Apolipoprotein E, Methylenetetrahydrofolate Reductase (MTHFR) Mutation and the Risk of Senile Dementia
—An Epidemiological Study Using the Polymerase Chain Reaction (PCR) Method

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We examined apolipoprotein E (Apo E) polymorphism and methylenetetrahydrofolate reductase (MTHFR) 677 C to T mutation by using the polymerase chain reaction (PCR) method in 100 elderly Japanese aged 60 or more, and assessed whether these genetic factors are associated with an increased risk for the clinical phenotypes of senile dementia, Alzheimer’s disease (AD) and vascular dementia (VD) by cross-sectional survey.

It was found that the Apo E ε 4 allele were associated with an increased prevalence of AD as previously reported. Although, it was not strongly related to the severity of senile dementia, a weak association between the ApoE genotype and the severity of dementia was suggested. The proportion of patients with senile dementia was higher in the group of carriers of MTHFR mutation than in the group of noncarriers. Furthermore, the proportion of male patients with senile dementia was higher in the group of homozygous for the mutation (+/+ ) than the group without the mutation (-/- ). Notably in VD patients, 5 of 7 males had the +/+ genotype.

The results suggest that the ApoE ε 4 genotype and the MTHFR mutation are associated with the clinical phenotype and the clinical onset of senile dementia.

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vascular dementia, Alzheimer’s disease, apolipoprotein E, methylenetetrahydrofolate reductase, polymerase chain reaction (PCR)

INTRODUCTION

In Japan the proportion of elderly people over 65 years old is about 16% and increasing. The prevalence of senile dementia among the total population aged 65 or more in 1995 was about 6.9% 1), which represents a social problem. Vascular dementia (VD) is the most common form of senile dementia in Japan, with Alzheimer’s disease (AD) next. Individuals affected with either or both of these diseases account for at least 80% of patients with senile dementia 2). The cause of VD is cerebrovascular atherosclerosis, whereas the cause of AD remains definitively unknown. However, recent molecular investigations have elucidated the genetic factors that play a significant role in the development of AD and vascular atherosclerosis 3,4. A close relation between apolipoprotein-E (apoE) alleles and AD has been reported by many investigators 5,6. It has also been reported that the C to T transition of 5, 10- Methylenetetrahydrofolate reductase (MTHFR) gene at nucleotide position 677 results in a decrease of enzymatic activity and increase of the plasma total homocysteine level, and that hyperhomocysteinemia is a risk factor for atherosclerotic cerebrovascular and coronary heart diseases 7,8. Since the association of atherosclerosis with senile dementia is worthy of consideration in not only VD but also AD, we examined both the apoE polymorphism and the MTHFR mutation in elderly people and assessed whether these genetic factors increase the risk for and modify the clinical phenotype of AD and VD.
MATERIALS AND METHODS

We investigated 100 elderly persons (25 men and 75 women) over 60 years old (average age: 76.8 ± 9.26 SD), who comprised residents of a special nursing home for the aged and the patients who were referred to the two hospitals for senile dementia or other diseases. Consent for participation in the study was provided by the subjects themselves or their legal guardians. Dementia was defined using the Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R) referring to the diagnosis of Computed Tomography (CT) or Magnetic Resonance Imaging (MRI). The grade of severity of dementia was diagnosed with the Hughes' clinical dementia rating (CDR). Among the subjects, 33 (average age: 73.0 ± 9.90 SD) were diagnosed as not having senile dementia and the residual 67 (average age: 78.6 ± 8.40 SD) were diagnosed as senile dementia. 35 patients had vascular dementia (VD, average age: 81.3 ± 6.41 SD), 24 had Alzheimer's disease (AD, average age: 75.7 ± 9.37 SD), and the residual eight corresponded to the mixed type of senile dementia (average age: 75.5 ± 9.96 SD). With informed consent for genetic examination, we took venous blood samples from all subjects and stored them separately at -80°C in 0.5-ml aliquots until the DNA analysis. Leukocyte DNA was obtained using the DNA Extractor WB Kit (Sodium iodide method, Wako).

