Table 5. The multiple logistic regression analysis on obesity defined as waist circumference 85+ cm in the 4th quartile.

Explanatory variables	Adjusted odds ratio(95% confidence interval)
Presence of the polymorphism of *ADRB3*	3.37 (1.12-10.16)
Age (per year)	1.04 (1.00-1.08)
Smoking (smoker/non-smoker)	0.48 (0.17-1.37)
Physical activity (per 1000 METs·minutes)	0.76 (0.40-1.46)

Presence of the polymorphism of *ADRB3*, age, smoking, and physical activity were considered as variables.

DISCUSSION

In this study, we classified the subjects into quartiles according to energy intake based on the replies to the FFQ. The values for energy intake based on the FFQ in this study were slightly lower than the National Nutrition Survey in 2002,[22] which were based on diet records. The discrepancy is thought to be due to a failure to list all food items consumed by the subjects in the period due to the limitation on the number of food items listed in the FFQ. The FFQ has been reported to underestimate the absolute level of consumption of nutrients and food groups compared to diet records.[23,24] However, it has been also reported that while the FFQ underestimates the absolute levels of consumption of nutrients and food groups, it is reasonably valid in ranking subjects and classifying them into quintiles according to the consumption of many nutrients and food groups.[23] Furthermore, the percentages of complete agreement, adjacent agreement, and complete disagreement according to tertile classification of daily energy intake based on diet records and FFQ have been found to be 56%, 36%, and 7%, respectively.[24] Thus, while energy intake based on the FFQ may only be a semi-quantitative index, it can be concluded to be an accurate means of classifying subjects into quartiles.

When the subjects were classified into two groups according to presence of the polymorphism, the total cholesterol values and PFC ratio (protein) of the subjects with the polymorphism were significantly lower than those of subjects without the polymorphism. Even if the function of the ADRB3 is considered,[7] these results are of unexplained origin.

The trend test showed progressive increases in waist circumference and BMI in the quartiles that paralleled increased levels of energy intake (Table 2). Furthermore, when subjects with a waist circumference 85+ cm [17] were defined as obese, the ratio in the group with the polymorphism was slightly but not significantly lower in the 1st and 3rd quartile, significantly lower in the 2nd quartile, and significantly higher in the 4th quartile (Table 3). These results indicate that increased energy intake may increase the risk of obesity without regard to the presence of the polymorphism of *ADRB3* and indicate that the combination of high energy intake and the presence of the polymorphism of *ADRB3* make this polymorphism a risk factor for obesity. Moreover, these results indicate that the combination of proper energy intake and the presence of the polymorphism of *ADRB3* may tend to reduce the risk of obesity. The hypothesis that the presence of the polymorphism of *ADRB3* alone does not affect the risk of obesity is supported by the finding that when the subjects were classified into two groups according to presence of the polymorphism, no significant difference in waist circumference or BMI was seen between the subjects with and without the polymorphism (84.1±9.19 vs 83.5±9.57, and 23.4±3.20 vs 23.2±3.47, respectively) (Table 1).

The results of the multiple logistic regression analysis showing that the presence of the polymorphism of *ADRB3* increases the risk of obesity in the 4th quartile alone (Table 5) also suggests interaction between high energy intake and the polymorphism of *ADRB3*.

A study comparing BMI, waist circumference, and waist-hip ratio (WHR) in regard to their respective associations with accumulation of abdominal visceral adipose tissue showed that the highest significant positive correlation was between waist circumference and abdominal visceral adipose tissue area measured by computed tomography in both men and women (r=0.77 and 0.87, respectively).[25] When obesity was defined according to waist circumference not BMI in the present study, the interaction between the polymorphism of *ADRB3* and high energy intake was found to increase the risk of obesity. This finding indicates that the polymorphism of *ADRB3* is associated with visceral-fat obesity, which is supported by the finding that in humans *ADRB3* is predominantly expressed in visceral fat.[6] The polymorphism of *ADRB3* has previously been reported to be associated with visceral-fat obesity,[13,26] and the results of the present study do not contradict these reports.

Adipose tissue functions as a secretory tissue producing various adipocytokines including leptin, tumor necrosis factor-alpha (TNF-α), plasminogen activator inhibitor type I (PAI-1), and adiponectin.[27-30] It is reported that adiponectin has anti-diabetic,[31] anti-atherogenic,[32] and anti-inflammatory biofunctions,[33] and that the plasma levels of adiponectin decreased with the accumulation of visceral adipose tissue.[34] It is also reported that intra-abdominal fat area was significantly associated with all of the metabolic syndrome criteria, including blood pressure, waist circumference, HDL cholesterol, triglyceride level, and fasting plasma glucose level, [35] independent of insulin sensitivity and subcutaneous fat area. In addition intra-abdominal fat area was independently associated with development of metabolic syndrome (adjusted OR=2.43, 95% CI=1.33-4.47).[36] Moreover, in the study based on 11 prospective European cohort studies involving 6156 men and 5356 women without diabetes, the overall hazard ratios for all-cause and cardiovascular disease mortality in subjects with the

metabolic syndrome compared with those without the syndrome were 1.44 (95% CI=1.17-1.84) and 2.26 (95% CI=1.61-3.17) in men and 1.38 (95% CI=1.02-1.87) and 2.78 (95% CI=1.57-4.94) in women after adjustment for age, blood cholesterol levels, and smoking.[37] Therefore, it is possible that prevention or treatment of visceral fat obesity, which is said to be associated with the polymorphism of *ADRB3*, could lead to reduced mortality associated with the metabolic syndrome.

The results of the present study demonstrated that the polymorphism of *ADRB3* alone does not increase the risk of obesity and that the environmental factor of high energy intake interacts with the polymorphism and leads to the significant increase in risk of obesity. This indicates the possibility that subjects with the Trp64Arg polymorphism can avoid the increase in the risk of obesity by proper energy intake control. Therefore, the Trp64Arg polymorphism may be a factor that we should take into consideration when we aim to tailor-made prevention or treatment of obesity. However, the findings that no significant difference in waist circumference or BMI was seen between the subjects with and without the polymorphism indicate that the effect of the polymorphism in the development of obesity is rather small and that it may be difficult to carry out tailor-made prevention or treatment of obesity based on genotyping of the Trp64Arg polymorphism of *ADRB3* alone. Further studies investigating the interaction between the Trp64Arg polymorphism of *ADRB3* and other polymorphisms involved in the development of obesity, will be needed.

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ORIGINAL ARTICLE

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Assessment of tailor-made prevention of atherosclerosis with folic acid supplementation: randomized, double-blind, placebo-controlled trials in each MTHFR C677T genotype

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Abstract This study aimed at assessing the effect of folic acid supplementation quantitatively in each MTHFR C677T genotype and considered the efficiency of tailor-made prevention of atherosclerosis. Study design was genotype-stratified, randomized, double-blind, placebo-controlled trials. The setting was a Japanese company in the chemical industry. Subjects were 203 healthy men after exclusion of those who took folic acid or drugs known to effect folic acid metabolism. Intervention was folic acid 1 mg/day p.o. for 3 months. The primary endpoint was plasma total homocysteine level (tHcy). In all three genotypes, there were significant tHcy decreases. The greatest decrease was in the TT homozygote [6.61 (3.47–9.76) μmol/l] compared with other genotypes [CC: 2.59 (1.81–3.36), CT: 2.64 (2.16–3.13)], and there was a significant trend between the mutated allele number and the decrease. The tHcy were significantly lowered in all the genotypes, but the amount of the decrease differed significantly in each genotype, which was observed at both 1 and 3 months. Using these time-series data, the largest benefit obtained by the TT homozygote was appraised as 2.4 times compared with the CC homozygote. Taking into account the high allele frequency of this SNP, this quantitative assessment should be useful when considering tailor-made prevention of atherosclerosis with folic acid.

Keywords Arteriosclerosis · Homocysteine · Folic acid · Dietary supplements · Methylenetetrahydrofolate reductase (NADPH2) · Polymorphism

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Introduction

In 1969, McCully reported the vascular pathology of severe, inherited, homocysteinemia for the first time (McCully 1969). Since then, many studies on hyperhomocysteinemia have been conducted, and elevated plasma total homocysteine (tHyc) level has become an established risk factor for atherosclerotic vascular diseases in many persuasive systematic reviews (Boushey et al. 1995; Welch and Loscalzo 1998; Hankey and Eikelboom 1999). Investigations at a genetic level have also progressed and have revealed not only considerably rare mutations that cause severe inherited homocysteinemia but also common polymorphisms, which moderately elevate the homocysteine level. The latter has attracted particular attention in the field of public health because of its high allele frequency in the general population.

