

MAOA, MTHFR, and TNF- β genes polymorphisms and personality traits in the pathogenesis of migraine

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Abstract Migraine is a multifactorial disease with various factors, such as genetic polymorphisms and personality traits, but the contribution of those factors is not clear. To clarify the pathogenesis of migraine, the contributions of genetic polymorphisms and personality traits were

simultaneously investigated using multivariate analysis. Ninety-one migraine patients and 119 non-headache healthy volunteers were enrolled. The 12 gene polymorphisms analysis and NEO-FFI personality test were performed. At first, the univariate analysis was performed to extract the contributing factors to pathogenesis of migraine. We then extracted the factors that independently contributed to the pathogenesis of migraine using multivariate stepwise logistic regression analysis. Using the multivariate analysis, three gene polymorphisms including monoamine oxidase A (MAOA) T941G, methylenetetrahydrofolate reductase (MTHFR) C677T, and tumor necrosis factor beta (TNF- β) G252A, and the neuroticism and conscientiousness scores in NEO-FFI were selected as significant factors that independently contributed to the pathogenesis of migraine. Their odds ratios were 1.099 (per point of neuroticism score), 1.080 (per point of conscientiousness score), 2.272 (T and T/T or T/G vs G and G/G genotype of MAOA), 1.939 (C/T or T/T vs C/C genotype of MTHFR), and 2.748 (G/A or A/A vs G/G genotype of TNF- β), respectively. We suggested that multiple factors, such as gene polymorphisms and personality traits, contribute to the pathogenesis of migraine. The contribution of polymorphisms, such as MAOA T941G, MTHFR C677T, and TNF- β G252A, were more important than personality traits in the pathogenesis of migraine, a multifactorial disorder.

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Introduction

Migraine is the most common type of neurovascular headache. In Japan, approximately 8.4% of people

experience migraine headaches [1], which are usually characterized by severe pain on one or both sides of the head and at times disturbed vision. Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter that regulates brain vascular constriction, release of neurotransmitter, and platelet aggregation [2, 3]. Between attacks, migraine patients had lower plasma levels of 5-HT and higher plasma levels of 5-hydroxyindoleacetic acid (5-HIAA), a 5-HT metabolite, than controls and patients with a tension-type headache [4]. Nagata et al. [5] also suggested that plasma 5-HT levels during attack free-periods in patients with migraine with an aura (MA) were significantly lower than in controls and patients with a migraine without an aura (MO). Moreover, intravenous injection of 5-HT aborted reserpine-induced and spontaneous migraine headaches [6, 7]. Thus, 5-HT has an important role in the pathophysiology of migraine; therefore, genetic association studies have been performed on 5-HT-related gene polymorphisms by many investigators [8–15]. However, recent reports have suggested that the pathogenesis of migraine is related not only to 5-HT-related gene polymorphisms but also to 5-HT-unrelated gene polymorphisms, such as angiotensin-converting enzyme (ACE) [16], methylenetetrahydrofolate reductase (MTHFR) [17, 18], estrogen receptor 1 (ESR1) [19, 20], and tumor necrosis factor beta (TNF- β) [21].

Although migraine is a multifactorial disease, most previous studies have focused on the contribution of a single factor. Interestingly, Park et al. [14] investigated a harm avoidance personality dimension and polymorphisms in 5-HT transporter protein gene in MO patients, and reported that two factors, including the harm avoidance personality and a variable number of tandem repeats polymorphism within intron 2 (VNTR) of 5-HT transporter (5-HTTVNTR) independently contributed to MO patients. To clarify the pathogenesis of migraine, more genetic factors including 5-HT-related and 5-HT-unrelated gene polymorphisms and personality traits need to be analyzed simultaneously. In addition, migraine and psychiatric disorders are known to associate with the some of commonness personality traits [22] and genetic factors [23]. Therefore, to show independent factors for pathogenesis of migraine, it is very important to exclude subjects with depression and other co-morbid psychiatric disorders.

In this study, using subjects without depression and other co-morbid psychiatric disorders, we simultaneously analyzed the contributions of gene polymorphisms and personality traits in the pathogenesis of migraine, and extracted independent contributing factors by multivariate stepwise logistic regression analysis.

