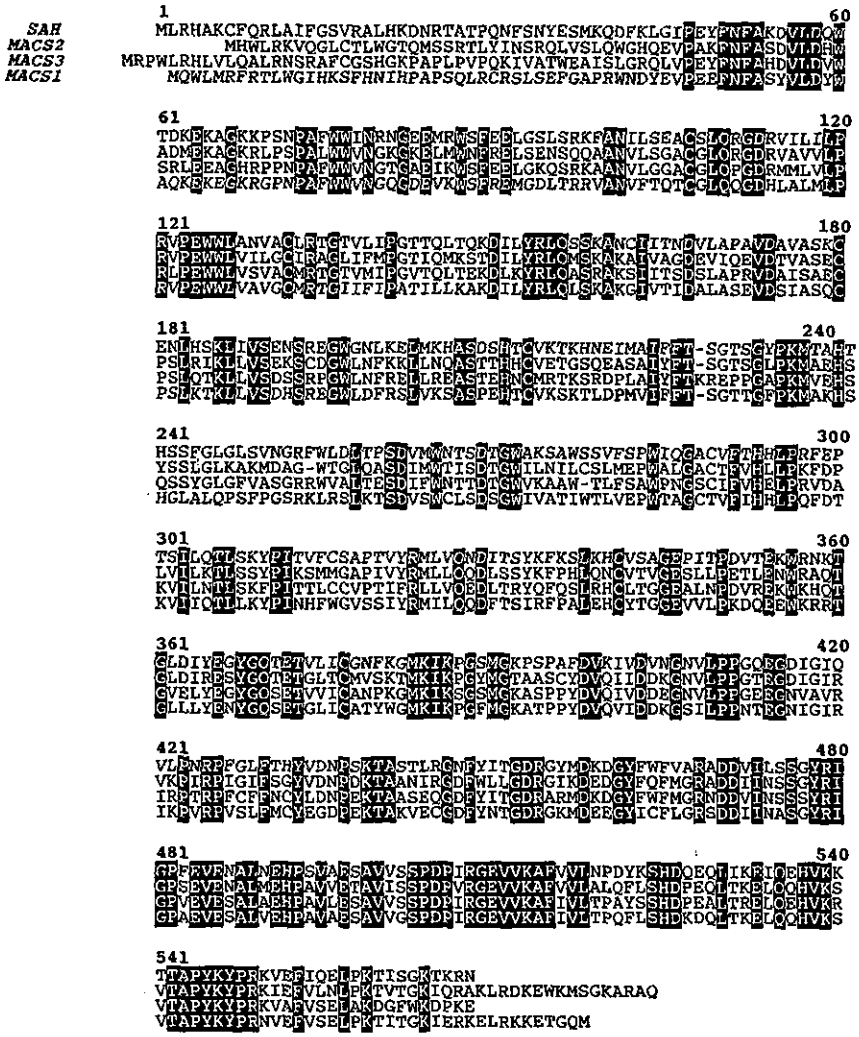

The *SAH* gene family. Amino acid sequences of *SAH* and *MACS1*, *MACS2*, and *MACS3* are shown. Identical amino acid residues among members are indicated. L513S polymorphism of *MACS2* is indicated by bold letter "**L**".

The expression levels of *MACS1*, *MACS2*, *MACS3*, and *SAH* mRNA were assessed by PCR, with the use of a human cDNA panel (Clontech) with 2 independent sets of primers.

Subjects

The selection criteria and design of the Suita Study have been described previously.[7] The genotypes were determined in 1976 consecutive subjects (written informed consent was obtained), who constituted the latter half of the study population in the preceding study. The study protocol was approved by the institutional ethics committee.

The characteristics of the subjects analyzed in the present study are summarized in Table 1, according to L513S polymorphism of *MACS2*. Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or the current use of antihypertensive medication. Total cholesterol and triglyceride levels were determined by enzymatic methods and kits (L-TC WAKO, Wako Pure Chemical, and Clinimate TG-2, Daiichi Chemicals). Homeostasis model assessment of insulin resistance (HOMA) was calculated as follows[9]: HOMA=[fasting insulin (μU/mL)× fasting glucose (mmol/L)]/22.5. Total immunoreactive insulin was measured by a kit (TOSOH), with the use of a 2-site immunoenzymometric assay.

Statistical Analysis

Values are expressed as mean±SEM. All statistical analyses were performed with the JMP statistical package (SAS Institute Inc). Multiple linear regression and multiple logistic analyses were performed with other covariates. Residuals of the waist-to-hip ratio and triglycerides were calculated by adjusting for age, gender, alcohol consumption (ethanol mL/d), and smoking (cigarettes/d). In some settings, the probability value was corrected (*Pc*) by the Bonferroni method. Principal component analysis was performed on the basis of correlations.

Linkage disequilibrium[10] and haplotype analyses were performed using the SNPAlyze statistical package (Dynacom Inc, http://www.dynacom.co.jp/; accessed March 5, 2003). Haplotype estimation was performed by the expectation-maximization algorithm.[11] To measure linkage disequilibrium between SNPs, Lewontin's D′ was calculated.[12]

Results

Confirmation of the *SAH* Gene Family

A BLAST search revealed the existence of 3 transcripts homologous to *SAH*, namely *MACS1* to *MACS3*. The complete genome structure of *MACS1* has been described previously.[8] The genome structure of *MACS3* and part of the genome structure of *MACS2* have not been reported, and we determined flanking sequences of coding exons of *MACS2* and *MACS3* for sequence screening of polymorphisms.

The polymorphisms found in the present study are summarized in Table 2. Polymorphisms in introns were not studied in detail and are not included in Table 2.

TABLE 1. Characteristics of the Study Population

Phenotype	SS (n=125)	LS (n=731)	LL (n=1120)	*P*
Men, %	54.4	47.2	47.5	NS
Age, y	60.1 (1.1)	59.6 (0.4)	60.1 (0.4)	NS
Alcohol consumption, mL/d	15.6 (2.0)	15.6 (0.8)	14.6 (0.8)	NS
Smoking, cigarettes/d	5.4 (0.9)	4.4 (0.4)	4.3 (0.3)	NS
HTN, %	42.4	37.4	38.7	NS
HDL, mmol/L	1.40 (0.04)	1.51 (0.02)	1.53 (0.01)	0.0025
TChol, mmol/L	5.42 (0.08)	5.42 (0.03)	5.45 (0.03)	NS
TG, mmol/L	1.74 (0.09)	1.42 (0.04)	1.40 (0.03)	0.0059
R-TG, mmol/L	+0.36 (0.01)	0.00 (0.01)	−0.04 (0.04)	0.0089
W/H	0.914 (0.006)	0.905 (0.003)	0.897 (0.002)	0.0034
R-W/H	+0.011(0.006)	+0.005 (0.002)	−0.004 (0.002)	0.0011
BMI, kg/m^2	23.4 (0.3)	22.9 (0.1)	22.6 (0.1)	0.0090
FBS, mmol/L	5.56 (0.09)	5.47 (0.04)	5.42 (0.03)	NS
HOMA	2.43 (0.17) (n=60)	1.90 (0.07) (n=422)	1.79 (0.05) (n=624)	0.0015
Insulin, μU/mL	9.5 (0.6) (n=60)	9.7 (0.2) (n=422)	7.3 (0.2) (n=624)	0.0036

Characteristics of the study population are shown according to the L513S polymorphism of the *MACS2* genotype. HTN indicates hypertensive subjects; HDL, HDL cholesterol; TChol, total cholesterol; TG, triglycerides; R-TG, residuals of TG; W/H, waist-to-hip ratio; R-W/H, residuals of W/H; BMI, body mass index; FBS, fasting blood glucose; HOMA, homeostasis model assessment of insulin resistance. R-TG and R-W/H were calculated by adjusting for age, gender, alcohol consumption, and smoking.

The expression of *MACS1* was not detected, as described below, which may downplay the importance of this gene. The AC repeat polymorphism in the promoter may not be suitable for high-throughput genotyping and was neglected in the present study. The polymorphisms in exons 8, 11, 12, and 13 were in complete linkage disequilibrium in the 36 subjects sequenced, and we selected the exon 12 polymorphism for the association study.

We found 3 polymorphisms in the coding region of *MACS2*, which were selected for the association study. The L513S polymorphism may have some functional meaning, since hydrophobic leucine is replaced by hydrophilic serine.

We found 4 polymorphisms in the coding region of *MACS3*. The Q159H (exon 3) and P353R (exon 7 to 1) polymorphisms were in complete linkage disequilibrium with the T534M (exon 12) and H361R (exon 7 to 2) poly-

TABLE 2. Polymorphisms in the Ch16p12 SAH Region

Gene	Region		Sequence	AA Change	Minor Allele Frequency
MACS1					
	Promoter		TGTTTAGAAA (CA)n TTGGAGAGGT	...	0.417
	Ex8		CTCCACCCTA[C/T]GACGTCCAGG	TAC(Y)/TAT(Y)	0.417
	Ex10		GGGACAGAGG[A/T]AAGATGGATG	GGA(G)/GGT(G)	0.070
	Ex11		AGGTTGAAAG[T/C]GCTTTGGTGG	AGT(S)/AGC(S)	0.417
	Ex12		ACCCAAGGAA[A/G]GTGAGTGAGG	AAA(K)/AAG(K)	0.417
	Ex13	3′UTR	CTGCACACCT[A/G]AGGCAAATCC	...	0.417
MACS2					
	Ex9		CACAGGGATT[G/A]ACTTGCATGG	TTG(L)/TTA(L)	0.222
	Ex11		GGGACGGGCA[G/A]ATGATATCAT	GAT(D)/AAT(N)	0.097
	Ex13		GTCCTGGCCT[T/C]GCAGTTCCTG	TTG(L)/TCG(S)	0.208
MACS3					
	Ex3		ACCGGCTGCA[G/C]GCGTCCAGGG	CAG(Q)/CAC(H)	0.167
	Ex7(1)		GCCCTCAACC[C/G]TGACGTGAGG	CCT(P)/CGT(R)	0.457
	Ex7(2)		AAGTGGAAAC[A/G]CCAGACCGGT	CAC(H)/CGC(R)	0.457
	Ex12		AGAGGCACTA[C/T]CGCGGGAACT	ACG(T)/ATG(M)	0.167

Polymorphisms in the SAH region are shown. Minor allele frequencies are obtained from the 36 subjects sequenced. Polymorphisms indicated by bold letters are used for genotyping of the study population.