In the metabolic pathway of homocysteine, 5,10-methylenetetrahydrofolate reductase (MTHFR, EC 1.5.1.20) is one of the key enzymes, as well as methionine synthase and cystathionine beta synthase. In the genes of these enzymes, some single nucleotide polymorphisms (SNPs) have been identified, and many studies are elu-

cidating the profiles of these polymorphisms. Among these SNPs, a common C to T substitution at position 677 of the MTHFR gene (MTHFR C677T polymorphism) results in an alanine to valine amino acid substitution in the protein and leads to a 30% decrease of enzyme activity in the heterozygote and a 60% decrease in the homozygote (Frosst et al. 1995). This homozygote (TT genotype) was known to cause mild hyperhomocysteinemia more frequently than the major allele homozygote. Furthermore, its allele frequency approaches 30% in many ethnic groups (Wilcken 2003). Thus, in the present study, we focused our attention upon this SNP as the target for tailor-made prevention of atherosclerosis.

There was uncertainty over whether the association between MTHFR C677T polymorphism and atherosclerotic diseases actually existed. In 1998, Brattstrom et al. reported a negative result in a meta-analysis of the relative risk of vascular disease associated with the TT genotype 1.12 (0.92–1.37) (Brattstrom et al. 1998). In 2002, however, this hypothesis came to be positively supported by two persuasive meta-analyses (Klerk et al. 2002; Wald et al. 2002) with more data included. [Klerk et al. reported CHD risk as 1.16 (1.05–1.28), and Wald et al. 1.21 (1.06–1.39).] Thus, it is inferred that the MTHFR C677T TT genotype is an independent risk factor for atherosclerosis via mild hyperhomocysteinemia. In addition, B vitamin supplementation such as folic acid has been shown to lower homocysteine levels (Homocysteine Lowering Trialists' Collaboration 1998; Vermeulen et al. 2000; Wald et al. 2001). The MTHFR C677T homozygote could therefore be a target of effective prevention by folic acid supplementation from the viewpoint of high-risk approach. However, quantitative data do not currently exist regarding the degree of response to folic acid in each genotype. This study thus aimed at assessing the effect of folic acid supplementation quantitatively in each MTHFR C677T genotype and considered the efficiency of tailor-made prevention of atherosclerosis in genotype-stratified, randomized, double-blind, placebo-controlled trials prepared in concordance with the CONSORT statement (Moher et al. 2001).

Materials and methods

Study subjects

Since we intended to examine the effect of folic acid supplementation as the primary prevention against atherosclerosis, we needed to recruit healthy people. Considering the higher incidence and mortality of atherosclerotic diseases in men than women, we focused on healthy men only. After the approval of the ethical committee of Keio University School of Medicine, we obtained the written informed consent from 210 healthy men for participation in the study. They were considered genetically unrelated according to the information by the company. Subjects in this trial finally consisted of 203 healthy men after exclusion of those who were taking folic acid or drugs known to effect folic acid metabolism. Mean age and body mass index (BMI) were 45.8 ± 11.5 years and 23.7 ± 3.66 kg/m^2 (mean ± SD), which are typical values in Japanese healthy male workers. The follow-up period was from December 2003 to March 2004. The flow diagram of the progress throughout the phases of randomized trials is shown in Fig. 1.

Study design

The study was designed around genotype-stratified, randomized, double-blind, placebo-controlled trials (nested RCTs). Intervention consisted of folic acid 1 mg/day per o.s. or an identical-looking placebo for 3 months since tHcy reduction is known to be maximal at a folic acid dosage of 1 mg/day (a minimum of 0.8 mg/day has been reported as necessary to achieve the maximum reduction in the tHcy level) (Vermeulen et al. 2000). The primary endpoint was the change in the plasma tHcy level, and the secondary endpoint was changes in the pulse wave velocity (PWV), ankle-brachial pressure index (ABI), and high-sensitive C-reactive protein (hsCRP) levels. All the measurements were done at baseline, 1, and 3 months after the start of intervention. As for deciding when intervention should be stopped in any subject, we made arrangements for monitoring and judging unexpected adverse events, including vitamin-B12-deficiency anemia, the masking of which is the only known side effect of folic acid supplementation. We decided that if any important adverse events judged by doctors in our group were observed, we would stop the trial quickly, and vitamin B12 levels were continuously monitored in all subjects to prevent vitamin-B12-deficiency anemia.

To generate the random allocation sequence, we used SAMPSIZE ver.2 software (Blackwell Science, London, UK) on a Windows workstation RX-65 (Sony, Tokyo, Japan). Randomization (blocking size set as four) was done after genetic stratification of three genotypes (i.e., independent three times randomization). Subjects were randomly allocated to the folic acid group and placebo group at a ratio of one to one. The doctor and nurses in the clinic had no information of the group assignment, and the nurses measuring PWV/ABI and the blood chemistry technicians also had no information of group assignment or any subject profiles. The success of blinding was evaluated by comparing the follow-up rate and the mean capsule-intake rate between both groups.

Measurements

Age and smoking status were self-reported, and medical history was acquired by interview. Height, weight, systolic and diastolic blood pressures, fasting blood glucose

level, serum lipid levels [total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol], aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (gamma-GTP), blood urea nitrogen (BUN), serum creatinine (Cr), and serum uric acid (UA) were measured in all subjects.

To monitor the intake of the folic acid or placebo capsules, we asked all participants to report the actual daily intake every month in a specific format and measured the serum folate level at baseline, 1, and 3 months after the start of intervention. We also monitored the vitamin B12 level at the same time to prevent vitamin-B12-deficiency anemia. Serum folate and vitamin B12 concentrations were measured using chemiluminescence enzyme immunoassay (CLEIA) on UniCel DxI 800 immunoassay systems (Beckman Coulter Inc., CA, USA).

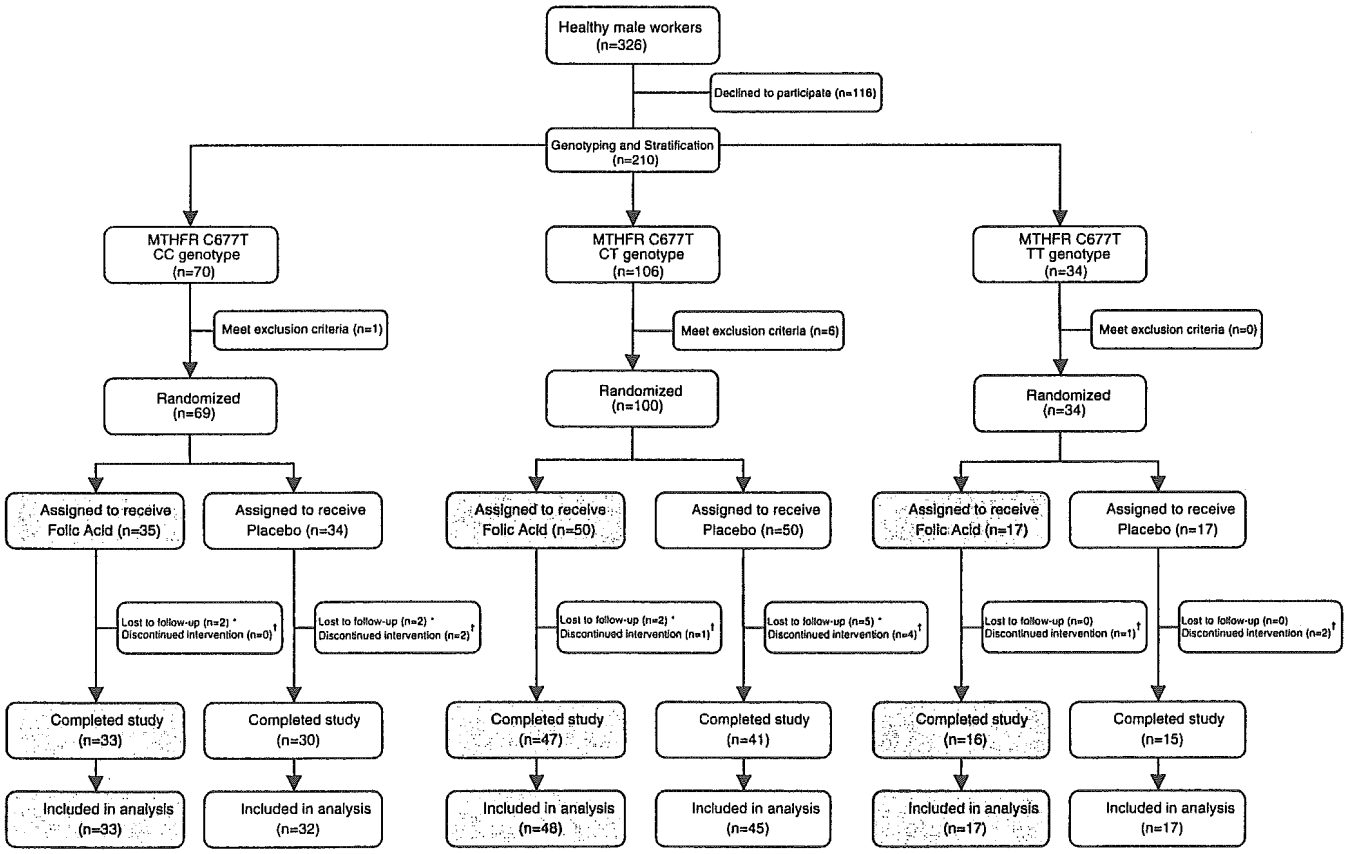
Fasting blood samples were drawn, and plasma for tHcy determination was separated with minimal delay and stored at −20°C until analysis. tHcy levels were assayed using the high-performance liquid chromatography (HPLC) method (Ubbink et al. 1991). The PWV and ABI were measured with the AT-form PWV/ABI (Nippon Colin, Aichi, Japan), which can monitor bilateral brachial and ankle pressure wave forms simultaneously using the volume plethysmographic method, with two optional tonometry sensors for carotid and femoral arterial wave measurements. The hsCRP was measured using the N-latex CRP II kit (Dade Behring Inc., IL, USA).

