The human homologue of *gob-5* is *hCLCA1*, which is expressed in intestinal epithelia, uterus, testes, and kidneys where *gob-5* is also expressed.[15,16] The human *hCLCA1* gene is one of the members of the Ca^{2+}-activated Cl^- channel family. The *hCLCA1* enhances mucus secretion by mediating the active transport of chloride ions.[10] Currently, the family includes two bovine homologues (*bCLCA1* and *bCLCA2*),[17–19] four murine homologues (*mCLCA1*, *mCLCA2*, *mCLCA3* (*gob-5*), *mCLCA4*),[20–24] and four human homologues (*hCLCA1*, *hCLCA2*, *hCLCA3*, *hCLCA4* (*hCaCC2*)).[15,16,25,26] All human *CLCA* genes identified are clustered on the short arm of chromosome 1 (1p22–31).[16,27] The *hCLCA1* gene is 31 902 bp in length, contains 15 exons, and encodes a protein of 914 amino acids.[16]

Mouse experiments on *gob-5*[10,11] and observations on bronchial epithelia from asthma patients[12] suggest that overexpression of *hCLCA1* in the bronchial epithelia exacerbates AHR and mucus hypersecretion in asthma patients. We therefore hypothesized that polymorphisms that affect expression or function of the *hCLCA1* gene would be associated with asthma phenotypes. In this study, we screened single-nucleotide polymorphisms (SNPs) in the *hCLCA1* gene and performed case-control and case-only association studies using clinically characterized Japanese patients with childhood or adult asthma.

Results

Characteristics of asthma patients

Clinical characteristics of the patients are described in detail elsewhere.[28] Briefly, 80% of the children with asthma were found to be positive for mite-specific IgE (atopic), 54% were found to have high serum IgE concentrations (>400 U/ml), and 46% were found to have atopic dermatitis. Of the adults with asthma, 28% were found to have high serum IgE concentrations and 22% experienced the onset of asthma before the age of 18 years.

Polymorphisms in the *hCLCA1* gene

During the course of the Japanese Single Nucleotide Polymorphisms project in Japan (http://snp.ims.u-tokyo.ac.jp/),[29,30] SNPs were identified in the *hCLCA1* gene. Two SNPs were located in exons. One (JST083354) was located in exon 6 and the other (JST046987 (SNP8 in this study)) in exon 15 (Table 1). Both SNPs were synonymous substitutions (Val215Val and Thr812Thr, respectively). Because the SNP of exon 6 showed complete linkage disequilibrium to SNP2 (JST120332) (Table 1) in 94 normal samples (data not shown), it was excluded from any further investigation. In addition to the exonic SNPs, seven SNPs found in the introns with a minor allele frequency of more than 20% were investigated in this study (Table 1). In the haplotype and diplotype analyses, PHASE and HAPLOTYPER programs showed essentially the same results. Results obtained with PHASE software are shown in Tables 2, 4, and 5.

To examine the linkage disequilibrium between identified SNPs, pairwise linkage disequilibrium coefficients D'[30] and r^2[31] and *P*-value were calculated using 592 controls (Table 2). Strong but not complete linkage disequilibrium was found between the following pairs: SNP1 and SNP3, SNP2 and SNP3, SNP4 and SNP5, SNP4 and SNP6, and SNP6 and SNP7. Weak linkage disequilibrium was found between the following pairs: SNP1 and SNP7, SNP2 and SNP7, SNP2 and SNP8, SNP4 and SNP8, and SNP5 and SNP7.

Association of each SNP with asthma and asthma related-phenotypes

Eight SNPs were genotyped in 384 patients with childhood asthma, 480 patients with adult asthma, and 672 controls. All genotype results of the SNPs in the control samples were in the Hardy–Weinberg equilibrium. The results of case–control and case-only association studies with significant ($P<0.01$) *P*-values are shown in Table 3.

In the case–control study, an association of SNP4 with the most severe cases of adult asthma was observed (odds ratio (OR) = 0.26, 95% confidence interval (CI) = 0.12–0.60, $\chi^2 = 11.43$, $P = 0.0032$, corrected OR (cOR) = 0.15). Both SNP2 and SNP8 showed significant association with aspirin-induced asthma (AIA) ($P = 0.0057$ and 0.0050, respectively). SNP8 showed some association with adult asthma cases that had a high (>10%) eosinophil count ($P = 0.0079$).

In the case-only study, associations between asthma-related phenotypes and the SNPs of *hCLCA1* were investigated within the group of asthma patients. There were significant differences in the genotype frequency of SNP2 and SNP8 between AIA and non-AIA asthma ($P = 0.0074$ and 0.0084, respectively). We found an association between SNP6 and the complication of atopic dermatitis in childhood asthma ($P = 0.0046$). There was no significant association

Table 1 SNPs in the *hCLCA1* gene

Name	*JSNP ID*	*SNP*[a]	*Position*[b]	*Amino acid*	*Minor allele frequency (%)*[c]
SNP1	IMS-JST049517	I5:908+246C>T	10047		42
SNP2	IMS-JST120332	I5:908+2199G>A	12000		43
SNP3	IMS-JST120333	I6:1086+589T>C	16220		45
SNP4	IMS-JST120334	I6:1087−754C>A	17837		20
SNP5	IMS-JST120335	I6:1087−705A>G	17886		33
SNP6	IMS-JST120341	I8:1533+997A>C	21007		42
SNP7	IMS-JST120342	I8:1534−808G>C	21445		33
SNP8	IMS-JST046987	E15:2787T>C	33001	T812T	49

[a]Numbering according to the cDNA sequence of *hCLCA1*.
[b]Numbering according to the genomic sequence of *hCLCA1*.
[c]SNPs were genotyped in this study.

Table 2 Pairwise linkage disequilibrium for all possible two-way comparisons among eight SNPs

	SNP1	*SNP2*	*SNP3*	*SNP4*	*SNP5*	*SNP6*	*SNP7*	*SNP8*
SNP1	*D'*	0.745*	0.749*	0.544*	0.480*	0.377*	0.337*	0.322*
	r^2	0.290*	0.489*	0.053*	0.079*	0.070*	0.040*	0.070*
SNP2		*D'*	0.987*	0.577*	0.409*	0.406*	0.271*	0.290*
		r^2	0.583*	0.060*	0.058*	0.081*	0.050*	0.064*
SNP3			*D'*	0.397*	0.386*	0.301*	0.322*	0.342*
			r^2	0.048*	0.059*	0.075*	0.063*	0.091*
SNP4				*D'*	1.000*	0.964*	0.474*	0.206*
				r^2	0.523*	0.340*	0.112*	0.010*
SNP5					*D'*	0.463*	0.212*	0.520*
					r^2	0.150*	0.043*	0.122*
SNP6						*D'*	0.914*	0.472*
						r^2	0.609*	0.144*
SNP7							*D'*	0.785*
							r^2	0.289*

*$P<0.05$.

Table 3 Association between SNPs of the *hCLCA1* gene and asthma or asthma-related phenotypes

Name	*Number*	*Genotype frequencies*			*OR (95% CI)*	χ^2 *(df=1)*	*P*
SNP2 (908+2199G>A)		AA	AG	GG			
Controls	640	0.166	0.512	0.322	Reference		
Adult asthma							
Aspirin-induced asthma	25	0.400	0.280	0.320	0.30 (0.13–0.68)[a] 0.29 (0.12–0.71)[b]	9.18[a]	0.0057[a]
Adult asthma							
With aspirin-induced asthma	25	0.400	0.280	0.320	Reference		
Without aspirin-induced asthma	441	0.170	0.485	0.345	0.31 (0.13–0.71)[a] 0.32 (0.14–0.73)[b]	8.39[a]	0.0074[a]
SNP4 (1087–754C>A)		CC	CA	AA			
Controls	642	0.629	0.340	0.031	Reference		
Adult asthma							
Severity=4	83	0.651	0.241	0.108	0.26 (0.12–0.60)[c] 0.15 (0.05–0.43)[b]	11.43[c]	0.0032[c]
SNP6 (1533+997A>C)		AA	AC	CC			
Childhood asthma							
With atopic dermatitis	169	0.278	0.556	0.166	Reference		
Without atopic dermatitis	203	0.424	0.419	0.157	1.91 (1.23–2.95)[d] 1.92 (1.24–2.97)[e]	8.50[d]	0.0046[d]
SNP8 (2787T>C)		TT	TC	CC			
Controls	636	0.285	0.465	0.250	Reference		
Adult asthma							
Eosinophil≥10%	71	0.296	0.591	0.113	2.63 (1.23–5.60)[f] 2.93 (1.34–6.43)[b]	6.68[f]	0.0079[f]
Aspirin-induced asthma	25	0.040	0.720	0.240	9.55 (1.28–71.10)[g] 6.69 (0.88–50.97)[b]	7.21[g]	0.0050[g]
Adult asthma							
With aspirin-induced asthma	25	0.040	0.720	0.240	Reference		
Without aspirin-induced asthma	441	0.277	0.521	0.202	9.18 (1.23–68.59)[g] 8.94 (1.19–66.88)[b]	6.82[g]	0.0084[g]

[a]GG+AG *vs* AA.
[b]OR adjusted for age and sex.
[c]CC+CA *vs* AA.
[d]CC+AC *vs* AA.
[e]OR adjusted for sex.
[f]TT+TC *vs* CC.
[g]CC+TC *vs* TT.

between atopy (positive mite-specific IgE) and any SNPs of *hCLCA1* among childhood asthma patients, suggesting that *hCLCA1* is primarily associated with asthma and/or dermatitis but not atopy.

Association between haplotypes of the *hCLCA1* gene and asthma

We used eight SNPs to construct the haplotype of the *hCLCA1* gene and estimated the frequency of each

haplotype in the control, childhood asthma, and adult asthma groups (Table 4). The frequency pattern of the haplotype differed between the control and childhood asthma groups ($P<0.0001$) and between the control and adult asthma groups ($P=0.0031$). Association with childhood asthma was observed with the TGCCAAGT haplotype (haplotype 6) (OR = 0.27, CI = 0.14–0.50, $\chi^2=19.742$, P_c (corrected P-value) = 0.00010) and the CATCAAGT haplotype (haplotype 18) (OR = 3.44, CI = 1.87–6.29, $\chi^2=17.800$, $P_c=0.0014$). Association between the CGCCACCT haplotype (haplotype 22) and adult asthma showed marginal significance ($P_c=0.014$).