Methods

Subjects

We enrolled 91 Japanese migraine patients who were admitted to the Department of Neurology, Showa University Fujigaoka Rehabilitation Hospital, Kanagawa (Japan), Pain Clinic in Showa University East Hospital, Tokyo (Japan), and Neurosurgery Clinic/Institute, Tokyo (Japan), between June 2006 and December 2010. Migraine was diagnosed according to the international classification of headache disorders, 2nd edition (ICHD-II) in 2004 [24]. Ninety-one patients with migraine were divided into patients with migraine with aura (MA, $n = 24$), and without aura (MO, $n = 67$). Non-headache healthy volunteers comprised the control group ($n = 119$). Controls were matched for age and sex with patients and recruited in the same geographic areas. Moreover, controls were checked by interview to be healthy. We also confirmed it using the ID migraine screener Japanese version [25], which included a four-item (headache exacerbation in daily performance, nausea, photophobia, and osmophobia). Patients and controls with depression and other co-morbid psychiatric disorders were excluded by their history. All patients and controls were Japanese and gave their informed consent to this study.

The clinical study was approved by the Ethics Committee for Genome Research of Showa University.

NEO-FFI

Personality traits were assessed by the NEO Five-Factor Inventory (NEO-FFI) personality questionnaire, including neuroticism, extraversion, openness, agreeableness, and conscientiousness [26]. Scores are summed totals and have a range of 0–48 for each of five personality traits.

Genotyping

The gene polymorphisms of serotonin (5-HT) transporter 5-HTTLPR (NG_011747) [27] and 5-HTTVNTR (NG_011747) [28], 5-HT_{2A} receptor T102C (rs6313) [29], 5-HT_{1B} receptor G861C (rs6296) [30], monoamine oxidase A (MAOA) VNTR (NG_008957) [31, 32] and MAOA T941G (rs6323) [33], methylenetetrahydrofolate reductase (MTHFR) C677T (rs1801133) [34], angiotensin-converting enzyme (ACE) insertion/deletion (I/D) (NG_011648) [35], estrogen receptor 1 (ESR1) G325C (rs1801132) [36] and G594A (rs2228480) [37], dopamine receptor 2 (DRD2) C939T (rs6275) [38], and tumor necrosis factor- β (TNF- β) G252A (rs909253) [21] were studied. Genomic DNA was

Table 1 Primers and restriction enzymes used for genotyping

Polymorphism	Primer	Restriction enzyme	Product size (bp)	Reference
5-HTTLPR (NG_011747)	5'-GGC GTT GCC GCT CTG AAT GC-3'	–	Short: 484	27
	5'-GAG GGA CTG AGC TGG ACA ACC AC-3'		Long: 528	
			Extra-long: 572	
5-HTTVNTR (NG_011747)	5'-GTC AGT ATC ACA GGC TGC GAG-3'	–	9: 250	28
	5'-CAT GTT CCT AGT CTT ACG CCA GTG-3'		10: 267	
			12: 301	
5-HT _{2A} T102C (rs6313)	5'-CAT CTG CTA CAA GTT CTG GCT TAG-3'	Msp I	T: 344	29
	5'-CTG CAG CTT TTT CTC TAG GG-3'		C: 217 and 127	
5-HT _{1B} G861C (rs6296)	5'-GAA ACA GAC GCC CAA CAG GAC-3'	Hinc II	G: 452 and 96	30
	5'-GCC AGA AAC CGC GAA AGA AGA-3'		C: 309, 143, and 96	
MAOAVNTR (NG_008957)	5'-ACA GCC TGA CCG TGG AGA AG-3'	–	2: 291 (short)	31,32
	5'-GAA CGG ACG CTC CAT TCG GA-3'		3: 321 (short)	
			3.5: 336 (long)	
			4: 351 (long)	
			5: 381 (long)	
MAOA T941G (rs6323)	5'-GAC CTT GAC TGC CAA GAT TC-3'	Fnu4H I	T: 130	33
	5'-CTT CTT CTT CCA GAA GGC C-3'		G: 65	
MTHFR C677T (rs1801133)	5'-TGA AGG AGA AGG TGT CTG CGG GA-3'	Hinf I	C: 198	34
	5'-AGG ACG GTG CGG TGA GAG TG-3'		T: 175 and 23	
ACE I/D (NG_011648)	5'-CTG GAG AGC ACT CCC ATC CTT TCT-3'	–	I: 490	35
	5'-GAC GTG GCC ATC ACA TTC GTC AGA T-3'		D: 190	
ESR1 G325C (rs1801132)	5'-AGC CCG CTC ATG ATC AAA CG-3'	Hinf I	C: 120	36
	5'-GGA TCA TAC TCG GAA TAG AGA AT-3'		G: 99 and 21	
ESR1 G594A (rs2228480)	5'-GAG GAG ACG GAC CAA AGC CAC-3'	Btg I	A: 227	37
	5'-GCC ATT GGT GTT GGA TGC ATG C-3'		G: 129 and 98	
DRD2 C939T (rs6275)	5'-ATC CTG CAG CCA TGG-3'	Nco I	C: 446	38
	5'-ATT GTC CGG CTT TAG-3'		T: 252 and 194	
TNF- β G252A (rs909253)	5'-GGT TTC CTT CTC TGT CTC TGA CTC TCC-3'	Nco I	A: 173	21
	5'-GAG AGA GAT CGA CAG AGA AGG GGA C-3'		G: 102 and 71	

