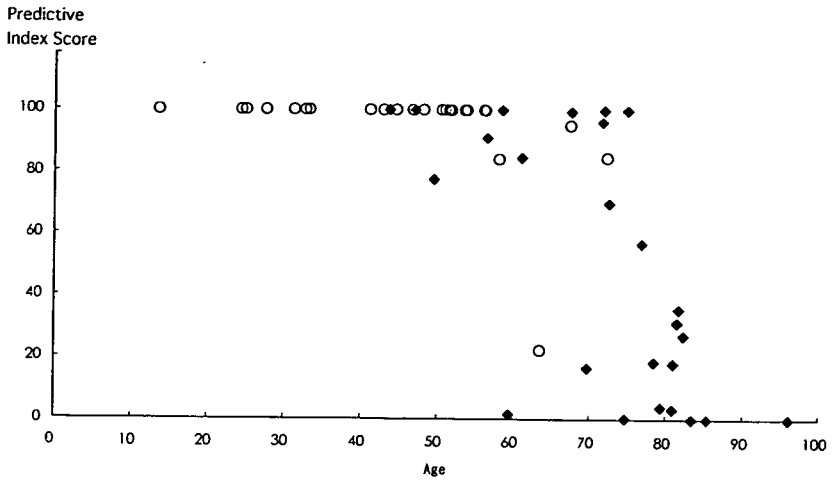

Fig. 6. Relationship between age and the predictive index score of Minamata disease (the 1st Kumamoto method).
○, Timing of Certification; ◆, Follow up (2003).

method, but dispersion by these methods was more marked than that by the first methods.

In Patient B, diagnosed as having typical MD (aged 47 years at the time of certification in Kumamoto Prefecture and 72 years at the survey in 2003), the discriminant score increased by the second Kumamoto method but was unchanged or decreased by the other methods. In a patient with fetal MD (aged 25 years at certification in Kagoshima Prefecture and 44 years at the survey in 2003), the discriminant scores obtained by the first and second methods were near 100 at the time of both certification and survey in 2003.

Discussion

In this study, we evaluated the present neurological findings and the temporal profile of the severity of MD in certified patients with MD using discriminants by multiple logistic analysis. Similar discriminant was produced by Igata in the certified patients of Kagoshima (Igata 1986), but non-certified ones in the same polluted area were used as controls. Our discriminants were obtained in certified patients in Kumamoto, Kagoshima, and Niigata using inhabitants in seaside area similar to Minamata district but not polluted by methylmercury as controls. The temporal profile in the severity of MD from the time of certification to the survey in 2003 and the influences of complications were evaluated. In addition, the status of complications was investigated, and the present status of patients with chronic MD 50 years after discovery was clarified. The mean age of the certified patients with MD in Minamata and Izumi district as the subjects was 70 years, showing the aging of patients with MD. Frequent disorders among the aged such as cerebral infarction, cervical spondylosis, cervico-omo-brachial syndrome, and spondylosis deformans were often observed, and the pathological findings of MD had been complicatedly modified by complications.

The probability of MD was evaluated using the discriminants established in this study. Because many patients with the most severe symptoms and signs with acute methylmercury intoxication died before our survey, only patients of lower severity might be included in this survey. In spite of such inevitable selection bias in our study design, the discriminant score was generally high by each method at the time of certification but had decreased in most patients at the time of the survey in 2003. This suggested alleviation of the symptoms and signs of MD after a course of about 25 years. The discriminant score and its temporal changes markedly differed among the discriminants used and were associated with weighted symptoms and signs. In other words, some symptoms and signs of MD such as sensory

disturbances were alleviated while others such as concentric contraction of visual fields and hearing impairment were not changed during the 25-year course.

Of the certified patients, only 1 patient showed definite aggravation of the discriminant score. This patient had developed LCCA in the previous year of certification, and the increase in the discrimination value may have been due to the progression of cerebellar ataxia associated with LCCA. It should be discussed whether this patient had MD complicated by LCCA or fulfilled the certification criteria (Director General of Environmental Health Department 1986) because sensory disturbances due to spondylosis deformans added to the cerebellar symptoms of LCCA. At least, the probability of MD is poor based only on the discriminant score at the time of certification.

These results suggest that MD is severest during the methylmercury exposure period and is alleviated with time after discontinuation of the exposure. When aggravation of neurological symptoms is observed, the influences of complications may be marked.

Evaluation of the age at the time of examination and the distribution of discriminant scores suggested changes in the pathological condition of MD at the age of 45 years. At the age of $\leqq 45$ years, the discriminant score was close to 100. At the age of > 45 years, the discriminant score indicates that the probability of MD is poor. One factor may be the modification of the pathological condition due to an age-associated increase in the incidence of complications.

Among the subjects in this study, a patient with classic MD was compared with a patient with fetal MD. The discriminant score in the patient with fetal MD did not change from the time of certification to the survey in 2003. In contrast, the patient with classic MD (certified at the age of 47 years) showed alleviation of symptoms and signs. It is unclear whether these findings reflect the development of irreversible neuronal damage induced by methylmercury exposure during the fetal period, or whether symptoms decrease after the age of 45 years even in the presence of fetal MD. Consideration should also be given to the time of methylmercury exposure (fetal period or after birth, childhood or adulthood), and the duration and amount of exposure. In patients with fetal MD, further continuous evaluation of neurological findings is necessary.

When the third Kumamoto method based only on 'glove-and-stocking type' sensory disturbances was used to evaluate the probability and severity of MD, all patients showed a very low discriminant score (< 20) at the time of survey in 2003. This suggests that the differentiation of chronic Minamata disease due to methylmercury based only on sensory disturbances is difficult. We believe that the precise diagnosis of MD based on the distribution of sensory disturbances is essentially unachievable, especially in the aged patients.

In conclusion, the established predictive index score of MD, which indicates the probability and the severity of MD, usually declined in 25 years. It was the age-related concomitant disorders that caused the deterioration of neurological findings in patients aged over 45 year.

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Galantamine

須藤慎治[*1]・中川正法[*2]

abstract

アルツハイマー病の治療薬として海外で使用されているgalantamineはアセチルコリンエステラーゼ阻害作用に加えて，ニコチン性アセチルコリン受容体に対してのアロステリック作用を併せもつユニークな薬剤である．ニコチン受容体の賦活によってモノアミンやグルタミン酸などの神経伝達物質の遊離も制御する効果が認められていることから，アルツハイマー病で低下した種々の神経伝達物質への改善効果が期待できる．さらに，galantamineはアミロイドβ蛋白による細胞死の抑制など神経保護作用も有している．海外で行われたgalantamineのアルツハイマー病患者を対象とした臨床試験の結果から認知機能に対して数カ月から年単位で有効であるとのエビデンスがメタ・アナリシスを含めて報告されている．認知機能改善効果以外に精神症状や日常生活動作の改善効果，介護時間の短縮効果なども報告されている．以上よりgalantamineは新規アルツハイマー病治療薬としての展開が期待される．

I　はじめに

アルツハイマー病（AD）は原因不明の進行性の神経変性疾患で，その原因に対する根本的な治療法の解明が期待されているが，現時点では対症療法が主体である．ADの脳内では種々の神経伝達物質の低下がみられるが，アセチルコリン（acetylcholine：ACh）作動性神経の低下が著しく，中核症状である記憶障害と密接に関係している[1]．現在わが国を含めて世界中で広く用いられているAD治療薬はAChの分解酵素（acetylcholinesterase：AChE）阻害薬によるACh作動性神経を賦活する治療である[2]．わが国で現在承認されているAD治療薬はドネペジル（アリセプト®）のみであるが，現在数種類の薬剤が臨床試験中である．本稿では海外ですでに市販され，わが国では現在臨床試験中のAChE阻害薬のgalantamine（海外商品名Reminyl®，Razadyne®）に関して概説する．

II　作用機序

Galantamineはヒガンバナ科の数種類の植物から抽出された天然成分であり，わが国で初めて単離され[3]オーストリアで最初に合成された．ドネペジルなどのAChE阻害薬と同様にAChの分解抑制作用によってAChレベルを上昇させてムスカリン性およびニコチン性アセチルコリン受容体を賦活する作用も有しているが，galantamineのAChE阻害作用は他の阻害薬と比較して弱く，*in vivo*ではドネペジルの4分の1程度と報告されている[4]．

Galantamineのユニークな点はニコチン性アセチルコリン受容体（nAChR）への刺激作用を併せもつdual actionを有することである（図1）．AD剖検脳

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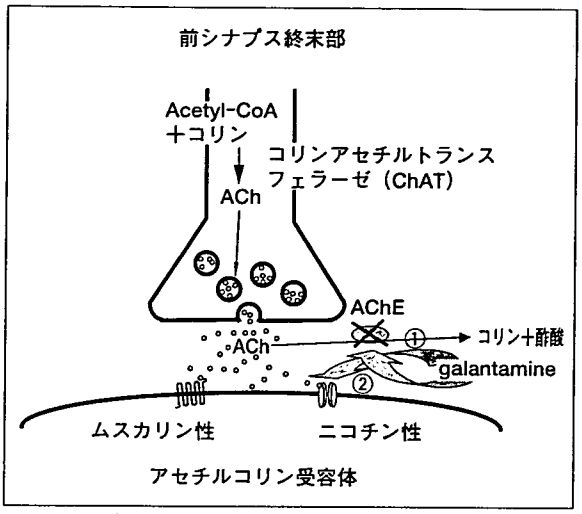