Table 4 Frequencies of haplotypes constructed from eight SNPs and ORs in the control group and in both asthma groups

Name	*SNP1 C/T*	*SNP2 G/A*	*SNP3 T/C*	*SNP4 C/A*	*SNP5 A/G*	*SNP6 A/C*	*SNP7 G/C*	*SNP8 T/C*	*Controls (1184)[a] Frequency*	*Childhood asthma (712)[a] Frequency*	*Adult asthma (878)[a] Frequency*	*OR (95% CI) Controls vs childhood asthma Controls vs adult asthma*	*χ^2 (df = 1)*	*P*
Haplotype 1	C	A	T	C	A	A	G	C	0.151	0.180	0.139	1.23 (0.96–1.58)	2.679	>1*
												0.91 (0.71–1.16)	0.605	>1*
Haplotype 2	T	G	C	C	A	A	G	C	0.123	0.112	0.091	0.90 (0.67–1.20)	0.508	>1*
												0.71 (0.53–0.95)	5.355	0.583*
Haplotype 3	T	G	C	C	A	C	C	T	0.084	0.118	0.109	1.47 (1.08–1.99)	6.021	0.414*
												1.35 (1.00–1.81)	3.896	>1*
Haplotype 4	C	A	T	C	A	C	C	T	0.065	0.052	0.071	0.79 (0.53–1.18)	1.344	>1*
												1.09 (0.77–1.55)	0.250	>1*
Haplotype 5	C	A	T	C	G	A	G	T	0.062	0.044	0.056	0.69 (0.45–1.07)	2.815	>1*
												0.90 (0.62–1.31)	0.310	>1*
Haplotype 6	T	G	C	C	A	A	G	T	0.060	0.017	0.058	0.27 (0.14–0.50)	19.742	0.00010*
												0.97 (0.67–1.40)	0.032	>1*
Haplotype 7	C	G	T	C	A	A	G	C	0.053	0.051	0.032	0.95 (0.62–1.44)	0.063	>1*
												0.59 (0.37–0.92)	5.432	0.583*
Haplotype 8	C	G	C	A	G	C	C	T	0.041	0.024	0.028	0.57 (0.32–0.99)	4.057	>1*
												0.68 (0.42–1.11)	2.429	>1*
Haplotype 9	C	G	T	A	G	C	G	C	0.032	0.032	0.035	1.01 (0.59–1.70)	0.001	>1*
												1.10 (0.68–1.79)	0.161	>1*
Haplotype 10	T	G	C	A	G	C	G	C	0.026	0.035	0.039	1.35 (0.79–2.31)	1.237	>1*
												1.50 (0.91–2.46)	2.598	>1*
Haplotype 11	T	G	C	C	G	A	G	T	0.025	0.044	0.022	1.75 (1.05–2.92)	4.731	0.833*
												0.85 (0.48–1.52)	0.297	>1*
Haplotype 12	C	A	T	A	G	C	C	T	0.024	0.031	0.025	1.27 (0.72–2.23)	0.697	>1*
												1.02 (0.58–1.80)	0.007	>1*
Haplotype 13	C	A	T	C	A	C	C	C	0.024	0.022	0.035	0.92 (0.49–1.70)	0.078	>1*
												1.46 (0.87–2.44)	2.087	>1*
Haplotype 14	T	A	T	C	A	A	G	T	0.019	0.024	0.016	1.23 (0.65–2.33)	0.426	>1*
												0.82 (0.42–1.60)	0.347	>1*
Haplotype 15	C	G	T	C	G	A	G	T	0.016	0.027	0.028	1.68 (0.88–3.19)	2.562	>1*
												1.80 (0.98–3.29)	3.728	>1*
Haplotype 16	T	G	C	C	A	A	C	T	0.015	0.014	0.015	0.92 (0.42–2.01)	0.041	>1*
												0.97 (0.47–2.00)	0.005	>1*
Haplotype 17	C	G	C	A	G	C	G	C	0.014	0.007	0.026	0.49 (0.18–1.32)	2.086	>1*
												1.85 (0.98–3.47)	3.714	>1*
Haplotype 18	C	A	T	C	A	A	G	T	0.014	0.045	0.017	3.44 (1.87–6.29)	17.800	0.0014*
												1.27 (0.62–2.58)	0.434	>1*
Haplotype 19	T	A	T	A	G	C	C	T	0.013	0.001	0.009	0.11 (0.01–0.83)	6.742	0.210*
												0.72 (0.30–1.70)	0.578	>1*
Haplotype 20	T	A	T	C	A	A	G	C	0.013	0.010	0.018	0.77 (0.31–1.91)	0.312	>1*
												1.45 (0.71–2.94)	1.050	>1*
Haplotype 21	T	G	C	A	G	C	C	T	0.013	0.004	0.022	0.33 (0.10–1.14)	3.380	>1*
												1.72 (0.87–3.41)	2.502	>1*
Haplotype 22	C	G	C	C	A	C	C	T	0.012	0.013	0	1.07 (0.46–2.49)	0.025	>1*
													10.453	0.014*
Haplotype 23	C	A	T	A	G	C	G	C	0.011	0.014	0.009	1.28 (0.56–2.94)	0.349	>1*
												0.83 (0.34–2.01)	0.175	>1*
Haplotype 24	C	G	C	C	A	A	G	T	0.011	0.008	0.015	0.77 (0.29–2.02)	0.292	>1*
												1.35 (0.62–2.93)	0.593	>1*
Haplotype 25	C	G	T	A	G	C	C	T	0.010	0.008	0.010	0.83 (0.31–2.22)	0.138	>1*
												1.01 (0.42–2.41)	0.001	>1*
Others									0.069	0.063	0.075			
Overall									1.000	1.000	1.000		75.088	<0.0001
													45.465	0.0031

Bonferroni-type adjustment is corrected with × 26. A total of 25 predominant haplotypes are listed. The 'Others' category includes 36 minor haplotypes (<1% frequency in controls).
*P-value corrected with Bonferroni correction (raw P-values were multiplied by 26).
[a]Analyzed allele number.

Table 5 Frequencies of diplotypes and ORs in the control group and in both asthma groups

Name	*Number*	*Diplotype frequencies*			*OR (95% CI)*	χ^2 *(df=1)*	P_c[a]
Haplotype 6		Homozygote	Heterozygote	Others[b]			
Controls	592	0.002	0.116	0.882			
Childhood asthma	356	0	0.034	0.966	0.26 (0.14–0.49)[c] 0.26 (0.14–0.50)[d]	20.11[c]	<0.0001[c]
Serum IgE≥400 IU/ml	193	0	0.016	0.984	0.12 (0.04–0.38)[c] 0.12 (0.04–0.39)[d]	18.20[c]	<0.0001[c]
Positive mite-specific IgE	289	0	0.024	0.976	0.19 (0.08–0.41)[c] 0.19 (0.09–0.42)[d]	21.52[c]	<0.0001[c]
Onset <3 years old	180	0	0.022	0.978	0.17 (0.06–0.47)[c] 0.17 (0.06–0.47)[d]	14.68[c]	0.00089[c]
Haplotype 18		Homozygote	Heterozygote	Others[e]			
Controls	592	0	0.027	0.973			
Childhood asthma	356	0	0.090	0.910	3.56 (1.92–6.58)[f] 3.61 (1.92–6.72)[d]	18.28[f]	0.0011[f]
Serum IgE≥400 IU/ml	193	0	0.093	0.907	3.70 (1.85–7.41)[f] 3.61 (1.79–7.23)[d]	15.41[f]	0.0081[f]
Severity≥2	162	0	0.099	0.901	3.95 (1.93–8.06)[f] 3.92 (1.90–8.06)[d]	16.11[f]	0.0064[f]
Haplotype 22		Homozygote	Heterozygote	Others[g]			
Controls	592	0	0.024	0.976			
Adult asthma	439	0	0	1.000		10.53[h]	0.013[h]

[a]*P*-value corrected with Bonferroni correction (raw *P*-values were multiplied by 26).
[b]Diplotype consists of haplotype other than haplotype 6.
[c]Haplotype 6/haplotype 6+haplotype 6/others *vs* others/others.
[d]OR adjusted for sex.
[e]Diplotype consists of haplotype other than haplotype 18.
[f]Haplotype 18/haplotype 18+haplotype 18/others *vs* others/others.
[g]Diplotype consists of haplotype other than haplotype 22.
[h]Haplotype 22/haplotype 22+haplotype 22/others *vs* others/others.

As shown in Table 5, we examined the association of the diplotypes of *hCLCA1* with asthma. The results suggested that a heterozygote of haplotype 6 showed a lower risk for childhood asthma (OR = 0.26, CI = 0.14–0.49, $\chi^2 = 20.11$, $P_c < 0.0001$, cOR = 0.26) and a heterozygote of haplotype 18 showed a higher risk for childhood asthma (OR = 3.56, CI = 1.92–6.58, $\chi^2 = 18.28$, $P_c = 0.0011$, cOR = 3.61) when compared to other diplotypes. We next studied the association of diplotypes with patients stratified by the asthma-related phenotypes. Significant associations were found between haplotype 6 and children with high IgE levels ($P_c < 0.0001$), positive mite-specific IgE results ($P_c < 0.0001$), and early age of asthma onset (<3 years old) ($P_c = 0.00089$). The association between haplotype 18 and childhood asthma was also significant when patients were limited to those with higher IgE levels ($P_c = 0.0081$) or those with severe symptoms ($P_c = 0.0064$). The significance of association between haplotype 22 and adult asthma was marginal ($P_c = 0.013$). In childhood asthma patients, there was no significant association between haplotype 6 or haplotype 18 and atopy, suggesting that these haplotypes are primarily associated with asthma development.

Discussion

We performed case–control and case-only association studies of SNPs in the *hCLCA1* gene using clinically characterized asthma patients. When each SNP in *hCLCA1* was studied for its association in all asthma samples, no association with childhood asthma or with adult asthma was suggested. However, when patients were stratified by asthma-related phenotypes, subgroups of patients showed associations with SNPs. The genotype frequencies of SNP2 and SNP8 in patients with AIA were different from those of the controls and those of patients without AIA. These data suggest an association of the *hCLCA1* gene with AIA and different pathophysiologies between patients with AIA and those without AIA. Because the number of patients with AIA is relatively small, an association study of *hCLCA1* with a larger number of patients with AIA may be required to confirm our observation. Adult patients with asthma and high eosinophil counts showed a significant association with SNP8. Because the SNP8 genotype frequency in adults with asthma and a low eosinophil count is similar to that of the controls (data not shown), only adult patients with high eosinophil counts showed different genotype frequencies from controls or other patient groups. The data may reflect different pathophysiologies between patients with eosinophilia and those without eosinophilia. An association was found between SNP6 and the complication of atopic dermatitis among childhood asthma patients. Because atopic dermatitis was closely related with total IgE levels and positive mite-specific IgE status, it is possible that *hCLCA1* is involved in atopy or the development of atopic dermatitis. Since

we could not detect significant association between *hCLCA1* and atopy in the case-only studies, it is likely that *hCLCA1* is primarily associated with dermatitis itself.

The evidence of association of the *hCLCA1* gene with asthma was shown more clearly by the haplotype analyses. The entire distributions of the haplotype frequency in childhood and adult asthma patients were different from that of the controls. The frequency of haplotype 6 was significantly lower in childhood asthma patients than in the controls. From the diplotype construction analysis, individuals who were heterozygous for haplotype 6 showed a lower risk for childhood asthma (OR = 0.26). These data suggest that it is a protective haplotype against asthma development. About 10% of Japanese individuals have this haplotype and they are expected to show a lower susceptibility to childhood asthma than those who do not. On the other hand, the frequency of haplotype 18 was significantly higher in childhood asthma patients than in the controls. From the diplotype analysis, individuals who harbor haplotype 18 showed a higher risk for childhood asthma (OR = 3.56). These data suggest that it is a high-risk haplotype for asthma development. About 3% of Japanese individuals have this haplotype and they are expected to show a higher susceptibility to childhood asthma than those who do not.