extracted from whole blood using NucleoSpin[®] Blood QuickPure (NIPPON Genetics Co., Ltd, Tokyo, Japan). The polymorphism of each gene was determined according to previous reports [21, 27–38]. Primer sequences, restriction enzymes, and expected fragment sizes of about 12 gene polymorphisms are shown in Table 1; Fig. 1.

The PCR products or restriction enzyme-treated PCR fragments with positive controls were run on 3% agarose gels and stained with ethidium bromide.

Statistical analysis

The genotype frequencies were tested using the public statistical web-tool <http://www.oege.org/software/hwe-mr-calc.shtml> for Hardy–Weinberg equilibrium (HWE). $P > 0.05$ was considered not deviate from the equilibrium. A power analysis was performed with the use of the Website

(http://www.dssresearch.com/toolkit/spcalc/power_a1.asp).

In this power analysis, we determined according to Cohen's criteria [39] the following: small effect size of ≥ 0.2 and < 0.5 , and medium effect size of ≥ 0.5 and < 0.8 , and large effect size of ≥ 0.8 , $\alpha = 5\%$. Results are expressed as mean \pm SD. We applied univariate analysis using Student's *t*-test for continuous variables, and χ^2 test or Fisher's exact test for categorical variables. Next, multivariate stepwise logistic regression analysis was performed to determine independent factors associated with the pathogenesis of migraine. Variables with $P < 0.1$ on univariate analysis were included in the multivariate model [40–42]. Odds ratio (OR) and its 95% confidence intervals (CI) were calculated to evaluate the effects of factors. Values of $P < 0.05$ were considered statistically significant. The analysis was performed using SPSS 11.0 J (SPSS, Tokyo, Japan).