Genotyping for the MTHFR C677T polymorphism was performed by the polymerase chain reaction (PCR) technique and restriction fragment length polymorphism (RFLP) analysis. We designed PCR primers 5′-CCCAGCCACTCACTGTTTTAG-3′ (sense) and 5′-TGGGAAGAACTCAGCGAACT-3′ (antisense) with DNASIS Pro Ver.2.0 (Hitachi Software Engineering Co. Ltd., Tokyo, Japan). Since the C to T transition at nucleotide 677 produces a Hinf I digestion site, the amplified 469-bp product derived from the mutant gene was cleaved into 393-bp and 76-bp fragments by Hinf I (TaKaRa Bio Inc., Shiga, Japan), which leaves the wild-type gene unaffected. After electrophoresis through 6% polyacrylamide gel, the digestion products were visualized by staining with ethidium bromide.

Statistics

We calculated the sample size according to the results of our pilot trial. We estimated the standard effect size in

Fig. 1 Process during phases of the randomized trials



Overall follow up rate after 3 months : 94.6% (CC : 92.8%, CT : 93.0%, TT : 100%)

* No post randomization contact

† Discontinued intervention means insufficient mean capsule intake rate (< 70%)

each genotype (CC, CT, and TT) as 0.5, 0.55, and 0.75 by the serum folate stratified analysis of the cross-sectional data in the pilot study. The planned enrollment of 68, 56, and 32 in each genotype gave an 80% power to detect a pairwise difference, with a two-sided test at $\alpha = 0.05$.

Analysis was based on the intention-to-treat policy. The distributions of triglyceride, HDL cholesterol, AST, ALT, γ-GTP, Cr, folate, vitamin B12, tHcy, and hsCRP concentrations were all skewed and regarded as log-normal. Therefore, these data were logarithmically transformed and Student's t test was applied. As to age, since the distribution was skewed and not log-normal, Wilcoxon's rank sum test was applied. The other variables were regarded as normally distributed and Student's t test was applied. As to cross-table analysis, the chi-square test or Fisher's exact test were applied. Hardy–Weinberg equilibrium was also assessed by the chi-square test. Comparisons with the baseline level were conducted with a paired t test. Differences in the above variables among different genotype subgroups (multiple comparisons) were assessed with Tukey's post hoc analysis of variance (ANOVA). Odds ratios (OR) and 95% confidence intervals (95% CIs) were calculated using logistic regression analysis. Changes were evaluated from the baseline level in each group. All statistical analyses were performed using SPSS for Windows version 11.0 (Statistical Product and Service Solutions, IL, USA), and statistical significance was accepted for a two-tailed $P < 0.05$. Interim analysis was done at 1 month after the start of intervention.

Role of the funding source

The sponsor of this investigator-initiated project (the Ministry of Economy, Trade and Industry, Japan) had no role in study design; in collection, analysis, or interpretation of data; in writing the paper; or in the decision to submit the paper for publication.

Results

Genotyping for the MTHFR C677T polymorphism in the 210 healthy Japanese male subjects showed that 70 were homozygous for the major allele (CC genotype), 106 were heterozygous (CT genotype), and 34 were homozygous for the minor allele (TT genotype). These results were in Hardy–Weinberg equilibrium and consistent with the results previously reported in another Japanese population (Nishio et al. 1996; Lwin et al. 2002). Seven the subjects (CC 6; CT 1; TT 0) who were taking folic acid or drugs known to effect folic acid metabolism were excluded.

The baseline characteristics of the study subjects in each of the MTHFR C677T genotypes (CC, CT and TT) in the folic acid and placebo groups are shown in Table 1.

Of the 203 people who were randomly allocated into the trial groups, 11 (folic acid group four, placebo group seven) were lost during the follow-up. Two subjects complained of a chilled sensation in the hands or numbness in the fingers and stopped taking the capsules. Both were in the folic acid group. Of the remaining 192 people, ten (folic acid group two, placebo group eight) discontinued intervention (less than 70% mean capsule intake rate), and 182 completed the study. Thus, the intention-to-treat population consisted of 192 people, and the follow-up rates in the folic acid and placebo groups were 96.1% and 93.1%, respectively. The flow of participants through each stage is shown in Fig. 1. Reviewing the records on the capsule intake revealed that the mean capsule-intake compliance rates were 91.8% and 92.8% in the folic acid and placebo groups, respectively.

As shown in Fig. 2, the serum folic acid level in the folic acid group was significantly higher than in the placebo group at both 1 and 3 months after the start of intervention (both $P < 0.001$). The plasma tHcy level in the folic acid group was significantly lower than in the placebo group at both 1 and 3 months (both $P < 0.001$).

There were no significant differences between the folic acid and placebo groups in regard to hsCRP, PWV, and ABI after the intervention. There were no significant differences between groups in regard to changes from the baseline level in hsCRP, PWV, and ABI. These are the results of the intervention trial without considering genotypes.

In addition to the significant increase of folic acid and significant decrease of tHcy, there were also significant changes in the folic acid groups in all three genotypes. As shown in Table 2, the TT homozygote group showed the largest decrease in tHcy, and there was a significant linear trend between the minor allele number and the degree of tHcy decrease. The PWV decreased in folic acid group at 1 month, but the trend with the allele number was not observed, and no significant change was observed at 3 months. The hsCRP significantly decreased in the folic acid group at 3 months, but no trend was observed in the allele number. The ABI did not change significantly in either group. In each of the MTHFR C677T genotypes (CC, CT, and TT), there were significant differences between the folic acid and placebo groups in regard to the decrease of plasma tHcy and the increase of serum folic acid, but there were no significant differences between the two groups in regard to the changes in hsCRP, PWV, and ABI after the intervention.

The significant differences between the MTHFR C677T genotypes in the decrease of plasma tHcy in the folic acid group are shown in Fig. 3. The TT homozygote group showed the largest decrease in plasma tHcy at both 1 and 3 months after intervention. Based on the stable results observed twice in the time course, the effect size of tHcy reduction in the TT homozygote subjects

Table 1 Baseline characteristics in each MTHFR C677T genotype. Mean (*SD*) or geometric mean (*GSD*) are shown. *AST/GOT* aspartate aminotransferase/glutamic oxaloacetic transaminase, *ALT/GPT* alanine aminotransferase/glutamic pyruvic transaminase, *γ -GTP* gamma-glutamyl transpeptidase, *hsCRP* high-sensitive C-reactive protein, *baPWV* brachial-ankle pulse wave velocity, *ABI* ankle-brachial pressure index