図1　シナプス部でのgalantamineのアセチルコリン作動性神経への作用の模式図

アセチルコリン（ACh）は神経終末からシナプス間隙へ放出された後，アセチルコリンエステラーゼ（AChE）によってコリンと酢酸に分解される．Galantamineは，①AChEの阻害作用によってAChの分解を抑制する一方，②ニコチン性アセチルコリン受容体への直接作用も有している．

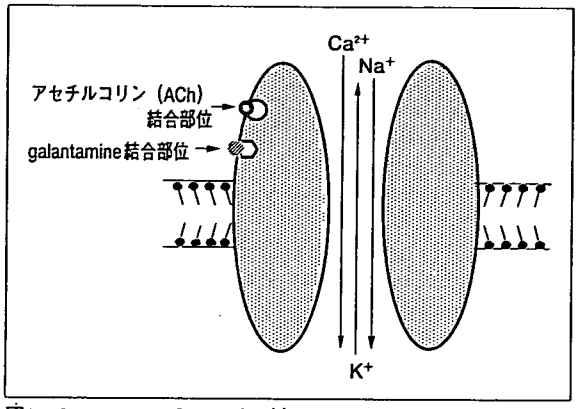

図2　Galantamineのニコチン性アセチルコリン受容体への作用

Galantamineはアセチルコリン（ACh）が結合する部位とは別のニコチン性アセチルコリン受容体の部位に結合してアロステリックリガンドとして作用を示す．

におけるnAChRの著明な減少が認められ[5]，PET（Positron Emission Tomography）を用いた研究ではAD患者でのnAChRの減少と認知機能障害の重症度との関連性も認められていることから[6]，nAChRを賦活することによってAD治療の可能性が期待される．Galantamineは本来AChないしAChのアゴニストやアンタゴニストが結合する部位とは別の部位（allosteric site）に結合し，nAChRの作用をアロステリックに増強するallosteric potentiating ligand（APL）として作用することが明らかにされた（図2）．臨床的濃度（0.1～1 μM）の範囲内ではgalantamineはAChE阻害作用とは別にAPLとしてnAChRを賦活してノルエピネフリン，グルタミン酸，GABAなどの神経伝達物質の遊離を促進する[7]．このAPL作用は他のAChE阻害薬のドネペジルやrivastigmineではみられなかった[7]．ニコチンアゴニストの場合には長期投与によって受容体の感受性が低下して薬剤耐性を生じる問題があるが，galantamineには直接受容体に対してのアゴニスト作用はなくAPLとして作用するため，持続的にnAChRの感受性を促進させる[8]．したがって，galantamineはAD脳で低下したnAChRのレベルないしは感受性を増大させて，認知機能と関連した神経伝達を長期にわたって回復させる可能性が基礎的研究の結果からは期待される．

ADの成因としてアミロイドβ蛋白（Aβ）の毒性が神経細胞障害を起こすアミロイド仮説が提唱されているが，nAChRの賦活はAβによる細胞毒性を防御する作用のあることが明らかにされた[9]．さらに最近，galantamineにはニコチンによるAβの細胞毒性からの保護効果を増強させ[10]，抗アポトーシス蛋白であるBcl-2の発現も増加させる作用のあることが示されている[11]．この保護効果の増強はnAChR拮抗薬のmecamylamineによって抑制されないことから，ACh結合部位への結合とは別にAPLとして作用している可能性がある[11]．このように，galantamineは神経変性に対しての保護薬としての側面も有していることから，病態の進展への予防効果も期待される．

III　ADに対するgalantamineの有効性と安全性

1　認知障害に対しての有効性

Galantamineの有効性が中等度のAD患者に対して多施設二重盲検無作為比較試験で検討されている[12]．636名の軽度から中等度のAD患者にプラセボ群ないし用量の異なる2つのgalantamine群（24mg/日，32mg/日）の3群に振り分けて6カ月間投与し，最終的に438名（69%）がこの試験を完了した．アルツハイマー病評価スケールとして世界中で広く用いられているADAS-cog/11（the 11-item Alzheimer's Disease

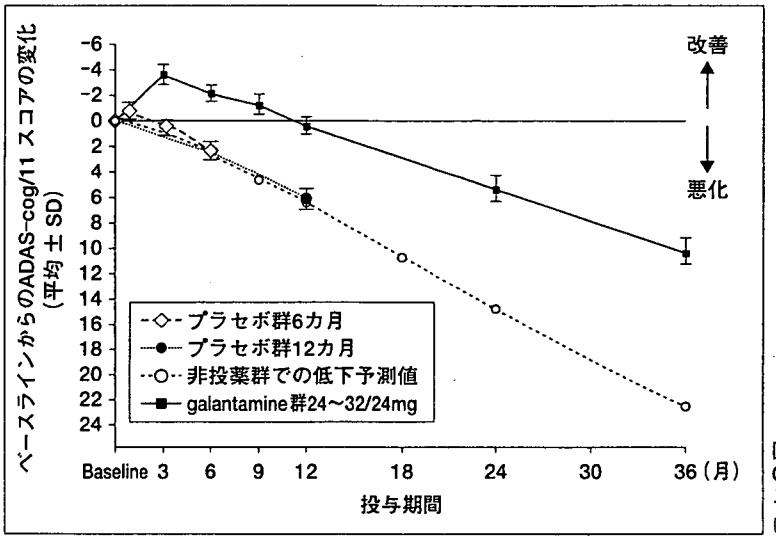

図3
Galantamine長期投与におけるADAS-cog/11スコアの経時変化
〔参考文献13)より引用改変〕

Assessment Scale cognitive subscale) はプラセボ群では6カ月目で平均2.2点悪化したのに対して，galantamine群では24mg/日投与群では平均1.7点，32mg/日投与群では1.6点の改善が認められ，プラセボ群と比較して有意であった．また，galantamineの長期臨床試験（6カ月の多施設二重盲検無作為比較試験とその後の6カ月のオープン試験に引き続いて行われた24カ月の延長投与）によれば[13]，36カ月間の試験を完了した119名のうち17%が依然として投与前のベースラインかそれ以上の認知機能を維持し，半数以上は悪化レベルが10点以下にとどまった．Sternらの計算式[14]から予想される未治療AD患者でのADAS-cog/11の悪化レベル（20.5～22点）より有意に軽度であった（図3）[13]．

ドネペジルとgalantamineの効果を比較した長期臨床試験の結果が報告されている[15]．Mini-Mental State Examination（MMSE）スコアが投与前に12～18点のgalantamine24mg/日投与群81名，ドネペジル10mg/日投与群75名で比較したところ，投与後13週では両群とも投与前のベースラインより改善効果を示したが，ドネペジル群と比較してgalantamine群のほうが有意に改善効果が大きかった．52週間の長期投与後にはドネペジル群では投与前のベースラインより悪化したのに対して，galantamine群では依然ベースライン上であり，galantamine群のほうが有意に悪化を抑制する効果がみられた（図4）．ADAS-cog/11による評価では投与後52週でのスコアは両群ともベースラインよりは悪化していたが，galantamine群のほうが悪化の程度は有意にドネペジル群より軽度だった．最新のCochrane Database of Systemic Reviewによる7編の臨床試験からのメタ・アナリシスの結果によれば，ADAS-cog/11はgalantamine 8～32mg/日のいずれの投与量でも3カ月と6カ月の時点において有意の改善効果を示したと結論づけている[16]．

以上のように，軽度から中等度のAD患者では，galantamineの服用により6～48カ月の長期にわたって認知機能に対して有効性を示すエビデンスが複数の臨床試験の報告から示され[12),17)]，メタ・アナリシスでも有効性が報告されていることから，galantamineはADにおける認知障害改善薬としての使用が推奨される．

2　認知障害以外の症状に対する効果

AD患者では進行とともに精神症状や行動障害がみられるようになるが，3編の臨床試験からの事後解析（post-hoc analysis）では，3ないし6カ月間のgalantamine投与は精神症状の評価尺度であるNPI (Neuropsychiatric Inventory) スコアの合計点でプラセボ群と比較して有意な改善を認めた．項目別では焦燥，不安，脱抑制などでプラセボ群との間で改善効果がみられた[18]．Galantamineによる精神症状などの改善効果にはnAChRへの賦活作用を介したモノアミンなど，種々の神経伝達物質の調節作用も関与している可能性が考えられる．ADが進行する