Our observations support the results from an earlier AHR-model mice study. The *gob-5* mRNA is strongly expressed in the airway epithelium, especially in the goblet cells, the function of which is to secrete mucins.[10] Mucus overproduction is thought to be responsible for the small-airway obstruction and lung dysfunction that are closely linked to morbidity and mortality in asthma cases.

This study is the first to investigate the association between SNPs of the *hCLCA1* gene and asthma in humans. It showed evidence of associations between particular haplotypes of the *hCLCA1* gene and asthma development in the Japanese population. None of the SNPs investigated in this study change the amino-acid sequence of the *hCLCA1* protein. Therefore, it is unlikely that the nucleotide changes *per se* affect the function of *hCLCA1*. Any nucleotide changes that are in linkage disequilibrium to this haplotype may exist in or near the *hCLCA1* gene. It will be necessary to extend the survey of polymorphisms in the promoter regions as well as introns and exons of the *hCLCA1* gene to identify any functional genetic changes. Several studies have reported that there was a significant association between promoter and intronic variants of a gene and a disease.[32,33] Demonstrating the alteration of gene functions as the result of polymorphisms is necessary to further validate the involvement of the *hCLCA1* gene in the pathogenesis of asthma.

While this manuscript was being prepared, the association of *hCLCA1* and chronic obstructive lung disease (COLD) in the Japanese population was published.[34] Among the eight SNPs in our study, only one SNP (SNP8) was also investigated in Hegab's study; therefore, the comparison of results between our studies is difficult. Association of *hCLCA1* with both asthma and COLD may imply a common pathogenic background between these two diseases.

Materials and methods

Subjects

We recruited 384 children with asthma and 96 adults with asthma from Osaka Prefectural Habikino Hospital and another 384 adults with asthma from the Miyatake Asthma Clinic. Details of these patients are described elsewhere.[28] All participants with asthma were selected according to the American Thoracic Society criteria.[35] Regarding the children with asthma, we recorded their age, sex, age at asthma onset, serum total IgE level, mite-specific IgE level, eosinophil count, clinical severity, incidence of atopic dermatitis, and the number of parents and siblings affected with asthma. Specific IgE was considered positive when values exceeded 0.35 U_A/ml (RAST score $\geqslant 1$) according to an enzyme immunoassay. The severity of childhood asthma was defined according to the degree of therapy required to control symptoms at the time of entry into this study. The grades were defined as follows: Grade 1, β stimulants only; Grade 2, sodium chromoglycate and/or theophylline; Grade 3, inhaled beclometasone, 400 μg/day or less; and Grade 4, inhaled beclometasone of more than 400 μg/day. Regarding the adults with asthma, we recorded their age, sex, age at asthma onset, serum total IgE level, eosinophil count, clinical severity, incidence of AIA, presence or absence of nasal polyps, and the number of relatives second-degree or closer with asthma. The severity of adult asthma was classified according to the system from the National Heart, Lung, and Blood Institute.[36] In this study, patients who were under 18 years of age at the time of entry were classified as having childhood asthma and those who were 18 years of age or older were classified as having adult asthma regardless of their age at the time of onset. We selected 672 control subjects who did not have atopy-related diseases from the general population in the Tokyo and Osaka areas. Genomic DNAs were prepared in accordance with standard protocols. All patients and volunteers provided written informed consent to participate in the study in accordance with the rules of the process committee at the SNP Research Center (RIKEN) and the Tohoku University School of Medicine.

SNP genotyping

Genotyping of SNPs was performed by using the Invader assay[37,38] or the TaqMan™ allele-specific amplification (TaqMan-ASA) method.[39] Specific primers were designed on the basis of genomic sequences obtained from the GenBank DNA database (accession number AF039401). The primer sequences and genotyping methods are shown in Table 6. The mixture for the Invader assay contained 5 ng of genomic DNA, 1.25 mM dNTPs, 5.9 mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl (pH 8.8), 10 mM β-mercaptoethanol, a pair of specific primers (12.5 pmol each), and *Ex-Taq* DNA polymerase (0.5 U; TaKaRa, Japan) in a final volume of 10 μl. Samples were amplified in the GeneAmp™ PCR system 9700 (Applied Biosystems, USA). Thermoprofiles were initial denaturation at 95°C for 2 min, followed by 37 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 3 min, with a final extension at 72°C for 10 min. The Invader assay was performed as previously described.[40]

The polymerase chain reaction (PCR) mixture for the TaqMan-ASA method contained 7.5 μl of 2 × TaqMan™

Table 6 Primers for genotyping

Primers for Invader assay			
Name	*Forward primer (5′–3′)*	*Reverse primer (5′–3′)*	
SNP1	GGGACCTTGTACACCATGTTGAG	CCCTGAAGGCACCGAAGAG	
SNP2	ATCAAGTGCACTGCTGCCTC	TGGCAGTGTCTCTAGTGTTTCCC	
SNP3	TGCTCCAAGGGAGAATCAAAG	CTGGAAGACAAGAGAGACTTCCC	
SNP4	TGAAGCTATAAAGGGTGGAATGG	CCCATCTGCTCCTCCTCCTC	
SNP5	TGAAGCTATAAAGGGTGGAATGG	CCCATCTGCTCCTCCTCCTC	
SNP6	AACCCTTGCTGTGCAAGACC	CCATAACTGCTGTGTGTCATGG	
SNP7	TTCTCTCACCTGCCAACACC	CAGGATACTCCTTCTGCCAATG	
Primers for TaqMan-ASA method			
Name	*Common forward primer (5′–3′)*	*Reverse primer (5′–3′)*	*TaqMan probe (5′–3′)*
SNP8	CAACAGCCTGAATAGCAATG	CAATGAATCTCTTCAAGTGAATACTAGT CAATGAATCTCTTCAAGTGAATACTAAC	ACTTCCTCAGAGTTGGCTTCCTTTGG

Universal PCR Master Mix (Applied Biosystems), 0.4 μM of each PCR primer, 0.12 μM of TaqMan probe, and 5 ng of template DNA in a final volume of 15 μl. The samples were analyzed with the GeneAmp™ PCR System 7700 (Applied Biosystems). The thermoprofiles were 50°C for 2 min, 95°C for 10 min, then 45 cycles of 95°C for 15 s, and finally 60°C for 1 min.

Statistical analysis

In all genotypic, haplotypic, and diplotypic distribution analyses, *P*-values were calculated using Fisher's exact test. Allele frequencies in asthma patients and controls were compared by the contingency χ^2 test. ORs were estimated according to Brown.[41] Corrections of ORs for age and sex were performed with logistic regression formula in the adult case analysis. Because the range of age of the controls (18–81 years) was not comparable with child cases (1–17 years), CI of OR becomes very broad in the childhood asthma analysis. Thus, ORs corrected for only sex were presented in childhood asthma analysis. As shown in Tables 3 and 5, the correction had little effects on the estimated OR values. Pairwise linkage disequilibrium between SNPs was estimated as D'[30] and r^2.[31] To infer the frequencies of haplotype in control and patient groups and diplotype in individuals with uncertain phases of genotypes, software programs PHASE™ version 2.0.2 (http://www.stat.washington.edu/stephens/software.html)[42] and HAPLOTYPER™ (http://www.people.fas.harvard.edu/~junliu/Haplo/docMain.htm)[43] were used. Haplotype frequencies between cases and controls were evaluated both by a whole distribution with Fisher's exact test and by χ^2 tests of one haplotype against others (haplotype-wise test). In the association study between a single SNP and asthma or an asthma-related phenotype, we performed many statistical tests; therefore, inflation of the false-positive results (type 1 error) is a concern. Because all eight SNPs were significantly in linkage disequilibrium (Table 2) and asthma-related phenotypes (seven variables for children and seven variables for adults) are significantly related,[28] the simple multiplication of *P*-values by the number of SNPs or phenotypes tested is too conservative and the appropriate value for the correction is not evident. Thus, to deal with the multiple comparisons, we did not apply Bonferroni corrections but rather set the significant *P*-value at 0.01 rather than 0.05. In the haplotype-wise test, comparisons were repeated as many as 26 times (25 haplotypes and 'another minor haplotype'; Tables 4 and 5). In this case, we assumed each comparison was independent and performed the Bonferroni correction (raw *P*-values were multiplied by 26). The corrected *P*-values were designated as P_c. The P_c-values less than 0.01 were judged to be significant. The software SPSS™ version 11.0J (SPSS Japan Inc., Tokyo, Japan) was used for all statistical analyses.

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Mutation analysis of the *MMAA* and *MMAB* genes in Japanese patients with vitamin B_{12}-responsive methylmalonic acidemia: identification of a prevalent *MMAA* mutation

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Abstract

Methylmalonic acidemia (MMA) is caused by the deficient activity of L-methylmalonyl-CoA mutase, which is a vitamin B_{12} (or cobalamin, Cbl)-dependent enzyme. MMA due to the effect of insufficient Cbl metabolism is classified into three forms (*cblA*, *cblB*, and *cblH*). Recently, the genes responsible for *cblA* and *cblB* were identified as *MMAA* and *MMAB*, respectively. The *MMAA* protein likely transports Cbl into the mitochondria for adenosylcobalamin synthesis, while the MMAB protein appears to be an adenosyltransferase. We performed a mutation analysis of 10 unrelated Japanese patients with vitamin B_{12}-responsive MMA. Seven patients had mutations in *MMAA*, whereas the other three patients showed no disease-causing substitutions in either *MMAA* or *MMAB*. Five novel mutations were identified in *MMAA* (R22X, R145X, L217X, R359G, and 503delC). The 503delC mutation was observed in five of the seven *MMAA* patients, suggesting that the mutation is prevalent in Japanese patients. This finding may facilitate the DNA diagnosis of vitamin B_{12}-responsive MMA within the Japanese population.

Keywords: Vitamin B_{12}-responsive methylmalonic acidemia; L-methylmalonyl-CoA mutase; Adenosylcobalamin; *MMAA*; *MMAB*

Introduction

Methylmalonic acidemia (MMA) is an autosomal recessive disorder of organic acid metabolism caused by a defect in the isomerization of L-methylmalonyl-CoA to succinyl-CoA [1]. The reaction is catalyzed by L-methylmalonyl-CoA mutase (MCM, EC 5.4.99.2) which requires adenosylcobalamin (AdoCbl) as a cofactor. MMA is subdivided into two forms. One is a defect in the MCM apoenzyne (*mut* MMA or vitamin B_{12}-unresponsive MMA, MIM 251000), and the other is a defect in the steps of AdoCbl synthesis (*cbl* MMA or vitamin B_{12}-responsive MMA) [2]. The patients with *cbl* MMA usually respond to pharmacological doses of hydroxycobalamin.

The intracellular synthesis of AdoCbl has been extensively investigated [2]. The cobalt atom of cob(III)alamin is reduced in steps; the first step is the reduction of cob(III)alamin to form cob(II)alamin, and the second step is the reduction of cob(II)alamin to cob(I)alamin. Within these reactions, a transport from the cytosol to the mitochondrial matrix occurs. The final step of AdoCbl synthesis is adenosylation by

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cob(I)alamin adenosyltransferase. Intact fibroblasts from patients with *cbl* MMA accumulated negligible amounts of AdoCbl [3].

