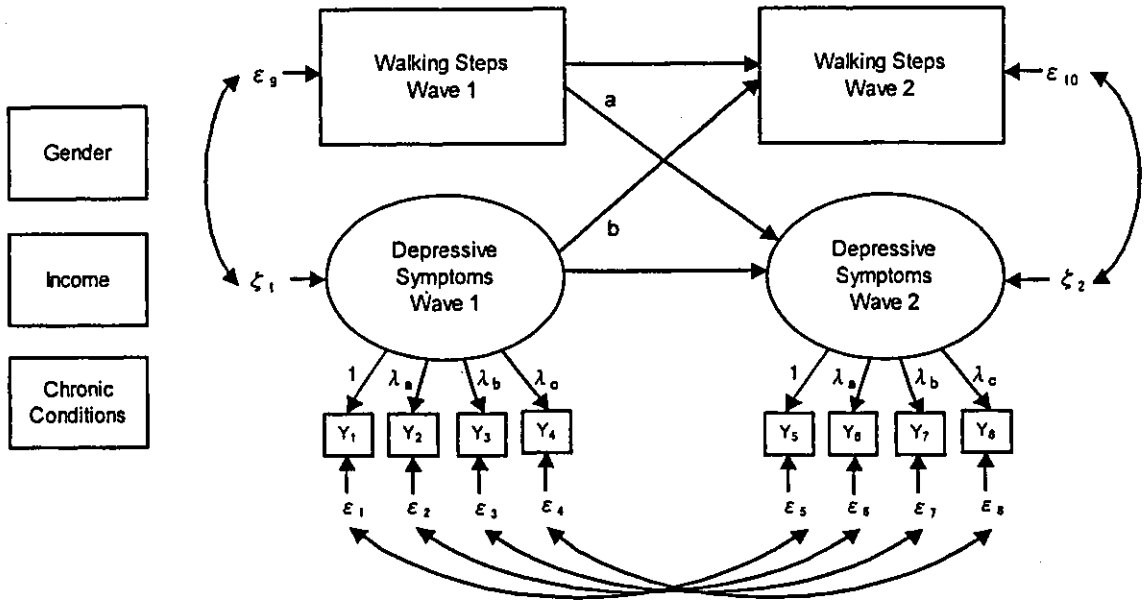

Figure 1. Cross-lagged regression model (saturated model) for testing longitudinal relations between walking steps and depressive symptoms. Y is the mean of each CES–D subscale (Y_1 and Y_5: Depressed Affect; Y_2 and Y_6: Positive Affect; Y_3 and Y_7: Somatic and Retarded Activity; and Y_4 and Y_8: Interpersonal). The epsilons and zetas signify error variables. The 12 direct effects of gender, income, and chronic conditions on walking steps and depressive symptoms at Waves 1 and 2 are not shown in the figure. CES–D = Center for Epidemiological Studies—Depression Scale. The letters a and b denote cross-lagged parameters.

symptoms at Waves 1 and 2 are enclosed in ellipses as latent variables on which the means of each CES–D subscale (Depressed Affect, Positive Affect, Somatic and Retarded Activity, and Interpersonal) loaded as indicators (see Radloff, 1977, for a fuller description of the factor structure of the scale). We constrained the indicators to load on the depression construct equally between the surveys and allowed their error terms to correlate across time. Other parameters were freely estimated, except two cross-lagged parameters (Parameters a and b in Figure 1), which were constrained in some way for model comparisons, as described below. The direct effects from exogenous variables (gender, income, and chronic conditions) on the walking steps and depressive symptoms at Waves 1 and 2 were also freely estimated in all analyses (arrows not shown in the figure).

To determine the most likely direction and time frame of the relationship between walking steps and depressive symptoms, we first developed the non-age-specific model for an overall sample. That is, using the data from all participants, we started the SEM procedure with a saturated model in which the two cross-lagged effects of walking steps on depressive symptoms and depressive symptoms on walking steps were both released (i.e., freely estimated). In the next step the following three models were statistically compared with the saturated model.

