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Association of *BDNF* Polymorphisms with the Risk of Epilepsy: a Multicenter Study

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Abstract Epilepsy is a common neurological disease characterized by recurrent unprovoked seizures. Evidence suggested that abnormal activity of brain-derived neurotrophic factor (BDNF) contributes to the pathogenesis of epilepsy. Some previous studies identified association between genetic variants of *BDNF* and risk of epilepsy. In this study, this association has been examined in the Hong Kong and Malaysian epilepsy cohorts. Genomic DNA of 6047 subjects (1640 patients with epilepsy and 4407 healthy individuals) was genotyped for rs6265, rs11030104, rs7103411, and rs7127507 polymorphisms by using Sequenom MassArray and Illumina HumanHap 610-Quad or 550-Duo BeadChip arrays techniques. Results showed significant association between rs6265 T, rs7103411 C, and rs7127507 T and cryptgenic epilepsy risk (p=0.00003, p=0.0002, and p=0.002, respectively) or between rs6265 and rs7103411 and symptomatic epilepsy risk in Malaysian Indians (TT vs. CC, p=0.004 and T vs. C, p=0.0002, respectively) as well as between rs6265 T and risk of cryptogenic epilepsy in Malaysian Chinese (p=0.005). The T_{rs6265}-$C_{rs7103411}$-$T_{rs7127507}$ was significantly associated with cryptogenic epilepsy in Malaysian Indians (p=0.00005). In conclusion, our results suggest that *BDNF* polymorphisms might contribute to the risk of epilepsy in Malaysian Indians and Chinese.

Keywords Epilepsy · Susceptibility · *BDNF* · Polymorphism

Introduction

Epilepsy is a common neurological disorder, with a prevalence of 1 % and is characterized by periodic and unpredictable occurrence of seizures [1]. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin super-family

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and is the most abundant neurotrophin in the brain. It plays an important role in the growth and differentiation of new neurons and synapses as well as in survival of existing neurons of the central and peripheral nervous system [2, 3]. Evidence suggested a potential contribution of BDNF and its receptor, tropomyosin-related kinase type B (TrkB), to the pathophysiology of epilepsy. In vivo and in vitro studies demonstrated that levels and activity of BDNF were increased during epileptogenesis [4–6]. In addition, decreased levels of TrkB in the hippocampus of adult mice impaired seizure; therefore, these markers were suggested as targets for therapeutic intervention [7].

Genetic studies identified associations of *BDNF* variants with some neurological or psychiatric disorders, including epilepsy, but the results were inconsistent [8–13]. The epilepsy populations included in the previous studies were very heterogeneous, consisting of febrile seizure, temporal lobe epilepsy, partial epilepsy, and childhood partial epilepsy. Out of seven studies, four were in Asians (Turkish, Japanese, and Taiwanese), two were in Caucasians (European Americans and Spanish), and one was Brazilian. No study of the association of *BDNF* variants with epilepsy has been performed on a Malaysian population, which is composed of three main ethnic groups (Malay, Chinese, and Indian). Therefore, we analyzed four polymorphisms of this gene (rs6265, rs11030104, rs7103411, and rs7127507) in the epilepsy cohort in Malaysia, as well as one in Hong Kong.

Materials and Methods

Study Population

A case control study was conducted through a collaboration of investigators from the University of Malaya Medical Centre (UMMC, Kuala Lumpur, Malaysia), Universiti Kebangsaan Malaysia Medical Centre (UKMMC, Kuala Lumpur, Malaysia), General Hospital Kuala Lumpur (GHKL, Kuala Lumpur, Malaysia), and the Chinese University of Hong Kong (CUHK, Hong Kong). The study protocol was reviewed and approved by the Medical Ethics Committees of the centers. A total of 1640 unrelated epilepsy patients were recruited from the epilepsy outpatient clinics of the centers involved, whereas 4407 control samples were obtained from healthy volunteer blood donors at the UMMC and the Hong Kong Red Cross. All patients from Malaysia and Hong Kong cohorts were thoroughly diagnosed through consensus by at least two experienced neurologists, according to the criteria for epilepsy, as based upon the International League Against Epilepsy (ILAE) [14].

Subjects were excluded from the study if they had any of the following: (a) unreliable record of seizure frequency, (b) significant psychiatric comorbidity, (c) history of pseudoseizures, (d) alcohol or drug abuse, or (e) presence of progressive or degenerative neurological or systemic disorders. Ethnicity of the cases and controls was determined by self-reporting. All patients gave written informed consent prior to the study, but only after they had been given a full explanation of the research outline.