TABLE 3. Linkage Disequilibrium Between Polymorphisms

Genotype	SAH12	M1/E12	M2/E9	M2/E11	M2/E13	M3/E7	M3/E12
SAH I/D	**−0.9999**	**0.5302**	−0.2939	−0.0415	0.1851	−0.0337	0.4589
	15.5322	**241.7198**	15.4449	0.1314	56.3709	0.6984	93.1298
SAH12		**−0.6890**	0.1353	**−0.7164**	0.1830	0.4778	**−0.7965**
		16.1148	4.0146	**2.1522**	4.4001	9.8528	**1.5742**
M1/E12			0.1697	0.4467	0.4434	0.2454	**0.9778**
			12.8313	46.1666	140.5322	101.3379	**181.6169**
M2/E9				**0.9732**	**−0.8225**	**0.9480**	0.3467
				983.5424	**97.3028**	**463.9159**	104.8876
M2/E11					**−0.7896**	**0.9397**	0.2992
					45.6709	**233.7887**	147.0224
M2/E13						**0.6942**	−0.2010
						409.5116	2.6518
M3/E7							0.3393
							25.5663

Linkage disequilibriums between polymorphisms are shown. D′ (upper) and χ^2 (lower) values are indicated. Bold letters indicate polymorphisms in strong linkage disequilibrium. The *SAH* I/D and intron 12 polymorphisms have been described previously.[7]

morphisms, respectively. Thus, we selected the H361R (exon 7 to 2) and T534M (exon 12) polymorphisms for the association study. We also determined 2 polymorphisms of *SAH*, I/D polymorphism in the promoter and A/G polymorphism in intron 12, which were concluded to be associated with multiple risk factors in the preceding study in 4039 subjects.[7]

Linkage disequilibrium among these polymorphisms is shown in Table 3. Although the locus for *MACS3* has not been clarified, strong linkage disequilibrium between the *MACS3* and *MACS2* polymorphisms indicates that *MACS3* may reside in this human chromosome 16p12 region near the *MACS2* locus.

Expression of the *MACS* Gene Family

RT-PCR analysis of expression levels of *MACS1*, *MACS2*, and *MACS3* and *SAH* revealed that *MACS2* and *MACS3* and *SAH* were expressed mainly in the kidney and liver. However, we could not detect PCR product from *MACS1* in any of the tissues examined including the spleen, thymus, prostate, testis, ovary, small intestine, colon, lymph node, heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas.

Association Study

Association studies between the polymorphisms in Table 2 and various phenotypes in the 1976 subjects revealed that the L513S polymorphism in *MACS2* strongly influenced triglycerides (TG), HDL cholesterol, waist-to-hip ratio (W/H), and body mass index (BMI) (Table 1). More intriguingly, an index for insulin resistance (HOMA) was influenced by the L513S polymorphism. Since members of the *SAH* gene family appear to have acyl-CoA synthetase activity toward fatty acids, it is likely that principal phenotypes influenced by this gene family may be the triglyceride level and/or visceral obesity (waist-to-hip ratio).

The effects of other polymorphisms on the triglyceride level and W/H ratio are indicated in Table 4. Residuals of the triglyceride level (R-TG) and W/H ratio (R-W/H) were calculated by adjusting for age, sex, alcohol consumption, and smoking. Residuals of the triglyceride level were also calculated after excluding subjects with hypolipidemic drugs to correctly assess the influence of polymorphisms on the triglyceride level (R-TG′). The influence of a *SAH* polymorphism on triglycerides and W/H ratio, which was evident in 4039 subjects in the preceding study, was weak in the present group of 1976 subjects, who comprised a subset (latter part) of the preceding 4039 subjects.

To avoid the problems of multiple testing, a principal component analysis was also performed. After performing a correlation analysis among TG, HDL, W/H, and BMI, the principal components were identified. The first principal component explained 50.5% of the total variance, and the influence of genotype on this component was analyzed by 1-way ANOVA (Table 4). The first principal component was defined as [0.422 (TG)+0.499 (HDL)+0.531 (W/H)+0.539 (BMI)]. Although the pathophysiological meaning of this component is difficult to discern at a glance, it was significantly affected by the L513S polymorphism (Table 4).

To clarify the possible contribution of polymorphisms other than the L513S polymorphism to triglycerides and W/H ratio, diplotypes defined by L513S and another polymorphism were determined in the study population. The effects of various haplotypes on triglycerides and W/H ratio were also evaluated.

There are 4 haplotypes defined by the L513S and I/D (SAH) polymorphisms: L513-D (haplotype1, allele frequency 0.559, 95% CI, 0.533 to 0.585), L513-I (haplotype2, allele frequency 0.200, 95% CI, 0.180 to 0.216), S513-D (haplotype3, allele frequency 0.136, 95% CI, 0.119 to 0.155), and S513-I (haplotype4, allele frequency 0.105, 95% CI, 0.086 to 0.120). The effects of the diplotypes defined by these 4 haplotypes on the triglyceride level are shown in Table 5. One-way ANOVA indi-

TABLE 4. Polymorphisms of SAH Gene Family and Triglycerides and W/H Levels

Phenotype	SAH I/D	SAH12	M1/E12	M2/E9	M2/E11	M2/E13	M3/E7	M3/E12	10D	D33
R-TG (n=1976)										
F value	2.1400	0.6510	0.9534	3.0167	1.2921	4.7360	0.3173	2.5916	**3.0537**	**18.3860**
P	0.1179	0.5216	0.3856	0.0492	0.2749	0.0089	0.7281	0.0752	**0.0012**	**<0.0001**
Pc	1.0000	1.0000	1.0000	1.0000	1.0000	0.2848	1.0000	1.0000	**0.0384**	**<0.0032**
df	2	2	2	2	2	2	2	2	**9**	**1**
R-TG′ (n=1898)										
F value	1.3562	0.6926	0.8046	4.2791	1.7569	**6.0809**	0.3752	3.0618	**3.5432**	**19.9302**
P	0.2579	0.5004	0.4474	0.0140	0.1713	**0.0023**	0.6872	0.0470	**0.0002**	**<0.0001**
Pc	1.0000	1.0000	1.0000	0.4480	1.0000	**0.0736**	1.0000	1.0000	**0.0064**	**<0.0032**
df	2	2	2	2	2	**2**	2	2	**9**	**1**
R-W/H (n=1976)										
F value	0.3093	0.8124	0.0952	1.0303	1.5800	**6.8456**	0.5537	0.8507	1.7752	2.4432
P	0.7340	0.4439	0.9092	0.3571	0.2062	**0.0011**	0.5749	0.4273	0.0681	0.1182
Pc	1.0000	1.0000	1.0000	1.0000	1.0000	**0.0352**	1.0000	1.0000	1.0000	1.0000
df	2	2	2	2	2	**2**	2	2	9	1
1st PC (n=1976)										
F value	0.4884	0.7813	2.5228	0.8158	2.0186	**9.7314**	1.1155	0.2028	**2.8083**	**9.7354**
P	0.6137	0.4580	0.0805	0.4424	0.1331	**<0.0001**	0.3279	0.8165	**0.0028**	**0.0018**
Pc	1.0000	1.0000	1.0000	1.0000	1.0000	**<0.0032**	1.0000	1.0000	**0.0448**	**0.0288**
df	2	2	2	2	2	**2**	2	2	**9**	**1**

The influence of polymorphisms on R-TG, R-TG′, R-W/H, and the first principal component (1st PC) were analyzed by 1-way ANOVA. R-TG and R-W/H were calculated by adjusting for gender, age, alcohol, and smoking (n=1976). R-TG′ was calculated after excluding subjects who were receiving hypolipidemic drugs (n=1898). The 1st PC was calculated as described in the text. The effects of the 10 diplotypes (10D) and the Diplotype 33 (D33) are also indicated. In D33, the 10 diplotypes (see TABLE 5) are recategorized into 2 groups, i.e., diplotype 33 and others. The haplotypes are defined in the text.

P values are corrected (*Pc*) by multiplying 32 [(8 genotypes+7 haplotypes+1 recategorization)×2 (possibly independent 2 phenotypes: triglyceride and waist-to-hip ratio)] (Bonferroni).

cated that the diplotype had significant effects on R-TG ($P=0.0012$) and R-TG′ ($P=0.0002$). As shown in Table 5, the diplotype 33 had significantly higher R-TG and R-TG′ levels. Thus, we recategorized the 10 diplotypes into 2 groups, that is, diplotype 33 and others. The influence of this diplotype 33 on the triglyceride level was highly significant even after correction by the Bonferroni method ($P<0.0001$ and $Pc<0.0032$, Table 4).

TABLE 5. Influence of Diplotype on Triglycerides Levels

Diplotype	n=1976 (n′=1898)	R-TG	*P*	R-TG′	*P*
11	632 (606)	0.01 (0.04)	<0.0001	−0.01 (0.04)	<0.0001
12	411 (393)	−0.12 (0.05)	<0.0001	−0.12 (0.05)	<0.0001
13	320 (306)	0.02 (0.06)	0.0001	0.01 (0.06)	<0.0001
14	332 (323)	0.02 (0.06)	0.0001	0.05 (0.06)	0.0001
22	75 (71)	0.13 (0.13)	0.0046	0.15 (0.13)	0.0074
23	4 (4)	0.41 (0.55)	NS	0.42 (0.54)	NS
24	81 (78)	−0.21 (0.12)	<0.0001	−0.23 (0.12)	<0.0001
33	46 (46)	0.69 (0.16)		0.70 (0.26)	
34	52 (49)	0.01 (0.15)	0.0023	0.03 (0.15)	0.0023
44	23 (22)	0.12 (0.23)	0.0444	0.19 (0.23)	0.0678

The influence of the diplotype on the triglyceride level was assessed by 1-way ANOVA. Haplotypes are defined in the text. The diplotype XY indicates the genotype with X and Y haplotypes. Thus, diplotype 23 indicates the genotype with one haplotype 2 and one haplotype 3. One-way ANOVA indicated that the diplotype had a significant influence on R-TG ($P=0.0012$) and R-TG′ ($P=0.0002$) (see TABLE 4).

P values indicate significant differences from the diplotype 33 group (by Fisher protected least significant difference test).

Discussion

We recently reported that genetic polymorphisms in *SAH* influenced multiple risk factors, including TG, HDL cholesterol, BMI, W/H ratio, and blood pressure status.[7] Since then, 3 other genes with high homology to *SAH* have been identified to cluster in the *SAH* region, chromosome16p12. Thus, it is possible either that the previously observed associations were due to linkage disequilibrium with truly important polymorphisms in other members of the SA gene family or that other polymorphisms in this gene family may also influence multiple risk factors.

In the present study, to evaluate the above-mentioned hypotheses, we performed extensive association studies between genetic polymorphisms in this region and multiple risk factors using a large cohort representing the general population in Japan. The L513S polymorphism in *MACS2* was shown to significantly influence TG, HDL, W/H, BMI, and HOMA index.