For isotyping of apolipoprotein E, leukocyte DNA samples were amplified by polymerase chain reaction (PCR) in a DNA thermal Cycler (Perkin Elmer) using oligonucleotide primers F4 (5'-ACAGAATTCGCCCCGGCCTGGTACAC3') and F6 (5'-TAAGCTTGGCAACGGGCTGTCACAAGGA-3') as previously described 10, 11. Each amplification reaction contained about 0.8 ~ 1 μl of leukocyte DNA, 17.2 pmol of each primer, 10% dimethyl sulfoxide, and 1.5 units of AmpliTaq DNA polymerase (Perkin Elmer) with PCR buffer and dNTP mixture in a final volume of 30 μl. The PCR reaction had an initial melting temperature of 95°C (5 min.) followed by 30 cycles of melting (95°C, 1 min.), annealing (60°C, 1 min.), extension (70°C, 2 min.), and denaturation (70°C, 10 min.). After PCR amplification, 5 units of HhaI (Wako, Nippon gene) was added directly to each reaction mixture for digestion of the apoE sequences (>3 hours at 37°C). Each digestion product was loaded onto 10% polyacrylamide gel (15 × 15 cm, 1-mm thick), electrophoresed at 30 mA for 1 hour and 20 minutes, stained with ethidium bromide, and visualized by UV light. HhaI cut the PCR products of ε 3 to generate 91-bp and 48-bp fragments. Fragments of 72-bp and 48-bp are produced in ε 4, and fragments of 91-bp and 83-bp are produced in ε 2. The genotypes were classified into 2/2, 2/3, 2/4, 3/3, 3/4 and 4/4.
To detect the mutant allele of Methylenetetrahydrofolate reductase (MTHFR), the same leukocyte DNA samples were examined. The PCR procedure was basically the same with for the isotyping of apoE, but the primers used for the analysis were 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and 5'-AGGACGGTGCGGAGTTGATG-3', and the restriction enzyme HinfI was used to identify the mutation, as described by Froost et al. These primers generate a fragment of 198bp. The substitution creates a HinfI recognition sequence which digests the 198-bp fragment into 175- and 23-bp fragments. As the latter fragment has been run off, the existence of a 175-bp fragment indicates the mutant allele. The mutant allele was designated as "+", and the wild-type that means absence of mutant allele was designated as "-". The three possible genotypes are +/+ homozygosity, +/- heterozygosity, and -/- homozygosity (Fig.2).

All the subjects were classified into Apo E ε 4-carriers or non-carriers. According to the three types of MTHFR genotypes, they were divided into "+/+", "+-/" and "-/-". Each group was examined by the proportion and the composition of senile dementia, average age, average age of onset, severity of dementia (CDR) and the rate of decline on dementia (CDR/years from onset of dementia). The difference in these information between the different genotypes of apoE and MTHFR were statistically analyzed with the Yates-corrected chi-square test, Welch's t-test, Pearson's correlation coefficient, and the multiple regression analyses using the computer software HALBAU (N88-Japanese BASIC) and Statistical Package for Social Sciences (SPSS, 7.5J).

**RESULTS**

The frequencies of Apo E genotypes and the frequencies of ε 4 carriers and noncarriers among non-dementia subjects, patients with AD, patients with VD, and patients with other forms of dementia are shown in Table 1. The frequency of ε 4 carriers was 12% in non-dementia, 58% in AD, 23% in VD, and 13% in mixed type. As shown in previous reports, the frequency of ε 4 carriers was significantly higher in AD patients than in both non-dementia (Odds ratio: 9.05, 95% confidence interval: 2.53-32.16, p<0.001) and VD patients (Odds

![Figure 2. Polyacrylamide gel is showing the polymerase chain reaction (PCR) products from amplification and restriction of the mutation region of MTHFR. HinfI digests the 198-bp fragment into 175-and 23-bp fragments. The 23-bp fragment was run off the gel. All three possible genotypes(+/-, +/- and -/-) are shown.](image-url)
Table 1. ApoE genotype and frequencies of ε 4 carriers and noncarriers.