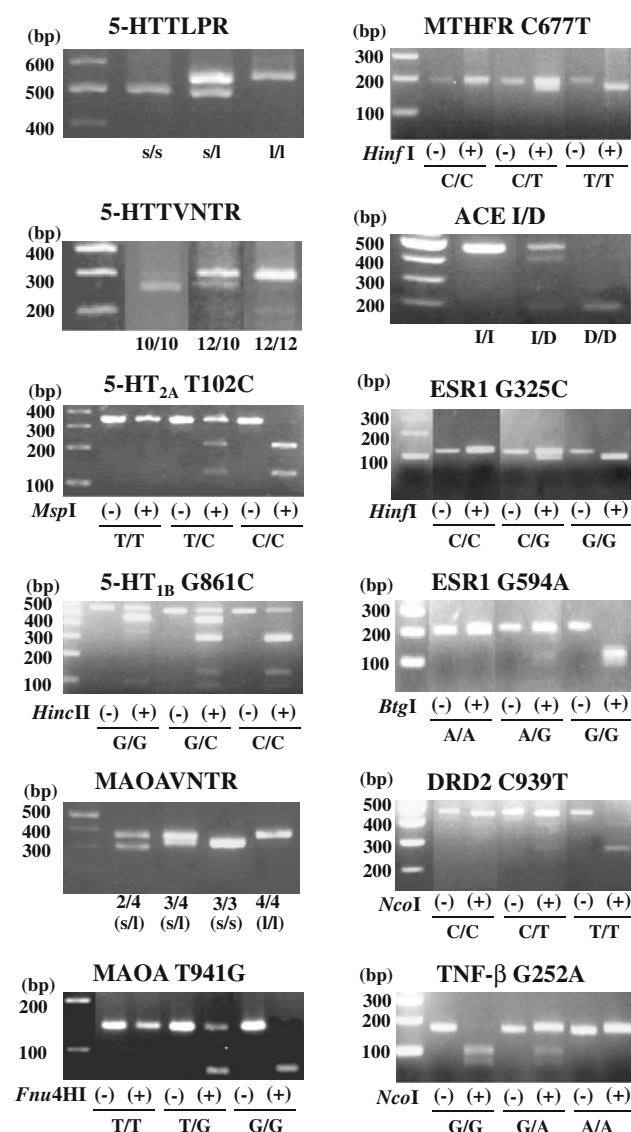


Fig. 1 PCR or PCR-restriction fragment length polymorphism (PCR–RFLP)-based genotyping of 5-HT-related and 5-HT-unrelated gene polymorphisms. PCR and digestion products were loaded on 3% agarose gel and stained with ethidium bromide (see Table 1)

Results

Characteristics of patients

The study population consisted of 91 migraine patients (age: 42.4 ± 10.2 years) including MA ($n = 24$) and MO ($n = 67$) (20 (22%) male and 71 (78%) female). One hundred and nineteen non-headache healthy Japanese controls (age: 40.7 ± 10.4 years) consisted of 31 (26%) men and 88 (74%) women. The migraine patients and healthy controls were age and sex matched.

Univariate analysis

Personality traits

As shown in Table 2, the neuroticism score was significantly higher in migraine patients (26.9 ± 8.0 , $P = 0.001$) than in controls (23.4 ± 7.2). The conscientiousness score was higher in migraine patients (29.3 ± 6.6 , $P = 0.068$) than in controls (27.7 ± 6.2). Extraversion, openness, and agreeableness scores were not different between migraine patients and controls.

In sub-group analysis, the neuroticism score was significantly higher in MA patients (28.6 ± 8.7 , $P = 0.002$) and in MO patients (26.3 ± 7.8 , $P = 0.011$) than in controls (23.4 ± 7.2). The extraversion score was lower in MA patients (21.3 ± 6.4 , $P = 0.052$) than in controls (24.3 ± 7.0). The conscientiousness score was higher in MO patients (29.3 ± 6.4 , $P = 0.096$) than controls (27.7 ± 6.2).

Gene polymorphisms

Table 3 shows the genotypic distributions in subjects. Only the genotype distribution of ESR1 G594A polymorphism was not consistent with HWE in controls ($P < 0.001$). It may be due to methodological reasons (e.g., non-population-based study and small sample size).

The genotypic distributions of MAOA T941G (T (male) plus T/T (female) plus T/G (female) vs G (male) plus G/G (female), $P = 0.048$), MTHFR C677T (C/C vs C/T plus T/T, $P = 0.026$), ACE I/D (I/I plus I/D vs D/D, $P = 0.082$), ESR1 G594A (G/G plus G/A vs A/A, $P = 0.001$), and TNF- β G252A (G/G vs G/A plus A/A, $P = 0.074$) were different between migraine patients and controls. On the other hand, no significant differences in genotypic distributions between migraine patients and controls were observed in the polymorphisms of 5-HTTLPR, 5-HTTVNTR, 5-HT_{2A} T102C, 5-HT_{1B} G861C, MAOAVNTR, ESR1 G325C, and DRD2 C939T.

In the results of sub-group analysis, between MA patients and controls, the genotypic distributions of 5-HTTLPR (s/s plus s/l plus s/l plus l/l plus l/l, $P = 0.004$), 5-HTTVNTR (12/12 vs 12/10 plus 12/9 plus 10/10, $P = 0.029$), 5-HT_{1B} G861C (G/G vs G/C plus C/C, $P = 0.040$), MAOAVNTR (s (male) plus s/s (female) plus s/l (female) vs l (male) plus l/l (female), $P = 0.077$), ACE I/D (I/I plus I/D vs D/D; $P = 0.025$) and TNF- β G252A (G/G vs G/A plus A/A, $P = 0.007$) were different. Between MO patients and controls, the genotypic distributions of MAOA T941G (T (male) plus T/T (female) plus T/G (female) vs G (male) plus G/G (female), $P = 0.068$), MTHFR C677T (C/C vs C/T plus T/T, $P = 0.008$), and ESR1 G594A (G/G plus G/A vs A/A, $P = 0.001$) were different.