Because the L513S genotype appeared to influence various phenotypes including TG, HDL, W/H, and BMI, a principal component analysis was performed to avoid the problems of multiple testing. The L513S polymorphism had a highly significant influence on the first principal component. However, the pathophysiological meaning of this component is difficult to discern.

The members of the *SAH* gene family seem to have acyl-CoA synthetase activity toward medium chain fatty acids.[6-8] Thus, it is logically highly likely from the biological viewpoint that principal phenotypes influenced by this gene family may be the TG level and/or visceral obesity. Therefore, we studied the influence of polymorphisms on the TG level and W/H ratio (an excellent index of visceral obesity) (Table 4). Diplotype 33 had a highly significant influence on the TG level and the L513S polymorphism of *MACS2* had a weak but significant influence on the W/H ratio. Therefore, most of the previously observed associations between a *SAH* polymorphism and multiple risk factors appear to be due to linkage disequilibrium with the L513S polymorphism and haplotype 3.

In conclusion, the present study confirmed the importance of this chromosomal region, especially *MACS2* and *SAH*, in the pathogenesis of hypertriglyceridemia and visceral obesity. Intriguingly, this locus has been reported to be one of the suggestive loci for body mass index in the Framingham Heart Study.[13]

Perspectives

Human MACS1, human SAH, and bovine counterparts have been reported to act as acyl-CoA synthetases for various fatty acids, especially medium-chain fatty acids (MCFA).[6-8,14] MCFA are abundant in milk, coconut oil, and various synthetic oils. The activation of MCFA takes place mostly in the mitochondrial matrix by acyl-CoA synthetase for MCFA. Most of the MCFA incorporated into hepatocytes is subject to β-oxidation. Some of the acyl-CoA produced during MCFA oxidation is directed toward ketone body production, and the rest is directed to de novo synthesis of long-chain fatty acids, which are then incorporated into triglycerides or other complex lipids.[15,16] Recently, it has been proposed that medium-chain triglycerides may help to prevent obesity.[17] Therefore, it is highly likely that members of the *SAH* gene family (possible acyl-CoA synthetases for MCFA) may play some important roles in triglyceride metabolism, energy expenditure, fat metabolism, and, therefore, insulin resistance. However, the precise in vivo functions of the members of this gene family and the functional properties of the L513S polymorphism remain to be clarified and await further investigation.

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Association of Methylenetetrahydrofolate Reductase Gene Polymorphism With Carotid Atherosclerosis Depending on Smoking Status in a Japanese General Population

Nozomu Inamoto, MD; Tomohiro Katsuya, MD, PhD; Yoshihiro Kokubo, MD, PhD; Toshifumi Mannami, MD, PhD; Takashi Asai, MD; Shunroku Baba, MD, PhD; Jun Ogata, MD, PhD; Hitonobu Tomoike, MD, PhD; Toshio Ogihara, MD, PhD

Background and Purpose—The association of the *methylenetetrahydrofolate reductase* gene (*MTHFR*) with carotid atherosclerosis remains inconsistent. This may be due to small sample size and inappropriate analysis. We investigated the association of *C677T/MTHFR* with blood pressure and carotid atherosclerosis in a Japanese general population.

Methods—Subjects (30 to 89 years of age; 1693 women, 1554 men) who gave informed consent were randomly selected from a general population in Suita, Japan. *MTHFR* genotypes were determined by TaqMan polymerase chain reaction. Carotid atherosclerosis was evaluated by high-resolution ultrasonography with atherosclerotic indexes of intimal-medial thickness (IMT), maximum IMT in the common carotid artery (CCA), plaque score, and stenosis (>50%).

Results—Age-adjusted diastolic blood pressure was significantly higher in women with the *TT* genotype than in those with the *CC* genotype. In a recessive model (*CC+CT* versus *TT*), all adjusted odds ratios for hypertension and >50% stenosis in women were 1.42 and 3.42 (95% confidence intervals, 1.01 to 1.99 and 1.23 to 9.53), respectively. In women, maximum IMT in CCA for smokers with the *TT* genotype was significantly higher than for smokers with the *CC* genotype and nonsmokers with the *TT* genotype ($P<0.05$).

Conclusions—Our study suggests that the *MTHFR TT* genotype is a risk factor for hypertension and carotid stenosis in women. Significant interactions between *C677T/MTHFR* and smoking on maximum IMT in CCA were observed in women but not in men. Smoking cessation for subjects with the *TT* genotype is important in the prevention of cerebrovascular disease. (***Stroke.* 2003;34:1628-1633.**)

Key Words: amine oxidoreductases ■ blood pressure ■ carotid arteries ■ Japan ■ risk factors

Hyperhomocysteinemia is associated with increased risk of atherosclerotic vascular disease.[1] The association of plasma total homocysteine concentration with atherosclerosis has been the subject of a number of clinical studies that have consistently linked moderate hyperhomocysteinemia with peripheral vascular disease, cerebrovascular disease, and coronary heart disease.[2–5]

Plasma total homocysteine levels are regulated mainly by 5,10-methylenetetrahydrofolate reductase, which is involved in the folate-dependent remethylation of homocysteine to methionine. Frosst et al[6] suggested that the *C677T* polymorphism in the *methylenetetrahydrofolate reductase* gene (*MTHFR*) is a candidate risk factor for vascular disease. The metabolic changes associated with *C677T/MTHFR* are postulated to modify the predisposition to diseases associated with folate deficiency.[7] Particular emphasis has been given to the role of *C677T/MTHFR* in cardiovascular[8] and cerebrovascular disease[9] and venous thrombosis.[10]

On the other hand, technical improvements in carotid ultrasonography have revealed new risk factors for stroke in its wide use. Some studies have demonstrated a close correlation between carotid ultrasound measurement, usually of carotid intimal-medial wall thickness (IMT), and the severity of extracranial carotid atherosclerosis.[11,12] Plasma total homocysteine levels have also been associated with more advanced carotid atherosclerosis in elderly subjects.[3,13] However, there have been controversies among their results. Most studies have failed to show an association between *C677T/MTHFR* and atherosclerotic disease.[14,15] These inconsistencies may be due to small sample size, combined-sex analysis, and lack of consideration of lifestyle. In this study, we examined the effect of *C677T/MTHFR* on carotid atherosclerosis and blood pressure (BP) in a large genetic epidemiological study, the Suita Study.

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From the Department of Preventive Cardiology, National Cardiovascular Center (N.I., Y.K., T.M., S.B., J.O., H.T.), and Department of Geriatric Medicine, Osaka University Graduate School of Medicine (N.I., T.K., T.A., T.O.),Suita, Osaka, Japan.

This article encompasses the doctoral dissertation of Nozomu Inamoto.

Correspondence to Tomohiro Katsuya, MD, PhD, Department of Geriatric Medicine, Osaka University Graduate School of Medicine, 2-2 No. B6, Yamada-Oka, Suita, Osaka 565-0871, Japan. E-mail katsuya@geriat.med.osaka-u.ac.jp

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Materials and Methods

Subject Population

The Suita Study was based on a random sample of 14 200 Japanese residents of Suita, a city located in the second-largest urban area in Japan, Osaka.[16] These 14 200 residents between 30 and 89 years of age were arbitrarily selected from the municipality population registry, stratified by sex and 10-year age groups. We sent these residents letters to ask if they were willing to participate in this study from 1989 with a cohort base; by February 1007, 51.7% of the subjects (n=7347) had paid an initial visit to the National Cardiovascular Center (NCVC). The participants have visited NCVC every 2 years since then for regular health checkups. In addition to routine blood examinations that included total serum cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, glycosylated hemoglobin A_{1C} (HbA_{1C}), systolic BP (SBP), and diastolic BP (DBP), DNA was extracted from an extra 5 mL blood withdrawn from those who underwent general examinations at NCVC between May 1996 and February 1998. Ninety percent of the subjects who visited NCVC during this period gave informed consent for genetic analysis of 13 genes including *MTHFR* and storage of a DNA sample and were enrolled in the present study. The study protocol of genetic analysis was approved by the ethics committee of Osaka University. Three physicians performed the carotid ultrasonic examinations. Finally, the subjects in the present study included 1693 women and 1553 men 30 to 89 years of age who attended regular health checkups and subsequently underwent ultrasonic examinations and genetic analysis.

Measurements

The subjects' BPs were measured after at least 10 minutes of rest in the sitting position. The mean value of 2 measurements of SBP or DBP obtained by a physician using a mercury sphygmomanometer (recorded >3 minutes apart) was used for the analysis. Hypertension was defined as a mean SBP of ≥160 mm Hg, a mean DBP of ≥95 mm Hg, or current use of antihypertensive medication.

The subjects were classified as current smokers or drinkers if they smoked or drank. Hypercholesterolemia was defined as serum total cholesterol levels ≥220 mg/dL or current use of antihyperlipidemic medication. Diabetes was defined as fasting plasma glucose levels ≥7.0 mmol/L (126 mg/dL) or nonfasting glucose levels ≥11.1 mmol/L (200 mg/dL), HbA1C ≥6.5%, or current use of antidiabetic medication. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared.

Blood samples drawn from the subjects after 12 hours of fasting were collected in EDTA-containing tubes. Total cholesterol and HDL cholesterol levels were measured with an autoanalyzer (Toshiba TBA-80) in accordance with the Lipid Standardization Program of the US Centers for Disease Control and Prevention through the Osaka Medical Center for Health Science and Promotion, Japan.[17] Among 3247 subjects, 1541 (820 women, 721 men) underwent measurement of fasting total plasma homocysteine levels by high-performance liquid chromatography.[18]

Carotid Ultrasound Measurements

Details of the carotid ultrasonic examination methods have been previously published.[16] We used a high-resolution B-mode ultrasonic machine with a 7.5-MHz transducer yielding an axial resolution of 0.1 mm. The regions from 30 mm proximal to the beginning of the dilation of the bifurcation bulb to 15 mm distal to the flow divider of both common carotid arteries (CCAs) were scanned. All measurements were made at the time of scanning with the electronic caliper and were recorded on photocopies. IMT was measured on a longitudinal scan of the CCAs at a point 10 mm proximal to the beginning of the dilation of each carotid artery bulb. IMT was defined as the mean of the IMT of the proximal and distal walls at the point of measurement. Maximum IMT in the CCA and maximum IMT were defined as the maximum IMT in the scanned CCA area and the maximum IMT in the entire scanned area, respectively. We defined a plaque, a focal IMT thickening, as an area where IMT ≥1.1 mm and calculated plaque score by totaling the maximum thickness of all the plaques in the scanned area. Finally, we defined stenosis as a condition in which a plaque occupied more than half of the lumen circumference of an artery on a cross-sectional scan. We performed color-flow Doppler examination to confirm the presence of stenosis.