| ApoE genotype | Non-dementia | AD     | VD     | Mixed type | Total |
|---------------|--------------|--------|--------|------------|-------|
| 4 / 4         | 0 / 33       | 2 / 24 | 0 / 35 | 0 / 8      | 2 / 100 |
| 3 / 4         | 4 / 33       | 12 / 24| 7 / 35 | 1 / 8      | 24 / 100|
| 3 / 3         | 27 / 33      | 10 / 24| 26 / 35| 7 / 8      | 70 / 100|
| 2 / 4         | 0 / 33       | 0 / 24 | 1 / 35 | 0 / 8      | 1 / 100 |
| 2 / 3         | 2 / 33       | 0 / 24 | 1 / 35 | 0 / 8      | 3 / 100 |
| 2 / 2         | 0 / 33       | 0 / 24 | 0 / 35 | 0 / 8      | 0 / 100 |

Frequency
ε 4 carriers 4 / 33 14 / 24 8 / 35 1 / 8 27 / 100
Non-ε 4 carriers 29 / 33 10 / 24 27 / 35 7 / 8 73 / 100

Table 2. Numbers of the phenotypes of senile dementia in Apo E ε 4 carriers and noncarriers.

|                  | ε 4 carriers | noncarriers | OR | 95% CI | p values |
|------------------|--------------|-------------|----|--------|----------|
| Total            | 27           | 73          |    |        |          |
| Non-dementia     | 4            | 29          |    |        |          |
| Dementia         | 23           | 44          | 3.46 | 1.14 - 10.49 | 0.03* |
| AD               | 14           | 10          | 9.05 | 2.53 - 32.16 | 0.0006** |
| VD               | 8            | 27          | 2.03 | 0.58 - 7.12 | 0.4    |
| Mixed type       | 1            | 7           | 1.31 | 0.18 - 9.82 | 1.0    |

OR: odds ratio
95% CI: 95% Confidence Interval
p values derived from Yates-corrected chi-square test.
* p<0.05, **p<0.001

The types of senile dementia in ε 4 carriers and non carriers are shown in Table 2. The prevalence of dementia was higher in the carriers (23/27) than in the noncarriers (44/73) (p<0.05). The difference was remarkable especially in the prevalence of AD (p<0.001). Table 3 shows the average age, average age of onset and the severity of senile dementia in ε 4 carriers and noncarriers. The average age of the ε 4 carriers in non-dementia was higher than that of noncarriers (p=0.05). In respect of the grade of severity of dementia, the average Hughes' clinical dementia rating (CDR) was higher in the carriers than in the noncarriers (p=0.07). We also examined the CDR per year from onset as the rate of decline. The average score of the CDR per year from onset was lower in the noncarriers (p=0.09). These results suggest that there is also an association between Apo E ε 4 and severity of senile dementia.

The frequencies of the three MTHFR genotypes are shown in Table 4. In our series, the frequencies of the MTHFR mutation in non-dementia were as follows: +/+ genotype, 15%, +/− genotype, 45%, −/− genotype, 39%, and these were similar to previous reports on both Japanese 12 and French-Canadian 9 populations. The MTHFR mutation was not associated with the senile dementia. Table 5 shows the odds ratio and 95% confidence interval for the phenotype of senile dementia in carriers and noncarriers of MTHFR mutation. The proportion of patients with senile dementia was slightly higher in the group of carriers. OR was higher in AD than VD patients. This suggests that the MTHFR mutation was more closely associated with the onset of AD than VD. The types of senile dementia and the different MTHFR alleles are shown in Table 6. The frequency of the +/+ genotype was higher and −/− genotype lower among the AD and VD than non-dementia subjects. The types of senile dementia and the different MTHFR alleles by sex are shown in Table 7. The prevalence of male patients with dementia was higher in the +/+ genotype (7/8) than in the −/− genotype (1/7) (p<0.05). The difference was more remarkable in the prevalence of VD patients (p=0.05) than AD patients (p=0.16), showing that 5 of 7 male patients with VD had the +/+ genotype. On the other hand, in the female patients there were no significant differences.