Genotyping

Genomic DNA was extracted from either peripheral white blood cells or buccal swabs. *BDNF* polymorphisms (rs6265, rs11030104, rs7103411, and rs7127507) were genotyped in subjects from Malaysia by using the MALDI TOF mass spectrometry (MassARRAY®, Sequenom, San Diego, CA, USA) method at the Hong Kong University Genome Research Centre (Pokfulam, Hong Kong). As part of a previously reported genome-wide association study [15], Hong Kong samples were genotyped for rs6265 and rs11030104 by deCODE genetics (Reykjavik, Iceland) on Illumina HumanHap 610-Quad or 550-Duo BeadChip arrays, and rs7103411 genotypes were imputed.

Statistical Analyses

Power of the study was estimated through the Power and Sample Size Calculation online software (3.0, available at http://ps-power-and-sample-size-calculation.software.informer.com/). The statistical power of our study was more than 80 % (OR=0.74, α=0.05, p_0=0.41, p_1=0.49, n=1179, m=3922). Genotype distributions were tested for Hardy-Weinberg equilibrium (HWE) using a goodness-of-fit χ^2 test with one degree of freedom. A p value of more than 0.05 indicates agreement with HWE. Allele associations were determined by binomial logistic regression. Association analysis was also performed separately by ethnicity, seizure type, and epilepsy type in order to avoid false discoveries due to population differences. Adjusted binomial logistic regression analysis for covariates (ethnicity, gender, age at recruitment, age at onset of epilepsy, seizure type, and epilepsy type) was used to obtain odds ratios with 95 % confidence intervals. Statistical analyses in this study were performed using the Statistical Package for the Social Sciences (SPSS) version 16.0 (Chicago, IL, USA). A p value of less than 0.05 in two-sided tests was considered statistically significant. However, the Bonferroni correction of multiple comparisons was used for testing each epilepsy subgroup. Haplotype and linkage disequilibrium (LD) analyses for the SNPs were performed with SHEsis, and each SNP and haplotype was corrected for multiple testing by using 1000 permutations.

Results

Table 1 shows the demographic and clinical data of 6047 patients and controls from Malaysia and Hong Kong. Of

Table 1 Demographic and clinical data of epilepsy patients and healthy controls from Malaysia and Hong Kong

Characteristics	Malaysia									Hong Kong (*N*=3363)			Total (*N*=6047)		
	Chinese (*N*=1061)			Indian (*N*=622)			Malay (*N*=1001)								
	Epilepsy	Control	*p*	Epilepsy	Control	*p*	Epilepsy	Control	*p*	Epilepsy	Control	*p*	Epilepsy	Control	*p*
Age at study, mean (SD)	36 (17)	35 (13)	0.4	35 (16)	33 (13)	0.4	33 (14)	30 (12)	<0.01	40 (19)	–	–	36 (17)	33 (13)	<0.01
Onset age of epilepsy, mean (SD)	18 (17)	–	–	16 (13)	–	–	15 (14)	–	–	26 (22)	–	–	19 (18)	–	–
Gender, *N* (%)															
Female	183 (43)	258 (40)	–	136 (46)	132 (41)	–	209 (49)	241 (42)	–	223 (46)	1964 (68)	–	751 (46)	2595 (59)	–
Male	240 (57)	380 (60)	0.4	162 (54)	192 (59)	0.2	222 (51)	329 (58)	0.1	265 (54)	911 (32)	<0.01	889 (54)	1812 (41)	<0.01
Seizure type, *N* (%)															
Generalized	185 (47)	–	–	150 (53)	–	–	194 (49)	–	–	24 (5)	–	–	553 (35)	–	–
Partial	210 (53)	–	–	134 (47)	–	–	199 (51)	–	–	464 (95)	–	–	1007 (65)	–	–
Unspecified[a]	28	–	–	14	–	–	38	–	–	–	–	–	80	–	–
Epilepsy syndrome, *N* (%)															
Cryptogenic	151 (36)	–	–	91 (31)	–	–	160 (38)	–	–	–	–	–	402 (25)	–	–
Idiopathic	120 (29)	–	–	125 (42)	–	–	142 (33)	–	–	–	–	–	387 (24)	–	–
Symptomatic	149 (35)	–	–	79 (27)	–	–	124 (29)	–	–	488 (100)	–	–	840 (51)	–	–
Unspecified[a]	3		–	3	–	–	5	–	–	–	–	–	11	–	–