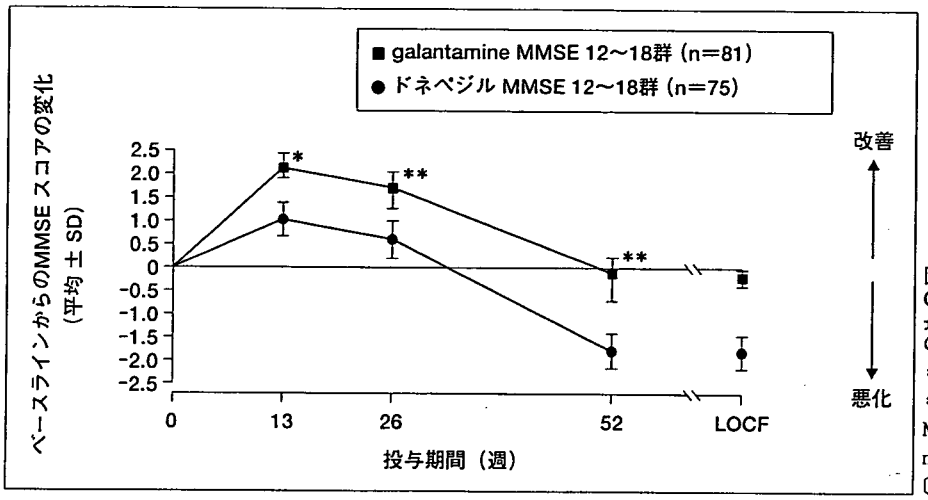

図4
Galantamineとドネペジルの長期投与におけるMMSEスコアの比較
*：p<0.05
**：p<0.005（vsドネペジル）
MMSE：Mini-Mental State Examination
〔参考文献15)より引用改変〕

と認知障害に加えて日常生活動作（Activities of Daily Living：ADL）の低下がみられるようになるが，galantamineはADLの評価スケールであるDAD（Disability Assessment for Dementia）スケールでは6カ月間の投与後に有意な改善がみられている[12]．このようなgalantamineの認知機能やADLにおける改善効果によって，介護者の負担軽減も認められている．Galantamine投与後6カ月において介護者（caregiver）がADL補助のために身体介護に費やした時間はプラセボ群と比較して1日あたり32分短縮していた[19]．また，この短縮効果は軽度より中等度のADでより効果的だった．さらに，AD患者を励ましたり危険のないようにするための見守り時間も半分以下に減らせたとの報告がある[20]．

3 安全性

臨床試験中に認められた副作用としては16mg/日投与で嘔気（galantamine群13%，プラセボ群5%，以下同様），下痢（12%，6%），嘔吐（6%，1%）の頻度がプラセボ群より有意に高く，24mg/日投与では嘔気，嘔吐，下痢，めまい，体重減少，食思不振，振戦がプラセボ群と比較して有意に（$p<0.05$）高かったがいずれも軽度から中等度であり，重篤なものはプラセボ群との間で有意差は認めなかった[17]．Galantamine 8mg/日以下の投与ではプラセボ群との間に有意差はみられなかった[17]．ドネペジルとの間で主要な副作用の発現率に有意差は認められておらず，これらの副作用の多くは他のAChE阻害薬と同様にgalantamineのAChE阻害作用によるコリン作動系神経の亢進作用によるものと考えられる．さらに，galantamineはAChE阻害作用に加えてnAChRの賦活作用を併せもつため，迷走神経刺激による洞不全症候群や心伝導障害，気管支喘息，消化性潰瘍などに関して特に注意が必要であろう．低用量（8mg/日）から開始して段階的に用量を増加する方法が推奨されている．

Ⅳ 各国での使用状況

Galantamineは海外ではすでに各国のヤンセン社より販売が開始されている．2000年3月にスウェーデンで最初に承認され，オーストリアをはじめとして現在では欧米諸国を中心に米国，英国，ドイツ，フランス，韓国等の69カ国以上で承認され，Reminyl®として広く使用されている．米国では2001年2月に認可され，軽度から中等度のADを対象に販売が開始されたが，糖尿病治療薬グリメピリド（アマリール®）との取り違えによるインシデンスのため，2005年7月よりRazadyne®と名称変更になっている．血中半減期は約7時間で短いため1日2回の内服が必要だったが，現在は1日1回の投与が可能な徐放剤も米国で発売されている．なお，わが国では2005年12月現在において国内未発売で，第Ⅲ相臨床試験が進行中である．

V おわりに

わが国では急速に高齢社会が進行し，AD患者も増加の一途を辿っている．第一線の臨床の現場において介護者も含めてAD治療薬に対するニーズは高いものがあるが，現在のところわが国ではドネペジルしか認可されていない．Aβに対するワクチン療法などの根本的治療法が仮に実用化された場合にも，神経伝達物質の調節薬による対症的治療法は記憶障害などの症状と密接に関連しているため依然として必要であろう．GalantamineはnAChRの賦活作用を介した神経保護作用などのユニークな作用機序を有しており，作用機序が異なる治療薬の登場によって治療の選択肢が広がる意味でも好ましいことである．AD患者のみならず，介護者の負担を少しでも軽減するためにも早急なAD治療薬の検討が望まれる．

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Association of a microsomal triglyceride transfer protein gene polymorphism with blood pressure in Japanese women

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Abstract. Genetic variants of the microsomal triglyceride transfer protein (MTP) have been associated with the serum concentration of low density lipoprotein-cholesterol, predisposition to coronary heart disease, or longevity. The relation of a -493G›T polymorphism in the promoter of *MTP* to blood pressure was examined in a population-based study. The subjects (1124 men, 1108 women) were aged 40-79 years and were randomly recruited to a population-based prospective cohort study of aging and age-related diseases in Japan. Blood pressure was measured at least twice with subjects in the sitting position. The serum lipid profile was determined after the subjects had fasted overnight. The -493G›T genotype of *MTP* was determined with a fluorescence-based allele-specific DNA primer assay system. There was no difference in the serum concentrations of total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, or triglycerides among *MTP* genotypes for men or for women. Systolic or diastolic blood pressure was not related to the -493G›T polymorphism in men. For women, however, systolic and diastolic blood pressures were significantly related to *MTP* genotype, with the *T* allele of the polymorphism being associated with low blood pressure. The relation between *MTP* genotype and the prevalence of hypertension was almost significant (P=0.055) for all women. Although *MTP* genotype was not associated with the prevalence of hypertension in premenopausal women, the relation between these parameters was significant (P=0.040) in postmenopausal women, with the *TT* genotype protecting against this condition. These results suggest that *MTP* genotype is a determinant of blood pressure in Japanese women.

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Introduction

Hypertension is a complex multifactorial and polygenic disorder that is thought to result from an interaction between an individual's genetic background and various environmental factors (1). Given that hypertension is a major risk factor for coronary heart disease, stroke, and chronic renal failure, personalized prevention of hypertension is an important public health goal. One approach to personalized prevention of and selection of the most appropriate treatment for hypertension is to identify disease susceptibility genes. Although genetic linkage analyses (2-6) and candidate gene association studies (2,7-10) have implicated various loci and genes in predisposition to hypertension, the genes that confer genetic susceptibility to this condition remain to be identified definitively. In addition, because of ethnic divergence of gene polymorphisms, it is important to examine polymorphisms related to hypertension in each ethnic group.

Microsomal triglyceride transfer protein is a heterodimeric lipid transfer protein that is essential for the assembly of apolipoprotein B-containing lipoproteins and their secretion from the liver and intestine (11). Mutations in the coding region of the gene for this protein (*MTP*) prevent the production of apolipoprotein B-containing lipoproteins, resulting in the rare genetic disorder abetalipoproteinemia (12). *MTP* is polymorphic, with several genetic variants existing in linkage disequilibrium (13). A common polymorphism (-493G›T) has been identified in the promoter region of *MTP* (located 493 base pairs upstream from the transcription start site), with the less prevalent *T* variant having been associated with a reduced plasma concentration of low density lipoprotein-cholesterol (14). The relation between *MTP* genotype and low density lipoprotein phenotype was confirmed in a large cohort of similar ethnic background (13), but conflicting results have been obtained with other cohorts (15,16). One possible explanation for this discrepancy might be that the phenotype associated with the -493G›T polymorphism of *MTP* is modulated by visceral obesity and hyperinsulinemia (17). The -493G›T polymorphism was recently shown to be associated with the prevalence of coronary heart disease (18). Furthermore, a haplotype marker of *MTP* was associated with longevity, suggesting that *MTP* might modify human life-span (19). Functional analysis of the -493G›T polymorphism with the use of promoter constructs revealed that

the promoter activity of the *T* variant was greater than that of the *G* variant (14).

We hypothesized that *MTP* might be a gene that confers susceptibility to hypertension. We have now performed a large-scale association study for the -493G>T polymorphism of *MTP* and both blood pressure and the prevalence of hypertension in community-dwelling Japanese men and women.

Materials and methods

Study population. The National Institute for Longevity Sciences - Longitudinal Study of Aging is a population-based prospective cohort study of aging and age-related diseases (20). The subjects are unrelated individuals stratified by both age and gender, and are randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan (21,22). The lifestyle of residents of this area is typical of that of individuals in most regions of Japan. The numbers of men and women recruited are similar and age at the baseline is 40-79 years, with similar numbers of participants in each decade (40s, 50s, 60s, 70s). The subjects will be followed up every 2 years. All participants are subjected at a special center to a detailed examination, which includes not only medical evaluation but also assessment of exercise physiology, body composition, nutrition, and psychology. Individuals with coronary heart disease, valvular heart disease, cardiomyopathies, or renal or endocrinologic diseases that cause secondary hypertension were excluded from the present study. Individuals receiving anti-hypertensive or lipid-lowering medication were also excluded. We examined the relation of the -493G>T polymorphism of *MTP* (NCBI, dbSNP, rs1800591) to blood pressure and the prevalence of hypertension in 1124 men and 1108 women. The study protocol complies with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of the National Institute for Longevity Sciences. Written informed consent was obtained from each subject.