The average age, average age of onset and the severity of senile dementia for the different MTHFR alleles are shown in Table 8. The average age was higher for the +/+ group than −/− group among non-dementia subjects (p<0.05). In respect to the grade of severity of dementia, the CDR score among the AD patients was higher in the −/− than +/+
Table 3. Average age and the severity of senile dementia in carriers of ε 4 and noncarriers.

|                | ε 4 carriers | Non-carriers | p values |
|----------------|--------------|--------------|----------|
| Average age    |              |              |          |
| Total          | 78.3 ± 8.14  | 76.2 ± 9.36  | 0.3      |
| Non-dementia   | 78.3 ± 3.30  | 72.3 ± 10.31 | 0.05 *   |
| Dementia       | 78.9 ± 8.38  | 78.8 ± 8.29  | 1.0      |
| AD             | 76.1 ± 7.59  | 75.2 ± 11.86 | 0.8      |
| VD             | 83.9 ± 7.74  | 80.6 ± 5.93  | 0.3      |
| Average age of |              |              |          |
| onset          |              |              |          |
| Dementia       | 73.4 ± 8.49  | 74.4 ± 7.90  | 0.7      |
| AD             | 72.1 ± 7.36  | 70.9 ± 10.89 | 0.8      |
| VD             | 78.0 ± 6.74  | 76.4 ± 5.82  | 0.6      |
| Average CDR    |              |              |          |
| Dementia       | 2.26 ± 0.81  | 1.85 ± 0.88  | 0.07 *   |
| AD             | 2.36 ± 0.74  | 1.95 ± 1.01  | 0.3      |
| VD             | 2.00 ± 0.93  | 1.72 ± 0.82  | 0.5      |
| CDR/year from  |              |              |          |
| onset          |              |              |          |
| Dementia       | 0.79 ± 0.67  | 0.52 ± 0.36  | 0.09 *   |
| AD             | 0.88 ± 0.73  | 0.59 ± 0.36  | 0.2      |
| VD             | 0.69 ± 0.59  | 0.47 ± 0.26  | 0.4      |

* p<0.1

p values derived from Welch’s t-tests.

Table 4. Frequencies of MTHFR genotypes.

| MTHFR | Non-dementia | AD | VD | Mixed type | Total |
|-------|--------------|----|----|------------|-------|
| − / − | 0.39 (13/33) | 0.17 (4/24) | 0.26 (9/35) | 0.25 (2/8) | 0.28 (28/100) |
| + / − | 0.45 (15/33) | 0.58 (14/24) | 0.49 (17/35) | 0.63 (5/8) | 0.51 (51/100) |
| + / + | 0.15 (5/33)  | 0.21 (6/24)  | 0.26 (9/35)  | 0.13 (1/8) | 0.21 (21/100) |

Table 5. Odds ratios for the phenotype of senile dementia and sex ratio in carriers and non-carriers of MTHFR mutation.

|                   | Carriers of mutation | Non-carriers of mutation | OR   | 95%CI     | p values |
|-------------------|----------------------|--------------------------|------|-----------|----------|
| No.               |                      |                          |      |           |          |
| Total             | 72                   | 28                       |      |           |          |
| Non-dementia      | 20                   | 13                       |      |           |          |
| Dementia          | 52                   | 15                       | 2.25 | 0.83 - 6.14 | 0.1      |
| AD                | 20                   | 4                        | 3.25 | 0.79 - 14.39 | 0.1      |
| VD                | 26                   | 9                        | 1.88 | 0.60 - 6.00 | 0.3      |
| Mixed type        | 6                    | 2                        | 1.95 | 0.30 - 16.6 | 0.7      |

OR: odds ratio
95% CI: 95% Confidence interval
p values derived from Yates-corrected chi-square test
Table 6. Numbers for the phenotype of senile dementia in different MTHFR genotypes.

|  | ++ | +/- | -/- | OR ++ vs. -/- | 95% CI | p values ++ vs. -/- | 95% CI |
|---|---|---|---|---|---|---|---|
| No. | | | | | | | |
| Total | 21 | 51 | 28 | | | | |
| Non-dementia | 5 | 15 | 13 | | | | |
| Dementia | 16 | 36 | 15 | 2.61 | 0.78 - 8.76 | 0.2 | |
| AD | 6 | 14 | 4 | 3.90 | 0.82 - 18.52 | 0.2 | |
| VD | 9 | 17 | 9 | 2.45 | 0.64 - 9.34 | 0.3 | |

OR: odds ratio
95% CI: 95% Confidence interval
p values derived from Yates-corrected chi-square test

Table 7. The phenotype of senile dementia in different MTHFR genotypes by sex.