Mahoney et al. [4] found that patients with *cbl* MMA were further subdivided into two classes by an AdoCbl synthesis assay using cell extracts. One type, *cblA* (MIM 251100), showed normal AdoCbl synthesis by cell extracts, in spite of a decreased accumulation of AdoCbl in intact fibroblasts. While the other type, *cblB* (MIM 251110), showed severe deficiency of AdoCbl synthesis in cell extracts. These two classes may also be distinguished by somatic cell complementation analyses [5]. It has been speculated that *cblB* is caused by a deficiency of adenosyltransferase [6], and that *cblA* mutants affect the activity of cob(III)alamin reductase or the mitochondrial transport of Cbl [4]. Watanabe et al. [7] reported that mitochondrial NADPH-linked aquacobalamin reductase was decreased in a cell line of *cblA*.

Laboratory diagnoses of *cbl* MMA have been hampered by complicated requirements for the biochemical assays, which are able to be offered by few laboratories worldwide. Recently, the genes responsible for *cblA* and *cblB* were identified as *MMAA* and *MMAB*, respectively, and the disease-causing mutations were reported [8,9]. The MMAA protein is speculated to be involved in the translocation of Cbl into mitochondria and the MMAB protein shows a similarity to bacterial adenosyltransferase. The identification of the two genes opened the way for the DNA diagnosis of *cbl* MMA. In this study, we performed a mutation analysis of ten Japanese patients with vitamin B_{12}-responsive MMA to examine the mutation spectrum within the population and to explore the possibility of a molecular diagnosis.

Methods

Patients

Ten apparently unrelated Japanese MMA patients were studied. There were no consanguineous marriages among the parents of these patients except for patient 2. All patients were symptomatic during their neonatal or infantile period. Diagnoses of MMA were confirmed by urinary organic acid analysis using gas chromatography/mass spectrometry. MCM activity was measured by isomerization of L-methylmalonyl-CoA to succinyl-CoA [10] and/or the incorporation of ^{14}C-propionate [11]. Since MCM activities in the cell cultures from the 10 patients recovered to normal ranges in the presence of AdoCbl or hydroxycobalamin, we assigned a diagnosis of vitamin B_{12}-responsive MMA to these patients. A complementation study was not performed.

The Ethics Committee of the Tohoku University School of Medicine approved this study.

Direct sequencing of the MMAA and MMAB genes

Genomic DNA was extracted from cultured fibroblasts, EBV-transformed lymphoblasts, or leukocytes with the aid of a Sepa Gene Kit (Sanko Junyaku, Tokyo, Japan). All coding exons including flanking introns in *MMAA* and *MMAB* were amplified by PCR (Table 1). To facilitate the cycle sequencing analysis, the M13 forward primer sequence or the M13 reverse primer sequence was attached to the 5′ end of the sense primers or the antisense primers, respectively. Direct sequencing of the PCR products was performed using a Big Dye Primer Cycle Sequencing kit and an ABI 310 Genetic Analyzer (PE Applied Biosystems, Foster city, CA, USA).

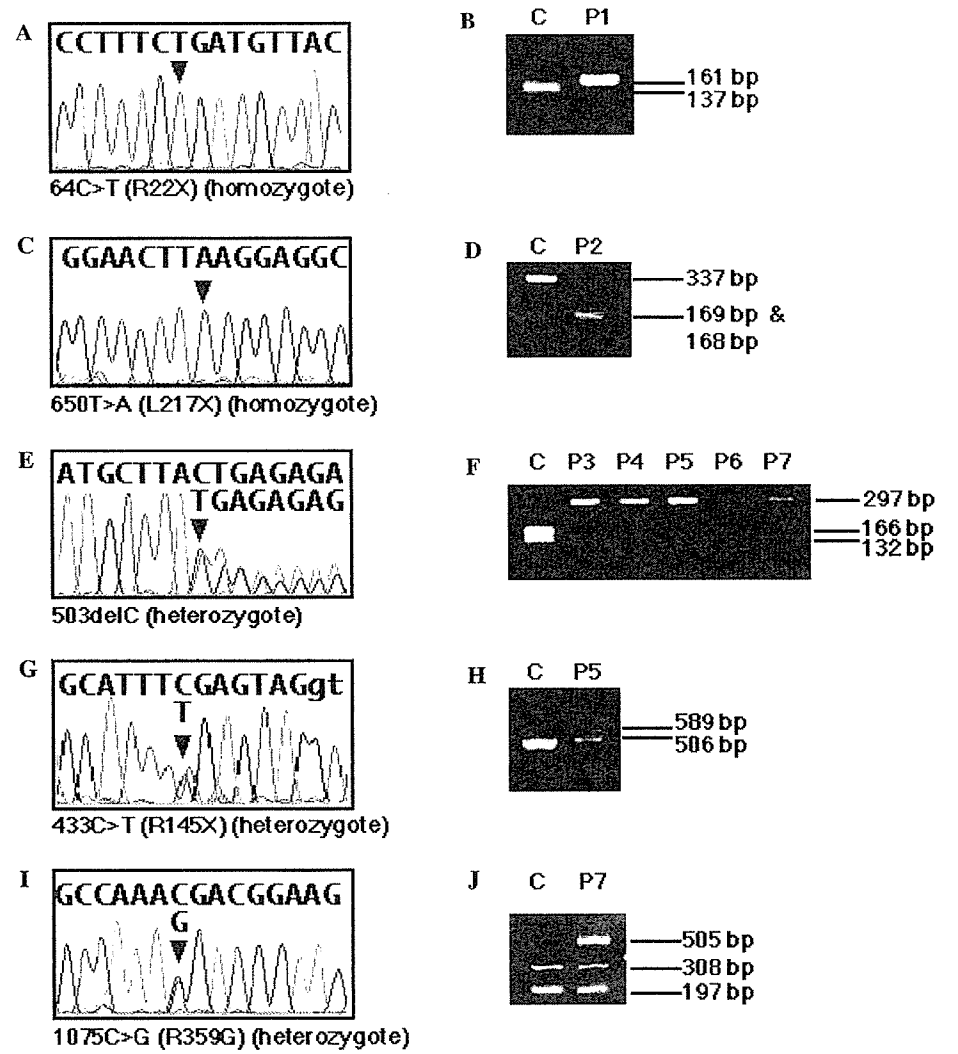
Table 1
Primers for the amplification of the *MMAA* and *MMAB* genes

Sense primers	Antisense primers
MMAA	
F-MMAA-Ex2: 5′-F-AATCACATTGAGCCAAAACG-3′	R-MMAA-Ex2: 5′-R-ACAGAATACAGAGAATTTGT-3′
F-MMAA-Ex3: 5′-F-CTCAGTAAAACTGATCGTAG-3′	R-MMAA-Ex3: 5′-R-TAGAGGTCACCCAACTGTGC-3′
F-MMAA-Ex4: 5′-F-GGAACTGGCTGATAATTGAC-3′	R-MMAA-Ex4: 5′-R-GTCACTCATCTTTATATAGC-3′
F-MMAA-Ex5: 5′-F-GTGACCATGAGTATGAGTAA-3′	R-MMAA-Ex5: 5′-R-GCCAACATGAATGATATTTC-3′
F-MMAA-Ex6: 5′-F-GATTCTTGGCATCCAGGGCT-3′	R-MMAA-Ex6: 5′-R-CTATCATCTTCACATAGAAG-3′
F-MMAA-Ex7: 5′-F-TAACTGGCAGGTATCAGCGT-3′	R-MMAA-Ex7: 5′-R-AGAAGACAAGAGCACCATAC-3′
MMAB	
F-MMAB-Ex1: 5′-F-GCCAGCTGTGGGTGGAGTCA-3′	R-MMAB-Ex1: 5′-R-CGACGACACCACGATTCACG-3′
F-MMAB-Ex2: 5′-F-AGGTTACAAGCAGCAAGCTG-3′	R-MMAB-Ex2: 5′-R-AAATGGTGTATGCCATGAGT-3′
F-MMAB-Ex3: 5′-F-CAGCATATCAGGAAAACAGA-3′	R-MMAB-Ex3: 5′-R-CATACTCGACTCAAACGCAA-3′
F-MMAB-Ex4: 5′-F-GCCTGCCACCTGAGAATCTA-3′	R-MMAB-Ex4: 5′-R-TGGATGCTGAGTCCCGTGAT-3′
F-MMAB-Ex5: 5′-F-TATTAGGTGGCGCCTCTGCA-3′	R-MMAB-Ex5: 5′-R-AGATGGTGACCCTAGGAGAG-3′
F-MMAB-Ex6: 5′-F-GTGATGGCCTCATGGCAGTT-3′	R-MMAB-Ex6: 5′-R-CATGTGTGTCTGTCACTGAA-3′
F-MMAB-Ex7: 5′-F-GGCTGGACTTCAGAGGAGCT-3′	R-MMAB-Ex7: 5′-R-TCAGAGATGGCCCTGCTGTA-3′
F-MMAB-Ex8: 5′-F-TGCTGCTCAAGGTTTAGGCC-3′	R-MMAB-Ex8: 5′-R-AATGCTGCCCCACACTGCTT-3′
F-MMAB-Ex9: 5′-F-GAAGACCCAGTTAGCGTTGA-3′	R-MMAB-Ex9: 5′-R-CTTTGAGCCTCTCTGGGTGA-3′

F, M13 Forward sequence (5′-TGTAAAACGACGGCCAGT-3′).
R, M13 Reverse sequence (5′-CAGGAAACAGCTATGACC-3′).

PCR with restriction fragment analyses for the identified mutations

PCR amplification of exons 2, 3, 4, and 7 in *MMAA* was performed as described above. PCR products were digested with *Taq*I (for R145X), *Dde*I (for 503delC), *Mse*I (for L217X), or *Hyp*99I (for R359G) (all from New England Biolabs, Beverly, MA, USA). Fragments were separated on agarose gels and visualized by ethidium bromide staining. The R22X mutation was detected by a PCR



K

Human	320	WEPEYIRISARSGEGISEMWDEMEDFQDLMLASGELTAERREQQEYW 366
Mouse	317	WRPKYIRISARSGEGITEMWDTMREFQHQMLASGELAAKRQTQHKYW 363
Caenorhabditis	302	WRPKYMRSSIMDKESYSEYCSSLYEFWDTIGESGDLERRRQDQMKKW 348
Mycobacterium	235	WRPPYLTMSAYEGRGLAELWDTYERHRQYLTGAGEFDARRRDQQYDW 281
Methylobacterium	233	WTPPYYTISGLHGKGLDSLWSRIEGHRSKLTATGEIAGKRREQDYKW 279
Escherichia	231	WQPRYLTCSALEKRGIDEIWHAIIDFKTALTASGRLQQYRQQQSYEW 277

Fig. 1. Mutation analysis by sequencing (A, C, E, G, and I) and PCR-restriction fragment analysis (B, D, F, H, and J). (A) Sequencing analysis in patient 1 (P1). A C-to-T substitution at nucleotide (nt) 64 (R22X) was found in a homozygous pattern. (B) The AS-MMAA-R22X primer, which includes one mismatch, creates an *Hpa*II site in the wild-type sequence. The left lane (C, control) showed a digested fragment. R22X abolishes this site. Patient 1 (P1) was homozygous for R22X. (C) In patient 2 (P2), a T-to-A substitution at nt 650 (L217X) was found in a homozygous pattern. (D) L217X creates an *Mse*I site. Patient 2 was homozygous for L217X. (E) Sequence of 503delC in a heterozygous pattern (patient 5, P5). (F) 503delC abolishes the *Dde*I site. Patients 3, 4, and 6 were homozygous, and patients 5 and 7 were heterozygous for 503delC. (G) Sequencing analysis in patient 5 (P5). A C-to-T substitution at nt 433 (R145X) was found in a heterozygous pattern. (H) R145X abolishes the *Taq*I site. Patient 5 was a heterozygote of R145X. (I) In patient 7 (P7), a C-to-G substitution at nt 1075 (R359G) was found in a heterozygous pattern. (J) R359G abolishes the *Hpy*99I site. Patient 7 was a heterozygote of R359G. (K) Comparisons between human MMAA protein and MMAA homologues of other species were analyzed by the Clustal W program. Arginine at 359 (arrow) is conserved in the MMAA homologues of other species (mouse, *C. elegans*, *M. tuberculosis*, *M. extorquens*, and *E. coli*).

method incorporating a restriction fragment length polymorphism using a mismatched primer. The PCR was conducted by a sense primer (F-MMAA-Ex2) and an antisense primer (AS-MMAA-R22X: 5′-GTGAAAGATGAAGT GGTAACAC-3′) that contained one mismatch (bold type) to create an *Hpa*II site with a wild sequence. After digestion of the PCR products with *Hpa*II (New England Biolabs), electrophoresis was performed using agarose gel.