Measurement of blood pressure and serum lipid profile. Blood pressure was measured at least twice with subjects in the sitting position according to the guidelines of the American Heart Association (23). Normal blood pressure was defined as both a systolic blood pressure of <140 mmHg and a diastolic blood pressure of <90 mmHg. Hypertension was defined as a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg, or both.

Venous blood was collected in the early morning after the subjects had fasted overnight. Blood samples were centrifuged at 1600 x g for 15 min at 4°C, and serum was separated and stored at -30°C until analysis. The serum concentrations of total cholesterol (24), high density lipoprotein-cholesterol (25), low density lipoprotein-cholesterol (26), triglycerides (27) were measured according to the methods previously described. Apolipoproteins A1, A2, B, C2, C3, and E were measured by corresponding turbidimetric immunoassay kits (Eiken Chemical, Tokyo, Japan) with Hitachi 7170 automatic analyzer (Hitachi, Tokyo, Japan).

Determination of genotype. Genotypes for *MTP* were determined with a fluorescence-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) (28). The polymorphic region of *MTP* was amplified by the polymerase chain reaction with allele-specific sense primers labeled at the 5' end with either fluorescein isothiocyanate (5'-ACATTATTTTGAAGTGATTGGXTG-3') or Texas red (5'-ACATTATTTTGAAGTGATTGGXGG-3') and with an antisense primer labeled at the 5' end with biotin (5'-AATT CACACTGAATTTTAGGATTTA-3'). The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 4.5 mmol/l $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol comprised initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 sec, annealing at 62.5°C for 30 sec, and extension at 70°C for 30 sec; and a final extension at 70°C for 2 min. The amplified DNA was then incubated in a solution containing streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature, and the plate was placed on a magnetic stand. The supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/l NaOH and were measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for fluorescein isothiocyanate and of 584 and 612 nm, respectively, for Texas red.

Statistical analysis. Quantitative data were compared among three groups by one-way analysis of variance and the Tukey-Kramer post hoc test, and between two groups by the unpaired Student's t-test. Blood pressure values were analyzed with adjustment for age and body mass index by the least squares method in a general linear model. Allele frequencies were estimated by the gene-counting method, and the Chi-square test was used to identify significant departure from Hardy-Weinberg equilibrium. The effect of *MTP* genotype on blood pressure was evaluated by multivariable regression analysis; P-values and R^2 were calculated from analysis including age, body mass index, and *MTP* genotype (*GG* = *GT* = 0, *TT* = 1). A P-value of <0.05 was considered statistically significant.

Results

The distribution of -493G>T genotypes of *MTP* was in Hardy-Weinberg equilibrium, and age and body mass index did not differ among genotypes, for men (Table I). Serum concentrations of apolipoprotein A2 were greater in men with the *GT* genotype or in men in the combined group of *GT* and *TT* genotypes than in those with the *GG* genotype. Serum concentrations of apolipoprotein C2 were also greater in men with the *GT* genotype than in those with the *GG* genotype. No differences in the serum concentrations of total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, triglycerides, or apolipoprotein A1, B, C3, or E were detected among *MTP* genotypes in men. Furthermore, systolic or diastolic blood pressure did not differ among *MTP* genotypes in men either with or without adjustment for age and body mass index.

For women, the distribution of -493G>T genotypes of *MTP* was in Hardy-Weinberg equilibrium, and age and body mass

Table I. Blood pressure and other characteristics for all male subjects (n=1124) according to MTP genotype.

Characteristic	*GG*	*GT*	*TT*	*GG + GT*	*GT + TT*
No. (%)	777 (69.1)	309 (27.5)	38 (3.4)	1086 (96.6)	347 (30.9)
Age (years)	57.7±0.4	56.6±0.7	55.1±2.0	57.4±0.4	56.5±0.6
Body mass index (kg/m^2)	22.7±0.1	23.0±0.2	23.4±0.5	22.8±0.1	23.0±0.2
Serum lipid profile (mg/dl)					
Total cholesterol	211.7±1.2	213.3±1.9	216.0±5.4	212.1±1.0	213.6±1.8
LDL-cholesterol	131.3±1.2	133.1±1.9	134.2±5.3	131.8±1.0	133.3±1.8
HDL-cholesterol	57.1±0.5	57.6±0.8	60.2±2.4	57.2±0.5	57.9±0.8
Triglycerides	135.9±3.5	131.0±5.5	132.1±15.5	134.5±2.9	131.1±5.2
Apolipoprotein A1	141.1±1.0	143.9±1.6	143.5±4.5	141.9±0.9	143.8±1.5
Apolipoprotein A2	37.7±0.2	38.5±0.3[a]	38.4±1.0	37.9±0.2	38.5±0.3[b]
Apolipoprotein B	107.7±0.9	110.0±1.4	110.9±3.9	108.4±0.7	110.1±1.3
Apolipoprotein C2	4.60±0.05	4.78±0.08[c]	4.62±0.22	4.65±0.04	4.76±0.07
Apolipoprotein C3	11.0±0.1	11.3±0.2	11.0±0.5	11.1±0.1	11.2±0.2
Apolipoprotein E	4.66±0.05	4.71±0.07	4.69±0.21	4.67±0.04	4.71±0.07
Blood pressure (mmHg)					
Systolic	120.8±0.7	120.7±1.1	126.0±3.3	120.8±0.6	121.3±1.1
Diastolic	75.2±0.4	75.6±0.7	77.1±2.0	75.3±0.4	75.7±0.6
Adjusted systolic	120.9±0.7	120.6±1.1	125.3±3.2	120.8±0.6	121.1±1.0
Adjusted diastolic	75.3±0.4	75.4±0.6	76.5±1.9	75.3±0.4	75.5±0.6

Data are means ± SE. LDL and HDL denote low and high density lipoprotein, respectively. Adjustment of systolic or diastolic blood pressure refers to correction for age and body mass index. Data in the combined group of the *GG* and *GT* genotypes (*GG + GT*) were compared with those in individuals with the *TT* genotype (recessive genetic model). Data in the combined group of the *GT* and *TT* genotypes (*GT + TT*) were compared with those in individuals with the *GG* genotype (dominant genetic model). [a]P=0.029, [b]P=0.025, [c]P=0.044 versus *GG*.

index did not differ among genotypes (Table II). There were no differences in serum concentrations of total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, triglycerides, or apolipoprotein A1, A2, B, C2, C3, or E among *MTP* genotypes for women. Systolic blood pressure was significantly lower in women with the *TT* genotype than in those with the *GG* genotype or in those in the combined group of *GG* and *GT* genotypes; the difference in systolic blood pressure between individuals with the *GG* genotype and those with the *TT* genotype (expressed as a percentage of the larger value) was 7.4%. Systolic blood pressure was also significantly lower in women in the combined group of *GT* and *TT* genotypes than in those with the *GG* genotype. Diastolic blood pressure was significantly lower in women with the *TT* genotype than in those in the combined group of *GG* and *GT* genotypes; the difference in diastolic blood pressure between these two groups was 5.9%. After adjustment for age and body mass index, systolic blood pressure was significantly lower in women with the *TT* genotype than in those in the combined group of *GG* and *GT* genotypes. Adjusted systolic blood pressure was also significantly lower in women in the combined group of *GT* and *TT* genotypes than in those with the *GG* genotype.

The effects of the -493G›T genotype of *MTP* on blood pressure were evaluated by multiple regression analysis including age, body mass index, and *MTP* genotype (Table III). The analysis revealed that *MTP* genotype significantly affected systolic and diastolic blood pressures for women.

To clarify the effect of the -493G›T polymorphism on blood pressure, we compared the distribution of genotypes between individuals with hypertension and those with normal blood pressure. One woman was excluded from this analysis because she was borderline hypertensive (hypertension and normal blood pressure at the first and second measurements, respectively). For men, no difference in the distribution of *MTP* genotypes was detected between hypertensive and normotensive groups (data not shown). There was an almost significant (P=0.055) relation between *MTP* genotype and the prevalence of hypertension for all women (Table IV). To examine the possible influence of menopause on the relation between *MTP* genotype and blood pressure, we analyzed the genotype distribution and prevalence of hypertension for premenopausal and postmenopausal women independently. Because of their small number (n=17), perimenopausal women were excluded from this analysis. There was no significant relation between *MTP* genotype and the prevalence of hypertension for premenopausal women. In postmenopausal women, however, *MTP* genotype was associated with the prevalence of hypertension, with the *TT* genotype protecting against this condition (Table IV).

Table II. Blood pressure and other characteristics for all female subjects (n=1108) according to *MTP* genotype.