|  | ++ | +/- | -/- | OR ++ vs. -/- | 95% CI | p values ++ vs. -/- | 95% CI |
|---|---|---|---|---|---|---|---|
| Men | | | | | | | |
| Non-dementia | 1 | 6 | 6 | | | | |
| Dementia (total) | 7 | 4 | 1 | 21.67 | 1.79 - 263 | 0.02** | |
| AD | 2 | 3 | 0 | 21.67 | 0.643 - 730 | 0.16 | |
| VD | 5 | 1 | 1 | 15.89 | 1.26 - 200 | 0.05* | |
| Women | | | | | | | |
| Non-dementia | 4 | 9 | 7 | | | | |
| Dementia (total) | 9 | 32 | 14 | 1.09 | 0.20 - 6.40 | 1.0 | |
| AD | 4 | 13 | 4 | 1.67 | 0.19 - 17.0 | 0.9 | |
| VD | 4 | 15 | 8 | 0.88 | 0.11 - 6.70 | 1.0 | |

OR: odds ratio
95% CI: 95% Confidence interval
p values derived from Yates-corrected chi-square test
*p=0.05
**p<0.05

group (p=0.08). Conversely, among VD patients, the CDR score was higher in the ++ than -- group.

The correlation matrix among Apo E, MTHFR subtypes and other variables was obtained only from dementia patients (n=67) and is presented in Table 9. Apo E has positive relationships with AD as well as the decline of severity of dementia (CDR/year from onset), while MTHFR ++ genotype was found to be correlated with sex of patients.

Table 10 shows the results of logistic regression analyses for each subtype of dementia. It revealed that Apo E ε 4 was a predictive factor for dementia patients (p<0.05). Specifically, Apo E ε 4 was a strong predictor for the onset of AD from this analysis (p=0.0001). On the other hand, the age of subject was the only factor associated with VD, and the VD was seen more in older patients (p<0.001).

Further, age, sex, and Apo E status were used to investigate the relationship with ++ MTHFR homozygous (Table 11). Male subjects with any subtypes of dementia were related with MTHFR (p<0.005). Interestingly, this relationship did not exist in non-dementia group.

DISCUSSION

Alzheimer disease (AD) and vascular dementia (VD), two major causes of senile dementia, have imposed a major burden on modern public health care. AD and VD, with the exception of very few familial entities with Mendelian inheritance, are regarded as multifactorial genetic disorders. Molecular investigations using the polymerase chain reaction (PCR) method have analyzed their genetic background. These approaches may be effective for the exploration of preventive methods for senile dementia.

In our study, the Apo E ε 4 allele was associated with an increased risk for AD, as currently suggested. According to Forno et al., the Apo E genotype does not strongly influence the rate of decline in AD. Our study suggested that the Apo E ε 4 allele worsens the severity of senile dementia. The effects of ε 4 need to be further investigated. Apolipoprotein
**Table 8.** Average age and the severity of senile dementia of the patients with different MTHFR genotypes.

|                  | +/+  | +/− | −/−  | p values |
|------------------|------|-----|------|----------|
| **Average age**  |      |     |      |          |
| **Total**        | 79.0 ± 9.01 | 77.0 ± 9.36 | 74.7 ± 9.36 | 0.1      |
| **Non-dementia** | 79.6 ± 3.65 | 72.2 ± 9.99 | 71.4 ± 10.90 | 0.04**   |
| **Dementia**     | 78.8 ±10.22 | 79.0 ± 8.27 | 78.8 ± 8.29 | 1.00     |
| **AD**           | 71.8 ±10.22 | 77.6 ± 9.36 | 75.0 ± 8.49 | 0.7      |
| **VD**           | 83.5 ± 8.29 | 82.1 ± 4.85 | 77.7 ± 6.10 | 0.1      |
| **Average age**  |      |     |      |          |
| **of onset**     |      |     |      |          |
| **Dementia**     | 74.5 ± 8.96 | 74.1 ± 9.36 | 73.4 ± 6.75 | 0.7      |
| **AD**           | 68.7 ± 9.56 | 73.2 ± 8.74 | 70.5 ± 8.89 | 0.8      |
| **VD**           | 78.3 ± 7.16 | 77.4 ± 5.21 | 73.9 ± 5.78 | 0.2      |
| **Average CDR**  |      |     |      |          |
| **Dementia**     | 1.81 ± 0.89 | 2.01 ± 0.89 | 2.13 ± 0.83 | 0.3      |
| **AD**           | 1.75 ± 0.88 | 2.21 ± 0.89 | 2.75 ± 0.50 | 0.08*    |
| **VD**           | 2.00 ± 0.87 | 1.74 ± 0.92 | 1.66 ± 0.71 | 0.4      |
| **CDR/year from** |     |     |      |          |
| **onset**        |      |     |      |          |
| **Dementia**     | 0.50 ± 0.26 | 0.59 ± 0.47 | 0.78 ± 0.72 | 0.2      |
| **AD**           | 0.61 ± 0.22 | 0.65 ± 0.41 | 1.36 ± 1.21 | 0.4      |
| **VD**           | 0.46 ± 0.27 | 0.53 ± 0.43 | 0.57 ± 0.33 | 0.5      |