MTHFR Genotype Determination With TaqMan Polymerase Chain Reaction Method

Genomic DNA was extracted from peripheral blood lymphocytes by standard procedures with a QIAamp DNA Blood Kit (Qiangen Inc). To deal with a large number of samples, we introduced the TaqMan polymerase chain reaction (PCR) method (Applied Biosystems). In the current investigation, we prepared 2 probes: C allele–specific probe, 5′ Tet-TCT GCG GGA GcC GAT TTC ATC ATC-Tamra-3′, and T allele–specific probe, 5′-Fam-TCT GCG GGA GtC GAT TTC ATC ATC-Tamra-3′. Primer design for PCR of the flanking region of *C677T/MTHFR* was as follows: forward, 5′-GGC TGA CCT GAA GCA CTT GAA-3′; reverse, 5′-GCG GAA GAA TGT GTC ATC CT-3′. PCR was carried out with a thermal cycler (GeneAmp, PCR System 9700, Applied Biosystems). PCR was performed according to the following conditions: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. The fluorescence level of PCR products was measured with an ABI PRISM 7200 and 7900 Sequence Detector (Applied Biosystems), resulting in clear identification of the 3 genotypes of *C677T/MTHFR*.

Statistical Analysis

The number of subjects was restricted to 3247 who had complete data, including *C677T/MTHFR* and carotid ultrasonographic measurements. Analysis of variance was used to compare mean values between groups, and if overall significance was demonstrated, the intergroup difference was assessed by means of a general linear model. Frequencies were compared by χ^2 analysis.

Associations of *C677T/MTHFR* with BP were investigated by sex through logistic regression analysis considering potential confounding risk variables, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), lifestyle (smoking and drinking), and antihypertensive medication. The genotype effect was examined according to a dominant (*TT*+*CT* versus *CC*) and a recessive (*TT* versus *CT*+*CC*) model. For multivariate risk predictors, the adjusted odds ratios (ORs) were given with the 95% confidence intervals (CIs). The relationships in men and women between *C677T/MTHFR* and hypertensive risk were expressed in terms of ORs adjusted for possible confounding effects. The association of *C677T/MTHFR* with carotid atherosclerotic index was also investigated by sex through logistic regression analysis considering potential confounding risk variables. Partial correlation coefficients between plasma total homocysteine and carotid atherosclerotic indexes by sex and *C677T/MTHFR* were determined. In addition, gene and environmental interactions were calculated with the following logistic regression model: logit $p=\beta_0+\beta_g x_g+\beta_e x_e+\beta_{ge} x_g x_e$, where x_g and x_e are genetic and environmental data, respectively; β_0 is an intercept term; β_g is the main effect due to genes; and β_e is the main effect of the environment. The coefficient β_{ge} of the product $x_g x_e$ estimates the gene and environmental interaction on the logit scale.[19] All analyses were performed with SAS statistical software (release 6.12, SAS Institute Inc).

Results

Basic Characteristics of Subjects in the Suita Study

As shown in Table 1, age, SBP, DBP, BMI, total cholesterol, HDL cholesterol, IMT, maximum IMT in CCA, plaque score, CCA stenosis (≥50%), percentage of current smokers, percentage of current drinkers, prevalence of hypertension, prevalence of diabetes mellitus, and total plasma homocysteine levels were significantly higher in men than in women.

TABLE 1. Basic Characteristics of Subjects in Suita, a Japanese Urban Population

	Women (n=1693)	Men (n=1554)
Age, y	58.2±12.2	60.4±12.8*
SBP, mm Hg	126.7±21.1	129.5±19.3*
DBP, mm Hg	78.0±11.0	80.7±11.0*
BMI, kg/m^2	22.3±3.2	23.0±2.8*
Total cholesterol, mmol/L	5.6±0.9	5.2±0.8*
HDL cholesterol, mmol/L	1.6±0.4	1.4±0.4*
IMT, mm	0.83±0.12	0.88±0.14*
Maximum IMT in CCA, mm	1.02±0.29	1.15±0.45*
Plaque score, mm	2.14±2.99	4.13±4.69*
Stenosis (≥50%), %	1.0	4.6†
Current smokers, %	8.0	39.6†
Current drinkers, %	28.2	70.6†
Present illness		
Hypertension	21.3	25.6†
Hyperlipidemia	48.0	30.2†
Diabetes mellitus	3.3	7.9†
Myocardial infarction	0.5	1.7†
Ischemic stroke	0.8	2.5†
Total plasma homocysteine, μmol/L	10.7±3.0 (n=820)	13.3±4.2* (n=721)

Values are mean±SD or percentage.

Hypertension indicates SBP ≥160 mm Hg and/or DBP ≥95 mm Hg or antihypertensive medication; hyperlipidemia, total cholesterol ≥5.68 mmol/L (220 mg/dL) or antihyperlipidemia medication; diabetes, fasting plasma glucose ≥7.0 mmol/L (126 mg/dL), nonfasting plasma glucose ≥11.1 mmol/L (200 mg/dL), or antidiabetic medication.

*P<0.05 between female and male by Student's t test.

†P<0.05 between women and men by χ^2 test.

Only the frequency of hyperlipidemia was significantly higher in women than in men.

C677T/MTHFR, Hypertension, and Plasma Homocysteine Levels

The frequencies of *C677T/MTHFR* in women were 37.5% for *CC*, 47.2% for *CT*, and 15.3% for *TT* genotypes, whereas those in men were 36.2% for *CC*, 47.8% for *CT*, and 16.0% for *TT* genotypes. There was no significant difference in allele frequencies between age groups (χ^2=1.07, df=2, P=0.59). The genotype distribution of *C677T/MTHFR* was not significantly deviated from Hardy-Weinberg's expectation in men or women. In women, SBP and DBP increased according to the number of *T677* alleles of *MTHFR*, but the association was not statistically significant. Only DBP in *TT* women was significantly higher in those with the *C677* allele after age adjustment. In the recessive model (*CT*+*CC* versus *TT*), however, *C677T/MTHFR* was significantly associated with the prevalence of hypertension, and the all adjusted OR for hypertension was 1.42 (95% CI, 1.01 to 1.99) in women (Table 2).

Figure 1 shows plasma total homocysteine levels according to genotype of *C677T/MTHFR* in men and women. Mean plasma total homocysteine levels in subjects with the *TT* genotype was significantly higher than that in subjects with the *CC* or *CT* genotype.

TABLE 2. ORs of Presence of Hypertension in Men and Women by *C677T/MTHFR*

	Dominant Model		Recessive Model	
	CC	*CT*+*TT*	*CC*+*CT*	*TT*
Women (n=1693)				
Hypertensive, %	19.5	22.4	20.7	24.7
All adjusted OR*	1	1.15 (0.88–1.49)	1	1.42 (1.01–1.99)†
Men (n=1554)				
Hypertensive, %	25.9	25.3	25.5	25.7
All adjusted OR*	1	0.93 (0.73–1.20)	1	1.00 (0.72–1.40)

*Conditional logistic analysis, adjusted for age, BMI, SBP, smoking, drinking, antihypertensive drug use, hypercholesterolemia, and diabetes.

†P<0.05 vs *CC* or *CC*+*CT* subjects.

Carotid Atherosclerotic Index and *C677T/MTHFR*

Carotid atherosclerotic indexes (IMT, maximum IMT in CCA, maximum IMT, and plaque score) were evaluated in men and women separately, according to *C677T/MTHFR* genotype (Table 3). In women with the *CT* genotype, age-adjusted IMT, maximum IMT in CCA, and all adjusted maximum IMT in CCA were significantly thicker than in those with the *CC* genotype. However, there was no difference between subjects with the *TT* and *CC* genotypes in any atherosclerotic indexes.

In contrast, *C677T/MTHFR* gave a significantly increased risk for stenosis (>50%) of CCA in women. In a recessive model (*CC*+CT versus *TT*), the all adjusted OR for stenosis (>50%) was 3.42 (95% CI, 1.23 to 9.53) in women and 1.41 (95% CI, 0.76 to 2.63) in men.

Partial correlation coefficients between plasma total homocysteine levels and carotid atherosclerotic index by *C677T/MTHFR* genotype are shown in Table 4. Positive relationships were found between plasma total homocysteine levels and IMT in men with the *CC* genotype and maximum IMT in CCA for men. These associations were stronger in men than in women.

Interaction Between *C677T/MTHFR* and Lifestyle on Carotid Atherosclerotic Index According to Sex

Figure 2 shows the association of IMT and maximum IMT in CCA with *C677T/MTHFR* according to smoking and drink-

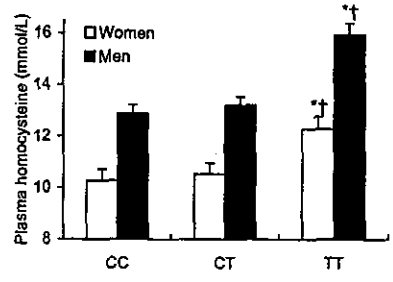

Figure 1. Plasma total homocysteine levels according to *C677T/MTHFR* by sex. Values are least-square mean±SE adjusted for age, BMI, smoking, drinking, antihypertensive drug use, hyperlipidemia, and diabetes. Bars indicate SE. *P<0.0001 vs CC subjects; †P<0.0001 vs CT subjects.

TABLE 3. Carotid Atherosclerotic Index in Men and Women by *C677T/MTHFR*

	MTHFR Genotype			
	CC	*CT*	*TT*	χ^2 *P*
Women (n=1693)				
IMT, mm				
Age adjusted	0.825±0.004	0.842±0.003‡	0.832±0.006	0.004
All adjusted*	*0.861±0.009*	*0.874±0.009‡*	*0.866±0.010*	*0.030*
Maximum IMT in CCA, mm				
Age adjusted	*1.004±0.011*	*1.035±0.010†*	*1.023±0.018*	*0.122*
All adjusted	1.075±0.026	1.100±0.025	1.094±0.029	0.231
Maximum IMT, mm				
Age adjusted	1.274±0.021	1.311±0.018	1.307±0.032	0.383
All adjusted	*1.415±0.050*	*1.441±0.049*	*1.444±0.056*	*0.586*
Plaque score, mm				
Age adjusted	1.990±0.108	2.259±0.096	2.141±0.169	0.178
All adjusted	2.915±0.262	3.114±0.254	3.026±0.293	0.369
Men (n=1554)				
IMT, mm				
Age adjusted	*0.886±0.005*	0.882±0.004	*0.889±0.007*	*0.668*
All adjusted	0.892±0.007	0.890±0.006	0.898±0.009	0.586
Maximum IMT in CCA, mm				
Age adjusted	1.162±0.020	1.140±0.017	1.144±0.030	0.713
All adjusted	1.173±0.027	1.163±0.026	1.165±0.036	0.916
Maximum IMT, mm				
Age adjusted	1.642±0.034	1.627±0.030	*1.721±0.051*	*0.273*
All adjusted	1.653±0.047	1.638±0.044	1.75±0.060	0.145
Plaque score, mm				
Age adjusted	4.201±0.178	4.010±0.155	4.308±0.268	0.550
All adjusted	*4.413±0.246*	*4.215±0.232*	*4.625±0.316*	*0.363*

*Values are least-square mean±SE adjusted for age, SBP, BMI, smoking, drinking, and medication (for hypertension, hyperlipidemia, or diabetes).