Characteristic	*GG*	*GT*	*TT*	*GG + GT*	*GT + TT*
No. (%)	763 (68.9)	313 (28.2)	32 (2.9)	1076 (97.1)	345 (31.1)
Age (years)	57.0±0.4	58.1±0.7	56.4±2.0	57.3±0.4	57.9±0.6
Body mass index (kg/m²)	22.8±0.1	22.6±0.2	22.2±0.6	22.7±0.1	22.6±0.2
Serum lipid profile (mg/dl)					
Total cholesterol	227.3±1.3	226.8±2.0	224.3±6.2	227.1±1.1	226.6±1.9
LDL-cholesterol	139.7±1.3	139.1±2.0	137.1±6.2	139.6±1.1	138.9±1.9
HDL-cholesterol	66.2±0.6	65.8±0.9	66.3±2.7	66.1±0.5	65.9±0.8
Triglycerides	108.5±2.2	110.3±3.5	110.9±10.8	109.0±1.9	110.3±3.3
Apolipoprotein A1	156.2±1.0	155.1±1.5	154.3±4.7	155.9±0.8	155.0±1.4
Apolipoprotein A2	37.8±0.2	37.9±0.3	36.0±1.0	37.8±0.2	37.7±0.3
Apolipoprotein B	110.3±0.9	110.0±1.4	109.8±4.5	110.2±0.8	110.0±1.4
Apolipoprotein C2	4.56±0.05	4.58±0.07	4.36±0.22	4.56±0.04	4.56±0.07
Apolipoprotein C3	10.8±0.1	10.9±0.1	10.9±0.5	10.8±0.1	10.9±0.1
Apolipoprotein E	5.00±0.04	5.00±0.07	5.05±0.21	5.00±0.04	5.01±0.06
Blood pressure (mmHg)					
Systolic	120.9±0.8	118.7±1.2	112.0±3.6[a,b]	120.3±0.6	118.0±1.1[a]
Diastolic	73.2±0.4	72.4±0.7	68.6±2.1[c]	72.9±0.4	72.0±0.7
Adjusted systolic	120.9±0.7	118.5±1.1	113.3±3.3[d]	120.2±0.6	118.0±1.0[e]
Adjusted diastolic	73.1±0.4	72.4±0.7	69.3±1.9	72.9±0.4	72.1±0.6

Data are means ± SE. LDL and HDL denote low and high density lipoprotein, respectively. Adjustment of systolic or diastolic blood pressure refers to correction for age and body mass index. Data in the combined group of the *GG* and *GT* genotypes (*GG + GT*) were compared with those in individuals with the *TT* genotype (recessive genetic model). Data in the combined group of the *GT* and *TT* genotypes (*GT + TT*) were compared with those in individuals with the *GG* genotype (dominant genetic model). [a]P=0.038 versus *GG*, [b]P=0.022 versus *GG + GT*, [c]P=0.041 versus *GG + GT*, [d]P=0.037 versus *GG + GT*, [e]P=0.020 versus *GG*.

Table III. Effects of *MTP* genotypes on blood pressure.

	Men		Women	
	P-value	R^2	P-value	R^2
Systolic blood pressure				
Age (years)	<0.001	0.060	<0.001	0.066
Body mass index (kg/m²)	<0.001	0.012	<0.001	0.098
MTP genotype	0.1276		0.0219	0.006
Diastolic blood pressure				
Age (years)	0.598		<0.001	0.026
Body mass index (kg/m²)	<0.001	0.079	<0.001	0.100
MTP genotype	0.381		0.0405	0.005

Data were analyzed by multiple regression analysis including age, body mass index, and *MTP* genotypes (*GG* = *GT* = 0, *TT* = 1).

Table IV. Distribution of *MTP* genotype in hypertensive or normotensive women.

	GG + GT (%)	*TT* (%)	P-value
All women (n=1107)			
Hypertensive	378 (98.4)	6 (1.6)	0.055
Normotensive	697 (96.4)	26 (3.6)	
Premenopausal women (n=279)			
Hypertensive	45 (97.8)	1 (2.2)	0.874
Normotensive	227 (97.4)	6 (2.6)	
Postmenopausal women (n=811)			
Hypertensive	329 (98.5)	5 (1.5)	0.040
Normotensive	458 (96.0)	19 (4.0)	

Discussion

The regulation of blood pressure involves both the integration of a variety of biological systems that control the structure and tone of the vasculature and the volume and composition of body fluid, as well as the adaptation of these systems to constantly changing physiological needs (29). Microsomal triglyceride transfer protein mediates the transport of triglycerides, cholesteryl esters, and phospholipids between

phospholipid surfaces (12). Polymorphisms of *MTP* have been associated with the serum concentration of low density lipoprotein-cholesterol (13,14) as well as with the susceptibility to coronary heart disease (18) and with longevity (19). We have now examined the relation of the -493G›T polymorphism of *MTP* to blood pressure and the prevalence of hypertension in community-dwelling Japanese men and women. Our results show that the *T* allele of this polymorphism is associated with low blood pressure in Japanese women and that the *TT* genotype is protective against hypertension in postmenopausal women.

We failed to detect an association of the -493G›T polymorphism of *MTP* with blood pressure in Japanese men. The reason for this gender difference in the relation of *MTP* genotype to blood pressure remains unclear. It might be attributable, however, at least in part, to the difference in the serum concentration of estrogen between men and women, given that estrogen exerts various favorable effects on vasomotor function, including stimulation of the production of nitric oxide and prostaglandin I_2 as well as inhibition of the release of endothelin-1 by vascular endothelial cells (30).

Given that selection bias can influence the results of association studies, it is important that study populations be genetically and ethnically homogeneous. Our study subjects were recruited randomly from individuals resident in the city of Obu and town of Higashiura in central Japan, where the population is thought to share the same ethnic ancestry and to possess a homogeneous genetic background. We also showed that the genotype distribution of the -493G›T polymorphism of *MTP* was in Hardy-Weinberg equilibrium both for men and for women in our study population. We thus appeared to avoid admixture and selection bias.

Although the -493G›T polymorphism of *MTP* has previously been associated with the serum concentration of low density lipoprotein-cholesterol (13,14), another study failed to detect such an association (15). In the present study, among men, serum concentrations of apolipoprotein A2 were greater in individuals with the *T* allele than in those with the *GG* genotype. Serum concentrations of apolipoprotein C2 were also greater in men with the *GT* genotype than in those with the *GG* genotype. Apolipoprotein A2 is a component of high density lipoprotein-cholesterol, and apolipoprotein C2 is a component of triglyceride-rich lipoproteins and high density lipoprotein-cholesterol. Although MTP has important roles in the assembly of apolipoprotein B-containing lipoproteins and their secretion from the liver and intestine (11), the effects of the -493G›T polymorphism of *MTP* on apolipoprotein A2 or C2 metabolism remain unclear. This polymorphism was also not associated with the serum concentrations of total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, triglycerides, or apolipoprotein A1, A2, B, C2, C3, or E in women. It is thus unlikely that the association of this polymorphism with blood pressure for women in the present study was attributable to an effect on serum lipid concentrations. The mechanism by which the -493G›T polymorphism of *MTP* affects blood pressure thus remains unclear.

The *T* variant of the -493G›T polymorphism was previously found to confer an excess risk of coronary heart disease in men that was independent of plasma lipids and eliminated by treatment with pravastatin (18). The effect of the *T* allele on blood pressure in the present study was also independent of serum lipid concentrations. The mechanism responsible for the association of the *T* variant with both an increased risk of coronary heart disease in men (18) and a reduced risk of hypertension in postmenopausal women (our study) remains to be elucidated. It is possible that the -493G›T polymorphism of *MTP* is in linkage disequilibrium with other polymorphisms of nearby genes that are actually responsible for the development of coronary heart disease and hypertension. Our present results, however, suggest that *MTP* genotype is a determinant of blood pressure in Japanese women.

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ORIGINAL ARTICLE

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Association of alcohol dehydrogenase 2*1 allele with liver damage and insulin concentration in the Japanese

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Abstract The Japanese have a polymorphism in the alcohol dehydrogenase 2 gene (*ADH2*). The alleles of *ADH2* (*ADH2*1* and *ADH2*2*) encode more active and less active forms for ethanol metabolism, respectively. We examined whether liver damage and the insulin–glucose axis vary according to *ADH2* genotype in the Japanese. The 2,232 subjects (1,126 men and 1,106 women) were recruited from a population-based prospective cohort study. Clinical evaluations including alcohol consumption, percentage of alcohol drinkers, plasma glucose, HbA1c, insulin, AST, ALT, γ-GTP, and prevalence of diabetes were compared among the *ADH2* genotypes. The percentage of drinkers, alcohol consumption, AST, ALT, and γ-GTP were higher in group *ADH2*1/1* than in group *ADH2*1/2* or *ADH2*2/2* (all $P < 0.05$). Hence, *ADH2*1/1* is associated with excess alcohol intake and liver disorders. However, the prevalence of diabetes did not differ among the three groups. For the glucose–insulin axis, we examined subjects who did not receive insulin therapy or oral anti-diabetes medication. While amounts of alcohol consumed and glucose levels were nearly the same between *ADH*1/2* and *ADH2*2/2*, insulin concentrations were lower in *ADH2*2/1* than in *ADH2*2/2* ($P < 0.05$ in men). This finding suggests that the *ADH2*1* allele is associated with a lower insulin concentration when alcohol intake is light or moderate. It also suggests that the genetic effect of *ADH2*1* plays an important role in alcohol drinking behavior and in the occurrence of liver injury, but the effect is so mild that it does not influence the glucose–insulin axis or prevalence of diabetes.