Results

Seven of the 10 patients with vitamin B_{12}-responsive MMA (patients 1–7) showed mutations in *MMAA*. No disease-causing substitutions were found in either *MMAA* or *MMAB* among the three remaining patients (patients 8, 9, and 10).

In patient 1, we found a C-to-T substitution at nucleotide (nt) 64 in a homozygous pattern (Figs. 1A and B). This substitution created a stop codon (R22X) within exon 2. Patient 2 showed a 650T > A substitution (L217X) in exon 4 in a homozygous pattern (Figs. 1C and D). One base (C) deletion at nt 503 in exon 3 was detected in five patients; patients 3, 4, and 6 were homozygotes and patients 5 and 7 were heterozygotes (Figs. 1E and F). Another mutation found in patient 5 was a C-to-T substitution at nt 433 in exon 2, which created a stop codon (R145X) (Figs. 1G and H). In patient 7, we found a C-to-G substitution at nt 1075 in a heterozygous pattern (Figs. 1I and J). The 1075C > G resulted in the replacement of arginine with glycine (R359G) in exon 7. The missense R359G mutation was not identified in DNA samples from 50 healthy volunteers (data not shown).

Patients 1, 2, 3, 4, and 6 showed a homozygous pattern for the mutations. A consanguineous marriage was found among the parents of patient 2 only. These patients were considered "presumptive" homozygotes because the possibility of being hemizygotes with a deleted second allele could not be ruled out.

We also found 1089G > C in *MMAA* (patient 9), and 56–57GC > AA (patients 2, 5, and 9) and 716T > A (patients 2, 5, 6, 7, 8, and 9) in *MMAB* (data not shown). In the database of Japanese Single Nucleotide Polymorphisms (JSNP) (http://snp.ims.u-tokyo.ac.jp/) [12], the 1089G > C (Q363H) in *MMAA* has been reported as a polymorphism (IMS-JST065247) (G; 0.9167, C; 0.0833). The 56–57GC > AA (R19Q) and 716T > A (M239K) substitutions have been reported in the dbSNP (http://www.ncbi.nlm.nih.gov/SNP). According to the dbSNP, the heterozygosity of 716T > A (rs9593) is 0.492 and that of 56–57GC > AA (rs10774774 and 10774775) is not reported.

Discussion

Three nonsense mutations (R22X, R145X, and L217X), one deletion (503delC), and one missense mutation (R359G) were identified in Japanese patients with *cbl* MMA. Dobson et al. [8] found three premature-termination mutations (260insATAAACTT, 592delACTG, and Q95X), and one missense mutation (Y207C). There was no overlap of the mutation spectrum between Caucasian and Japanese patients.

Among seven patients diagnosed as *cblA* in this study, three were homozygotes and two were heterozygotes of 503delC; thus the allelic frequency was 57%. It suggests that the 503delC mutation is the predominant *MMAA* gene mutation among Japanese vitamin B_{12}-responsive MMA patients (see Table 2). A similarity in the mutation spectrum among Japanese patients and other Asian patients has been reported in other single-gene disorders, such as propionic acidemia [13–15], phenylketonuria [16] and glycogen storage disease type Ia [17,18]. It would be interesting to study whether the 503delC mutation is also prevalent among vitamin B_{12}-responsive MMA patients in other Far East Asian countries.

The missense mutation R359G observed in patient 7 was not found in 100 alleles in healthy volunteers. Arginine at 359 is conserved in MMAA homologues of other species (mouse, *Caenorhabditis elegans*, *Mycobacterium tuberculosis*, *Methylobacterium extorquens*, and *Escherichia coli*) (Fig. 1K). These data suggest that R359G is likely to be a disease-causing mutation.

Table 2
Mutations in the *MMAA* gene identified in this study

Patient	Mutation 1		Mutation 2	
	Nucleotide change	Effect on coding sequence	Nucleotide change	Effect on coding sequence
1	64C > T	R22X	64C > T	R22X
2	650T > A	L217X	650T > A	L217X
3	503delC	Frame shift	503delC	Frame shift
4	503delC	Frame shift	503delC	Frame shift
5	433C > T	R145X	503delC	Frame shift
6	503delC	Frame shift	503delC	Frame shift
7	503delC	Frame shift	1075C > G	R359G
8	Not detected		Not detected	
9	Not detected		Not detected	
10	Not detected		Not detected	

In the other three patients (patients 8, 9, and 10), no mutations except polymorphisms were detected in either *MMAA* or *MMAB*. Because we had not done complementation studies, it was not possible to know whether these three patients were *cblA*, *cblB*, or belong to another complementation group (*cblH*) [19,20].

In conclusion, we have identified five novel *MMAA* mutations in seven of ten Japanese patients with vitamin B_{12}-responsive MMA. The 503delC mutation in *MMAA* was observed in five patients. The identification of the prevalent mutation would facilitate DNA diagnoses of vitamin B_{12}-responsive MMA within the population.

Acknowledgments

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The value of self-measured home blood pressure in predicting stroke

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Home blood pressure measurements more accurately and reliably reflect target organ damage and the prognosis of cardiovascular disease than conventional blood pressure measurements. All evidence of the value of home blood pressure measurement in predicting stroke is currently derived from a population-based prospective study in Japan (the Ohasama study). The authors demonstrated that home blood pressure measurement provides more useful prognostic information on stroke than conventional blood pressure measurements. The predictive value of home blood pressure measurement increased progressively with the number of measurements. Even the initial-first home blood pressure values (one measurement) showed a significantly greater relation with stroke risk than conventional blood pressure values (mean of two measurements). Home blood pressure measurement increased the predictive power of categorizations of guidelines compared with conventional blood pressure measurement. Home blood pressure measurement is a useful tool to predict future risk of stroke.

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Home blood pressure measurement

Devices for the self-measurement of blood pressure (BP) at home (home BP measurement) are produced worldwide at a rate of more than 10 million a year, and 30 million such devices have already been distributed in Japan [1]. The clinical significance of home BP measurement is obvious; not only doctors but also patients can monitor the effects of antihypertensive treatment and obtain objective information on medication response. Patients can also recognize elevations of BP when they discontinue or fail to take routine doses of medication. The immediate feedback of home BP measurements encourages medication compliance, follow-up clinic visits and active participation in medical treatment, thus resulting in improved management of hypertension.

Recent guidelines for the treatment of hypertension, such as the Sixth and Seventh Reports of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC-VI and 7) [2,3], the 1999 WHO–International Society of Hypertension (WHO–ISH) Guidelines for the Management of Hypertension [4], the 2003 European Society of Hypertension–European Society of Cardiology (ESH–ESC) Guidelines for the Management of Arterial Hypertension [5] and the Japanese Society of Hypertension (JSH) Guidelines for the Management of Hypertension [6], have all emphasized the importance of home BP measurements in clinical applications of practice, research and epidemiology. The Working Group of JSH established the JSH Guidelines for Self-Monitoring of Blood Pressure at Home in 2003 [7]. Home BP measurements more accurately and reliably reflect target organ damage and the prognosis of cardiovascular disease than conventional (casual, screening, office) BP measurements [7–9]. Current evidence of the value of home BP measurement in predicting stroke is derived from our population-based prospective study in Japan (the Ohasama study) [8–13]. This review will refer to the value of home BP measurement in predicting stroke based on the results of the Ohasama study.

The Ohasama study

Since 1986, we have been conducting an epidemiological survey of hypertension using home BP measurement in Ohasama, in the northern part of Japan [14]. Ohasama initially

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had a population of 9400, but this has now dropped to 6800. Over the past 18 years, we have obtained home BP data from 5000 participants aged 7 years and over, as well as long-term clinical outcomes and information on risk factors and predictors. This study project was approved by the Institutional Review Board at Tohoku University School of Medicine and by the Department of Health of Ohasama.

Ohasama town

Ohasama is located 100 km north of Sendai, the central city of the northern part of Japan, and 500 km north of Tokyo, the capital of Japan. Most of the adult population is employed in fruit growing and the remainder work in offices or factories in Ohasama or neighboring towns. Ohasama is a typical rural town. The per capita income in northeastern Japan, where Ohasama is located, is approximately US$20,000 a year. The climate and nutritional habits of the region are similar to those in other areas of northern Japan. Urinary excretion of sodium chloride was measured during the study period in some participants in the home BP program. The mean ± standard eviation (SD) value was 14.9 ± 4.4 g/day (n: 190; mean ± SD age: 52.8 ± 8.4 years) [14]. The most common cause of death among the residents of this town was cerebrovascular disease, followed by cancer and heart disease. Compared with the mortality in Japan, the standardized mortality ratio (SMR) of the residents in Ohasama during 1988–1992 was 0.98 for all cause, 1.31 for cerebrovascular disease, 0.99 for cancer and 0.59 for heart disease [15].

BP measurement in the Ohasama study

Home BP

Since 1987, we have been distributing home BP measurement devices to the residents of Ohasama [14]. We used the following procedure to ascertain the accuracy of home BP measurement. Briefly, health education classes were conducted by physicians and well trained public health nurses to inform the population of the significance of home BP recording and to teach them how to measure their own BP. Approximately 80% of household members living in Ohasama attended the classes; public health nurses visited all of the remaining households to provide instruction on home BP measurement. All participants were asked to hold their cuff-covered arms at heart level during home BP measurements. The participants were asked to perform each step in the procedure while being observed by a nurse. Individuals attended an education class for systematic retraining only once during home BP measurement; however, the procedure is comparably easy for individuals with an average educational background in Japan. After their ability to measure home BP was verified, participants were asked to measure their own home BP in a sitting position once every morning within 1 h of awaking, after urination, before breakfast and after 2 or more minutes of rest, and to record the measurements for 4 weeks. If individuals were taking antihypertensive drugs, home BP was measured before medication was taken.