Table 2 NEO-FFI

NEO-FFI	Control <i>n</i> = 119	Migraine <i>n</i> = 91	<i>P</i> value	MA <i>n</i> = 24	<i>P</i> value	MO <i>n</i> = 67	<i>P</i> value
Neuroticism	23.4 ± 7.2	26.9 ± 8.0	0.001*	28.6 ± 8.7	0.002*	26.3 ± 7.8	0.011*
Extraversion	24.3 ± 7.0	23.1 ± 6.7	0.214	21.3 ± 6.4	0.052	23.8 ± 6.7	0.614
Openness	28.8 ± 5.1	28.9 ± 5.4	0.919	30.6 ± 5.0	0.129	28.3 ± 5.5	0.515
Agreeableness	29.5 ± 5.4	29.7 ± 4.4	0.729	29.0 ± 5.2	0.722	29.9 ± 4.0	0.494
Conscientiousness	27.7 ± 6.2	29.3 ± 6.6	0.068	29.4 ± 7.2	0.235	29.3 ± 6.4	0.096

Mean ± SD, *P* value below 0.10 is in *boldface*

* *P* < 0.05

Multivariate analysis

The multivariate stepwise logistic regression was performed with neuroticism score, conscientiousness score, MAOA T941G, MTHFR C677T, ACE I/D and TNF- β G252A; and neuroticism score (*P* < 0.001), conscientiousness score (*P* = 0.004), MAOA T941G (*P* = 0.010), MTHFR C677T (*P* = 0.034), and TNF- β G252A (*P* = 0.027) were selected as significant factors that independently contributed to the pathogenesis of migraine. ESR1 G594A was excluded from multivariate analysis, because the genotype distribution of ESR1 G594A polymorphism was not consistent with HWE in controls. Table 4 lists each OR and 95% CI. The ORs (per unit increase) were 1.099 (95% CI = 1.050–1.149), 1.080 (95% CI = 1.025–1.139), 2.272 (95% CI = 1.215–4.250), 1.939 (95% CI = 1.051–3.576) and 2.748 (95% CI = 1.121–6.736).

Using an alpha error of 5%, post hoc analysis, for neuroticism score and conscientiousness score in personality traits, and for MAOA T941G, MTHFR C677T, and TNF- β G252A polymorphisms, showed that our sample size had the power of 0.91 (large), 0.43 (small), 0.48 (small), 0.61 (medium), and 0.49 (small), respectively.

Discussion

In this report not only gene polymorphisms but also personality traits are involved in the pathophysiology of migraine using multivariate stepwise logistic regression analysis. The personality traits, neuroticism and conscientiousness, and gene polymorphisms, such as MAOA T941G, MTHFR C677T, and TNF- β G252A, were selected as factors that independently contributed to the pathogenesis of migraine in Japanese population.

Previous reports have shown the association between migraine and neuroticism [22, 43]. The neuroticism score of NEO-FFI in this study was significantly high in migraine patients. The harm avoidance (HA) score in the Temperament and Character Inventory (TCI), another personality

test, was elevated in migraine patients [14, 44]. Since the HA score is known to be correlated with the neuroticism score of NEO-FFI [45], neuroticism is an important personality trait in migraine. Moreover, in this study, we found for the first time that the conscientiousness is independently related with the pathogenesis of migraine. Thus, these personality traits including neuroticism and conscientiousness contributed to the pathogenesis of migraine.