N number, *SD* standard deviation

[a] Excluded from analysis

1152 patients from Malaysia, 423, 298, and 431 were Chinese, Indians, and Malays, respectively, while 488 epilepsy patients were from the Han Chinese population in Hong Kong. Out of the 4407 controls, 2875 were from Hong Kong and 1532 from Malaysia. Amongst the demographic and clinical results, the mean age at study of epilepsy Malaysian patients was significantly more than healthy controls in the pooled subjects, mainly among Malays ($p<0.01$). Moreover, there was significant difference of gender distribution between epilepsy patients and healthy controls in the pooled subjects and in the Hong Kong cohort. In either patients or controls, none of the genotype distributions deviated from Hardy-Weinberg equilibrium. Unadjusted binomial regression was carried out for the genotypes and alleles by epilepsy syndrome or seizure type and ethnicity for each polymorphism: rs6265, rs11030104, rs7103411, and rs7127507 (Tables 1, 2, and 3 in supplementary). Furthermore, adjusted analysis was performed to identify the effect size of the covariates such as age, gender, and ethnicity on the results (Tables 1, 2, and 3 in supplementary). Finally, associations of each polymorphism with epilepsy were analyzed, using the Bonferroni correction for multiple comparisons.

Adjusted analysis demonstrated significant association for rs6265, rs7103411, and rs7127507 in Indians; the association was significant for either generalized or partial seizure except for rs7127507 with generalized seizure (Table 2). Significant association was also observed for rs7103411 in Chinese from Malaysia; the association held for either generalized or partial seizure (Table 2). After Bonferroni correction, associations remained significant in Indians with generalized or partial seizure for rs6265 (T vs. C, $p=0.004$ and $p=0.002$; TT vs. CC, $p=0.007$ and $p=0.002$, respectively) and rs7103411 (T vs. C, $p=0.001$ and $p=0.0003$; TT vs. CC, $p=0.004$ and $p=0.001$, respectively). Hence, T_{rs6265} and $C_{rs7103411}$ were more likely risk alleles in Indians for both types of seizures. Adjusted analysis data by epilepsy syndrome showed significant association in Indian with cryptgenic epilepsy for rs6265, rs7103411, and rs7127507 or with symptomatic epilepsy for rs6265 and rs7103411. Similarly, there was significant association in Chinese from Malaysia with cryptogenic epilepsy for rs6265 and rs7103411 (Table 3). After Bonferroni correction, associations remained significant in Indians with cryptgenic epilepsy for rs6265 (T vs. C, $p=0.00003$; TT vs. CC, $p=0.0004$; CT vs. CC, $p=0.001$ and CT+TT vs. CC, $p=0.0001$, respectively), rs7103411 (T vs. C, $p=0.0002$ and TT vs. CC, $p=0.001$, respectively), and rs7127507 (C vs. T, $p=0.002$ and CC vs. TT, $p=0.005$, respectively) or with symptomatic epilepsy for rs6265 (TT vs. CC, $p=0.004$) and rs7103411 (T vs. C, $p=0.0002$; TT vs. CC, $p=0.0002$ and CT+TT vs. CC, $p=0.001$, respectively). Significant association was also remained in Chinese from Malaysia with cryptogenic epilepsy for rs6265 (T vs. C, $p=0.005$ and TT vs. CC, $p=0.006$, respectively). Therefore, T_{rs6265} was a risk allele in both Indians and Chinese from Malaysia with cryptogenic epilepsy, whereas the $C_{ra7103411}$ and $T_{rs7127507}$ were risk variants in Indians from Malaysia with cryptogenic and symptomatic epilepsy, respectively.

Haplotype analysis by epilepsy syndrome or seizure type was performed for the rs6265, rs11030104, rs7103411, and rs7127507 polymorphism in each ethnicity, and results were corrected with 1000 permutations (Tables 2 and 3). Then, Bonferroni procedure were used for correction of multiple comparisons. Haplotype results of association analysis after 1000 permutations were significant in Indians with generalized or partial seizure for TCT ($p=0.001$ and $p=0.005$, respectively) and with partial seizure for CTC ($p=0.02$). After Bonferroni correction for covariates, all associations were lost. Therefore, combination of rs6265, rs7103411, and rs7127507 loci is not a risk factor for both types of seizures. Results of haplotype association study, after 1000 permutations, were significant in Indians with cryptogenic epilepsy for CTC and TCT ($p=0.002$ and $p=0.00005$, respectively). After Bonferroni correction for multiple comparisons of covariates, association remained significant for TCT haplotype. To sum up, T_{rs6265} and $C_{rs7103411}$ and T_{rs6265}-$C_{rs7103411}$-$T_{rs7127507}$ might be risk factors for cryptogenic and/or symptomatic epilepsy in Indians and T_{rs6265} risk allele for cryptogenic epilepsy in Chinese from Malaysia.