	MTHFR C677T genotypes					
	CC		CT		TT	
	Folic acid group n = 35	Placebo group n = 34	Folic acid group n = 50	Placebo group n = 50	Folic acid group n = 17	Placebo group n = 17
Demographics						
Age (years)	45.5 (11.5)	46.1 (12.2)	44.9 (12.1)	46.2 (11.2)	45.2 (10.1)	48.4 (11.4)
Gender (male/female)	35/0	34/0	50/0	50/0	17/0	17/0
Body-mass index (kg/m2)	24.5 (4.98)	23.3 (2.9)	23.8 (3.33)	23.7 (3.46)	23.1 (3.2)	22.7 (3.83)
Smoking	17 (48.6%)	20 (58.8%)	29 (58.0%)	30 (60.0%)	10 (58.8%)	12 (70.6%)
Alcohol intake (g/week)	189.7 (185.0)	129.2 (179.0)	118.5 (175.8)	171.7 (214.4)	322.4 (303.6)	134.2 (152.0)
Blood pressure						
Systolic (mmHg)	136.4 (19.1)	132.5 (14.7)	137.1 (20.1)	131.5 (18.9)	135.8 (13.6)	133.7 (17.3)
Diastolic (mmHg)	83.2 (12.4)	78.5 (10.1)	83.6 (14.4)	81.0 (13.0)	85.6 (13.9)	81.4 (12.4)
Laboratory values						
Total cholesterol (mg/dl)	215.3 (34.5)	204.1 (39.0)	207.1 (42.2)	200.4 (37.5)	208.8 (41.4)	210.8 (31.3)
Triglyceride (mg/dl)	131.8 (1.82)	135.9 (1.82)	108.8 (1.66)	122.0 (1.85)	102.2 (1.48)	111.1 (1.56)
HDL cholesterol (mg/dl)	55.5 (1.27)	49.6 (1.24)	52.8 (1.27)	51.7 (1.23)	60.0 (1.27)	51.4 (1.33)
AST/GOT (IU/L)	23.4 (1.29)	22.8 (1.35)	23.5 (1.37)	24.0 (1.57)	23.7 (1.26)	21.3 (1.21)
ALT/GPT (IU/l)	26.4 (1.55)	23.0 (1.69)	27.1 (1.75)	25.7 (1.88)	22.7 (1.41)	20.6 (1.53)
γ-GTP (IU/l)	47.6 (1.92) *	34.5 (1.90)	49.0 (2.08)	38.5 (1.96)	41.5 (2.08)	30.5 (1.66)
Blood urea nitrogen (mg/dl)	13.7 (3.31) *	15.4 (2.33)	14.4 (2.83)	13.8 (2.38)	13.4 (2.75)	13.7 (3.12)
Serum creatinine (mg/dl)	0.87 (1.13)	0.90 (1.16)	0.82 (1.14)	0.87 (1.16)	0.82 (1.14)	0.81 (1.12)
Serum uric acid (mg/dl)	5.86 (1.09)	5.74 (1.16)	5.53 (1.14)	5.68 (1.20)	5.72 (1.31)	5.54 (1.03)
Serum Vitamin B_{12} (pg/ml)	548.9 (1.75)	503.7 (1.31)	483.4 (1.33)	475.2 (1.44)	468.1 (1.30)	535.6 (1.35)
Serum folic acid (ng/ml)	6.09 (1.39)	6.68 (1.44)	5.97 (1.56)	6.26 (1.72)	5.61 (1.83)	5.36 (1.50)
Outcome index						
Plasma homocysteine (μmol/l)	9.36 (1.28)	8.70 (1.23)	9.46 (1.20)	9.15 (1.25)	12.8 (1.66)	14.5 (1.72)
hsCRP(mg/dl)	0.44 (2.80)	0.51 (2.70)	0.63 (3.16)	0.48 (3.19)	0.42 (2.71)	0.49 (3.02)
baPWV (cm/s)	1410 (235.8)	1367 (231.9)	1407 (239.6)	1393 (211.5)	1381 (152.4)	1349 (228.4)
ABI (no dimension)	1.14 (0.09)	1.13 (0.09)	1.11 (0.07)	1.11 (0.08)	1.15 (0.07)	1.12 (0.08)

* $P < 0.05$ by t test for comparison with the placebo group

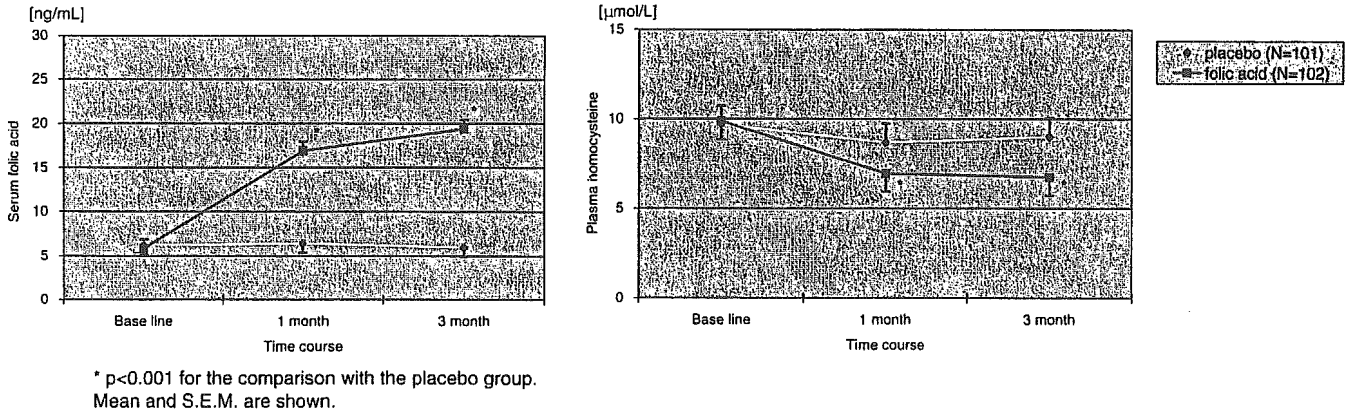


Fig. 2 Serum folic acid and plasma total homocysteine (tHcy) levels in folic acid and control groups

was estimated as 2.4× compared with the wild type. There were significant linear trends between the allele number and the decrease of plasma tHcy at 1 and 3 months (both $P < 0.01$). Both these results of the trend tests were statistically significant, even after being adjusted for age, BMI, smoking status, and alcohol consumption at 1 and 3 months (both $P < 0.01$). Tukey's post hoc test of ANOVA demonstrated a statistically larger decrease in the TT genotype group than seen in the CC and CT genotype groups (both $P < 0.01$ at 1 and 3 months).

All of the analytical strategies were prespecified, including subgroup analyses and adjustment (no ancillary analysis), and based on the intention-to-treat analysis.

Table 2 Changes of outcome variables in each MTHFR C677T genotype

	MTHFR C677T genotypes					
	CC		CT		TT	
	Folic acid group (n = 35)	Placebo group (n = 34)	Folic acid group (n = 50)	Placebo group (n = 50)	Folic acid group (n = 17)	Placebo group (n = 17)
Change in plasma homocysteine (mol/l)[a]						
After 1 month	−2.59 (2.27)**	−0.40 (1.65)	−2.64 (1.68)**	−1.02 (1.18)	−6.61 (6.42)	−2.09 (6.96)
After 3 months	−3.05 (2.13)**	−0.90 (1.51)	−2.85 (1.28)**	−0.83 (1.40)	−6.84 (8.42)*	4.87 (16.2)
Change in serum folic acid (ng/ml)[a]						
After 1 month	12.8 (5.28)**	0.23 (2.25)	11.6 (6.4)**	−0.08 (4.23)	7.85 (9.27)**	0.16 (1.33)
After 3 months	13.6 (4.42)**	−0.38 (2.45)	14.8 (4.86)**	−1.19 (4.74)	9.87 (7.94)**	−0.49 (2.22)

[a] Mean (*SD*) is indicated
*P <0.05; ** P < 0.01 for t test versus placebo group. Changes are evaluated from the baseline level in each group

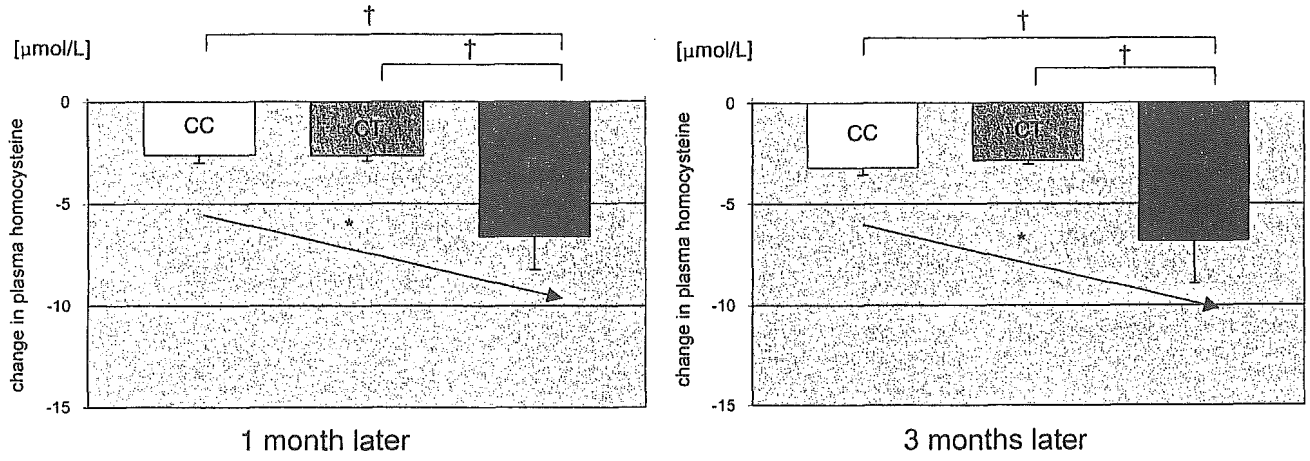


Fig. 3 Differences between MTHFR C677T genotypes in the decrease of plasma total homocysteine (tHcy) in the folic acid group

Discussion

We have shown that a significant lowering effect of tHcy was observed in the folic acid group in all the MTHFR C677T genotypes, and the degrees of lowering from the baseline level were almost the same at 1 and 3 months after the start of intervention. This indicates that tHcy lowering effect by folic acid supplementation 1 mg/day begins to plateau after 1 month or so. The main result of our study is that the effect size of tHcy reduction in the minor allele homozygote (TT genotype) is estimated to be 2.4× compared with that in the major allele homozygote. Since there are significant differences in baseline homocysteine levels between the TT genotype and the others, it is possible the baseline level per se influences the degree of change after the intervention. But the greater decrease of tHcy in the TT genotype was not due to the higher baseline level. The change was still larger in the TT genotype, even if the difference of baseline was concerned (52.5% reduction in the TT versus 30.1% in the CC and 29.0% in the CT genotype). This observation suggests that not only the folate level but also the MTHFR genotype per se influences the responsiveness to folate supplementation. Since this polymorphism is thought to change the affinity of MTHFR to folate (Guenther et al. 1999), this speculation seems reasonable from the viewpoint of molecular mechanisms.