*p values derived from Welch’s t-tests

**p<0.05, *p<0.1

**Table 9.** Correlation Matrix for Pearson’s coefficient (N=67, dementia patients only).

|              | Apo E | MTHFR |
|--------------|-------|-------|
| **AD**       | 0.378** | 0.075 |
| **VD**       | -0.253*  | -0.023 |
| **Mixed type** | -0.169  | -0.076 |
| **MTHFR**    | 0.030 | -     |
| **ApoE**     | -     | 0.030 |
| **Age**      | -0.032 | 0.048 |
| **Sex**      | 0.154 | 0.333 ** |
| **Age of onset** | -0.056  | 0.045 |
| **CDR**      | 0.224 | -0.127 |
| **CDR/years from onset** | 0.255*  | -0.191 |

*p<0.05

**p<0.01

**Table 10.** Logistic regression analysis for subtypes of dementia (n=100).

| Variable      | Dementia | AD | VD |
|---------------|----------|----|----|
| **B and OR**  |          |    |    |
| **P**         |          |    |    |
| **P**         |          |    |    |
| **OR**        |          |    |    |
| **P**         |          |    |    |
| **OR**        |          |    |    |
| **P**         |          |    |    |

| **B** | **OR** | **P** | **B** | **OR** | **P** | **B** | **OR** | **P** |
|-------|--------|-------|-------|--------|-------|-------|--------|-------|
| Age   | 0.047  | 1.048 | 0.081 | -0.057 | 0.945 | 0.070 | 0.105  | 1.111 | 0.001** |
| Sex   | -1.12  | 0.325 | 0.053 | -1.357 | 0.258 | 0.054 | 0.297  | 1.346 | 0.634 |
| ApoE ≤ 4 | 1.47  | 3.41  | 0.022*  | 2.231 | 9.307 | 0.0001*** | -0.589 | 0.555 | 0.267 |
| MTHFR++/+ | 0.68  | 3.977 | 0.283 | 0.429 | 1.535 | 0.501 | 0.204  | 1.226 | 0.722 |

B: Regression coefficients
OR: Odds ratio
p: p value

* p<0.05, **p<0.001, ***p=0.0001
E polymorphism was originally studied as a risk factor for atherosclerosis of heart disease. Apo E is one of three common alleles of the Apo E isoforms termed ε 2, ε 3, and ε 4, and was elucidated to relate with atherosclerosis of heart disease in patients with type III hyperlipidemia. Apo E plays a key role in the transport of cholesterol from peripheral tissues to the liver; thus the modification of lipid metabolism in Apo E polymorphism can account for its relation to an increased risk for atherosclerosis. Apo E ε 4 is known to increase the levels of total cholesterol and betalipoprotein, which are responsible for atherosclerosis and eventually the development of VD. However, the mechanism by which Apo E ε 4 operates to provoke the onset and worsen the clinical symptoms of AD remains unknown. Atherosclerosis may partially play a role in the development and phenotypic modifications of AD. In fact, a study in Rotterdam suggested that both AD and VD were associated with atherosclerosis and that there was an interaction between Apo E and atherosclerosis in the etiology of AD.