This scheme (multiple single measurement) was introduced to establish the most generalizable methods for home BP measurement. The reasons are as follows:

1. Since home BP must be measured over a long period, a minimal demand on participants, i.e., at least once on each occasion, may improve compliance with measurement. A permanent requirement for multiple (two or more) measurements of home BP on each occasion for a long period creates too large a burden for participants, which lowers compliance.
2. If the measurement frequency is not regulated, participants will measure their own BP with a voluntary measurement frequency, for example, three consecutive measurements on one morning and only one measurement on the following morning. Consequently, practitioners would be unsure of which measurements should be evaluated for clinical decision-making.
3. Consecutive and multiple measurements of home BP on each occasion provide different measurement results, which may cause confusion in some participants. Generally, participants tend to value the lowest BP obtained from multiple measurements. As a result, the lowest BP value may be reported to the practitioner, introducing selection bias.
4. Home BP would be evaluated as a mean value averaged for a certain period. However, averaging procedures are very time-consuming, and neither participants nor practitioners tend to work constructively at this procedure, such that there is often a selection bias in the values chosen for evaluation, so that not all values can be evaluated.
5. Averages of the first measurement of the home BP on each occasion for a certain period are comparable among patients/participants from many institutes or clinics, even though the number of home BP measurements on each occasion is different. The first measurement on each occasion might provide the most generalizable information.

Conventional BP

Annual health check-ups are available to all Japanese citizens aged 40 years or over, where conventional BP is measured. Participants are seated at rest for at least 2 min and then conventional BP is measured by well trained nurses or technicians. In Ohasama, BPs were measured twice consecutively during the health check-up, using a semi-automatic BP measuring device based on the microphone method. For conventional BP, we used data measured at an annual check-up that occurred within the same time period when home BP was first initiated as part of the study protocol.

BP measuring devices

Home BP was measured with a semiautomatic BP measuring device (HEM401C; Omron Healthcare Co. Ltd, Kyoto, Japan), based on the cuff-oscillometric principle, which generates a digital display of systolic and diastolic BP. Conventional BP was measured using a semi-automatic BP measuring device (USM700F; Ueda Electronic Work Co. Ltd, Tokyo, Japan), based on the microphone method.

The devices for measurement of conventional BP and home BP were calibrated before the start of the study [14]. The mean difference (SD) between HEM401C and USM700F was -0.4 (6.0) mmHg for systolic BP and +1.2 (5.8) mmHg for diastolic BP. All devices met the criteria set by the Association for the Advancement of Medical Instrumentation [16].

Follow-up & analysis in the Ohasama study

The incidence and past history of stroke and transient ischemic attack (TIA) were investigated by use of the Stroke Registration System of Iwate Prefecture, death certificates, receipt of National Health Insurance, and questionnaires sent to each household at the time of home BP measurement. This was then confirmed by checking the medical records of Ohasama Hospital, which is the only hospital in the town. Computed tomography (CT) scan and magnetic resonance imaging (MRI) of the brain are available and more than 90% of the participants have their regular check-ups at this facility.

We used the definitions of stroke or TIA according to the Classification of Cerebrovascular Diseases III described in the Special Report from the National Institute of Neurological Disorders and Stroke (NINDS III) [17]. At first, we classified the cases of clinical disorder with focal brain dysfunction for TIA or stroke. Then stroke incident cases were subclassified by types of stroke–brain hemorrhage, subarachnoid hemorrhage (SAH), intracranial hemorrhage from arteriovenous malformation (AVM, which was not observed in the current study), and brain infarction. CT scan and MRI were used to determine the clinical definition of stroke. For 3% of stroke cases, death certificates were the only source of information [18].

The analysis in our previous studies that investigated the association between stroke risk and BP values included only the first event for those who had multiple nonfatal events. Information for individuals, such as height, weight, habitual smoking, use of antihypertensive medication at baseline, history of heart disease, hypercholesterolemia or diabetes mellitus, was obtained from questionnaires sent to each household at the time of home BP measurements, from records of annual health check-ups and from medical records at Ohasama Hospital. Participants using lipid-lowering drugs or those with serum cholesterol levels of ≥5.68 mmol/l (220 mg/dl) were considered to have hypercholesterolemia. Participants with a fasting glucose level of ≥7.77 mmol/l (140 mg/dl) or nonfasting glucose level of ≥11.11 mmol/l (200 mg/dl) or those using insulin or oral antihyperglycemic drugs were defined as having diabetes mellitus. A past history of CVD included a history of myocardial infarction, angina pectoris, atrial fibrillation or cardiac failure.

The estimated relative hazard (RH) and the 95% confidence interval (95% CI) of variables were derived from the coefficient and standard error as determined by the Cox proportional hazards model adjusted for age, sex and smoking status, for the use of antihypertensive medication at baseline, and for history of heart disease, diabetes mellitus and or hypercholesterolemia. Data are shown as mean ± SD. A two-tailed p value of less than 0.05 was accepted as indicative of statistical significance. All statistical analyses were conducted using the SAS package (Version 8.2, SAS Institute, Inc., Cary, NC, USA).

Study population for prospective analyses

To prospectively investigate the association between home BP levels and subsequent risk of outcomes (mortality, stroke incidence), we excluded participants under the age of 40 years at the time of home BP measurement from the analysis because death or stroke occurrence was less common among these younger people. The total population of the three districts of Ohasama was 4774. Of those, 2716 were aged 40 years and over at baseline. Among them, 575 participants working outside the town were considered ineligible and were excluded from the study. This exclusion was necessary since our project also included ambulatory BP measurement [18]. In order for us to attach a device for ambulatory BP monitoring to the study participants, it was necessary that they stayed in the town during working days. Out of the remaining 2141 participants, hospitalized participants (n: 121) and demented or bedridden participants (n: 31) were not invited into the study.

In total, 1989 participants were eligible for the study. Of those, 1957 (96%) participants gave their informed consents and participated in the study. The data were obtained from 1913 participants (98% of the above participants; mean age: 60.9 years, male: female = 42:58) who measured their home BP during 3 days or more during the 4-week study period. This criteria was justified by our previous observation that the mean BP value obtained for the first 3-days was not significantly different from the values obtained for the entire study period [14]. Preliminary analysis showed that the mean ± SD home systolic BP (systolic BP) in 458 participants for the first 3 days of the 21-day period (123.2 ± 18.4 mmHg) was similar to that for the entire period (122.8 ± 17.5 mmHg). There was no significant difference among home diastolic BPs (diastolic BP) throughout the 21 days of the study. Therefore, data from participants who measured their home BP three or more times during the 4-week study period were used for analysis. The mean number of home BP measurements among the study participants was 20.8 ± 8.3 (mean ± SD).

Those who participated in the study (n: 1913) and those who did not (owing to the above reasons; n: 803) were not significantly different in terms of educational background. SMR of the participants during 1988–1992 compared with the nonparticipants was 1.11 for overall mortality, which was not statistically significant [15]. Thus, selection bias seemed to be remote and the study participants were considered to represent the people in this community well.

Casual BPs were not obtained from those members of the 1913 participants who did not participate in annual health check-ups (n: 124). A total of 87 individuals had a previous history of stroke, so they were excluded from the present analysis to show the relationship between the first onset of stroke and BP. Therefore, the study sample consisted of 1702 individuals.

The mean age was 61 ± 11 years and the ratio of men to women was 37:63. The home BP values (125 ± 15/75 ± 10 mmHg) were significantly lower than the conventional BP levels (133 ± 19/76 ± 12 mmHg). Of the 1702 study participants, 370 (22%) were classified as current or ex-smokers and 507 (30%) were treated with antihypertensive medication at baseline, while 16 (1%), 218 (13%) and 207 (12%) were classified as having a history of heart disease, diabetes mellitus or hypercholesterolemia, respectively.

Prediction of stroke by home BP measurement

In published studies that investigated the association between stroke risk and BP values in the Ohasama study, we accumulated follow-up data until December 31, 2001 [10–13]. Mean duration of follow-up was 10.6 ± 3.0 years. A total of 32 participants (2%) moved away and were lost to follow-up, while 79 cardiovascular deaths (5%) and 180 noncardiovascular deaths (11%) were recorded.

Of the 1702 participants, 153 (9%) had a first onset of stroke or TIA. This was due to cerebral infarction in 106 (69%), intracerebral hemorrhage in 28 (18%), subarachnoid hemorrhage in 12 (8%), TIA in four (3%) and unknown causes in three (2%).

Association between home, conventional BP values & stroke risk

At first, to investigate the association between BP values and stroke risk nonparametrically, we subdivided the participants into four groups according to individual home BP values, and then compared their risk for total, hemorrhagic, and ischemic stroke and TIA [10]. In the analysis, the group with the lowest BP values were treated as the reference group. For home systolic BP measurements, the risks for total and ischemic stroke among those with systolic BP ≥135 mmHg were significantly higher than that among those with systolic BP <115 mmHg (RH = 2.22 and 3.44, respectively, both $p < 0.004$), with a significant observable linear trend (all $p < 0.001$). The higher systolic BP category also tended to be associated with the risk of hemorrhagic stroke, although there was no statistically significant difference (RH: 1.55, $p = 0.2$). Similarly, home diastolic BP values were significantly related to stroke risk (all p for linear trend <0.005). There were nonsignificant linear trends between conventional systolic BP, diastolic BP and the risk for total, ischemic and hemorrhagic stroke, except for a significant association between conventional systolic BP and the risk of ischemic stroke ($p = 0.03$).

Comparison of predictive values between home & conventional BP

Since the association between BP and stroke was log-linear, we parametrically compared the predictive values between home and conventional BP as continuous variables (FIGURE 1) [10]. A 10-mmHg elevation of home systolic BP values was significantly associated with 29, 32 and 30% increases in the risk for total, hemorrhagic and ischemic stroke, respectively. A 5-mmHg elevation of home diastolic BP was also significantly associated with 18, 28 and 17% increases in the risk for total, hemorrhagic and ischemic stroke, respectively. Although all conventional systolic BP and diastolic BP values showed a tendency for linear association with the risk of stroke and subtypes, the predictive power was less remarkable compared with those of home BP values.

When home and conventional BP values were simultaneously included in a Cox model, only home BP was significantly related with total stroke risk. The model including both home and conventional BP lost 'goodness of fit' when home BP was removed (systolic BP likelihood ratio: 16.4; diastolic BP likelihood ratio: 11.9; both $p < 0.001$). However, the goodness of fit of the model including both home and conventional BP did not change significantly when conventional BP was removed (systolic BP likelihood ratio: 0.035; diastolic BP likelihood ratio:0.001; both $p > 0.8$). Similarly, home BP values showed a significantly greater relation with the risk of hemorrhagic and ischemic stroke than the conventional BP values (all $p < 0.02$).

This finding is the first demonstration that home BP has a stronger predictive power of ischemic and hemorrhagic strokes than conventional BP.

How many times should BP be measured at home for better prediction of stroke risk?

Home BP is generally self-measured several times over a particular period but it has not, to date, been determined how many measurements are needed to provide reliable information in terms of the prognostic significance. Although previous studies of short duration proposed the optimum schedule of home BP measurements to be an average of at least 3 days, these studies were based merely on the repeatability and the stability of home BP measurements [19,20].