In addition to the fact that the MTHFR C677T polymorphism is very common in many ethnic groups [allele frequency is about 0.30–0.35 (Wilcken et al. 2003)], it is more important that the mild hyperhomocysteinemia caused by this polymorphism can be improved with folic acid supplementation. Folic acid intake can easily be fortified by trying to take more green vegetables or taking supplements. Venn et al. proved the natural folate intake (350 μg folate derived from food) significantly changed the serum folate status

in a randomized controlled trial in free-living subjects (Venn et al. 2002). This interaction between the MTHFR genotype and folic acid on the tHcy level is a typical gene–environmental interaction. Thus, this polymorphism is one of the ideal targets of tailor-made prevention from the viewpoint of a high-risk approach.

Since hyperhomocysteinemia is suspected as being a risk factor for many diseases (oral clefts, Down syndrome, placenta-mediated diseases, colorectal neoplasias, Alzheimer's disease, etc.) in addition to vascular diseases and neural-tube defects (Ray and Laskin 1999; Ames 1999; Botto and Yang 2000; Ueland et al. 2001; Ames et al. 2002; Quadri et al. 2004), this information is useful not only for the prevention of atherosclerosis but also for that of other diseases when considering a tailor-made preventative approach.

As for our secondary endpoints, we could not find clear results on PWV and ABI in 3 months of folic acid supplementation. There was a significant difference in the PWV at 1 month, demonstrated by the paired t test compared with the baseline in the folic acid group (P = 0.021) but not at 3 months. The paired t test showed a significant difference in the ABI at 3 months compared with the baseline in the folic acid group (P = 0.015) but not at 1 month. Furthermore, there were no significant differences between the folic acid and placebo groups in regard to changes in PWV and ABI after the intervention.

Since the PWV and ABI are not fine indices of atherosclerosis and vascular stenosis respectively, to detect any change in such a short period seems difficult. It is compatible with the previous report by Mangoni et al. (Mangoni et al. 2002) that 4 weeks of folic acid intervention (5 mg/day) did not change the PWV significantly in healthy cigarette smokers. It is also compatible with the previous report by Vermeulen et al. that 2 years of folic acid intervention (5 mg folic acid + 250 mg vitamin B6/day) did not change the ABI significantly in healthy siblings of patients with premature atherothrombotic disease (Vermeulen et al. 2000). Via lowering the tHcy (Homocysteine Lowering Trialists' Collaboration 1998; Vermeulen et al. 2000; Wald et al. 2001) or directly improving endovascular dysfunction (Mangoni et al. 2002), folic acid may improve the PWV and ABI, but 3 months does not seem long enough to detect any significant changes. Longer observation will be needed to clarify this association in Japanese.

Although Doshi et al. reported that folic acid reduces intracellular endothelial superoxide (Doshi et al. 2001) and Nakano et al. reported folic acid protects against oxidative modification of human LDL (Nakano et al. 2001), we could not find any clear results with folic acid supplementation on hsCRP. Although there was a significant decrease in the hsCRP level in the folic acid group at 3 months (paired t test, P = 0.041) compared with the baseline level, the changes from baseline in the folic acid group and those in the placebo group were not different significantly (data not shown). As has been pointed out by Folsom et al., the B vitamin status is not presumed to be a strong correlate of circulating levels of inflammatory markers (Folsom et al. 2003).

As for generalizability (external validity), these findings were based on the results of a study of healthy Japanese male workers. Whereas the allele frequency of this polymorphism is not so different among ethnic groups, the response to folic acid can be to some extent different. In addition, the baseline level of folic acid will be a more important factor. Dividing our data into the two groups using baseline folate levels (more or less than the median value), the baseline homocysteine levels were significantly different between the TT and CC genotypes only in the lower folate group but not in the higher folate group. So, it is possible the baseline level of folic acid influences the individual response to folate supplementation. The Food and Drug Administration in the United States mandated the fortification of cereal-grain products with folic acid to prevent neural-tube defects in 1996 (effective by January 1998) (US Food and Drug Administration 1996). According to the report of Jacques et al. (Jacques et al. 1999), plasma folate level changed from 4.6 to 10.0 ng/ml before and after the fortification (No B vitamin supplements users) using the data of the Framingham Offspring Study Cohort. The baseline level of our study subjects was 6.09 ng/ml (Table 1). Although such a national intervention is rather rare in the world, the difference in the baseline level of folic acid is very important, as is shown by significant heterogeneity between the results obtained in European populations compared with North American populations in the meta-analysis by Klerk et al.(Klerk et al. 2002).

As for side effects from folic acid supplementation, nothing remarkable was observed. In the dropouts from the folic acid group, one complained of a chilled sensation in the hands and one had numbness in the fingers. Since these complaints were not reported as far as we know, the association is unclear. None of the subjects showed any sign of vitamin B12 deficiency during the serum vitamin B12 monitoring. This finding has verified that oral folic acid supplementation is quite safe, at least for 3 months or so.

In conclusion, we report that the MTHFR C677T minor allele homozygote obtained a 2.4-fold beneficial decrease in tHcy levels compared with the major allele homozygote. Considering the high allele frequency of this polymorphism, the results of this quantitative assessment should be useful when considering tailor-made prevention of atherosclerosis with folic acid.

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genetic analyses, participated in data collection, and verified all results. Haruhito Kikuchi, Izumi Takei, and Kiyoaki Watanabe advised on important intellectual content for the concept of the study. Takeo Nakayama gave much valuable advice regarding study design. Kazuyuki Omae was responsible for the study design and organization, data interpretation, and overview of the project. All authors were involved in the data interpretation and contributed to the writing of the paper. The first author (Koichi Miyaki) had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. No conflicts of interest exists, for any author.

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第24回　血小板機能シリーズ No.7

PFA-100による血小板凝集能測定

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PFA-100とは

PFA-100 (Platelet Function Analyzer)は，1995年にDade Behring社から発売された比較的新しい血小板機能評価機であり，簡便かつ高感度を特徴とする(図1)[1)-3)]．血管壁への血小板の粘着および凝集，そして血小板血栓の形成を*in vitro*で再現するとされている．その測定原理を図2に示すが，高シア状態下(5000～6000/s)キャピラリーで吸引されて若干活性化した血小板[4)]が，2種類の凝集誘起剤［コラーゲンとエピネフリン(Col/EPI)もしくはコラーゲンとADP (Col/ADP)］の塗布された膜に接触し，血小板血栓を形成することで膜孔を閉塞させる[3)]．形態観察によると，その際にできる血栓は，一部脱顆粒した血小板主体の血栓であり[5)]，血小板同士の接着にはフィブリノーゲンよりもVWFが大きな役割を果たしている[5)6)]．

Platelet rich plasma (PRP)を用いた血小板凝集機能検査の煩雑さや感度を改善するため，これまでにPA-200，RPFA，Cone-plate法といった新しい血小板機能評価法が開発されてきた．それらの中でPFA-100は，特に欧米で最も研究されている評価法の1つである．(なお，2005年8月本稿作成時，日本では販売されていない)

使用方法および正常範囲

測定は採血後4時間以内に行う．1/10容の3.8％(もしくは3.2％)クエン酸添加全血を1回の評価で800 μL使用する．専用カートリッジは使い捨てで，凝集誘起剤の組み合わせにより，Col/EPIカートリッジ，Col/ADPカートリッジの2種