Keywords Alcohol dehydrogenase 2 · ADH2 · Diabetes · Insulin resistance · Liver dysfunction · Alcohol · Prospective cohort study

Abbreviations: ALDH: Aldehyde dehydrogenase · ADH: Alcohol dehydrogenase · PCR: Polymerase chain reaction

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Introduction

A reduced incidence of type 2 diabetes has been observed among drinkers in several large prospective studies. Conigrave et al (2001) reported a 12-year prospective study in a cohort of 46,892 US male health professionals, in which 1,571 new cases of type 2 diabetes were reported. The frequency of alcohol consumption was inversely associated with diabetes. Hu et al (2001) reported a large cohort study of 84,941 female nurses from 1980 to 1996, in which abstinence from alcohol use was associated with a significantly increased risk of diabetes. In contrast, other studies (Holbrook et al 1990) have shown an increased risk of diabetes among a proportion of subjects in the top alcohol consumption category. In Japanese men, Tsumura et al (1999) reported that heavy drinking is associated with an increased risk of type 2 diabetes, while moderate drinking is associated with a decreased risk of type 2 diabetes, showing a U-shaped relationship.

The genotypes involved in ethanol metabolism are now known to be associated not only with drinking, but also with longevity and oxidative stress parameters (Ohsawa et al 2003). In Japanese, the pharmacokinetics of alcohol metabolism have been well studied. Alcohol dehydrogenase (ADH) is one of the key enzymes in alcohol metabolism. Class I ADH isoenzymes, encoded

by *ADH1*, *ADH2* and *ADH3*, form dimers among the isoenzymes and oxidize ethanol and other small aliphatic alcohols (Borson et al 1988). About 85% of the Japanese population are carriers of the β2-subunit encoded by the *ADH2*2* allele, while isoenzymes with the β2-subunit have been found in only 5% or less of Europeans and white Americans. The β1- and β2-subunits differ by only one amino acid residue: Arg-47 in the NAD(H) pyrophosphate-binding site is substituted with His-47 in the β2-subunit. ADH2 functions as a dimer and the β2β2 dimer exhibits about 100 times more catalytic activity for ethanol oxidation than the β1β1 dimer at physiological pH (Borson et al 1988), whereas the β1β2 heterodimer exhibits nearly the same activity as the β1β1 homodimer. Thus, relative enzymatic activities of *ADH2*1/1:ADH2*1/2:ADH2*2/2* can be estimated as 1:26:100 if a dimer were to form between the subunits of ADH2*1 and ADH2*2 (Borson et al 1988; Yoshida et al 1981).

Several studies (Higuchi et al 1996; Yamauchi et al 2001) have reported that the *ADH2* genotype is associated with excess alcohol intake and alcohol-related disorders in the Japanese population. We have previously reported that the *ADH2* genotype affected LDL-cholesterol levels and the occurrence of cerebral infarction in a community-dwelling Japanese population (Suzuki et al 2004). We therefore examined whether the glucose–insulin axis or prevalence of diabetes is associated with the *ADH2* genotype in the same Japanese population.

Research design and methods

The National Institute for Longevity Sciences–Longitudinal Study of Aging (NILS–LSA), a population-based prospective cohort study of aging and age-related diseases, was begun in 1997 (Ohsawa et al 2003; Shimokata et al 2000; Yamada et al 2002). All participants were independent residents of the Aichi prefecture in Japan. Residents aged 40–79 years old were randomly selected from the register in co-operation with the local government.

The area of study is located in the south of Nagoya City. It is a commuter town and contains an industrial area belonging to the Toyota group, but it has many orchards and farms, so it has both urban and rural characteristics. This area is geographically located in the center of Japan, and its climate is average for Japan. We examined a representative sample of the area's population via a national postal questionnaire of prefecture-stratified random samples of 3,000 households from all prefectures in Japan, and previously showed that the lifestyle of people in this area was the most typical of all areas in Japan.

The sample consisted of 2,232 subjects (1,126 men and 1,106 women) who were randomly recruited. We refer to them as "subjects-1." Subjects-1 was stratified by both age and sex. Randomly selected men and women were invited, by mail, to attend an explanatory meeting. At the meeting, the procedures for each examination and follow-up schedule were fully explained. Written informed consent to the entire procedure was obtained from each participant. Participants in the present study were recruited from subjects examined in 1997–1999. The study protocol was approved by the Committee on the Ethics of Human Research of National Chubu Hospital and the National Institute for Longevity Sciences.

Descriptions of the physical examinations performed have been published before (Ohsawa et al 2003; Shimokata et al 2000; Yamada et al 2002). In brief, lifestyle, medical history and prescribed drugs were examined by questionnaire. Anthropometric measurements were taken by a physician. A drinker is defined as a subject who has drunk more than 5 g of alcohol on average per day during the past year. Amounts of alcohol consumed were carefully examined by taking pictures before and after drinking as well as with questionnaires. The percentage of non-smokers to smokers was also noted.

Venous blood was collected early in the morning after at least 12 h fasting. The mean of two determinations of blood chemistry data was obtained for each participant. Clinical evaluations included gender, age, height, body-mass index, smoker status, alcohol consumption, percentage of alcohol drinkers, and blood chemistry (fasting plasma glucose (FPG), HbA1c, insulin, AST, ALT, and γ-GTP levels). Diagnosis of diabetes was based on medical records, or it was defined as a FPG concentration greater than 126 mg/dl or an HbA1c of more than 6.5%, and/or if medication was taken to lower the blood glucose level. Namely, not all subjects whose FPG level was greater than 110 mg/dl did not receive the 75 g oral glucose tolerance test according to the criteria of the Japan Diabetes Society. In the analysis of glucose–insulin associated parameters, to exclude the effect of medications, the diabetic patients who received insulin therapy or oral medications for diabetes were excluded from subjects-1, and the remaining subjects were defined as the "subjects-2" group.

Genotyping of *ADH2*

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). To determine the genotype with the G214A substitution (Arg-47-His), the polymorphic region of *ADH2* was amplified by polymerase chain reaction (PCR) with an antisense primer labeled at the 5′ end with biotin (5′-GATGGTGGCTGTAGGAATCTG-3′) and a G allele-specific sense primer labeled with FITC (5′-CCACGTGGTCATCTGTNCG-3′) or A allele-specific sense primer labeled with Texas red (5′-AACCACGTGGTCATCTGTNTG-3′).

Table 1 Comparison of parameters among three groups of men (subjects-1), divided according to *ADH2* genotype. Right columns indicate *P*-values of statistical differences between two groups

Variables	Men			*P*-value		
ADH genotype	2/2	1/2	1/1	2/2 vs. 1/2	2/2 vs. 1/1	1/2 vs. 1/1
Subjects-1 $n=$	689	378	59			
Age (years)	59.5 ± 0.4	58.9 ± 0.6	58.0 ± 1.4	n.s.	n.s.	n.s.
Height (cm)	164.4 ± 0.2	164.7 ± 0.3	164.6 ± 0.8	n.s.	n.s.	n.s.
BMI	23.0 ± 0.1	22.8 ± 0.1	22.9 ± 0.4	n.s.	n.s.	n.s.
Smoking (%)	61/39	63/37	63/37	n.s.	n.s.	n.s.
Alcohol (g/day)	28.8 ± 1.4	29.5 ± 1.9	44.5 ± 4.8	n.s.	0.0049**	0.0102**
Drinkers (%)	67.0	67.1	85.5	($P < 0.0175$)		
AST (IU/l)	26.6 ± 0.7	26.6 ± 0.9	33.6 ± 2.3	n.s.	0.0038**	0.0049**
ALT (IU/l)	27.1 ± 0.9	26.8 ± 1.2	34.3 ± 3.0	n.s.	0.02*	0.02*
γ-GTP (IU/l)	58.2 ± 3.1	57.3 ± 4.1	80.3 ± 10.5	n.s.	0.04*	0.04*
Diabetics (%)	13.3	13.3	13.6	n.s.	n.s.	n.s.

AST 2/2 ± 1/2 vs. 1/1, $P < 0.0033$; ALT 2/2 ± 1/2 vs. 1/1, $P < 0.02$; γ-GTP 2/2 ± 1/2 vs. 1/1, $P < 0.04$; drinkers 2/2 ± 1/2 vs. 1/1, $P < 0.005$; alcohol 2/2 ± 1/2 vs. 1/1, $P < 0.005$
*$P < 0.05$
**$P < 0.01$

Statistical analysis

Data are presented as means ± SE. The statistical significance of any difference in mean values and frequencies was determined with the Student's *t*-test or the Tukey–Kramer test. We used a one-way analysis of variance to test for overall differences among multiple groups, and the Fisher LSD post hoc test to identify which group differences accounted for the significant *P*-value. The significance of deviation from Hardy–Weinberg equilibrium was analyzed using the chi-square test. A *P*-value of < 0.05 was considered statistically significant.

Results

Influence of *ADH2* genotypes on drinking behavior and liver function

Among the 2,232 subjects, 1,355 (men 689, women 666) had the *ADH2*2/2* genotype, 759 (men 378, women 381) had the *ADH2*2/1* genotype, and 118 (men 59, women 59) had the *ADH2*1/1* genotype. The *ADH2*2/2*, *ADH2*2/1*, and *ADH2*1/1* genotypes were in Hardy–Weinberg equilibrium. There was no gender difference.

First, we compared the percentage of drinkers dependent upon *ADH2* genotype. The percentage of drinkers was significantly higher in both men and women in the *ADH2*1/1* group, showing overall differences among the groups (Table 1 and Fig. 1a). The difference was statistically significant according to the Fisher LSD post hoc test in men ($P < 0.0175$), women ($P < 0.0166$), and total subjects-1 ($P < 0.0033$) (Table 1). Moreover, amounts of alcohol consumed were much higher in the *ADH2*1/1* group than the other *ADH2* groups in men and total subjects-1 ($P < 0.01$ in *ADH2*2/2* vs. *ADH2*1/1* and $P < 0.05$ in *ADH2*1/2* vs. *ADH2*1/1*) (Tables 1, 3 and Fig. 1b). On the other hand, no significant difference in alcohol consumption among *ADH2*1/1* and the other groups was found in women, probably because much less alcohol was consumed by women than men (Table 2 and Fig. 1b). For smoking (percentage of non-smokers to smokers), there was no difference according *ADH2* genotype in men and in women.