†$P<0.05$ vs *CC* subjects; ‡$P<0.005$ vs *CC* subjects.

ing status. In women with the *CC* or *CT* genotype, IMT in smokers was significantly higher than in nonsmokers. In women with the *TT* genotype, maximum IMT in CCA in smokers and drinkers was significantly higher than that in nonsmokers and nondrinkers, respectively (Figure 2–A2, $P<0.05$ for interaction; Figure 2–B2). In men with the *CC* or *TT* genotype, IMT and maximum IMT in CCA were significantly higher in smokers than in nonsmokers (Figure 2–A1).

TABLE 4. Partial Correlation Coefficient Between Plasma Total Homocysteine and Carotid Atherosclerotic Index by Sex and *C677T/MTHFR*

	CC	*CT*	*TT*
IMT			
Women	0.056 (0.334)	0.014 (0.784)	−0.027 (0.758)
Men	0.167 (0.001)	0.056 (0.300)	0.111 (0.253)
Maximum IMT in CCA			
Women	0.058 (0.398)	0.005 (0.935)	−0.098 (0.355)
Men	0.218 (0.003)	0.146 (0.016)	0.363 (0.002)

Figures in parentheses indicate *P* value adjusted for age, BMI, drinking, smoking, SBP, and medication for hypertension, hyperlidemia, and diabetes mellitus.

Discussion

The present study showed that the *TT* genotype of *C677T/MTHFR* was significantly associated with the prevalence of hypertension (OR, 1.15) and carotid stenosis (<50%) in women but not in men. In addition, the specific genotype of *C677T/MTHFR* affected maximum IMT in CCA in the interaction with smoking in women. These results show an association of *C677T/MTHFR* with BP and carotid atherosclerosis on the basis of gene and environmental interaction, which has not been previously reported.

Although previous studies showed that subjects with the *TT* genotype of *C677T/MTHFR* are associated with an increased risk of cardiovascular disease via an increase in plasma homocysteine levels,[2,6,20] the conclusion is still controversial.[7,14,15,21,22] The inconsistencies may be attributed to small sample size, combined-sex analysis, and no inclusion of lifestyle factors such as smoking and drinking. One should be aware that detecting gene and environmental interactions

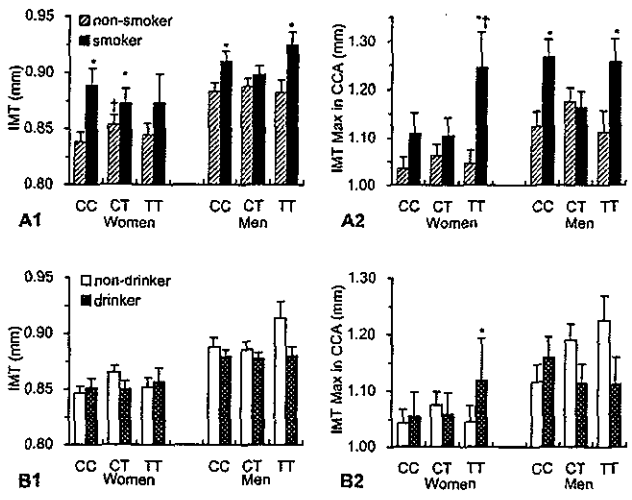

Figure 2. Association between *C677T/MTHFR* and carotid atherosclerotic indexes (IMT and maximum IMT in CCA) according to smoking (A) and drinking (B) status in men and women. Data are shown as the least-square mean±SE adjusted for age, BMI, SBP, smoking, drinking, and medication (for hypertension, hyperlipidemia, and diabetes). *$P<0.05$ vs nonsmokers (or nondrinkers) in subjects with the same genotype; †$P<0.05$ vs *CC* subjects with the same lifestyle (for smoking and drinking).

could require a substantially larger sample size than the sample size necessary for detecting genetic or environmental effects alone.[23] Thus, we examined the effect of *C677T/MTHFR* in a large general population with various phenotypes that included plasma homocysteine levels, atheromatous indexes, smoking and drinking status, and relevant basic characteristics.

It can be questioned why the *TT* genotype of *C677T/MTHFR* is not unequivocally associated with increased cardiovascular risk,[5] based on the argument that the gene is a strong predictor of hyperhomocysteinemia in general populations.[6,24,25] It could be attributed to the close relationship between plasma homocysteine levels and folate metabolism. Several reports revealed that plasma total homocysteine levels become elevated only in folate-deficient subjects with the *TT* genotype[7,25,26] and that the slope of regression lines relating total homocysteine to folate increases in the order of *CC*, *CT*, and *TT* genotypes.[15,24] In other words, if folate intake is sufficient, subjects with the *TT* genotype would not have increased risk of cardiovascular disease via hyperhomocysteinemia.

Under stratification by sex, we observed that the *TT* genotype was independently associated with DBP and carotid stenosis in women and showed a greater disadvantage in female smokers and drinkers. Even though homocysteine would injure the endothelium of small arteries at an early stage[27] and endothelial dysfunction plays a critical role in the early events of atherosclerosis,[28] we currently have no definitive answer to explain the results. However, it seems to be an important finding that most of the positive results in the present study were obtained only in women. As supporting data of our results, a female-specific significant association with the *TT* genotype was also reported in the predisposition to ischemic stroke[29] and asymptomatic carotid atherosclerosis.[30] Motti et al[31] reported that sex differentiation is independently associated with homocysteine. Plasma homocysteine levels are significantly higher in healthy men than in women, which is consistent with our results (Table 1). In addition, homocysteine levels are reported to be lower in premenopausal women than in men and postmenopausal women. Furthermore, a recent report suggested that total homocysteine levels were significantly correlated with fat-free mass and testosterone and inversely with estradiol. The sex difference with regard to total homocysteine levels was explained primarily by differences in fat-free mass but also by estradiol concentration. Those results might be a feasible explanation for the lack of association in men.[32] However, there was no association between *C677T/MTHFR* and carotid atherosclerosis in premenopausal and postmenopausal women (data not shown). This result suggests that estrogen might have a protective effect against homocysteinemia but not atherosclerosis via *C677T/MTHFR*. Indeed, previous reports did not find such a specific advantage in the relationship between *C677T/MTHFR* and coronary artery disease in young woman in a small Caucasian population.[33,34]

Disadvantages of our study design were that only half of the subjects had their total plasma homocysteine levels analyzed. This is not a serious limitation, however, because the association between *C677T/MTHFR* and plasma homocysteine levels has already been demonstrated in several large studies.[7,26] Another disadvantage was that we had no data on the physical activity and nutrition of the subjects, but these data were also supported by previous studies. The dietary intake of folate, vitamin B_6, and B_{12} is inversely (negatively) correlated with plasma homocysteine[35,36]; physical activity is also inversely associated with plasma homocysteine.[37] There is a need for additional prospective studies with data on relevant confounders that have sufficient power to examine the association between homocysteine concentration and stroke risk, whether linear or threshold, and to study interactions between homocysteine, other dietary markers, and established stroke risk factors such as smoking and hypertension. Similarly, the evidence linking hyperhomocysteinemia with hypertension is limited and inconsistent. Ultimately, the case for a causal role of elevated homocysteine levels in vascular disease, including hypertension and stroke, will depend on data from randomly controlled trials of homocysteine-lowering interventions.

In summary, the present study shows that the homozygous *T677* allele of *C677T/MTHFR* is a risk factor for hypertension and carotid stenosis in women. In addition, smoking increased IMT in CCA in women with the *TT* genotype. In the near future, physicians might use the genotypic data of *C677T/MTHFR* to modify their patients' lifestyles to prevent cardiovascular disease.

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医師主導の治験および臨床試験

佐瀬一洋　　国立循環器病センター　心臓内科／治験管理室

要約

平成15年度には改正薬事法施行および臨床研究倫理指針告示という大きな変化がある。臨床研究はトランスレーショナル・リサーチの最終段階であり，その支援体制整備や人材育成が急務である。薬事法上の治験や医師主導の治験は臨床試験の一部に過ぎないものの，ひと足先に導入された世界標準のGCPにより育成された組織や人材を活用しつつ，治験や臨床試験全体の充実へ向けて，被験者保護を充実し，スピード・質・コストを改善するためのシステムを創る必要がある。ただし，医師および実施医療機関の長への負担はこれまでになく大きくなることが予想され，費用負担と利益相反，委員会審議の形骸化等，解決すべき問題点はまだまだ多い。

1　はじめに

ポスト・ゲノム時代を迎え，創薬R&D（研究開発）は世界規模での競争となっている[1-3]。基礎実験や探索的研究の結果から導かれた仮説がしばしば検証的試験により覆されてきた事実[4-7]を見ても，トランスレーショナル・リサーチの最終段階である臨床試験研究は患者にとっての真の利益を評価するために必要不可欠といえる。

わが国では臨床研究のスピード・質・コストに問題が残る[8-10]。平成14年12月6日に発表されたBT（バイオテクノロジー）戦略会議の最終答申[11]では臨床研究を促進するための体制整備を図るべきと提言され，平成15年4月30日に発表された文部科学省，厚生労働省の「全国治験活性化3カ年計画」[12]でも基盤整備がうたわれている。

平成15年度には改正薬事法施行および臨床研究指針告示という大きな変化がある。世界的な被験者保護の流れとともに注目が必要である。

2　治験と臨床試験

「医師主導の治験」という言葉を耳にする機会が多くなった。しかしながら，現時点では一般の医療機関において薬事法や治験という単語の意味が十分に理解されているとは言い難い。

「治験」とは，厚生労働大臣に治験届けを提出したうえで，医薬品・医療機器の承認のための科学的な見地からの審査に必要な実証データの収集を目的として，ヒトを対象に実施する臨床試験である（表1）。すなわち，医学研究⊃臨床試験⊃治験という関係である（図1）。