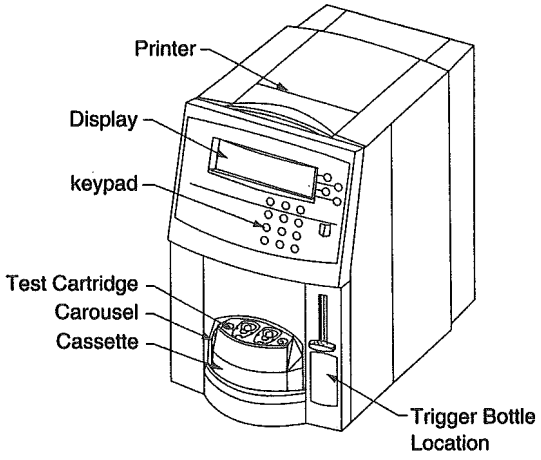

図1　PFA-100

(文献1より改変引用)

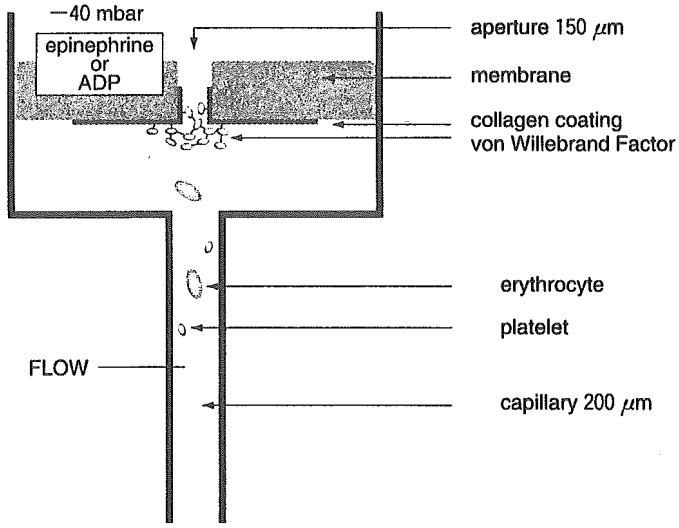

図2　カートリッジの断面図
（Dade Behring 社資料より改変引用）

類がある．最初に Col/EPI カートリッジを使用し，正常値から外れた場合，Col/ADP カートリッジを用いて原因を精査する，という使い方を製造元は提示している．

測定は非常に簡便で，特に訓練は必要ない．採血管からカートリッジに全血を移す際ピペット操作が必要であるものの，遠心分離操作の必要はなく，PRP を用いた一般的な血小板凝集検査に比べ使い勝手がよい．Dade Behring 社が発表している平均閉塞時間［90％範囲］は Col/EPI カートリッジが 124 秒［85～165 秒］，Col/ADP カートリッジが 92 秒［71～118 秒］であり，他グループの報告でも大きな違いはない[7]．なお，300 秒がカットオフ時間として規定されており，1 回の測定（2 サンプルの測定が可能）は 5 分から 15 分で完了する．試験誤差（CV％）は，duplicate 測定間で 12％（Col/EPI），10％（Col/ADP）程度である[8]．

PFA-100 の臨床応用 —出血傾向の評価法として—

出血傾向の評価にはこれまで出血時間法が汎用されているが，必ずしも出血傾向と相関せず[9]，感度の低さが問題となっている．そのため近年では本人および家族の出血歴の問診が重要視されている．

PFA-100 は一次止血を *in vitro* で再現する評価法とされており，出血時間法の代替になるかどうか検討されてきた．これまでの報告を総合すると，PFA-100 は，von Willebrand 病（VWD）のスクリーニングとして出血時間法よりも検出感度がよい．ただし，PFA-100 の結果のみで診断に用いることはできず，PT，APTT など他の止血学的検査を含め様々な試験結果からの総合的な判断が必要である．一方，デスモプレッシン（DDAVP）治療の薬効モニタリングの用途では，PFA-100 は感度がよく，その有用性が認識されている（表 1）[7][10][11]．

PFA-100 の臨床応用 —薬効モニタリング法として—

PFA-100 は，血小板機能を抑制する薬剤の薬効モニタリングとしても汎用されている．一般的な用量・用法の結果，閉塞時間に影響を及ぼすかどうか，各々の薬剤の場合について表にまとめた（表 2）．

1．アセチルサリチル酸（アスピリン®）

血小板凝集抑制剤として最も使用されている薬剤の 1 つである．アスピリンは，Col/EPI カートリッジの閉塞時間を延長させ，その一方 Col/ADP カートリッジの閉塞時間は延長させない，という報告が多い[12][13]．Col/EPI カートリッジ閉塞時間の反応性が高感度であり，血小板凝集抑制の程度を簡便に数値化できることから，服薬状況および薬効のモニタリングに有効である．また近年，適切な服用にもかかわらず Col/EPI カートリッジ閉塞時間が延長しないケースをアスピリン不応症とした研究が多い[14][26]．

2．チクロピジン（パナルジン®），クロピドグレル（Plavix® ：本邦未承認）

チエノピリジン系抗血小板薬は $P2Y_{12}$ 受容体を非可逆的に阻害する．多くの報告では Col/EPI，Col/ADP いずれのカートリッジでも閉塞時間は延長しない[15]．その一方，4 週間連続投与の結果，閉塞時間の延

表1　PFA-100による抗血小板/抗血栓作用の薬効モニタリング

薬剤名	薬効/阻害様式	Col/EPI カートリッジ	Col/ADP カートリッジ	参考文献
アスピリン	COX-1阻害	＋	－	12, 13
チクロピジン	P2Y_{12}受容体阻害	－	－	15, 18
クロピドグレル	P2Y_{12}受容体阻害	＋[16] or －[25]	＋[8] or －[25]	16, 25
アブシキシマブ	GPⅡb/Ⅲa阻害	ND	＋	17, 18
インドメタシン	COX-1阻害	＋	ND	19, 21
メロキシカム	COX-2阻害	＋[21] or －[19]	ND	19, 21
ヘパリン	抗凝固作用	－	－	7, 17, 18
ワルファリン	抗凝固作用	－	－	7

＋：閉塞時間延長，－：閉塞時間延長せず
ND：参考文献に記載なし

表2　PFA-100の臨床応用と利点/欠点

臨床応用	利点	欠点
(1)出血時間の代替法(スクリーニング)	出血時間より高感度，高信頼性，短時間で評価可能	単独で診断に用いることができない 原因疾患の特定はできない
	簡便	条件が固定されおり変更不可 2カートリッジ(2条件)のみ
	低侵襲，0.8 mL全血で評価可能	クエン酸が必要
	4時間後まで評価可能	真空採血管での採血が結果に影響を与えることがある
	VWD，血小板機能欠損に高感度	Type I VWDに偽陰性も
	ばらつきが小さい (健常人サンプルではCV＜10％)	患者サンプルではばらつき大 カットオフ値付近の評価が難しい
	高シア依存で生理的条件に近い	血管壁の影響は考慮されない
	正常値の場合，VWDおよび血小板無力症やベルナール・スーリエ症候群といった重篤な血小板機能障害の可能性を排除できる	異常値の場合，その他の試験と組み合わせて判断が必要
	薬物による血小板機能異常検出の感度が高い	異常値出現のためしばしば再検査が必要
(2)止血療法のモニタリング	DDAVPによる血管内皮由来VWF分泌に感受性有り	血小板VWFや高分子量VWFの影響有り
	血小板輸血に感受性有り	しばしば血小板数に影響する
	凝固系異常の影響を受けず血小板機能異常を評価できる	血小板機能低下時のFactor Ⅶa補充療法のモニタリング不可
(3)抗血小板薬のモニタリング	アスピリンやNSAIDsがCol/EPI閉塞時間を延長する	クロピドグレルの薬効確認ができない場合あり

(文献10より改変引用)

長がみられたとの報告もある[16]．

3．アブシキシマブ(ReoPro®：本邦未承認)

GPⅡb/Ⅲa阻害薬であるアブシキシマブは，0.25 mg/kgボーラス投与，10 μg/h 12時間持続投与の結果，Col/ADPカートリッジ閉塞時間を延長させる[17][18]．

4．インドメタシン

インドメタシンは可逆的COX-1阻害薬である．50 mgの1回投与でCol/EPIカートリッジ閉塞時間の延長が観察される[19]．

5．メロキシカム(モービック®)

血小板に普段発現しているCOXのアイソザイムはCOX-1であり，血小板におけるトロンボキサンA_2産生はCOX-1による．メロキシカ

ムはCOX-2阻害薬の1つであり，COX-1阻害作用は弱い[20]．そのため，アスピリンやインドメタシンと比べ，閉塞時間の延長は軽微[21]または延長しない[19]と報告されている．

