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Lack of association between methylenetetrahydrofolate reductase gene variants & essential tremor in Han Chinese

Sir,

Essential tremor (ET) is the most common pathologic tremor and a common, age-dependent, neurological disorder in adults[1,2]. The approximated prevalence (all ages) is 0.4 per cent in New Guinea and Italy. It increases to 4.6 per cent in people ≥65 yr old and 21.7 per cent in those ≥95 yr old[2,3]. The epidemiological data are relatively limited in China, with the estimated prevalence (age ≥50 yr) of 0.3 per cent[4]. The clinical characteristics of ET are bilateral action (postural and kinetic) tremors which mostly affect upper limbs, less frequently the head and voice and rarely the legs[2,5]. The main pathological findings are unknown. Decreased gamma-aminobutyric acid (GABA) receptors and Purkinje cell loss have been observed in cerebellum, suggesting that ET is probably a cerebellar disorder. Lewy body pathology related with Parkinson's disease (PD) and Alzheimer's disease was observed in brain, supporting a neurodegenerative aetiology for ET[2,6].

Although genetic factors are considered to play a major role in ET development, a primary genetic base remains elusive[2,7]. At least eight gene loci and five pathogenic genes have been reported to be associated with ET. Variants in some genes have been described as conferring susceptibility to ET[2,8]. Three new loci have been also identified to be associated with ET in a genome-wide association study[9].

The human methylenetetrahydrofolate reductase (*MTHFR*) gene, a 12-exon gene, encodes a 656-amino acid protein. MTHFR is one of the three key enzymes involved in the homocysteine and folic acid metabolic pathways and catalyzes 5,10-methylenetetrahydrofolate conversion to 5-methyltetrahydrofolate[10], the major circulating form of folic acid and a methyl group donor in the remethylation process of homocysteine to methionine[11]. Genomic DNA methylation, an epigenetic characteristic of DNA regulating gene expression and genomic integrity, is thought to directly correlate to folate status and inversely to plasma homocysteine levels[12,13]. Elevated homocysteine level elicits neurotoxic effects, associated with neurodegenerative disorder[11]. In 2004, two *MTHFR* gene variants, rs1801131 (c.1286A>C, p.E429A, previously named A1298C) and rs1801133 (c.665C>T, p.A222V, previously named C677T), were found to confer susceptibility to ET in Turkey[12]. In our previous study, the *MTHFR* gene variant was found to be related to PD in Han Chinese[11], a disorder that overlaps with ET in clinical and pathological features[14]. Epigenetically controlled regulations of genes related to production of tremorogenic substances, and the homocysteine-induced neurotoxic effects, were suspected to be the mechanisms by which *MTHFR* gene variants confer susceptibility to ET[11,12,15]. This study was aimed to evaluate whether these two variants of *MTHFR* were associated with ET in Han Chinese.

A total of 200 ET patients (100 males/100 females, mean age 50.6±15.1 yr and mean age at onset 41.2±18.2 yr) as well as 430 ethnicity-matched unrelated Han Chinese normal controls (215 males/215 females, mean age 51.8±16.5 yr) were consecutively enrolled from the Third Xiangya Hospital of Central South University, Changsha, China between December 2007 and July 2015. Cases were enrolled from the Department of Neurology, and normal controls were healthy volunteers with no symptoms or family history of neurological disorders, examined by two independent neurologists. With data on clinical medical history and family history, clinical and auxiliary examinations, ET cases without symptoms or family history of other movement diseases were collected and recorded. The diagnosis of ET was established according to the common clinical criteria for inclusion[16]. Cases with bradykinesia, any other sign of

parkinsonism, isolated tremors, or atypical symptoms were excluded. The clinical characteristics of the patients are shown in Table I. Of the 200 patients, 142 (71%) were negative for coding variants in the *FUS* gene, a reported monogenic pathogenic gene of ET, and 94 (47%) were negative for coding mutations in the *LINGO1* gene, a susceptibility gene of ET[2,17,18]. This study was approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University and written informed consent was obtained from all participants.

Genomic DNA was extracted from peripheral lymphocytes[17]. Genotyping of the two *MTHFR* gene variants, rs1801131 and rs1801133, was performed by Bioyong Technologies (Beijing, China) utilizing the Sequenom MassARRAY Analyzer as previously described[2,11]. The genotyping assay was conducted by investigators blinded to the sample status. Duplicate samples, as well as positive and negative controls, were applied for quality control. Sanger sequencing was conducted on 10 per cent of the randomly selected samples to validate method reliability and accuracy[2,11].