わが国においては従来治験にまつわる金銭面の疑惑やデータの信頼性等が問題とされ，更に業績として認められ

表1　「治験」

薬事法（昭和三十五年八月十日法律第百四十五号）第二条第七号 「この法律で「治験」とは，第十四条第三項（同条第七項，第十九条の二第四項及び第二十三条において準用する場合を含む。）の規定により提出すべき資料のうち臨床試験の試験成績に関する資料の収集を目的とする試験の実施をいう。」

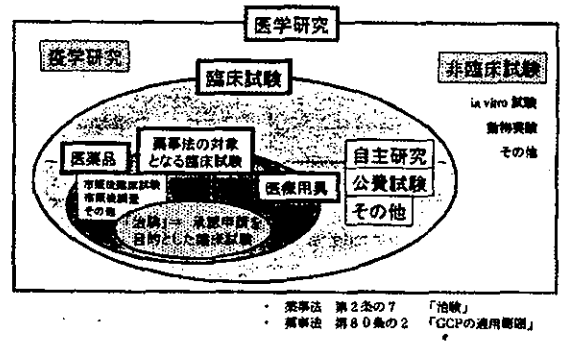

図1　臨床研究と治験

にくい等のインセンティブの欠如も含め，医療機関の関心はあまり高いとは言えなかった。

近年，黒船に例えられるICH（日米欧三極医薬品規制ハーモナイゼーション国際会議）による一連のガイドライン（表2），特に新GCP（ICH-E6）や海外データ受け入れ（ICH-E5）により，まず治験についていち早く国際化が進んだ[13]。しかしながら，治験を欧米で先行させるケースがみられたり，治験届の数が減少していることから「治験の空洞化」を懸念する声がある。

表2　ICHガイドラインの例

E1A	「致命的でない疾患に対し長期間の投与が想定される新医薬品の治験段階において安全性を評価するために必要な症例数と投与期間について（平成7年5月24日薬審第592号）」
E2A	「治験中に得られる安全性情報の取り扱いについて（平成7年3月20日薬審第227号）」
E2B	「個別症例安全性報告の伝達のためのデータ項目」
E2C	「市販医薬品に関する定期的安全性最新報告（PSUR）（平成9年3月27日薬安第32号）」
E3	「治験の総括報告書の構成と内容に関するガイドライン（平成8年5月1日薬審第335号）」
E4	「新医薬品の承認に必要な用量-反応関係の検討のための指針（平成6年7月25日薬審第494号）」
E5	「外国臨床データを受け入れる際に考慮すべき民族的要因についての指針（平成10年8月11日医薬審第672号）」
E6	「医薬品の臨床試験の実施の基準に関する省令（平成9年3月27日厚生省令第28号）」，及び「医薬品の臨床試験の実施の基準に関する省令の施行について（平成9年3月27日薬発第430号薬務局長通知）」
E7	「高齢者に使用される医薬品の臨床評価法に関するガイドライン（平成5年12月2日薬新薬発第104号）」
E8	「臨床試験の一般指針（平成10年4月21日医薬審第380号）」
E9	「臨床試験のための統計的原則」について（平成10年11月30日医薬審第1047号）
E10	「臨床試験における対照群の選択とそれに関連する諸問題」について（平成13年2月27日医薬審第136号）
E11	「小児集団における医薬品の臨床試験に関するガイダンスについて」（平成12年12月25日医薬審第1334号）
E12	「降圧剤の臨床評価に関する原則」（平成12年5月29日医薬審第738号）
M1	「『ICH国際医薬用語集日本版（MedDRA/J）』の使用について」（平成11年12月28日医薬安第164号，医薬審第1843号」
M3	「医薬品の臨床試験のための非臨床安全性試験の実施時期についてのガイドライン（平成10年11月13日医薬審第1919号）」

長期的に見れば，治験の国際化は必ずしも国内治験の空洞化を意味しないが，治験を推進するためには臨床研究全体を推進する必要がある[12]。医療の質向上のための科学的根拠づくりを円滑に実施し，国際水準の臨床研究への参加も可能となるような方向性を模索するべきである。

3　薬事法改正と医師主導型の治験

平成15年7月30日に改正薬事法が（一部を除き）施行される。平成9年の前回改正時には，薬害エイズの教訓やICHによる国際整合化を踏まえ，新GCP導入や海外データ相互利用が可能となった。今回の改正は，（1）「バイオ・ゲノムの世紀」への対応，（2）承認・許可制度の見直し，（3）医療機器規制の見直し，（4）「治験型臨床研究」制度の導入を特徴としている（表3）。

いわゆる「医師主導型の治験」とは，従来治験とそれ以外に二分されてきた臨床試験について，「自ら治験を実施しようとする者による治験届制度」として新たに位置づけられたものである（図2）。厚生労働大臣に対する治験届の提出とGCPの適用を条件に，未承認薬剤・機械器具の提供や特定療養費制度による治験期間中の保険適用に道が開かれるという。現時点ではまだ費用負担や被験者保護の具体的方策等の議論が進行中のようであるが，ICH-GCPにおける「sponsor-investigator」に似た概念とも受け取れ，今後の関連諸通知や諸規定，標準的業務手順書等の整備に注目があつまっている（表4）。

4　改正GCP

いわゆる新GCP（ICH-E6（表2））では，科学性・倫理性・信頼性の向上を目的に，治験依頼者，治験審査委員会，治験実施医療機関の長，治験責任医師の各実施主体の要件と責務を明示するとともに，品質管理・品質保証活動としてのモニタリング・監査や，治験協力者として治験コーディネーター（CRC）等による治験責任医師補助等の臨床試験支援業務が導入された。

平成15年7月30日から「医薬品の臨床試験の実施の基準に関する省令の一部を改正する省令」（以下「改正GCP」）が施行された。改正GCPの施行については，平成15年6月12日付の医薬局長通知（以下「局長通知」）等，関連諸通知（表4）のなかで具体的な留意点についての記述がなさ

表3　薬事法改正の主なポイント

◎医療機器に係る安全対策の抜本的な見直し
　医薬品以上に多様な技術・素材が用いられる医療機器の特性に配慮
◎「バイオ・ゲノムの世紀」に対応した安全確保対策の充実
　生物由来製品の安全確保に向けての法的整備が急務
◎市販後安全対策の充実と，承認許可制度の見直し
　企業の安全対策責任の明確化と，国際整合性を踏まえた製造承認制度の見直し
◎「自ら治験を実施する者」に対する治験の計画届出制を拡大
　いわゆる医師主導の治験

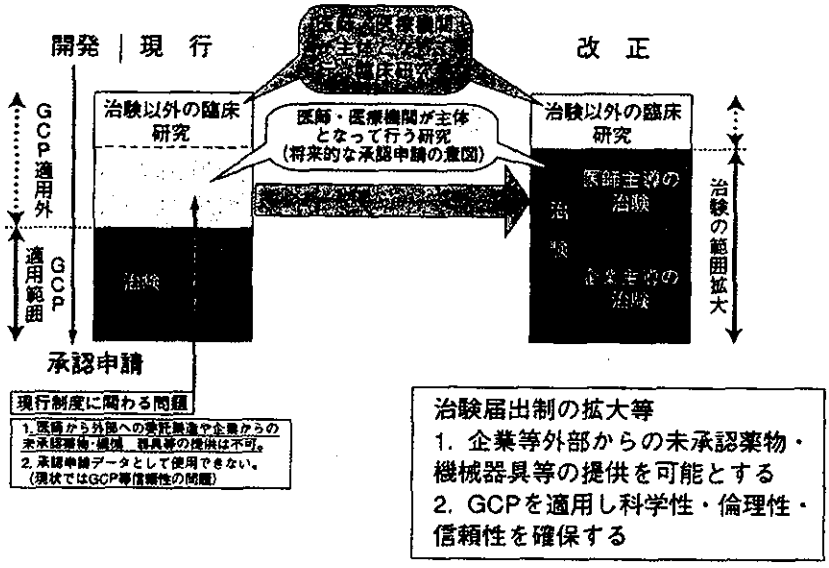

図2　医師主導の治験の位置づけ

表4　医師主導の治験に関連する法令・諸通知

◎薬事法及び採血及び供血あっせん業取締報の一部を改正する法律
　（平成14年法律第96号）
◎薬事法及び採血及び供血あっせん業取締報の一部を改正する法律の一部の施行について
　（平成15年5月15日医薬発第0515017号厚生労働省医薬局長通知）
◎医薬品の臨床試験の実施の基準に関する省令の一部を改正する省令
　（平成15年厚生労働省令第106号）
◎医薬品の臨床試験の実施の基準に関する省令の一部を改正する省令の施行について
　（平成15年6月12日医薬発第0612001号厚生労働省医薬局長通知）
◎自ら実施する薬物に係る治験の計画の届出等に関する取扱いについて
　（平成15年6月12日医薬審発第0612001号厚生労働省医薬局審査管理課長通知）
◎「薬物に係る治験の計画の届出等に関する取扱いについて」の一部改正について
　（平成15年6月12日医薬審発第0612004号厚生労働省医薬局審査管理課長通知）

れているが，新　GCPと同様，今後審査課長通知等の更に詳細な文章が発出されるものと予想される。適用対象は，改正前のGCPの適用対象（製薬メーカーや輸入販売業者）に加え，医師主導型治験を実施する医師・医療機関が新たに加わった。

改正GCPの作成にあたっては，厚生労働科学特別研究班「医師主導の治験の実施の基準のあり方に関する研究（主任研究者：上田慶二）」において平成14年7月から12月まで計7回の会議で検討された[10]。

基本原則として，（1）企業が主体となって行う治験と同様に，現行のGCPと同様の水準と内容を網羅し，国際標準（ICH-GCP）との整合性にも配慮すること，（2）現行GCPにおける治験依頼者の責務に関して，原則として，「自ら治験を実施しようとする者」又は「自ら治験を実施する者」（医師，医療機関）が「治験依頼者」と同等の責務を負うものとすること，の2点が示された。具体的には「治験の依頼をしようとする者」は「自ら治験を実施しようとする者」に，「治験依頼者」は「自ら治験を実施する者」に読み替えたものが基礎となっている。

改正前のGCPと比較すると，（1）言葉の定義（自ら治験を実施しようとする者」，「自ら治験を実施する者」，「治験薬提供者」等），（2）医療機関の長が治験実施を承認する旨の規定，（3）モニタリング・監査の実施について医療機関の長と治験審査委員会がチェックを行う旨の規定，（4）治験施設支援機関（SMO）に係る規定，（4）被験者のプライバシーと秘密の保全に関する規定などがそれぞれ追加された。