The low level of plasma 5-HT seems to promote vasodilation, which is known to be one of the origins of headache. Oxidase deamination of 5-HT is known to be catalyzed by monoamine oxidase A (MAOA). MAOA is located on the X chromosome at Xp.11.3-Xp11.4 [46]. Several polymorphisms, including MAOA T941G and MAOAVNTR, have been identified in the MAOA coding sequence [31, 47]. Although the T941G polymorphism did not express the change in coding protein, it was reported to be significantly associated with low (T) and high (G) catalytic activity [31]. MAOA T941G polymorphism has been shown to be related with mood disorders, including major depressive or bipolar disorders [48]; however, we could not find a report showing the relation of MAOA T941G polymorphism in migraine. Hotamisligil and Breakfield [33] showed that MAOA activity is lower in T941 MAOA polymorphism than in G941 MAOA polymorphism. Our study was shown that G and G/G polymorphisms are higher in migraine patients than controls; therefore, it is possible that the plasma 5-HT levels in migraine patients, especially MO patients, are lower than in controls.

High plasma levels of homocysteine are related to a high risk for cardiovascular disease, cerebrovascular disease, and peripheral vascular disease [49, 50]. MTHFR is a key enzyme in homocysteine metabolism, catalyzing the production of 5-methylenetetrahydrofolate from 5,10-methylenetetrahydrofolate [51–53]. The T/T genotype of MTHFR C677T polymorphism, which changes an alanine to a valine residue in the catalytic domain, has been shown to decrease enzyme activity [53]. Moreover, the reduction of MTHFR leads to increased homocysteine levels [53]. In a Japanese study, Kowa et al. [17] reported that the T/T

Table 3 Gene polymorphisms

		Control (<i>n</i> = 119)		Migraine (<i>n</i> = 91)		<i>P</i> value	MA (<i>n</i> = 24)		<i>P</i> value	MO (<i>n</i> = 67)		<i>P</i> value	
		<i>n</i>	(%)	<i>n</i>	(%)		<i>n</i>	(%)		<i>n</i>	(%)		
5-HTTLPR	s/s	73	(61)	53	(58)		13	(54)		40	(60)		
	s/l	38	(32)	29	(32)		6	(25)		23	(34)		
	l/l	4	(3)	8	(9)		5	(21)		3	(4)		
	s/xl	3	(3)	1	(1)		0	(0)		1	(1)		
	l/xl	1	(1)	0	(0)		0	(0)		0	(0)		
	s/s, s/l, s/xl l/l, l/xl	114	(96)	83	(91)	0.171	19	(79)	0.004*	64	(96)	1.000	
5-HTTVNTR	12/12	98	(82)	72	(79)		15	(63)		57	(85)		
	12/10	18	(15)	15	(16)		8	(33)		7	(10)		
	12/9	3	(3)	2	(2)		0	(0)		2	(3)		
	10/10	0	(0)	2	(2)		1	(4)		1	(1)		
	12/12 12/10,12/9,10/10	98	(82)	72	(79)	0.554	15	(63)	0.029*	57	(85)	0.633	
	21	(18)	19	(21)		9	(38)		10	(15)			
5-HT _{2A} T102C	T/T	27	(23)	22	(24)		8	(33)		14	(21)		
	T/C	67	(56)	52	(57)		13	(54)		39	(58)		
	C/C	25	(21)	17	(19)		3	(13)		14	(21)		
	T/T T/C, C/C	27	(23)	22	(24)	0.801	8	(33)	0.269	14	(21)	0.777	
92	(77)	69	(76)		16	(67)		53	(79)				
5-HT _{1B} G861C	G/G	34	(29)	18	(20)		2	(8)		16	(24)		
	G/C	63	(53)	60	(66)		21	(88)		39	(58)		
	C/C	22	(18)	13	(14)		1	(4)		12	(18)		
	G/G G/C, C/C	34	(29)	18	(20)	0.144	2	(8)	0.040*	16	(24)	0.488	
85	(71)	73	(80)		22	(92)		51	(76)				
MAOAVNTR	Female	s/s	34	(39)	28	(39)		8	(40)		20	(39)	
		s/l	38	(43)	35	(49)		12	(60)		23	(45)	
		l/l	16	(18)	8	(11)		0	(0)		8	(16)	
	Male	s	23	(74)	14	(70)		3	(75)		11	(69)	
		l	8	(26)	6	(30)		1	(25)		5	(31)	
		s, s/s, s/l l, l/l	95	(80)	77	(85)	0.372	23	(96)	0.077	54	(81)	1.000
24	(20)	14	(15)		1	(4)		13	(19)				
MAOA T941G	Female	T/T	26	(30)	13	(18)		2	(10)		11	(22)	
		T/G	44	(50)	30	(42)		10	(50)		20	(39)	
		G/G	18	(20)	28	(39)		8	(40)		20	(39)	
	Male	T	10	(32)	6	(30)		1	(25)		5	(31)	
		G	21	(68)	14	(70)		3	(75)		11	(69)	
		T, T/T, T/G G, G/G	80	(67)	49	(54)	0.048*	13	(54)	0.221	36	(54)	0.068
39	(33)	42	(46)		11	(46)		31	(46)				