1. The stability model specified that both cross-lagged effects (Parameters a and b in Figure 1) were constrained to be zero.

2. The depression-to-step model specified that the effect of depressive symptoms on steps (Parameter b) was released, and the effect of steps on depressive symptoms (Parameter a) was constrained to be zero.

3. The step-to-depression model specified that the effect of steps on depressive symptoms was released, and the effect of depressive symptoms on steps was constrained to be zero.

Table 2 presents the summary statistics. The chi-square goodness-of-fit tests suggested that all models provided good fits with the observed data; however, the fitness indices of the step-to-depression model (goodness-of-fit index [GFI] = .975, adjusted GFI = .954, comparative fit index = .997, Akaike information criterion = 136.833) suggested that this model was the most likely to be equivalent with the saturated model.

Differences in fit between the models were also examined to determine which model provided the best representation of the data. The results indicated that the stability model and the depression-to-step model provided significantly worse fits than the saturated model. The step-to-depression model, however, was not significantly different from the saturated model, and it provided a significantly better fit than the stability model, $\chi^2(1, N = 1{,}151) = 5.87$, $p < .05$. Although the step-to-depression model and the depression-to-step model are not nested and cannot be directly compared, the pattern of findings favored the step-to-depression model.

On the basis of the aforementioned model-testing procedure, we applied a multigroup analysis to the step-to-depression model to test whether the effect of walking steps on depressive symptoms is consistent between the middle-aged and the older groups. This procedure is similar to the process used to evaluate the overall predictive model. That is, the saturated model (having no constraints of the cross-lagged effect of walking steps at Wave 1 on

Table 2
Chi-Squares and Fit Indices of All Models, Controlling for Gender, Income, and Chronic Conditions

Model	Parameter constraints[a]	χ^2	*df*	χ^2_{diff}[b]	GFI	AGFI	CFI	AIC
Saturated		50.96	48		.976	.954	.997	136.963
Stability	a = b = 0	58.70	50	7.74*	.973	.950	.992	140.702
Depression-to-step	a = 0	.56.83	49	5.86*	.973	.951	.993	140.827
Step-to-depression	b = 0	52.83	49	1.87	.975	.954	.997	136.833

Note. GFI = goodness-of-fit index: AGFI = adjusted goodness-of-fit index; CFI = comparative fit index; AIC = Akaike information criterion.
[a] The letters a and b denote cross-lagged parameters. [b] Comparison with the saturated model.
* $p < .05$.

depressive symptoms at Wave 2 in both age groups) was compared with the following three possible models:

1. The non-age-specific model stipulated that the cross-lagged effect of steps on depressive symptoms was identical across age groups (Parameter a in Figure 1 was constrained to be equal between the middle-aged and the older groups).

2. The middle-aged-specific model stipulated that the steps were effective only on the middle-aged group (Parameter a in the older group was constrained to be zero, whereas Parameter a in the middle-aged group was freely estimated).

3. The older-specific model stipulated that the steps were effective only on the older group (Parameter a in the middle-aged group was constrained to be zero, whereas Parameter a in the older group was freely estimated).

Table 3 indicates the results of testing. Although all models, including the saturated model, could be rejected as statistically different from the observed data, the fit indices indicated that each model provided a good fit. Differences in fit among the models revealed that the non-age-specific model, $\chi^2(1, N = 1{,}151) = 5.70$, $p < .05$, and the middle-aged-specific model, $\chi^2(1, N = 1{,}151) = 5.73$, $p < .05$, provided significantly worse fits than the saturated model. The older-specific model, however, was statistically comparable to the saturated model, $\chi^2(1, N = 1{,}151) = 0.53$ (*ns*). The inference from these results is that the older-specific model fits the data best.