To determine the optimum number of home BP measurements in relation to their predictive value for stroke risk, we therefore analyzed the association between the number of home BP measurements and the risk of stroke in 1491 individuals of the 1702 participants who measured their home BP for a period of 2 weeks or more [11]. The initial 1-day, 2-day, 1-week, 1-week (mean of one, two, seven and 14 measurements, respectively) and multiple (mean of more than 14 measurements; average number of measurements: 25) home BP values were calculated for each individual.

The predictive value of home BP increased progressively with the number of measurements, showing the highest predictive value with multiple home BP measurements: 10-mmHg elevations of 1-day, 2-day, 1-week, 2-week and multiple home systolic BP values were associated with respective 19, 20, 27, 31 and 35% increases in the risk of stroke and TIA (all $p<0.01$) (FIGURE 2) [11]. Similarly, 5 mmHg elevations of 1-day, 2-day, 1-week, 2-week and multiple home diastolic BP values were associated with respective 10, 12, 16, 20 and 21 increases in the risk (all $p < 0.01$).

When one-day home and conventional BP values were included simultaneously in a Cox model, only one-day home BP was significantly related to stroke/TIA risk, as follows. 1-day home

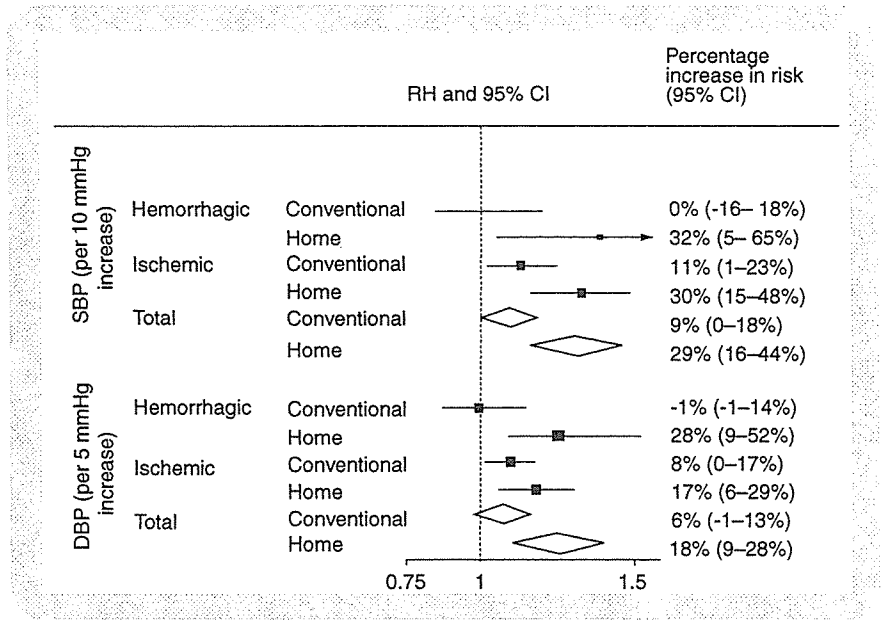

Figure 1. Predictive values of home and conventional blood pressure for the risk of stroke and subtypes. RH and 95% CI of home and conventional SBP (above) and DBP (below) levels adjusted for age, gender, smoking status, the use of antihypertensive medication history of heart disease, hypercholestrolemia, and diabetes for the risk of total (total stroke or transient ischemic attack [TIA], hemorrhagic (cerebral hemorrhage or subarachnoid hemorrhage) and ischemic (cerebral infarction or TIA) stroke. RH is expressed as in increase in stroke risk per 10 mmHg elevation of SBP and per 5 mmHg elevation of DBP. Boxes are centred on the point estimates of RH in the relevant stroke subtypes and are sized in proportion to the number of events recorded. The diamond represents the results for total stoke and is centred on the point of the RH. The horizontal lines and the tips of the diamond represent 95% CI. CI: confidence intervals; DBP: diastolic blood pressure; RH: Relative hazard; SBP: systolic blood pressure. Adapted with permission from [10].

systolic BP: RH per 10-mmHg elevation of 1.18, p = 0.002; screening systolic BP: RH of 1.02, p = 0.6; 1-day home diastolic BP: RH per 5-mmHg elevation of 1.09, p = 0.03; conventional diastolic BP: 1.05, p = 0.3 [11]. The model including both 1-day home and conventional BP lost 'goodness of fit' when 1-day home BP was removed (systolic BP likelihood ratio: 9.70, p < 0.01; diastolic BP likelihood ratio: 4.91, p < 0.03). However, the goodness of fit of the model including both 1-day home and conventional BP did not change significantly when conventional BP was removed (systolic BP likelihood ratio = 0.21, p > 0.5; diastolic BP likelihood ratio = 1.31, p > 0.1). Similarly, in this analysis, the 2-day, 1-week, 2-week and multiple home BP values showed a significantly greater relation to the risk of stroke and TIA than the conventional BPs (all p < 0.03).

In this analysis, multiple home BP measurement (average of 25) was the strongest predictor of stroke or TIA, indicating that the predictive power of home BP was dependent partly upon the number of measurements. A threshold for the minimum number of home BP measurements within the range of 1–14 measurements for increasing the predictive power of stroke risk could not be determined, suggesting that as many measurements as possible and preferably more than 14 should be recommended for adequate prediction of stroke risk. Initial one-day home BP was a stronger predictor of stroke or TIA risk than conventional BP, even for a lower number of measurements. These results suggest that, in addition to the number of measurements, other factors, such as the lack of the white-coat effect, may be associated with superior predictive power. A recent report from an Italian general population [21] has partly confirmed these results. The study demonstrated that an average of only two home BP values obtained in the morning and in the evening within a day had a similarly strong predictive power for cardiovascular mortality to 24-hour ambulatory BP values [21], although shortcomings have been pointed out on the methods of statistical analysis [22]. These results also suggest that the view that 'the measurement of the initial-single day should be excluded' [19,20] is not necessarily applicable from the viewpoint of prognostic significance.

Prediction of stroke by home versus conventional BP measurement in relation to the JNC-7 classification

The JNC-7 is based on recent, up-to-date evidence for handling hypertension [3]. The JNC-7 classification has two distinctive features in comparison with the past JNC-VI [2] and the 1999 WHO/ISH guidelines [4]: risk stratification is simplified to four grades on the basis of BP; a new category, 120–139 mmHg of systolic BP or 80–89 mmHg of diastolic BP, is defined as prehypertension. The JNC-7 recommended that participants who had a past history of cerebrovascular disease should be treated intensively to prevent the recurrence of stroke. However, there was no specific description of strategies to prevent first stroke. Although the applicability of JNC-VI was demonstrated in the Japanese population [23], it is still uncertain whether or not the newer classification could be similarly useful in Asian populations.

Accordingly, we examined whether the JNC-7 classification was applicable in predicting the first-stroke risk among Japanese and compared the predictive power of home BP and conventional BP measurement for stroke risk in relation to the conventional BP-based classification of JNC-7 [12].

According to the JNC-7 criteria, the participants were classified into four groups on the basis of either home BP or conventional BP (TABLE 1). The conventional BP classification was equal to JNC-7 criteria. In the present analysis, 135/85 mmHg of home BP was the cut-off value for hypertension, according to the JNC-VI and JNC-7 guidelines. To define stage 2 hypertension and normotension based on home BP, we postulated that 95 mmHg and 75 mmHg of diastolic home BP are equivalent to 100 mmHg and 80 mmHg of diastolic conventional BP (i.e., a parallel shift of conventional to home BP). Systolic BP levels of home BP were introduced

from the rate of participants with normotension (group 1), prehypertension (group 2), stage 1 hypertension (group 3) and stage 2 hypertension (group 4) of conventional BP classification. According to this classification method for home BP, home BP in Group 1 was defined as systolic BP < 115 mmHg and diastolic BP < 75 mmHg; Group 2 as 115 ≤ systolic BP < 135 mmHg and/or 75 ≤ diastolic BP < 85 mmHg; Group 3 as 135 ≤ systolic BP< 150 mmHg and/or 85 ≤ diastolic BP < 95 mmHg; and Group 4 as 150 ≤ systolic BP and/or 95 ≤ diastolic BP.

After classification on the basis of either conventional or home BP, groups 2–4 were further divided into two subgroups (a and b): those without and those with cardiovascular disease risks (diabetes mellitus, hypercholesterolemia, habitual smoking or history of cardiovascular diseases), respectively. According to JNC-7 criteria, these cardiovascular disease risks are a compelling indication for antihypertensive drugs. Therefore, all participants were assigned to one of seven categories (groups 1, 2a, 3a, 4a, 2b, 3b and 4b). Participants classified according to conventional BP and home BP were analyzed separately.

The relative hazards for first stroke or TIA in subgroup b, with cardiovascular disease risks, on the basis of both home BP and conventional BP increased linearly with the elevation of BP grade [12]. In groups 4b and 4a, the predictability of home BP (4b-RH: 6.41, CI: 2.81–14.6, p < 0.0001; 4a:RH 2.88, CI: 1.09–7.60, p = 0.03), in terms of magnitude of RH, was higher than that of conventional BP (4b-RH 2.94, CI 1.32–6.55, p = 0.009; 4a–RH 2.06, CI 1.02–4.15, p = 0.04). On the other hand, the risk in Groups 2a and 3a was not significantly different from that in group 1 by the conventional BP-based classification (group 2a– RH: 0.94, CI: 0.50–1.77, p = 0.8; Group 3a-RH 0.75, CI 0.35–1.62, p = 0.5), and no stepwise increase in risk was observed (trend p = 0.1). However, when based on home BP classification, a significant increase in risk was clearly observed even in Group 3a (RH 2.40, CI 1.09–5.29, p = 0.03), and the stroke or TIA risk was increased linearly (trend p = 0.01).

We found that JNC-7 classification by home BP had stronger predictive power than by conventional BP for stroke or TIA risk in this prospective cohort study. Risk for stroke was also predicted when home BP was used for classification, irrespective of the presence of cardiovascular disease risks, but not necessarily when conventional BP was used. These data also support the intervention strategy for primary prevention of cardiovascular disease in prehypertensive individuals. This intervention might be based on home BP information, since stroke risk in patients without cardiovascular disease risks is predictable only when based on home BP, not on conventional BP.

Use of 2003 ESH–ESC guidelines for predicting stroke using home BP measurement

The 2003 ESH–ESC [5] followed the concepts of the 1999 WHO/ISH guidelines [4], stating that comprehensive risk stratification is the essential strategy for the management of hypertension. The 2003 ESH–ESC guidelines emphasized the importance of individualized treatment.

Although the 2003 ESH–ESC guidelines would possibly be applicable even for populations outside Europe, the usefulness of the guidelines in non-European countries has not yet been established. Therefore, we examined whether the 2003 ESH–ESC classification was applicable to predicting the risk of first stroke incidence in Japan [13]. We also compared the predictive power of home and conventional BP for stroke risk in relation to the stratification system of the 2003 ESH–ESC guidelines. Finally, we compared the prediction of first stroke based on the simplified risk stratification suggested by JNC-7 [3], with prediction based on the comprehensive risk stratification from the 2003 ESH–ESC guidelines.