Table 3 continued

		Control (n = 119)		Migraine (n = 91)		P value	MA (n = 24)		P value	MO (n = 67)		P value
		n	(%)	n	(%)		n	(%)		n	(%)	
MTHFR C677T	C/C	37	(31)	42	(46)		8	(33)		34	(51)	
	C/T	61	(51)	31	(34)		12	(50)		19	(28)	
	T/T	21	(18)	18	(20)		4	(17)		14	(21)	
	C/C	37	(31)	42	(46)	0.026*	8	(33)	0.829	34	(51)	0.008*
	C/T, T/T	82	(69)	49	(54)		16	(67)		33	(49)	
ACE I/D	I/I	49	(41)	39	(43)		10	(42)		29	(43)	
	I/D	48	(40)	43	(47)		14	(58)		29	(43)	
	D/D	22	(18)	9	(10)		0	(0)		9	(13)	
	I/I, I/D	97	(82)	82	(90)	0.082	24	(100)	0.025*	58	(87)	0.375
	D/D	22	(18)	9	(10)		0	(0)		9	(13)	
ESR1 G325C	G/G	14	(12)	16	(18)		3	(13)		13	(19)	
	G/C	62	(52)	43	(47)		12	(50)		31	(46)	
	C/C	43	(36)	32	(35)		9	(38)		23	(34)	
	G/G	14	(12)	16	(18)	0.233	3	(13)	1.000	13	(19)	0.156
	G/C, C/C	105	(88)	75	(82)		21	(88)		54	(81)	
ESR1 G594A	G/G	78	(66)	64	(70)		16	(67)		48	(72)	
	G/A	26	(22)	26	(29)		7	(29)		19	(28)	
	A/A	15	(13)	1	(1)		1	(4)		0	(0)	
	G/G, G/A	104	(87)	90	(99)	0.001*	23	(96)	0.310	67	(100)	0.001*
	A/A	15	(13)	1	(1)		1	(4)		0	(0)	
DRD2 C939T	C/C	29	(24)	15	(17)		2	(8)		13	(19)	
	C/T	61	(51)	49	(54)		14	(58)		35	(52)	
	T/T	29	(24)	27	(30)		8	(33)		19	(28)	
	C/C	29	(24)	15	(17)	0.164	2	(8)	0.105	13	(19)	0.437
	C/T, T/T	90	(76)	76	(84)		22	(92)		54	(81)	
TNF- β G252A	G/G	11	(9)	16	(18)		7	(29)		9	(13)	
	G/A	56	(47)	39	(43)		8	(33)		31	(46)	
	A/A	52	(44)	36	(40)		9	(38)		27	(40)	
	G/G	11	(9)	16	(18)	0.074	7	(29)	0.007*	9	(13)	0.376
	G/A, A/A	108	(91)	75	(82)		17	(71)		58	(87)	

P value below 0.10 is in *boldface*

* $P < 0.05$

genotype of MTHFR is increased in MA patients. Moschiano et al. [54] suggested that homocysteine plasma levels were higher in MA patients than in healthy controls. Recent meta-analysis showed that the MTHFR T/T genotype may be a marker of MA [55]; however, a German study of 656 MA patients and 625 controls [56], and a

Finnish study of 898 MA patients and 900 controls did not confirm an association [57]. On the other hand, Schurks et al. [18] suggested that the risk of MA is reduced among carriers of the T/T genotype of MTHFR C677T polymorphism. Oterino et al. [58] also reported that the genetic distribution of the C/C genotype of MTHFR C677T is