Discussion

BDNF is a crucial factor for growth, differentiation, and maintenance of neurons and synaptogenesis in the brain [16, 17]. The *BDNF* gene has a complex structure with multiple regulatory elements (Fig. 1). This gene is composed of 11 exons and 9 alternative, tissue-specific promoters [18]. The rs6265 polymorphism is located in the 5′ pro-region, which encodes a prodomain form (pro-BDNF) which is cleaved to generate mature BDNF [20]. This cleavage is a fundamental step in regulation of BDNF function in the brain [21, 22]. Substituation of C to T in rs6265 changes valine to methionine in the 66th residue (V66M) of the BDNF protein. This locus contributes to pathogenesis of various diseases [23–27]; however, evidence for an association with epilepsy is conflicting. In the present case-control study, the association of epilepsy with rs6265 and three intronic SNPs, rs11030104, rs7103411, and rs7127507, in different types of seizure and epilepsy syndrome was examined in cohorts from Malaysia and Hong Kong. Our results showed that T_{rs6265} and $C_{rs7103411}$ might increase risk of cryptogenic or symptomatic epilepsy in Indians or of cryptogenic epilepsy in Chinese from Malaysia, while $T_{rs7127507}$ might increase risk of cryptogenic epilepsy in Indians. BDNF exhibits a potential role in hippocampal function. This protein is widely expressed in the hippocampus, where seizures originate in many types of epilepsy,

Table 2 Significant association of *BDNF* polymorphisms with seizure type in Malaysian subjects, adjusted for covariates

SNP	Allele/genotype	Generalized									Partial					
		Chinese			Indian			Malay			Chinese			Indian		
		OR	95 % CI	*p*	OR	95 % CI	*p*	OR	95 % CI	*p*	OR	95 % CI	*p*	OR	95 % CI	*p*
rs6265	T vs C	–	–	–	0.64	0.48–0.87	0.004[a]	–	–	–	–	–	–	0.61	0.45–0.84	0.002[a]
	TT vs CC	–	–	–	0.35	0.16–0.75	0.007[a]	–	–	–	–	–	–	0.29	0.14–0.63	0.002[a]
	CT+TT vs CC	–	–	–	0.62	0.42–0.90	0.01	–	–	–	–	–	–	0.61	0.41–0.91	0.02
rs7103411	T vs C	1.32	1.06–1.65	0.02	1.60	1.21–2.14	0.001[a]	–	–	–	1.29	1.04–1.60	0.02	1.74	1.29–2.34	0.0003[a]
	TT vs CC	1.72	1.11–2.65	0.02	2.50	1.34–4.68	0.004[a]	–	–	–	1.64	1.07–2.53	0.02	2.97	1.54–5.71	0.001[a]
	CT vs CC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	CT+TT vs CC	1.56	1.09–2.24	0.02	1.96	1.09–3.51	0.03	–	–	–	–	–	–	2.20	1.20–4.05	0.01
rs7127507	C vs T	–	–	–	–	–	–	–	–	–	–	–	–	1.44	1.06–1.94	0.02
	CC vs TT	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	TC vs TT	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	TC+CC vs TT	–	–	–	–	–	–	–	–	–	–	–	–	1.61	1.08–2.40	0.02
rs6265-rs7103411-rs7127507[b]																
	CTC	–	–	–				–	–	–	–	–	–	0.7	0.5–0.9	0.02
	TCT	–	–	–	1.7	1.2–2.3	0.001	–	–	–	–	–	–	1.6	1.2–2.3	0.005

OR odds ratio, *95 % CI* 95 % confidence interval

[a] After Bonferroni correction for multiple comparisons, results remained significant

[b] Haplotypes with frequency less than 3 % were excluded from analysis

Table 3 Significant association of *BDNF* polymorphisms with epilepsy syndrome in Malaysian subjects, adjusted for covariates