Using the Power and Sample Size Calculations program (version 3.1.2, *http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize*), the power analysis was conducted to detect association with the disorder at a significance level of 0.05, based on the minor allele frequencies of 0.295 (rs1801131) and 0.304 (rs1801133), accessed from single nucleotide polymorphism database (*https://www.ncbi.nlm.nih.gov/snp/*). Hardy-Weinberg equilibrium test was carried out to assess the normal heterozygosity of the participants. A Pearson's Chi-square test was applied to evaluate differences in the frequencies of the genotypes and alleles between the ET and control groups. Two-sided $P<0.05$ was considered as statistically significant. Haplotype analysis and statistical analysis were carried out using SHEsis Online Version (*http://analysis.bio-x.cn/*) and Predictive Analytics Software Statistics 18.0 (SPSS Inc., Chicago, IL, USA), as previously described[2].

Table I. Clinical characteristics of the participants

Clinical characteristics	ET (n=200) n (%)	Control (n=430) n (%)
Upper limb tremor	200 (100.0)	0 (0.0)
Head tremor	16 (8.0)	0 (0.0)
Leg tremor	16 (8.0)	0 (0.0)
Jaw tremor	9 (4.5)	0 (0.0)
Voice tremor	12 (6.0)	0 (0.0)
Taking of tremor-inducing drugs (*e.g.* metoclopramide, chlorpromazine and perphenazine)	0 (0.0)	0 (0.0)
Excessive alcohol consumption	0 (0.0)	0 (0.0)
Bradykinesia	0 (0.0)	0 (0.0)
Rigidity	0 (0.0)	0 (0.0)
Kayser-Fleischer ring	0 (0.0)	0 (0.0)
Encephalatrophy (brain magnetic resonance imaging)	0 (0.0)	0 (0.0)
Thyroid malfunction (thyroid function test)	0 (0.0)	0 (0.0)

ET, essential tremor

The power was estimated to be over 0.8 (0.843 for rs1801131 and 0.848 for rs1801133) with an assumed odds ratio of 1.7. Genotypic distributions of patients and controls were within Hardy-Weinberg equilibrium. No significant differences were found in either genotypic or allelic distributions between cases and controls though the minor allele frequency of variant rs1801131 was marginally higher in cases than controls ($P = 0.062$). No haplotypes showed a possible ET association (Table II) though the C-C haplotype of rs1801131-rs1801133 showed a marginally significant association with ET ($P = 0.072$). With an odds ratio of 1.3, a low power (0.306 for rs1801131 or 0.309 for rs1801133) was obtained with the limited sample size.

In-depth genetic studies involving a larger sample size and different ethnic populations with a defined diagnostic gold standard may help understand potential associations between *MTHFR* gene variants and ET, as well as any underlying aetiopathogenesis, especially those overlapped with PD. In addition, omics technologies applied in different studies will greatly help explore and confirm the genetic risk or protective factors for ET[9,19].

In conclusion, the present study showed no evident association between these two *MTHFR* variants (rs1801131 and rs1801133) and ET in Han Chinese though a plausible role of rs1801131 on ET could not be excluded with marginal *P* values. Further studies need to be done for confirmation and implications.

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Table II. Genotype, allele and haplotype distributions of the methylenetetrahydrofolate reductase (*MTHFR*) gene variants (rs1801131 and rs1801133) in Han Chinese essential tremor patients and controls

dbSNP ID	Genotype/ allele/haplotype	Patients (%) (n=200)	Controls (%) (n=430)	χ^2	*P*	OR (95% CI)
rs1801131	AA	121 (60.5)	288 (67.0)			
	AC	66 (33.0)	125 (29.0)			
	CC	13 (6.5)	17 (4.0)	3.437	0.179	
	A	308 (77.0)	701 (81.5)			
	C	92 (23.0)	159 (18.5)	3.484	0.062	1.317 (0.986-1.759)
rs1801133	CC	85 (42.5)	154 (35.8)			
	CT	89 (44.5)	213 (49.5)			
	TT	26 (13.0)	63 (14.7)	2.594	0.273	
	C	259 (64.7)	521 (60.6)			
	T	141 (35.3)	339 (39.4)	2.012	0.156	0.837 (0.654-1.071)
rs1801131-rs1801133	A-C	42.2	42.3	0.000	0.997	1.000 (0.786-1.271)
	A-T	34.8	39.2	2.166	0.141	0.831 (0.649-1.064)
	C-C	22.6	18.3	3.241	0.072	1.306 (0.976-1.749)
	C-T	0.4	0.2	-	-	-

dbSNP, single nucleotide polymorphism database; OR, odds ratio; CI, confidence interval

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