Based on the 2003 ESH–ESC risk stratification system, the participants were first classified into six BP categories (TABLE2) [13]. Home and conventional BP-based criteria were defined as follows: optimal (home BP < 115/75, conventional BP < 120/80 mmHg); normal (home BP 115/75–124/79, conventional BP 120/80–129/84 mmHg); high normal (home

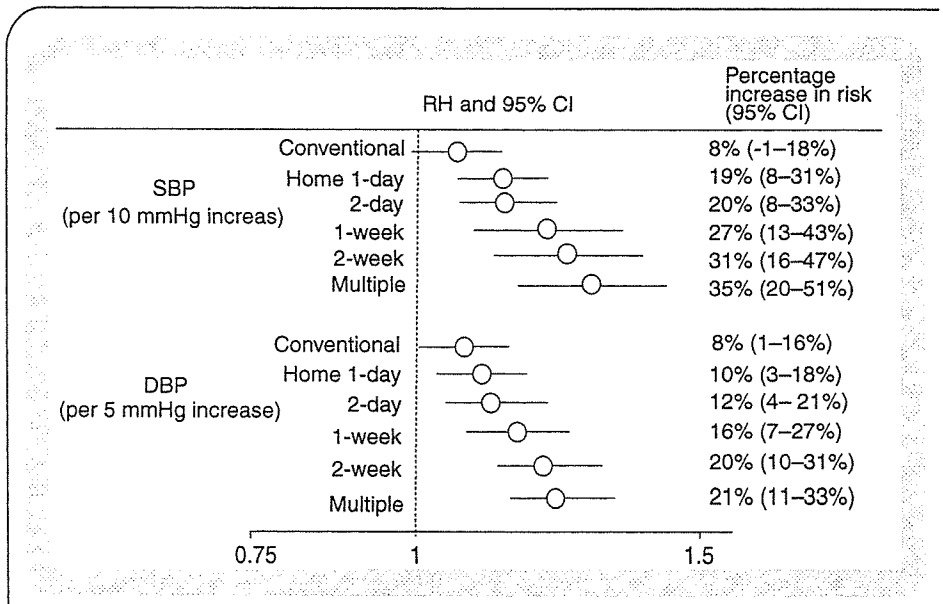

Figure 2. Predictive values of home and conventional blood pressures. RH and 95% CI of 1-day home, 2-day home, 1-week home, 2-week home, multiple home, and screening SBP and DBP levels adjusted for age, gender, smoking status, the use of antihypertensive medication, history of heart disease, hypercholesterolemia and diabetes for first symptomatic stroke. Open cicrles are RH expressed as an increase in stroke risk per 10 mmHg elevation of SBP and per 5 mmHg elevation of DBP. Horizontal lines represent 95% CI.
CI: confidence intervals; DBP: diastolic blood pressure; RH: Relative hazard; SBP: systolic blood pressure.
Adapted with permission from [11].

Table 1. Classification of groups according to HBP or CBP. Values and cardiovascular risks.

Groups	Category definitions	Systolic BP (mm Hg)		Diastolic BP (mm Hg)	Cardiovascular disease risks*
HBP					
Group 1	Normotension	<115	and	<75	Yes or no
Group 2a	Prehypertension	115–134	or	75–84	No
Group 2b					Yes
Group 3a	Stage 1 hypertension	135–149	or	85–94	No
Group 3b					Yes
Group 4a	Stage 2 hypertension	≥150	or	≥95	No
Group 4b					Yes
CBP					
Group 1	Normotension	<120	and	<80	Yes or no
Group 2a	Prehypertension	120–139	or	80–89	No
Group 2b					Yes
Group 3a	Stage 1 hypertension	140–159	or	90–99	No
Group 3b					Yes
Group 4a	Stage 2 hypertension	≥160	or	≥100	No
Group 4b					Yes

The higher category was used when a subject's systolic and diastolic BPs fell into different categories.
*Cardiovascular disease risks: diabetes mellitus, hypercholesterolemia, smoking habit, or history of cardiovascular disease.
BP: Blood pressure; CBP: Conventional blood pressure; HBP: Home blood pressure. Adapted with permission from [12].

BP 125/80–134/84, conventional BP 130/85–139/89 mmHg); grade 1 (mild hypertension: home BP 135/85–149/94, conventional BP 140/90–159/99 mmHg); grade 2 (moderate hypertension: home BP 150/95–164/104, conventional BP 160/100–179/109 mmHg); grade 3 (severe hypertension: home BP ≥165/105, conventional BP ≥180/110 mmHg). The conventional BP classification was equal to the 2003 ESH–ESC criteria. In the present analysis, hypertension was defined as home BP ≥135/85 mmHg, according to the JNC-VI, JNC-7 and 2003 ESH–ESC guidelines, and home BP of 135/85 mmHg is equivalent to conventional BP of 140/90 mmHg. To define other BP levels based on home BP, we postulated that 75, 80, 95 and 105 mmHg of diastolic home BP were equivalent to 80, 85, 100 and 110 mmHg of diastolic conventional BP, respectively. Systolic BP levels for home BP were introduced from the rate of participants from each level of conventional BP classification. In the present analysis, we did not include the concept of pure systolic hypertension.

The individuals were then stratified into four classes based on the extent of cardiovascular risks: 1st class (no risk factors), 2nd class (one or two risk factors), 3rd class (more than two risk factors or diabetes mellitus), and 4th class (past history of cardiovascular disease [CVD]). Risk factors were defined as follows: an age of over 55 for males and 65 for females, body mass index (BMI) greater than 25 kg/m^2, habitual smoking and hypercholesterolemia. Finally, study participants were assigned to one of five groups, according to the 2003 ESH–ESC criteria: average risk, low added risk, moderate added risk, high added risk, and very high added risk (TABLE 2). Participants with an optimal BP (optimal) who were not described in the risk stratification table of the original ESH–ESC guidelines were assigned to the average- low- or moderate-risk group according to their classes. The average risk group was used as the reference group in the analysis. Participants classified according to conventional BP and home BP were analyzed separately. In addition to these criteria, the classification system based on the JNC-7 guidelines as reported earlier (TABLE 1), was also used [12].

The risk of first stroke was increased linearly, with the increase in grade of stratified risk based on home BP as well as conventional BP (FIGURE 3). Even in the low-risk group, the risk for stroke was significantly higher than in the average-risk group. In the low-risk group, there was no difference in the stroke risk between home BP classification and conventional BP classification (home BP–RH 2.24, 95% CI 1.32–3.80, p = 0.003; conventional BP–RH 2.76, 95% CI 1.63–4.66, p = 0.0001). The stroke risk in the very high-risk group was extremely high when participants were classified by home BP

Table 2. Stratification of risk to quantify prognosis.

Category definition	Optimal	Normal	High normal	Grade 1 hypertension	Grade 2 hypertension	Grade 3 hypertension
CBP-based	≤120/80 (n = 370)	120/80–129/84 (n = 387)	130/85 – 139/89 (n = 396)	140/90 – 159/99 (n = 375)	160/100 – 179/109 (n = 136)	≥180/110 (n = 38)
HBP-based	≤115/75 (n = 432)	115/75–124/79 (n = 390)	125/80 – 134/84 (n = 378)	135/85 – 149/94 (n = 362)	150/95 – 164/104 (n = 111)	≥165/105 (n = 29)
No other risk factors	Average	Average	Average	Low	Moderate	High
1–2 risk factors	Average	Low	Low	Moderate	Moderate	Very high
≥3 risk factors or DM	Low	Moderate	High	High	High	Very high
PHCVD	Moderate	High	Very high	Very high	Very high	Very high

Average: Average risk; DM: Diabetes mellitus; High: High added risk; Low: Low added risk; PHCVD: Past history of cardiovascular disease; Moderate: Moderate added risk; Very high: Very high added risk.
CBP: conventional blood pressure; HBP: Home blood pressure. Adapted with permission from [13].

(RH 14.4, 95% CI: 6.92–29.8, p <0.0001). The predictive power decreased when participants were classified by conventional BP (RH 5.30, 95% CI: 2.23–12.6, p = 0.0002). The statistically significant linearity among the groups was observed for both home BP and conventional BP classifications (trend p < 0.0001). When the low risk group was designated as a reference category in the Cox model, the stroke risk in the moderate-risk group was significantly high for home BP (RH 2.04, 95% CI: 1.34–3.09, p = 0.0009), whereas the moderate-risk group was not significantly different from the low risk group using the conventional BP classification (RH 1.33, 95% CI: 0.90–1.97, p = 0.2). When both classifications were treated as continuous variables and were simultaneously included in the model, only home BP classification was significantly related to stroke risk (home BP classification–RH 1.88, 95% CI: 1.55–2.28, p < 0.0001; conventional BP classification-RH 0.98, 95% CI: 0.81–1.20, p = 0.9). The model, including both home BP and conventional BP classifications, lost 'goodness of fit' when home BP was removed (likelihood ratio: 40.2; p < 0.001), whereas no significant changes occurred when conventional BP was removed (likelihood ratio: 0.028; p = 0.9).

Further analysis was conducted comparing the JNC-7 guideline-based classification (including SAH and excluding TIA, which was a modified analysis from the previous analysis) and the 2003 ESH–ESC guideline-based classification (FIGURE 3). The stroke risk in group 4b (highest) was significantly elevated for home BP (RH 4.54, 95% CI: 2.16–9.54, p < 0.0001) as well as for conventional BP classification (RH: 2.81, 95% CI: 1.31–6.04, p = 0.008). However, for the magnitude of RH, the stroke risk based on the 2003 ESH–ESC classification was clearly more dramatic than that based on the JNC-7 classification.

We found that the 2003 ESH–ESC classification was useful and applicable for a general Japanese population in predicting future stroke incidence. Furthermore, the risk stratification system became extremely powerful for the prediction of stroke incidence when home BP was used instead of conventional BP.

In comparison with the 2003 ESH–ESC guidelines, the JNC-7 classification adopts a simplified risk stratification that consists of four grades based on conventional BP [3]. Individuals who have hypertension and at least one risk factor are considered to be candidates for antihypertensive drugs and intensive treatment. Thus, their cardiovascular risks are not thoroughly considered in JNC-7. In the previous study it was reported that the JNC-7 classification is applicable for the general Japanese population [12]. However, when based on the risk stratification system proposed in the 2003 ESH–ESC guidelines, the measurements of home BP as well as conventional BP would predict the first stroke incidence more accurately than those based on the simplified risk stratification in JNC-7, as shown in the current study [13]. It is a reasonable assumption that a comprehensive risk stratification system could be used for individualized BP management. Furthermore, we would like to emphasize that the stroke risk in the moderate-risk group was significantly higher than that in the low-risk group when based on home BP, whereas no significant differences were observed between two risk groups when based on conventional BP in this study; these findings support the assertion that BP management should be based on home BP information.

Expert commentary

Although home BP measurement is now acknowledged worldwide in the major guidelines as a useful tool for clinical practice, a lack of information on the prognostic significance has limited its use in clinical decision-making. In the Ohasama study, we demonstrated that home BP measurements provide more useful prognostic information on cerebrovascular disease than conventional BP measurements. An increased number of measurements improves the predictive