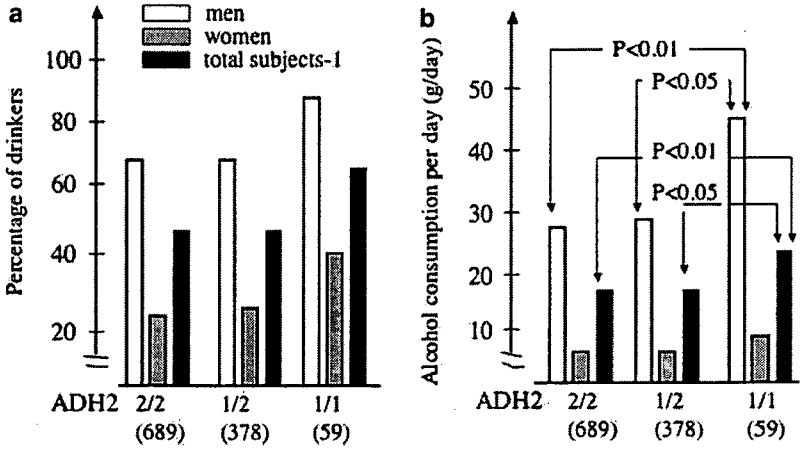

Fig. 1a, b Correlation of *ADH2* genotype with alcohol drinking behavior. **a** Percentage of drinkers in three groups based on *ADH2* genotype. Values in parentheses indicate the total number of subjects (*white bars* men, *gray bars* women, and *black bars* total subjects). **b** Average amounts of alcohol consumed per day. Subjects in the *ADH2*1/1* group drink more alcohol than those in the *ADH2*2/2* and *ADH2*1/2* groups

Table 2 Comparison of parameters among three groups of women (in subjects-1), divided according to the three *ADH2* genotypes. Right columns indicate *P*-value of statistical difference between each two group

Variables	Women			*P*-value		
ADH genotype	2/2	1/2	1/1	2/2 vs. 1/2	2/2 vs. 1/1	1/2 vs. 1/1
Subjects-1 $n=$	666	381	59			
Age (years)	59.4 ± 0.4	59.1 ± 0.6	60.0 ± 1.4	n.s.	n.s.	n.s.
Height (cm)	151.3 ± 0.2	151.1 ± 0.3	151.1 ± 0.8	n.s.	n.s.	n.s.
BMI	23.0 ± 0.1	22.7 ± 0.2	23.1 ± 0.4	n.s.	n.s.	n.s.
Smoking (%)	93/7	93/7	92/8	n.s.	n.s.	n.s.
Alcohol (g/day)	5.2 ± 0.6	5.4 ± 0.8	6.4 ± 2.0	n.s.	n.s.	n.s.
Drinkers (%)	22.9	25.5	39.7	< 0.0166		
AST (IU/l)	24.5 ± 0.6	23.5 ± 0.7	23.3 ± 1.8	n.s.	n.s.	n.s.
ALT (IU/l)	21.2 ± 0.8	20.1 ± 1.0	18.9 ± 2.5	n.s.	n.s.	n.s.
γ-GTP (IU/l)	27.9 ± 1.1	28.5 ± 1.4	29.4 ± 3.6	n.s.	n.s.	n.s.
Diabetics (%)	9.16	10.5	6.78	n.s.	n.s.	n.s.

Drinkers 2/2 ± 1/2 vs. 1/1, $P < 0.01$

Next, we compared blood parameters of liver function, namely AST, ALT, and γ-GTP activities. In men, levels were significantly higher in the *ADH2*1/1* group than the other two *ADH2* groups (Table 1, AST; $P < 0.01$ in *ADH2*2/2* vs. *ADH2*1/1* and $P < 0.01$ in *ADH2*1/2* vs. *ADH2*1/1*. ALT; $P < 0.05$ in *ADH2*2/2* vs. *ADH2*1/1* and $P < 0.05$ in *ADH2*1/2* vs. *ADH2*1/1*. γ-GTP; $P < 0.05$ in *ADH2*2/2* vs. *ADH2*1/1* and $P < 0.05$ in *ADH2*1/2* vs. *ADH2*1/1*), indicating that more alcohol intake in the *ADH2*1/1* group causes damage to the liver. On the other hand, no significant difference was found in women (Table 2); nevertheless the *ADH2*1/1* group consumed more alcohol than the other groups, probably because women drink less than men.

In subjects-1, the percentage of those with diabetes was compared among the three ADH2 genotypic groups. However, there was no statistical difference in the prevalence of diabetes among the three groups (men; *ADH2*2/2*:13.3%, *ADH2*1/2*:13.3%, and *ADH2*1/1*:13.6%, women; *ADH2*2/2*:9.2%, *ADH2*1/2*:10.5%, and *ADH2*1/1*:6.8%, total subjects-1; *ADH2*2/2*:11.2%, *ADH2*1/2*:11.9%, and *ADH2*1/1*:10.2%) (Tables 1, 2, 3).

Influence of *ADH2* genotype on fasting insulin concentration

We tried to clarify the correlation of insulin concentration with *ADH2* genotype. To exclude the effect of medication, subjects were limited to those (subjects-2) not treated with insulin therapy and/or with oral medications for diabetes. Although habits or behaviors generally depend upon genetic factors, we would like to distinguish the genetic effects from the secondary results of alcohol consumption. Since the frequency of drinking and the amountsof alcohol consumed were the same in the *ADH2*1/2* and *ADH2*2/2* groups (Fig. 1 and Tables 1, 2, 3), we compared fasting insulin concentrations between these two groups. Insulin levels were lower in the *ADH2*1/2* than *ADH2*2/2* group in total subjects-2 ($P < 0.02$). In men, insulin levels were lower in the *ADH2*1/2* than *ADH2*2/2* group ($P < 0.05$), while in

Table 3 Comparison of parameters among three groups of total subjects-1 divided according to *ADH2* genotype. Right columns indicate *P*-values of statistical differences between two groups

Variables	Total (men + women)			*P*-value		
ADH genotype	2/2	1/2	1/1	2/2 vs. 1/2	2/2 vs. 1/1	1/2 vs. 1/1
Subjects-1 $n=$	1,352	756	118			
Age (years)	59.4 ± 0.3	59.0 ± 0.4	59.0 ± 1.0	n.s.	n.s.	n.s.
Height (cm)	158.2 ± 0.2	158.1 ± 0.3	156.8 ± 0.8	n.s.	n.s.	n.s.
BMI	23.0 ± 0.1	22.7 ± 0.1	23.1 ± 0.3	n.s.	n.s.	n.s.
Smoking (%)	77/23	78/22	78/22	n.s.	n.s.	n.s.
Alcohol (g/day)	17.2 ± 0.9	17.6 ± 1.1	24.9 ± 2.8	n.s.	0.0089**	0.0158**
Drinkers (%)	45.4	45.6	62.0	< 0.0033		
AST (IU/l)	25.6 ± 0.4	25.0 ± 0.6	28.3 ± 1.4	n.s.	n.s.	0.0383**
ALT (IU/l)	24.2 ± 0.6	23.4 ± 0.8	26.5 ± 2.0	n.s.	n.s.	n.s.
γ-GTP (IU/l)	43.3 ± 1.7	42.9 ± 2.3	54.4 ± 5.7	n.s.	n.s.	n.s.
Diabetics (%)	11.2%	11.9%	10.2%	n.s.	n.s.	n.s.

Drinkers 2/2 ± 1/2 vs. 1/1, $P < 0.001$; alcohol 2/2 ± 1/2 vs. 1/1, $P < 0.01$
* $P < 0.05$
** $P < 0.01$

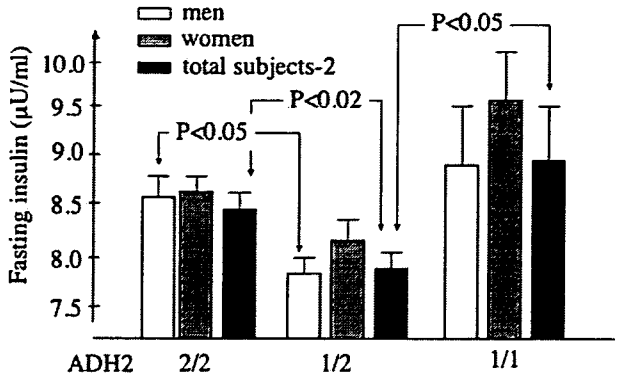

Fig. 2 Correlation of *ADH2* genotype with fasting insulin concentration in subject-2 group. Fasting insulin concentration (μU/ml): a significant difference was found between *ADH2*2/2* and *ADH2*1/2* in men (8.56±0.24 vs. 7.77±0.32, $P<0.05$), and between *ADH2*2/2* and *ADH2*1/2* in total subjects-2 (8.44±0.15 vs. 7.84±0.20, $P<0.02$). A significant difference was found between *ADH2*1/2* and *ADH2*1/1* in total subjects-2 (7.84±0.20 vs. 8.92±0.50, $P<0.05$)

women, the *ADH2*1/2* group tended to have lower insulin concentrations (Fig. 2 and Table 4). This suggests that the *ADH2*1* allele has a lowering effect on the concentration of insulin.