6．ヘパリン，ワルファリン

凝固系の抑制は，上記のような抗血小板薬と異なり，閉塞時間への影響が少ないことが報告されている[7][17][18]．

既存評価系との比較

VWD検出の目的では，PFA-100はテンプレート出血時間法よりも高感度にVWDを検出できる[7][12][22]．これは，PFA-100の特徴として，VWF抗原量やVWFリストセチンコファクターへの感受性が高いためと考えられる．ただし，重篤度やVWDの型によって検出できないケースもあり，例えば，Type Iで比較的症状の軽いケースや，VWF-GPIbの正常な結合能を保つType 2Nでは閉塞時間は延長しないことが報告されている．トータルでは9割程度のVWD検出成功率を示す[7]．

また，VWDの止血療法としてDDAVPが汎用される．その治療効果はPRPを用いた血小板凝集検査では確認できないが，PFA-100では閉塞時間の短縮がみられ，薬効の確認が可能とされている[23][24]．これもPFA-100がVWFに感度よく反応するためと考えられている．

一方，薬剤感受性で既存評価系よりも鈍い場合がある．例えば，チクロピジンやクロピドグレルは，血小板凝集検査においてADP刺激による最大凝集率を抑制するが，PFA-100の閉塞時間は延長しない場合が多い[15][18][25]．

測定値の解釈の注意点―閉塞時間に影響を与える因子・与えない因子

PFA-100は比較的新しい評価系であり，測定値に影響を及ぼす因子について注意深く認識しておく必要がある．閉塞時間に最も影響を与える因子として，VWF抗原量およびVWFリストセチンコファクター[11]，採血時のクエン酸濃度[8][26]が報告されている．低血小板数(＜80×10^9/l)や低ヘマトクリット(＜30％)では閉塞時間が長くなる[1][26]．年齢[27]，喫煙[28][29]は弱い相関があるとする報告もある．性差はほとんど影響を与えない[27][28]．多少食い違う報告も散見されるが，評価条件や対象母集団(健常人or患者)の違いにより影響度が異なるからと考えられる．興味深いことに，フィブリノーゲンや第Ⅴ，Ⅷ，Ⅸ，Ⅺ凝固因子の影響はみられない[7][26]．そのため，低フィブリノーゲン血症，異常フィブリノーゲン血症，血友病AおよびBの評価に用いることはできない．

今後の展望

PFA-100の臨床応用の1つとして抗血小板薬の薬効モニタリングを先に挙げた．それでは薬物によってどの程度の血小板機能抑制を維持すれば最大の効果(つまり将来におけるイベント再発の予防)を得ることができるだろうか？ 残念ながらPFA-100を用いた大規模な臨床研究は未実施であり，今後の研究が期待される．

現在，ワルファリンなど一部の特別な薬剤を除き，薬効モニタリングは臨床の現場で一般的でない．PFA-100は，操作が非常に簡便でかつモニタリング可能な薬剤も多いことから，血栓・循環領域での使用が一般的になるポテンシャルを持っていると考えられる．今後，薬効モニタリング研究が発達し，科学的根拠に基づいたより良い治療の結果，患者さんが恩恵を享受できるようになることを祈ってやまない．

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1. 抗血小板療法の基礎

C. 血小板機能と遺伝子多型（分子疫学的立場）

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THROMBOSIS and Circulation

§論文のポイント

[1] 血栓症には多くの後天的要因が関与するが，遺伝的要因を示唆する所見も少なくない．血小板機能の個体差の一部も遺伝的に決定されるといわれる．最近，血小板凝集能の家族性を示した興味深い報告がみられる．

[2] 血小板膜糖蛋白は，凝集や粘着など血小板の重要な機能を担う受容体であり，これら膜糖蛋白にみられる遺伝的多様性（多型）は血小板機能の個体差と関連する可能がある．

[3] GPIa/IIa の多型はコラーゲン粘着能と，GPIb/IX/V 複合体の多型は VWF との反応性に，GPIIb/IIIa の多型は血小板凝集能や抗血小板薬の効果と，そして ADP 受容体 $P2Y_{12}$ の多型は血小板凝集能に影響する可能性が示唆されている．

[4] 血小板受容体の遺伝子多型と血栓症発症率，重症度，治療効果などとの関連が報告されているが，いずれも確立された事実とはいいがたい．

[5] 遺伝子多型が血小板機能の個体差やアスピリンをはじめとする抗血小板薬の感受性に影響する可能性が十分に考えられ，そのメカニズム解明に期待が寄せられる．

§キーワード

血小板膜糖蛋白／遺伝子多型／血栓症／抗血小板薬／アスピリン不応症

遺伝的要因による血栓形成能の個体差

日本人には従来，血栓症が少ないとされてきた．しかし最近のデータでは日本での術後の深部静脈血栓症は整形外科領域で 8.0 ～ 31.3 %，婦人科領域では 10.8 %にみられており[1]，欧米と比較してそれほど大きな差とは考えられない．血栓症には，多くの後天的要因が関与するが，若年発症者，家族内集積，ほかに危険因子を伴わない血栓症患者など，遺伝的要因を示唆する所見も少なくない．血小板機能の個体差の一部も遺伝的に決定されるといわれる．最近，血小板凝集能の家族性を示した興味深い報告がみられる．例えば健常人の ADP，エピネフリン，コラーゲン凝集能を測定してみると，どの凝集惹起物質でも同胞では男－男，男－女，女－女，いずれの組み合わせでも凝集率はよく相関するが，同じ環境に生活する夫婦間では凝集率の相関は全くみられない(図 1)[2]．

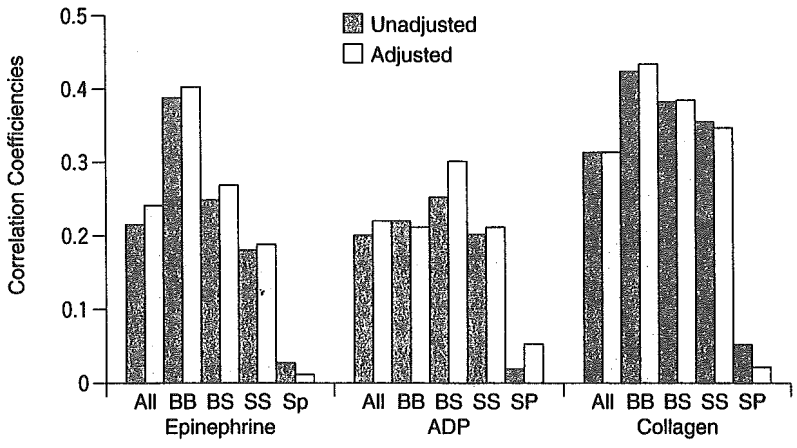


図 1　血小板凝集能には遺伝的要因が強く関与する

BB ： brother-brother，BS ： brother-sister，SS ： sister-sister，Sp ： spouse pairs.

(文献 2 より引用)

血小板膜蛋白の遺伝子多型と血小板機能

血小板膜糖蛋白の遺伝子多型の多くは血小板同種抗原（human platelet antigen ： HPA)の原因として広く知られている．例えば GPIIb の 843 Ile/Ser は Bak，GPIIIa の 33 Leu/Pro は PI^A，143 Arg/Gln は Pen，GPIbα の 145 Thr/Met は Sib (Ko)などの抗原名称が付けられている(図 2)．いずれも凝集や粘着など血小板の重要な機能を担う受容体であるため，これら遺伝子多型が血小板機能の個体差に影響する可能性がある．遺伝子多型と血栓性疾患の関係は疫学的によく検討されているが，ここでは血小板機能に及ぼす影響を中心に述べる．

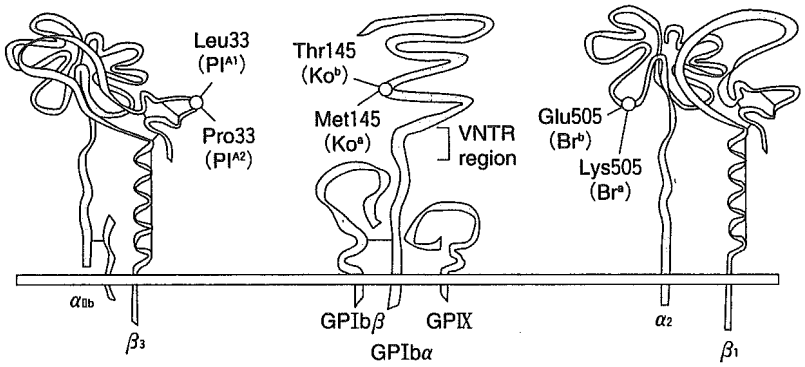
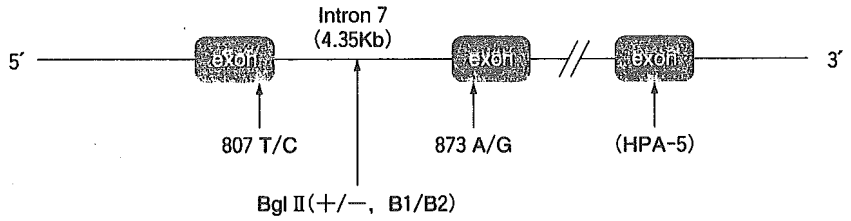