Parameter estimates of the older-specific model indicated that the two lagged effects (autoregressive paths from walking steps at Wave 1 to walking steps at Wave 2, and depressive symptoms at Wave 1 to depressive symptoms at Wave 2) were strong in both age groups, indicating that walking steps and depressive symptoms remained fairly stable over the 2 years (βs = .64 and .71 for walking steps and .62 and .74 for depressive symptoms in middle-aged and older groups, respectively, $p < .01$ in each case). Additionally, the older group showed a significant cross-lagged effect ($\beta = -.11$, $p < .05$) of walking steps at Wave 1 on depressive symptoms at Wave 2, suggesting that older participants who walked more at baseline reported fewer depressive symptoms at follow-up.

Discussion

In this study we used longitudinal data from a large community sample in Japan to examine the relationships between physical activity and psychological well-being. Cross-lagged panel analyses demonstrated that the baseline daily walking activity estimated by a pedometer was associated with depressive symptoms at the 2-year follow-up in older adults, even after adjusting for potential confounding variables. This is in line with the results of other studies conducted in Western countries (e.g., Camacho et al., 1991; Hassmen et al., 2000; Herzog et al., 1998, Lampinen et al., 2000; Ross & Hayes, 1988). In contrast, the analyses did not support the antidepressant effect of physical activity in middle-aged adults. Thus, as we predicted, the findings provide evidence for age

Table 3
Multigroup Testing of the Step-to-Depression Model, Controlling for Gender, Income, and Chronic Conditions

Model	Parameter constraints[a]	χ^2	*df*	χ^2_{diff}[b]	GFI	AGFI	CFI	AIC
Saturated		145.43	95		.981	.964	.987	319.43
Non-age-specific	m = o	151.13	96	5.70*	.981	.963	.986	323.13
Middle-aged-specific	o = 0	151.16	96	5.73*	.981	.963	.986	323.16
Older-specific	m = 0	145.96	96	0.53	.981	.964	.988	317.96

Note. GFI = goodness-of-fit index; AGFI = adjusted goodness-of-fit index; CFI = comparative fit index; AIC = Akaike information criterion.
[a] Alphabetic notations indicate age groups: m = middle-aged, o = older. [b] Comparison with the saturated model.
* $p < .05$.

differences in the beneficial effect of physical activity on psychological well-being.

In general, people become less active as they age (Shephard, 1997), as demonstrated in our study: Participants from the older group accumulated fewer walking steps than participants from the middle-aged group. However, it is unclear whether a decrease in habitual activity is a normal part of aging (Shephard, 1997), because studies have also reported that older adults can achieve excellent exercise adherence and maintenance (Emery, Hauck, & Blumenthal, 1992; McAuley, Jerome, Elavsky, Marquez, & Ramsey, 2003). In other words, it is likely that older adults' physical capacity may be underestimated because they are ostensibly inactive in their daily lives. Consequently, one possible explanation for the age difference we saw in this study is that individuals who are far below physical capacity in fitness (i.e., older adults) gain greater benefit from exercise. Our results may also suggest that the daily walking activity was too mild to affect the depressive symptoms of midlife adults. In fact, some studies (McGowan, Pierce, & Jordan, 1991; Rehor, Dunnagan, Stewart, & Cooley, 2001) have reported that vigorous aerobic exercise (e.g., running) or resistance training (e.g., weight lifting) improves psychological well-being in younger persons. Thus, the results in the present study suggest that a reasonable match between age and the type of exercise may be necessary for there to be an antidepressant effect.

Although some studies have suggested that older people can improve their psychological well-being by vigorous aerobic and resistance training (Blumenthal et al., 1991, 1999; Singh et al., 1997), too much physical activity can provoke cardiac risks or cause musculoskeletal injury, especially for an older person (Shephard, 1997). In addition, Penninx et al. (2002) found that a walking exercise intervention was more effective than a resistance exercise intervention for reducing depressive symptoms in older adults. In this regard, modest activity such as walking would be a secure and effective exercise for preventing depressive symptoms in older persons.