「自ら治験を実施する者」が「治験依頼者」と同一主体であることから，品質管理・品質保証（モニタリング・監査），副作用被害に対する補償，治験実施計画書の作成，治験薬概要書の作成，重篤な有害事象報告，治験薬管理，治験薬提供，総括報告書作成，治験審査委員会（IRB）の審査機能の確保，「自ら治験を実施する者」と医療機関の長および治験責任医師の関係，その他（治験届の範囲・内容，治験の妥当性の確保，治験データの所有権および譲渡，多施設で行う場合の留意点等）についての修正がなされている。

5 医師主導治験における臨床試験の計画

5.1 治験届

医師主導の治験の実施で実施医療機関がまず認識しなければいけない重要なポイントは，本制度は医薬品の承認申請の資料とする目的で行うものであり，厚生労働大臣に対する治験計画の届出が必要なことである（表5）。更に，治験届の提出に先立ち，治験審査委員会の承認を踏まえた実施医療機関の長の承認が必要とされている（改正GCP第15条の7）。従って，医師主導型の試験の流れとしては，まず治験薬概要書の作成，治験実施計画書等の作成，施設における治験審査委員会の承認を踏まえた実施医療機関の長の承認，その後厚生労働省への治験計画届提出の順となる。業務の一部はCROやSMOに外部委託することができる（第39条の2関係）。

5.2 治験実施計画書

治験実施計画書（Protocol）は，「自ら治験を実施する者」が，必要な情報を契約等により企業から入手し，作成する（改正GCP第15条の3，同第15条の4）。計画段階では試験の内部妥当性，外部妥当性についての検討と独立した治験審査委員会などの審査が必要である。

いわゆる外部妥当性としては，文献調査および治験薬概要書等の資料をあらかじめ用意する必要があるが，パブリケーション・バイアス，すなわち開発中止届けや有害事象報告など一般には公表されていない情報が存在する可能性があるため，企業や行政当局との十分な検討が必要である。

一方，内部妥当性としては，臨床試験の目的，デザイン，

表5　医師主導の治験を行うに当たって

前提：医師主導であっても，治験とは医薬品の承認申請の資料とする目的で行う臨床試験であることに留意が必要
↓
自ら治験を実施しようとする者（治験責任医師）は，
（1）　治験計画作成
（2）　実施医療機関の長に承諾を求めることを通じて院内の治験審査委員会に諮る
（3）　厚生労働大臣に治験届を提出する
（4）　GCPを遵守（被験者保護，データの信頼性保護のため）

実施，解析，報告について，個々の臨床試験のフェーズおよび試験の目的に応じて，ICH-E9等の各種ガイドライン（表2）を参考に十分検討し，試験開始前に治験実施計画書に明確に規定しなければならない。

5.3 治験薬概要書

予定されている臨床試験が十分安全であることを示すために，自ら治験を実施する者は，非臨床試験又は先行する臨床試験の結果を検討しなければならない。

「自ら治験を実施する者」は，必要な情報を契約により企業等から入手し，治験薬概要書（Investigator's Brochure）を作成する（改正GCP第15条の5）。治験薬提供者は，必要な資料又は情報を提供する（局長通知，第15条の5関係）。

治験薬概要書は，いわゆる非臨床試験や臨床試験の結果をまとめたもので，被験薬の物理的，化学的および製剤学的性質，薬理，毒性，薬物動態，薬物代謝に関連する理化学試験，動物試験，他の臨床試験等の結果が含まれる。

5.4 多施設共同試験

多施設共同試験の計画は，医師主導の治験においても有り得るとされているが，検証的試験においてはむしろ大半を占める可能性もある。

医師主導の治験では，多施設共同試験の場合の特例として，各施設の治験責任医師が連名で一つの治験の計画を提出しても差し支えないとされている（審査管理課長通知第0612004号，表4参照）。その場合，各治験責任医師が「自ら治験を実施する者」となるが，治験計画の届出，厚生労働大臣や各実施医療機関への副作用報告に関する調整業務を，治験調整医師又は治験調整委員会に委嘱できる（改正GCP第26条の4）。

治験実施計画書，症例報告書，同意説明書（案）の作成，薬剤の準備，データ・マネージメント等，治験調整医師のもとに業務が集中することが予想される。臨床試験の中央事務局／治験調整委員会業務について，手順書の整備等の支援体制について，臨床研究センターの整備やCROへの委託も含め検討が必要である。

5.5 倫理的配慮

治験および臨床試験を実施する場合には，ヘルシンキ宣言に基づき，必要な情報の十分な提供や説明の下での被験者の同意と協力（インフォームド・コンセント）が必要不可欠である。同意説明文書は自ら治験を実施しようとする者が作成する（改正GCP第15条の6）。

世界的には利益相反（conflict of interest）問題が注目されている。治験審査委員会の審議資料のなかで「治験の費用に関する事項」として実施医療機関以外の者が治験の費用の一部を負担する場合（治験薬を提供する場合を含む。）の負担に関する具体的な取り決めの内容等が含まれること（局長通知，第15条の7関係）とされているが，透明性の確保と情報公開が必要となろう。

また，治験審査委員会における審議の公正性確保が必要である。自ら治験を実施する者の上司・部下，当該治験薬提供者と密接な関係を有する者等（局長通知，第29条関係）は関与委員として審議および採決に加わることができないことを手順書に明示する必要がある。

5.6 生物統計の重要性

臨床試験の計画と解析における生物統計学の役割は欠くことのできないものと認められている（ICH-E9，E10各ガイドライン（表2）参照）。

自ら治験を実施しようとする者による治験の準備等に関する基準（改正GCP第15条の2）第2項の「治験の実施の準備及び管理に係る業務を行うことにつき必要な専門的知識を有する者」とは，治験に関する医学的な問題について適切な助言を行う医学の専門家，並びに治験実施計画書，治験薬概要書等の作成・改訂，データの取り扱い，統計解析の実施，総括報告書の作成等，治験の全過程を通じて活用されるべき実施医療機関内部および外部の専門家（例：生物統計学者，臨床薬理学者等）を含むもの（局長通知，第15条の2関係）と明記されていることに注目が必要である。

5.7 品質管理・品質保証

治験の品質管理・品質保証として治験依頼者によるモニタリングおよび監査は特に重要である。医師主導の治験では「自ら治験を実施する者」の責任となるが，実施主体はノウハウを有する第三者（実施医療機関，IRB（実施医療機関のIRB，他のIRB），製薬企業，CRO等）が行ってもよいとされており，公正性確保と守秘義務に留意して計画すべきである（改正GCP第26条の7，8，9）。

5.8 治験薬の提供

医師主導型治験において，どのように治験薬の品質を確保するかは問題である。「治験薬提供者」とは，自ら治験を実施するものに対して薬物を提供する者と定義されている（改正 GCP 第2条）が，治験薬 GMP（Good Manufacturing Practice）適合について，文書等により明確な取り決めが義務付けられた（局長通知第26条の3関係）。

更に，治験外の患者に対する利用防止策，特に薬事法違反回避のため治験薬の提供を治験計画届受理後に限ることが明記された（局長通知，第26条の2関係）。

プラセボと実薬との割り付けについては，実施する者が自らの責任で割り付けをすることが必要で，治験薬提供者の責務ではないといわれている[14]。

6 医師主導治験における臨床試験の実施

6.1 治験審査委員会

医師主導の治験においては，治験審査委員会（IRB）がより重要な役割を担う（改正 GCP 第32条）。新たに厚生労働大臣への治験届提出に先立つ審査（第15条の7），モニタリング・監査報告の審査（改正 GCP 第31条第3項）等の規定が追加されている。厚生科学特別研究班では，IRB の審査機能確保のため，IRB を指導・監督することが必要であるとして，治験計画届の受理の際に，IRB の審査体制や IRB 委員の教育体制などを審査すること，IRB のレベル向上のため，IRB に係るガイドラインの策定およびその周知，IRB 委員の教育および研修の実施を行うことを提言している[14]。

6.2 実施医療機関の長

わが国では，治験の実施にあたっては医療機関の長による承認が必要（改正 GCP 第10条及び第13条）とされている。更に，医師主導の治験においては，厚生労働大臣に治験届を提出する前に，実施医療機関の長の承認を得ておくことが必要とされた（改正 GCP 第15条の7）。

また，治験開始後は，副作用報告（改正 GCP 第31条関係），治験薬管理（同第39条），モニタリング・監査（同第36条の3，第37条），治験の中止等（同第40条），緊急回避のための逸脱（同第46条）等に関連し，治験を中止させることを含め，実施医療機関の長は必要な措置を講ずることが求められている（同第32条第3項）。通常実施医療機関の長は多忙であることが予想されるため，あらかじめ業務手順を明確化しておく必要がある。

6.3 治験責任医師

治験実施中の治験責任医師の要件と責務は，分担医師・協力者の一覧表作成（改正 GCP 第43条），被験者のスクリーニング（同第44条），被験者に対する説明や措置（同第45条），プロトコル遵守（同第46条），症例報告書作成（同第47条），副作用報告（同第48条），治験の中止（同第49条），ならびにインフォームド・コンセント（同第50条～第55条）と多岐にわたる。

更に，医師主導の治験では，「自ら治験を実施しようとする者」として，治験薬の管理，副作用情報の収集・検討，モニタリング・監査の実施と報告，治験中止の判断等（同第26条の1～12）多くの業務が発生する。従って，治験の準備として標準的業務手順書の作成（局長通知，第15条の2関係）が求められている。

なお，治験の実施の準備および管理に係る業務の一部を，医療機関外部（CRO および SMO）に委託することができる（同第15条の8関係）が，国公立病院では契約の主体や費用負担をどうするか等，実際の運用にあたっては関係各方面の調整が必要となろう。

6.4 安全性情報

被験者保護の観点から，治験中に得られる安全性情報は重要であり，医師主導の治験においても迅速かつ適切な措置を講じることは有益なことである。実際，「自ら治験を実施する者」は，改正薬事法第80条の2の規定により薬事法施行規則第66条の7に沿って副作用等に厚生労働大臣（実際には審査センター宛）に報告しなければならない。ICH-E2A（表2）には用語の定義（有害事象，副作用，予測できない副作用など），取り扱い（報告すべきもの，報告期限，報告方法など）についての国際的な合意が示されている。

「自ら治験を実施する者」は，外部からの重篤な有害事象の報告（改正 GCP 第20条），特に海外副作用情報の把握について，国内企業が治験実施中の場合以外は自ら把握すべきとされている。把握の方法や治験薬提供者の姿勢が課題として残されている[14]。

また，治験責任医師は，医師主導の治験実施中に治験薬の副作用によると疑われる死亡その他の重篤な有害事象の発生を認めたとき，直ちに実施医療機関の長，他の実施医療機関の治験責任医師（多施設共同治験の場合）および治験薬提供者に対しても通知しなければならない（改正GCP第48条）。