Table 4 Multivariate analysis of factors in the pathogenesis of migraine

Variable	β	OR	95% Confidence interval	P value
Neuroticism	0.094	1.099 ^a	1.050–1.149	< 0.001
Conscientiousness	0.077	1.080 ^a	1.025–1.139	0.004
MAOA T941G	0.821	2.272 ^b	1.215–4.250	0.010
MTHFR C677T	0.662	1.939 ^c	1.051–3.576	0.034
TNF- β G252A	1.011	2.748 ^d	1.121–6.736	0.027

OR Odds Ratio = exp (β)

^a Per point

^b Genotype T, T/T, T/G vs G, G/G

^c Genotype C/T, T/T vs C/C

^d Genotype G/A, A/A vs G/G

higher in MO patients than in controls. In the present study, we found that the C/C genotype of MTHFR C677T may be one of the contributing factors of MO. Interestingly, the T/T genotype at MTHFR C677T polymorphism [59] is known to be a risk factor for depression. Since most previous studies did not exclude the subjects with depression and other co-morbid psychiatric disorders, it seems to be one of the reasons for the difference of results between previous studies and ours. MTHFR activities are known to be involved in the activity of dihydropteridine reductase [60], which has a key role in recycling tetrahydrobiopterin, a coenzyme for the rate-limiting enzymes of monoamine and nitric oxide (NO) biosynthesis [61]. Miwa et al. [62] reported that the increase of tetrahydrobiopterin content in the brain enhances 5-HT biosynthesis. Since plasma 5-HT levels during migraine attacks were substantially higher than during attack-free periods, it is possible that the reduction of activity in C/C carriers of MTHFR may stimulate not only the recycling of tetrahydrobiopterin but also 5-HT biosynthesis during migraine attacks. In C/C carriers of the MTHFR C677T polymorphism, vasoconstriction and platelets aggregation, in response to 5-HT which is a major trigger of migraine may be stimulated.

This is the first report from Japan on genetic association of the G allele for TNF- β G252A polymorphism with migraine, especially MA. On the other hand, Trabace et al. [63] suggested that carrying the A allele for TNF- β G252A polymorphism is a risk factor for the development of MO, but not MA. Asuni et al. [21] also reported that the G allele for G252A TNF- β gene polymorphism is associated with MO. Ghosh et al. [64] showed that no association of TNF- β G252A polymorphism is determined between patients with migraine and controls. Since these researches were performed in Europe and India, racial differences may have caused this discrepancy. Interestingly, Abraham et al. [65] showed that the G allele is associated with higher TNF- α production in lymphoblastoid cell lines. On the other hand,

the A/A genotype for TNF- β G252A polymorphism is known to be associated with higher TNF- α production [66]. Moreover, Messer et al. [67] reported that the A allele is related to increased TNF- β production. Thus, the association between TNF- β G252A polymorphism and TNF production is controversial. Cytokines are important mediators of inflammatory pathways, and the serum levels of cytokines such as TNF- α during attacks are higher than their levels upon outside attacks in patients with migraine [68]. Since the G252A polymorphism of TNF- β is a silent mutation, it can be stated that the TNF- β G252A gene polymorphism may have linkage disequilibrium with other functional mutations.

Genetic polymorphisms in the voltage-dependent P/Q-type Ca²⁺ channel alpha 1A subunit (CACNA1A) [69] and the alpha 2 subunit of the sodium/potassium pump (ATP1A2) caused migraine [70], and these familial hemiplegic migraines are rare. Almost all migraine types are caused by multifactorial factors, including genetic factors and environmental factors. In our study, by excluding the subjects with depression and other co-morbid psychiatric disorders, we could select purely many independent contributing factors, including personality traits and gene polymorphisms, to the pathogenesis of migraine using logistic regression analysis. However, because of small sample size, we did not have enough power for conscientiousness score, MAOA T941G and TNF- β G252A.

Although the sample size is the biggest limitation of the study, we could provide that the multiple factors, such as gene polymorphisms and personality traits, contribute to the pathogenesis of migraine. The contributions of polymorphisms, such as MAOA T941G, MTHFR C677T, and TNF- β G252A, seem to be more important than personality traits in the pathogenesis of migraine, a multifactorial disorder. Future studies are needed to collect more subjects and determine the pathogenesis of MA and MO, since the contributing factors differed between controls and MA or MO patients by univariate analysis.

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