One of the greatest limitations in the present study is that the antidepressant effect of walking was not extremely strong ($\beta = -.11$). It should also be noted that the model comparisons using SEM revealed that the step-to-depression model was better, but the stability and depression-to-step models also fit the observed data well. Future analyses should attempt to refine our findings. For example, according to Williamson and Schulz (1992, 1995), illness and its resultant pain are related to depression because they restrict patients' routine activities. Although we excluded possibly disabled individuals from the data and adjusted for the effect of chronic health conditions in the analyses, prior health factors may still play an important role in the relationship between walking and depressive symptoms. It should also be noted that although the final model, predicting depression from baseline walking scores, fit the data the best, all of the models provided an adequate fit to the data on the basis of the goodness-of-fit indices. As such, the final model was based on the best-fitting model, within the context of a number of good-fitting statistical models.

It would also be valuable to consider the factors that mediate the relationship between walking and depressive symptoms. Both physiological and psychosocial pathways have been hypothesized as mediating the antidepressant effect of physical activity (Brown, 1992; Paluska & Schwenk, 2000). For example, physical activity is believed to have an effect by enhancing brain aminergic synaptic transmission (Ransford, 1982) or by reducing activity of the hypothalamic–pituitary–adrenal axis (van der Pompe, Bernards, Meijman, & Heijnen, 1999). The psychosocial explanation posits that individuals who exercise improve their psychological well-being by increasing self-efficacy (McAuley, Blissmer, Katula, Duncan, & Mihalko, 2000) or because the activity acts as a diversion from unpleasant stimuli (Hill, 1987). Modeling complex multistep pathways that consider these and other factors (e.g., gender) would be a fruitful approach for improving the predictive effect of physical activity on psychological well-being.

Nevertheless, an advantage of the present study is that it showed the availability of a simple measure of walking steps for predicting depressive symptoms in community-dwelling adults. Although there is increasing evidence regarding the importance of physical activity in maintaining mental health in older people, past findings were generally based on subjective, self-report measures of physical activity (e.g., Herzog et al., 1998; Lampinen et al., 2000) or on experimental works using small samples (e.g., McNeil et al., 1991; Singh et al., 1997). The use of an objective, low-cost, and user-friendly measure such as a pedometer makes assessing physical activity easier and thus more feasible to consider as a mental health factor in large community surveys.

Furthermore, a recent study (Talbot, Gaines, Huynh, & Metter, 2003) reported that a pedometer-driven walking program with a self-management educational program increased physical activity, muscle strength, and functional performance in older adults with osteoarthritis, as opposed to the educational program alone. This implies that a pedometer can also be used as a motivator for exercise adherence. From this viewpoint the results in the present study are small but important steps that warrant further research to clarify the availability of this tool to promote psychological well-being in older adults.

In conclusion, the present study partially confirmed the protective effect of physical activity on depressive symptoms in community-dwelling adults. The findings suggest that age should be taken into account when incorporating a walking exercise in research and daily practice for mental health.

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Received March 11, 2003
Revision received January 29, 2004
Accepted January 30, 2004 ■

American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 127B:78–80 (2004)

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

Grant sponsor: Grant-in-Aid for Scientific Research (to K.M.); Grant number: B-12470131; Grant sponsor: Research Grants for Comprehensive Research on Aging and Health (to K.M.); Grant number: 10C-4; Grant sponsor: The Ministry of Health and Welfare (Research Grants for Longevity Sciences to A.F.); Grant number: 12-01.

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Received 25 April 2003; Accepted 16 September 2003

DOI 10.1002/ajmg.b.20160

Published online 13 January 2004 in Wiley InterScience (www.interscience.wiley.com)

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.

ACKNOWLEDGMENTS

We thank Dr. H. Amono for his help on statistical analysis.

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