6.5 モニタリング・監査

医師主導の治験において，「モニタリング」とは，治験が適正に行われることを確保するため，「監査」とは，治験により収集された資料の信頼性を確保するため，自ら治験を実施する者が実施医療機関に対して特定の者を指定して行わせる調査であるが，中立かつ公平に実施されるべきであり，モニタリング・監査を治験責任医師自身が実施することはできない点に注意が必要である。

医療機関外部の第三者機関を利用することができるが，当該実施医療機関内の者を指定する場合は，当該治験に従事していない第三者で，監査は更にモニタリングと独立した者が実施するべきであるとしてモニターになるべきでない者等が明示された（改正GCP第26条の7）。

また，自ら治験を実施する者からの独立性を更に担保するため，モニタリング・監査に関する計画書，業務手順書の作成（改正GCP第26条の7及び9），モニタリング・監査の報告書（改正GCP第31条第3項）等について，今回新たに治験審査委員会の審議が必要とされた。

なお，直接閲覧に際して被験者の個人情報を保護するため，実施医療機関の長は必要な措置を講じなければならない（局長通知，第36条関係）。

6.6 効果安全性評価委員会

効果安全性評価委員会は，治験の継続の適否又は治験実施計画書の変更について審議するための委員会であり，治験の進行，安全性データおよび重要な有効性エンドポイントを適切な間隔で評価する（局長通知，第26条の5関係）。統計学を含む適切な学識を持った臨床試験の専門家から構成されるべきである（ICH-E6）。独立性を保つため，自ら治験を実施する者，治験責任医師等，治験調整医師，治験審査委員会の委員，治験薬提供者および実施医療機関の長は効果安全性評価委員会の委員になることはできない。

6.7 記録の保存

記録の保存等では，自ら治験を実施する者は実施医療機関の長と契約をするわけではないことを踏まえ，契約書に代わり実施医療機関の長による承認書が必須文書とされる（改正GCP第26条の12）。

6.8 補償と賠償

治験の副作用被害に対する補償（改正GCP第14条）については，厚生科学審議会専門委員会における議論[14]では原則的には医師個人の責任であるが，IRBの審査を受けた上で医療機関の長が治験の実施を承認していることから医療機関の長も責任を有するとの意見があった。局長通知では，補償措置については，被験者に生じた副作用被害について金銭的な補償ではなく医療の提供という形での補償や副作用被害等を想定した保険などを利用することが一つの方策として示されている（局長通知，第15条の9関係）。

有過失責任である賠償については，被験者保護の観点から責任の所在が不明瞭にならないよう，契約関係や治験薬GMP適合性の確認等，あらかじめ十分に検討しておくことが重要になろう。

7 医師主導の治験とその研究結果

総括報告書の作成（改正GCP第25条）は，ICH-E3（表3）に基づき，「自ら治験を実施する者」が作成すべきとされている。その内容については，承認申請に必要な最低限の情報を網羅する必要がある。

治験データの所有権および譲渡について，薬事法上，承認申請を行う者は企業であることから，医師主導の治験で得られたデータの提供等については，「自ら治験を実施する者」と企業が契約を締結する必要がある。TLO（Technology Licensing Organization）の整備は試験実施医療機関へのインセンティブとして重要である。

知的所有権は製薬企業の生命線ともいえることから，秘密保持契約の締結・遵守等は重要である。一方，有害事象や期待に反する試験解析結果の取り扱いでは利益相反問題が生じる可能性がある。当該治験により収集された臨床試験成績に関する資料が承認申請書に添付されないことを知り得た場合，その旨およびその理由を実施医療機関の長に文書により通知することが求められる（局長通知第26条の

10関係)。

8 臨床研究の指針

ヘルシンキ宣言では，人を対象とした臨床研究の実施に際して，インフォームド・コンセント取得と研究者から独立した委員会（IRB：Institutional Review Board またはHEC：Hospital Ethical Committee）による審査を求めている。米国では国家研究法（National Research Act）やCommon Rules（45CFR46）により委員会審査の法的根拠が明確であるが，我が国では倫理審査委員会の構成員や審査資料の施設差がある。これまで臨床研究全般を対象として，その倫理性や科学性を担保する指針がなく，それが臨床研究の進まない一因との指摘があり，また，被験者の権利擁護についても十分なされていないとの指摘もある[12]。

厚生労働省は，厚生科学審議会科学技術部会専門委員会（高久史麿委員長）を中心に，「臨床研究の倫理指針」とその細則が作成[14]され，指針については平成15年7月16日の厚生労働省告示第255号により平成15年7月30日施行とされた。

指針案は（1）被験者の人権擁護，（2）被験者への説明と同意，（3）医師の責任，研究協力者の業務の明確化，（4）倫理審査委員会の機能などを中心に，ヘルシンキ宣言に沿った内容となっている。

指針の適用範囲は，ヒトを対象とした臨床研究のうち，（1）患者本人の診断，治療のみを目的とした医療行為，（2）他の法令および指針（ヒトゲノム・遺伝子解析研究，遺伝子治療の臨床研究，疫学研究，ヒト幹細胞を用いた臨床研究）の適用範囲に含まれる研究を除いたものとされる。かなり広範囲に及ぶ可能性があるが，審議項目の増加と合わせ，事務局機能の強化が必要となろう。

9 国際的な被験者保護の流れ

被験者保護についての原則，とくにヘルシンキ宣言は，ヒトを対象とする全ての臨床試験を実施するにあたって遵守されなければならない。

国際的には，被験者保護への関心が増大している。ペンシルベニア大学での遺伝子治療患者 Jesse Gelsinger（17歳）や，ジョンスホプキンス大学での健常ボランティア Ellen Roche（24歳）の死亡事件は，各マスコミにより大きな社会問題として報道され，法体系の見直し（Common Rules 改訂）と体制整備（OHRP：Office for Human Research Protection）が行われた[15-18]。

今後，被験者保護として，IRB の充実や有害事象への適切な対応が特に重要である。

IRB の充実については，米国科学アカデミー医学研究所では，2002年10月に「Responsible Research」と題する報告書[18]を発表し，審議の形骸化を防ぐために利益相反（Conflict of Interest）審査委員会，科学審査委員会，研究倫理委員会（Research ERB）の役割分担を提唱している。

有害事象については，因果関係の有無を問わずに「有害事象は必ず起こる」「発生してから対応すると混乱する」ことを前提に，被験者への対応，記録の保存，迅速な報告をあらかじめ手順化しておくことが重要である。記録に際しては，情報源，内容，重篤度，因果関係などについてチェックリストの作成が有用である。

10 臨床研究支援体制の必要性

治験の国際化と空洞化の議論の中で，治験依頼者が実施国／医療機関を選択する場合，質・スピード・コストの改善が焦点とされ，更に国際的には被験者保護の充実が急務である。従って，臨床研究支援体制の必要性は益々高まっている。

文部科学省，厚生労働省による「全国治験活性化3カ年計画」では，（1）治験のネットワーク化の推進，（2）治験実施体制の充実，（3）患者の治験参加を支援する施策が示されている。まず，治験のネットワーク化の推進として，大規模治験ネットワークの構築，オーファンドラッグ等の治験の推進，地域ネットワーク等への支援を進めるという。次に，医療機関の治験実施体制の充実等として，治験コーディネーター（CRC）の養成確保（2005年までに5,000人），実施研究者等のインセンティブの向上，医療機関における治験実施施設等の整備，医療関係者への治験に関する理解の促進，国立病院等における治験実施体制の充実，SMO や CRO の養成を進めるという。更に，患者の治験参加支援として，国民に対する普及啓発，被験者に対する治験実施状況の情報提供，医療機器治験の充実，企業の治験負担軽減，そして臨床研究全体の推進が示されている。

新 GCP 施行後の治験推進策は，医療機関における試験実施段階，すなわち，SMO（Site Management Organiza-

tion）的機能に重点が置かれてきた。具体的には，治験事務局・治験審査委員会事務局の諸規程・手順書整備や記録保存，治験責任医師業務の責務であるインフォームド・コンセント，プロトコル遵守，症例報告書作成等に対する治験コーディネーター（CRC）による補助等が充実してきた（図3）。

今後，試験の計画段階や解析段階，すなわち，ARO（Academic Research Organization）的機能に焦点を当てた臨床研究支援体制整備が必要である。具体的には，医師・生物統計家，データマネージャー，リサーチ・ナース，臨床薬理専門家，生命倫理専門家，規制担当，法律顧問を含め，主要学会とも連携しつつ質の高い臨床試験をデザイン・管理できる臨床研究センターを育成しなければならない（図4）。そのための費用負担について，医療機関がどのように対応するかは今後の課題である。

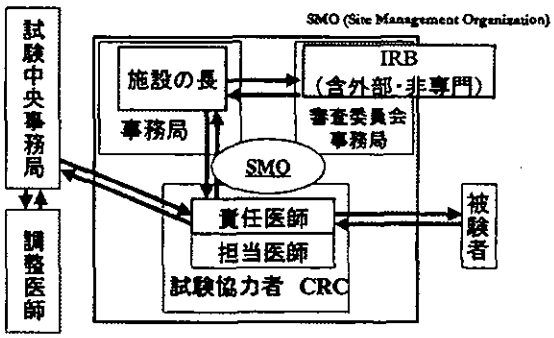

図3　実施医療機関と責任医師

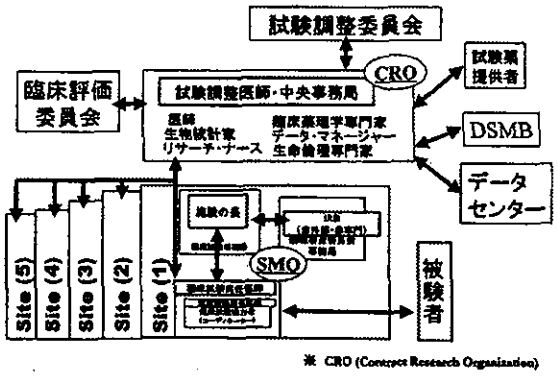

図4　中央事務局と調整医師

11 結語

改正薬事法の施行と臨床研究指針の告示を契機に，被験者保護に配慮しつつ質の高い臨床試験を計画・実施できる臨床研究チームの育成が進むことが期待されている。新GCPで育成された人材を活用し，簡素で標準的なシステムを作ることは，治験の活性化・空洞化防止策にもつながると思われる。社会に対する説明責任を果たしつつ産学官が連携することが重要である。特に，医師主導の治験については，費用負担や危機管理など，まだまだ不確実な要素が多い。説明責任と透明性をキーワードに，今後の展開に注目する必要がある。

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