Next, we compared the concentration of insulin between *ADH2*1/2* and *ADH2*1/1*. The concentration tended to be higher in the *ADH2*1/1* group than the *ADH2*1/2* group in men, women and total subjects-2, but a significant difference was only found in total subjects-2 (insulin, *ADH2*1/2*: 7.84±0.20 μU/ml, *ADH2*1/1*: 8.92±0.50 μU/ml, $P<0.05$, Table 3 and Fig. 2). Because the *ADH2*1/1* group is small, the difference may have become statistically insignificant in men or in women.

In subjects-2, while the difference was statistically insignificant, the average level of HbA1c tended to be lower in the *ADH2*1/2* group than the *ADH2*1/1* or *ADH2*2/2* group (Fig. 3 and Table 4). For instance, in total subjects-2, HbA1c was 5.20±0.02%, 5.17±0.02%, and 5.23±0.05%, respectively, in the *ADH2*2/2*, *ADH2*1/2*, and *ADH2*1/1* groups. Therefore, low insulin levels in the *ADH2*1/2* group seem to parallel low HbA1c levels, showing a U-shaped relationship with *ADH2* genotype as in Figs. 2 and 3.

Discussion

By examining the correlation between *ADH2* genotype and drinking behavior, we confirmed the previous observation that *ADH2* genotype influences the amount of alcohol consumed in a Japanese population (Higuchi et al 1996). In addition to alcohol consumption and percentage of drinkers, men from the *ADH2*1/1* group had the highest levels of AST, ALT, and γ-GTP, suggesting that they drink so much alcohol that their livers become damaged. This coincides with the observation of Tanaka et al (1996), supporting the idea that *ADH2* polymorphisms play an important role in alcoholic liver diseases.

In terms of the mechanism involved, since carriers of *ADH2*1/1* have less enzymatic activity for ethanol than carriers of *ADH2*2/1* or *ADH2*2/2*, the slow rate of ethanol clearance could damage the liver, but this is unlikely because ethanol is less toxic than acetaldehyde. Alternatively, it is possible that the slow rate of ethanol clearance protects the subjects from the uncomfortable feeling caused by acetaldehyde, thereby causing them to drink too much alcohol and leading to liver damage.

Interestingly, concentrations of insulin were higher in the *ADH2*1/1* than the *ADH2*1/2* group. Onishi et al (2003) reported that excess alcohol intake can induce insulin resistance with enhanced PI3-kinase activation. Therefore, in the *ADH2*1/1* group, excess alcohol intake may cause insulin resistance, resulting in hyperinsulinemia. Otherwise, some liver dysfunction caused by excess alcohol intake may cause a high glucose output from liver, thereby inducing hyperinsulinemia.

Table 4 Comparison of glucose-insulin axis parameters among three groups of subjects-2 divided according to the three *ADH2* genotypes

Variables				*P*-value		
ADH genotype	2/2	1/2	1/1	2/2 vs. 1/2	2/2 vs. 1/1	1/2 vs. 1/1
Men $n=$	640	346	57			
FPG (mg/dl)	103.3±0.7	102.6±0.9	103.3±2.2	n.s.	n.s.	n.s.
HbA1c (%)	5.24±0.02	5.22±0.03	5.27±0.08	n.s.	n.s.	n.s.
Insulin (μU/ml)	8.46±0.22	7.69±0.31	8.47±0.75	0.0452*	n.s.	n.s.
Women $n=$	623	354	57			
FPG (mg/dl)	98.6±0.6	99.3±0.8	99.2±2.1	n.s.	n.s.	n.s.
HbA1c (%)	5.15±0.02	5.11±0.03	5.17±0.06	n.s.	n.s.	n.s.
Insulin (μU/ml)	8.42±0.19	8.00±0.26	9.36±0.65	n.s.	n.s.	n.s.
Total $n=$	1,263	700	114			
FPG (mg/dl)	101.0±0.46	101.0±0.6	101.2±1.5	n.s.	n.s.	n.s.
HbA1c (%)	5.20±0.02	5.17±0.02	5.23±0.05	n.s.	n.s.	n.s.
Insulin (μU/ml)	8.44±0.15	7.84±0.20	8.92±0.50	0.018*	n.s.	0.045*

*$P<0.05$
**$P<0.01$

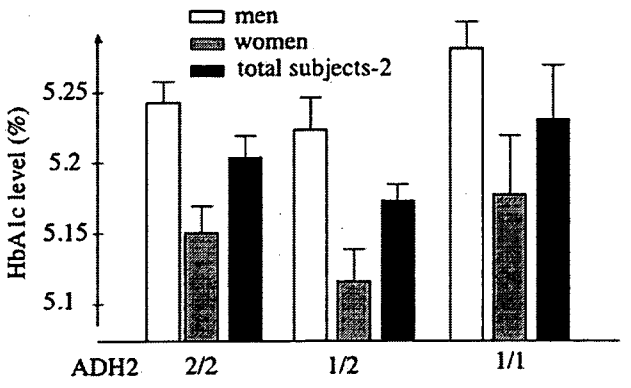

Fig. 3 Correlation of *ADH2* genotype with HbA1c level in subject-2. A significant difference was not found between the three groups. However, the HbA1c level showed a U-shaped relationship as if correlated to the insulin level

Next, we tried to focus on the *ADH2*'s genetic effects on the insulin–glucose axis. Because alcohol produces complicated effects, it is generally difficult to distinguish the genetic effects from the influence of alcohol drinking behavior. Interestingly, alcohol consumption or percentage of drinkers did not differ between the *ADH2*1/2* and *ADH2*2/2* groups (Tables 1, 2, 3 and Fig. 1a, b). This enabled us to compare the insulin concentration, dependent upon the difference in ADH2 activity itself, based on the *ADH2* polymorphism, almost independently from alcohol intake. Among subjects-2, we found that fasting insulin concentrations were significantly lower in the men and total subjects-2 with the *ADH2*1/2* genotype than those with the *ADH2*2/2* genotype (Table 4 and Fig. 2). A similar trend was seen in women, suggesting that this trend is reproducible irrespective of gender.

Thus, this study suggests that *ADH2*1* has a biphasic effect on the insulin concentration, a lowering effect with *ADH2*1/2*, and a raising effect with *ADH2*1/1* on excess alcohol intake. Interestingly, the average levels of HbA1c in subjects-2 tended to be lower in the *ADH2*1/2* group than the *ADH2*1/1* or *ADH2*2/2* groups. These two parameters seem to exhibit a U-shaped relationship (Figs. 2, 3). In nondiabetic subjects, a low insulin concentration together with a low HbA1c level usually coincides with low insulin resistance. Therefore, the above relationship suggests that light-to-moderate drinkers with the *ADH2*1* allele are likely to have reduced insulin resistance. Interestingly, this coincides with numerous other observations (Conigrave et al 2001; Hu et al 2001; Tsumura et al 1999) in terms of the notion that light drinking could benefit glucose tolerance.

Alcohol dehydrogenase catalyzed the first step in the metabolism of ethanol but has a wide range of substrates, including both aliphatic and aromatic alcohols, aldehydes, sterols, and ω-hydroxy fatty acids. We previously reported that, in the same population study, the *ADH2*1* allele is associated with increased levels of LDL-cholesterol and high blood pressure, and an increased risk of cerebral infarction (Suzuki et al 2004). The concentration of insulin or resistance to insulin could be affected by sex hormones, sex hormone-binding globulin or obesity (Falkner et al 1999; Collison et al 2000). Therefore, as another possibility, the interaction of the *ADH2*1* allele with several hormones associated with sex or lipids may decrease the insulin resistance in target tissues (Harada et al 1998).

However, in this study, the prevalence of diabetes did not differ among the three *ADH2* genotypes in subjects-1. Therefore, the effect of *ADH2* genotype on insulin resistance may be so mild or complex that it did not influence the prevalence of diabetes in the community-dwelling Japanese population. Alternatively, since all of the subjects whose FPG levels were higher than 110 mg/dl were not confirmed by the oral glucose tolerance test, if the subjects who had postprandial hyperglycemia had been included in subject-1, the result could have been different. To clarify this, a further study will be needed.

It is well known that drinking behavior is influenced more by *ALDH2* (aldehyde dehydrogenase 2) genotype than *ADH2* genotype (Higuchi et al 1996). However, although a similar investigation was performed on the correlation between *ALDH2* genotypes and their phenotype, no genetic effect of *ALDH2* was found in insulin–glucose axis and liver dysfunction (Ohsawa et al 2003). Thus, amounts of alcohol consumed would not simply depend upon insulin level.

In conclusion, this is the first paper to propose an effect of *ADH2* genotype on insulin concentrations in the Japanese. The effect seems small, although it was statistically significant due to the large number of subjects. The effect is possibly too small to have a significant bearing on the prevalence of diabetes. However, this finding provides several insights into the complex relationship between alcohol metabolism, genetic background, change in alcohol drinking behavior, the insulin–glucose axis, and the prevalence of diabetes and liver dysfunction.

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