The interrelation of Apo E ε 4 to other risk factors for AD also remains unclarified. Mutations of the very low density lipoprotein receptor gene, encoding one of the Apo E receptors, were reported to have a genetic association with sporadic AD. This would also suggest that impaired lipid metabolism contributes to the onset of AD.

Severe MTHFR deficiency gives rise to homocystinuria, which is characterized by neurological abnormalities and premature atherosclerotic changes with thromboembolism. The thermolabile variant of MTHFR does not create homocystinemia and neurological abnormalities. The genotypes of the thermolabile variant mutation of MTHFR, comprising +/+, +/−, and −/−, lead to significantly different enzyme thermolability as described by Frooss et al. It was reported that the frequency of the +/+, +/−, and −/− genotype in Japanese middle-aged males was 11, 54, and 35%, respectively, which is consistent with the results for a French Canadian population: 12, 51, and 37%, respectively. In the present study, the frequencies of MTHFR mutation in non-dementia were similar to those previously reported. The MTHFR mutation was initially proposed to be associated with the development of coronary artery disease. However, this proposal is controversial among some researchers. According to Catteneo et al., the homozgyous MTHFR gene mutation is not a risk factor for deep-vein thrombosis but increases the risk associated with factor V, a common risk factor for deep-vein thrombosis. A study by Rees et al. suggested that neither factor V nor thermolabile MTHFR were risk factors for premature death among the very elderly.

The possible association of the MTHFR mutation with vascular diseases led us to hypothesize that it is a candidate for a risk factor for VD and AD. A causal relation between the MTHFR mutation and the onset of AD and VD was suggested in our study and the grade of severity of dementia was paradoxically higher in the −/− than +/+ group. There was no strong association between the MTHFR mutation and senile dementia; nevertheless, the mutation was considered to modify the clinical phenotype of senile dementia. Perhaps the most remarkable result from our study was that the proportion of male patients with senile dementia was higher in the group homozygous for the mutation (+/+ ) than in the group without the mutation (−/−). This suggests that the MTHFR mutation is more influential in men than women in causing the onset of VD. In the Hisayama study by Yoshitake et al., the incidence of VD was higher for men than for women in Japan. Deloughey et al. reported that the mean homocysteine levels increased by MTHFR homozygotes in the vascular disease subjects were significantly higher than in healthy age-matched subjects and it was slight more apparent for men. But there were no reports for the MTHFR genotypes by sex.
Therefore, further epidemiological study is necessary to establish the role of the MTHFR mutation as a risk factor for senile dementia. Alzheimer's disease (AD) is the major cause of dementia in most western countries, whereas vascular dementia (VD) is more common in Japan. According to the Honolulu-Asia aging study, the prevalence of AD among elderly Japanese-American men in Hawaii was higher than that in elderly Japanese men, and was comparable to that in an European-ancestry population. These observations suggest that environmental factors play a major role in the onset and clinical variations of senile dementia in addition to genetic risk factors. It can be readily understood that vascular atherosclerosis and eventual VD are affected by life style such as alcohol consumption and smoking. The proposed relation of lipid metabolism to AD, as mentioned above, also implies the importance of life style in preventing the onset of AD.

Our study revealed that Apo E may be a risk factor and a phenotypic modification factor for senile dementia, and MTHFR is slightly associated with the onset of senile dementia, especially among men. These results suggest that cerebral atherosclerosis contributes to the development of not only VD, but also AD. Investigations on a larger number of senile dementia patients will elucidate the relation of these genetic factors to senile dementia in more detail. Through this research, a way to prevent senile dementia might be found.