図 2　血小板膜蛋白の代表的遺伝子多型

GPIa/IIa 複合体（インテグリン $\alpha_2\beta_1$）

GPIa/IIa（インテグリン $\alpha_2\beta_1$）はコラーゲン受容体の 1 つで血小板のコラーゲンに対する粘着を司る．この受容体は主要な血小板膜受容体のなかでは血小板膜上の発現量が少なく，しかも正常人での発現量の個体差が大きい．1997 年，Kunicki らは GPIa/IIa の膜発現量の多様性が α_2 サブユニットに存在する少なくとも 3 つの遺伝子多型と関連していると報告した[3]．蛋白コード領域の 807 T/C，873 A/G と，イントロン 7 の Bgl II 認識部位(+/−)であり，3 者は連鎖不均衡にある(図 3)．807 T や Bgl II(+)対立遺伝子を有



807(T/C)，Bgl Ⅱ(+/−)，873(A/G)の3つの多型は連鎖不均衡にある．

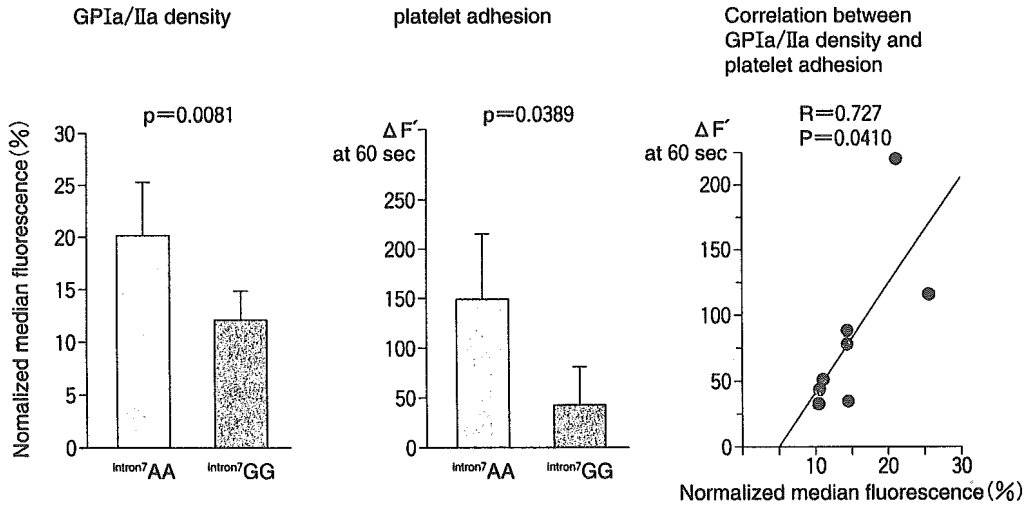


図3　血小板コラーゲン受容体($\alpha_2\beta_1$ インテグリン)の α_2 遺伝子多型と，これらが蛋白発現量，血小板コラーゲン粘着に与える影響

(文献3、4より引用)

する個体は膜発現量が高く，807 C や Bgl Ⅱ(−)対立遺伝子を有する個体は膜発現量が低い．これらの血小板機能に及ぼす影響が検討されたが，予想どおり流動状態下での血小板コラーゲン粘着量は 807 T や Bgl Ⅱ(+)対立遺伝子を有する血小板で高いことが示された(図3)[4)5)]．

Ia/Ⅱa 複合体遺伝子多型は，❶出血性疾患有病者における出血症状の程度，❷血栓性疾患や血管障害の易罹病性，の2点から検討されている．前者では type 1 von Willebrand 病患者の出血症状は Ia/Ⅱa 複合体遺伝子多型に一部依存すると報告されている．後者に関して，いくつかの報告があるが，心筋梗塞や糖尿病性細小血管症との関連が示唆されている．

GPIb/Ⅸ/V 複合体

GPIb/Ⅸ/V 複合体は VWF の膜受容体である．特に流速が速い血液中では，GPIb/Ⅸ/V 複合体は血小板が内皮下組織への接触とその後の血小板活性化を引き起こす tethering molecule として必須であり，血小板血栓形成の初期段階を制御する分子として重要だと考えられている．

GPIb/Ⅸ/V 複合体には複数の遺伝子多型が知られている．なかでも GPIbα に存在する3つの多型(145 Thr/Met，#399-411 の 13 アミノ酸配列の 1-4 回の反復多型，− 5 T/C 多型)，がよく研究されている．

われわれは 145 Thr/Met および #399-411 の 13 アミノ酸反復多型と CAD との関連について検討し，

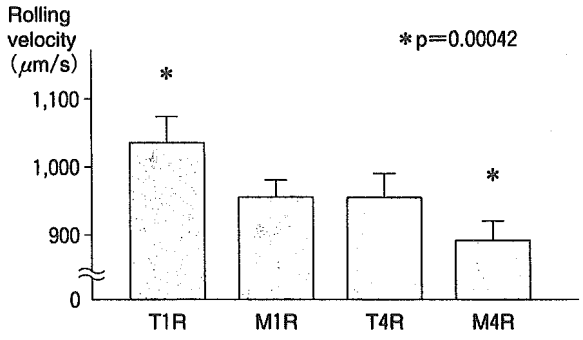


図4　種々遺伝子型のGPIbαを発現するCHO細胞が，固相化VWF上をrollingする速度(ずり速度114 s^{-1})

T1R：145 Thr, VNTR 1 repeat
M1R：145 Met, VNTR 1 repeat
T4R：145 Thr, VNTR 4 repeat
M4R：145 Met, VNTR 4 repeat

(文献7より引用)

Met-alleleまたは4-repeat alleleがCADの有病率，重症度と関連があることを報告した[6]．

通常の血小板機能検査でGPIb/IX/5のこれら遺伝子多型による血小板機能の差は報告されていない．しかし*in vivo*では反復の多い分子は受容体の背が高いため高分子マルチマーであるVWFと結合しやすい，長い分子ほど発生する血小板内シグナルが強い，などのメカニズムが働いている可能性がある．最近われわれは145 Thr/Met多型と1-repeat/4-repeat多型をCHO細胞で発現し，そのVWF結合能が異なる可能性を示した(図4)[7]．すなわち，心血管リスクとされる145 Metまたは4-repeat alleleを有するGPIbαでは，CHO細胞のずり速度下依存性VWF上rollingの速度が低下することを明らかにした．

GPIIb/IIIa複合体(インテグリン $\alpha_{IIb}\beta_3$)

GPIIb/IIIa複合体(インテグリン$\alpha_{IIb}\beta_3$)は血小板フィブリノゲン受容体であり，血小板凝集に必須である．GPIIb/IIIaは血小板血栓形成におけるkey moleculeであることから，抗血栓薬の標的となっており，多数の抑制物質が開発されている．

33 Leu/Pro多型の血小板機能に及ぼす影響については依然議論のあるところだが，Framingham Offspring Studyでは1,442人の血小板凝集能が測定されており，33 Proを有する個体では，凝集惹起に必要なepinephrine濃度が有意($p < 0.001$)に低く，この傾向は33 Proをホモで有するものでより強かったとされている[8]．このほか，遺伝子型によるアスピリン効果の違いや抗IIb-IIIaモノクローナル抗体Reo Proの効果の違いが報告されているが，いずれも確立された事実とはいえない．またこの多型は白人では血小板機能との関連で着目されているが，日本人では対立遺伝子頻度は非常に低く，臨床上問題とされることは少ない．

ADP受容体の遺伝子多型と血小板機能

血小板にはいくつかのADP受容体が知られているが，このうち$P2Y_{12}$は抗血小板薬チクロピジンやクロピドグレルの標的であり，その機能の個体差は臨床的に重要と思われる．健常人におけるADP凝集率が$P2Y_{12}$遺伝子型に影響されると最近報告された(図5)[9]．

いわゆるアスピリン不応症の成因

抗血小板薬は動脈血栓症の再発予防や一部のハイリスク患者の一次予防に用いられ，その地位はEBMとして確立されたものと考えられる．しかし一方では，その予防効果は不十分であり薬効は必ずしも満足できるものではない．抗血小板薬の臨床効果が不十分な原因の1つとして，いわゆる「アスピリン不応症」なる状態が認められることが近年判明している．事実，アスピリン不応症患者では実際に冠状動脈疾患での再発率が高いことが最近報告された．不応のメカニズムとしてアスピリン吸収過程の個人差，別のNSAIDsとの競合，血小板のturnoverの促進，アスピリンへの反応性を弱めるCOX-1のSNPの存在，特定の病態下でのCOX-2の発現，ADP刺激時のGPIIb/IIIaの活性化反応性の上昇，コラーゲンへの反応性の上昇，など