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REFERENCES

1. Miyanaga K. Changes in the disease which cause dementia-comparison between Japan and the world. J Sen Dement, 1999;13:129-141 (in Japanese).
2. Tanzi RE, George-Hyslop PH, Haines JL et al. The genetic defect in familial Alzheimer's disease is not tightly linked to the amyloid beta-protein gene. Nature, 1987;329:156-157.
3. Kamino K, Orr HT, Payami H, Wijsman EM et al. Linkage and mutational analysis of familial Alzheimer disease kindreds for the APP gene region. Am J Hum Genet, 1992;51:998-1014.
4. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene Dose of Apolipoprotein E Type 4 Allele and the Risk of Alzheimer's Disease in Late Onset Families. Science, 1993; 261: 921-923.
5. Poirier J, Davignon J, Bouthillier D, et al. Apolipoprotein E polymorphism and Alzheimer's disease. Lancet, 1993; 342:697-699.
6. Saunders AM, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele ε4 with late-onset familial and sporadic Alzheimer's disease. Neurology, 1993; 43: 1467-1472.
7. Froos R, Blom HI, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase, Nature Genet, 1995; 10: 111-113.
8. Wilcken DEL, Wang XL, Sim AS, et al. Distribution in healthy and coronary population of the methylenetetrahydrofolate reductase (MTHFR) C677 T mutation: Arteriosclerosis, thrombosis, and Vascular Biology, 1996; 16,878-882.
9. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised. Washington, DC: American Psychiatric Association, 1987.
10. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with Hind J. Lipid Res, 1990; 31:545-548.
11. Emi M, Wu LL, Robertson MA, et al. Genotyping and sequence analysis of apolipoprotein E isoforms. Genomics, 1988; 3: 373-379.
12. Nishio H, Lee MJ, Fujii M, et al. A common mutation in methylenetetrahydrofolate reductase gene among the Japanese population. Jpn J Human Genet, 1996; 41:247-251.
13. Forno GDF, Rasmusson DX, Brandt, et al. Apolipoprotein E genotype and rate of decline in probable Alzheimer's disease. Arch Neurol. 1996;53:345-350.
14. Zannis VI, Breslow JL. Human VLDL Apo E isoprotein polymorphism is explained by genetic variation and post-translational modification. Biochemistry, 1981; 20:1033-1041.
15. Breslow JL, Zannis VI, SanGiacomo TR, et al. Studies of familial type III hyperlipoproteinemia using as a genetic marker the Apo E genotype E2/2. J Lipid Res, 1982; 23: 1224-1235.
16. Boerwinkle E, Visvikis S, Welsh D, et al. The use of measured genotype information in the analysis of quantitative phenotypes in man. II. The role of the apolipoprotein E polymorphism in determining levels, variability, and covariability of cholesterol, betalipoprotein, and triglyceride in a sample of unrelated individuals. Am J Med Genet, 1989; 27:567-582.
17. Hoffman A, Ott A, Breteler MMB, et al. Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam study. Lancet, 1997; 349:151-154.
18. Okuizumi K. Genetic association of very low density
lipoprotein (VLDL) receptor gene with sporadic Alzheimer's disease, Nature Genet. 1995; 11:207.
19. Kang SS, Wong PWK, Susmano A, et al. Thermolabile methylenetetrahydrofolate reductase: An inherited risk factor for coronary artery disease. Am J Hum Genet, 1991; 48,536-545.
20. Anderson JL, King GJ, Thomson MJ, et al. A mutation in the methylenetetrahydrofolate reductase gene is not associated with increased risk for coronary artery disease or myocardial infarction. Am Colleg Cardiol, 1997;30:1206-1211.
21. Cattaneo M, Tsai MY, Buicciarelli P, Taiolo E, et al. A common mutation in the methylenetetrahydrofolate reductase gene (C677T) increases the risk for deep-vein thrombosis in patients with mutant factor V (Factor V:Q<sup>69</sup>). Arterioscler Thromb Vasc Biol. 1997; 17:1662-1666.
22. Rees DC, Liu YT, Cox MJ, et al. Factor V Leiden and thermolabile methylenetetrahydrofolate reductase in extreme old age. Thromb Haemost, 1997; 78:1357-1359.
23. Yoshitake T, Kiyohara Y, Kato I, et al. Incidence and risk factors of vascular dementia and Alzheimer's disease in a defined elderly Japanese population: The Hisayama Study. Neurology, 1995; 45:1161-1168.
24. Deloughery TG, Evans A, Sadeghi A, et al. Common mutation in Methylenetetrahydrofolate reductase. Correlation with Homocysteine metabolism and late-onset vascular disease. Circulation, 1996:94:3074-3078.
25. White L, Petrovitch H, Ross W, et al. Prevalence of dementia in older Japanese-American Men in Hawaii. The Honolulu-Asia aging study. J Am Med Association, 1996;276(12):955-960.