SNP	Allele/genotype	Cryptogenic epilepsy						Symptomatic epilepsy		
		Chinese			Indian			Indian		
		OR	95 % CI	*p*	OR	95 % CI	*p*	OR	95 % CI	*p*
rs6265	T vs C	0.70	0.54–0.90	0.005[a]	0.48	0.34–0.68	0.00003[a]	0.64	0.43–0.93	0.02
	TT vs CC	0.50	0.30–0.82	0.006[a]	0.21	0.09–0.50	0.0004[a]	0.27	0.11–0.66	0.004[a]
	CT vs CC	–	–	–	0.44	0.26–0.72	0.001[a]	–	–	–
	CT+TT vs CC	–	–	–	0.39	0.24–0.64	0.0001[a]	–	–	–
rs7103411	T vs C	1.33	1.04–1.72	0.03	1.94	1.37–2.76	0.0002[a]	1.99	1.39–2.87	0.0002[a]
	TT vs CC	1.74	1.06–2.85	0.03	3.61	1.70–7.66	0.001[a]	4.09	1.93–8.68	0.0002[a]
	CT vs CC	–	–	–	–	–	–	2.36	1.14–4.91	0.02
	CT+TT vs CC	1.55	1.04–2.33	0.03	2.42	1.23–4.76	0.01	3.10	1.56–6.15	0.001[a]
rs7127507	C vs T	–	–	–	1.82	1.25–2.63	0.002[a]	–	–	–
	CC vs TT	–	–	–	5.86	1.71–20.05	0.005[a]	–	–	–
	TC+CC vs TT	–	–	–	1.89	1.18–3.02	0.01	–	–	–
rs6265-rs7103411-rs7127507[b]										
	CTC	–	–	–	0.5	0.4–0.8	0.002			
	TCT	–	–	–	2.1	1.5–3.1	0.00005[a]	1.7	1.1–2.6	0.01

E epilepsy case, *C* healthy control, *OR* odds ratio, *95 % CI* 95 % confidence interval

[a] After Bonferroni correction for multiple comparisons, results remained significant

[b] Haplotypes with frequency less than 3 % were excluded from analysis

such as temporal lobe epilepsy (TLE) [28]. At the initial stage of epileptogenesis in TLE, seizures can increase production of BDNF to foster neurogenesis, decrease neuronal cell death, and exert anti-inflammatory effects leading to protection of neurons against seizure-induced damage [29, 30].

Mouse model studies of the V66M polymorphism showed that despite normal expression of BDNF protein in the brains of M carriers (either heterozygotes or homozygotes), the depolarization-induced secretion of this protein was decreased at least in part due to a failure of BDNF protein containing M66 to undergo normal intracellular trafficking and localize to secretory granules or synapses [31, 32]. In our cohorts, the M66 allele was more common in the epilepsy patients than in controls, and this effect was ethnicity dependent. Perhaps, positive selection in some ethnicities has altered the frequency of V66 and its linkage disequilibrium (LD) with other polymorphisms that may affect *BDNF* expression [33]. Figure 2 shows differences of LD among polymorphisms genotyped in

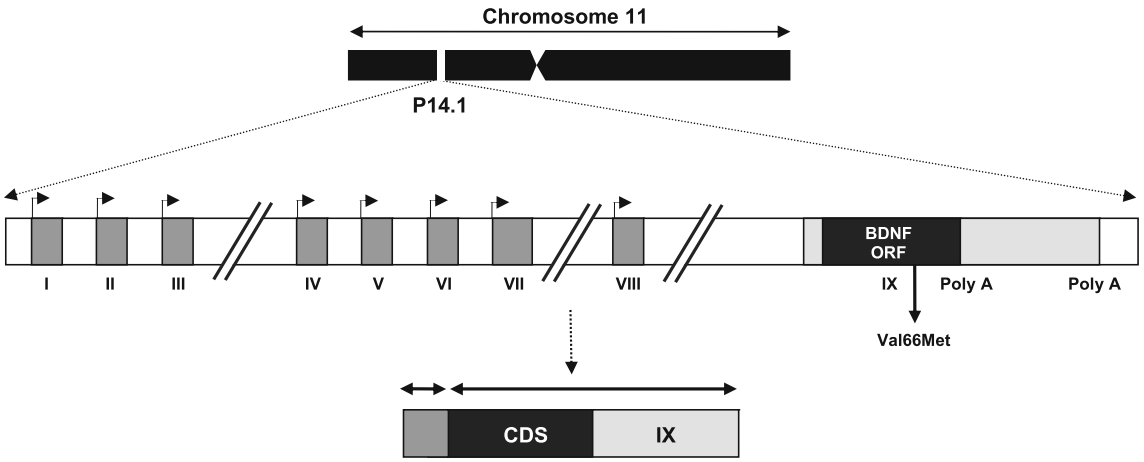

Fig. 1 *BDNF* gene and the structure of encoded protein. *BDNF* consists of at least eight distinct exons (I–VIII) (*dark boxes*) and promoters (*arrows*) that initiate the transcription of multiple distinct *BDNF* mRNAs, each of which contains an alternative 5′ exon spliced to a common coding exon (exon IX) that employs either of two polyadenylation sites (poly A). The rs6265 polymorphism is a nonsynonymous G to A variant located at position 196 of exon IX, which results in valine (val) to methionine (met) substitution at codon 66 (*val66met*), changing the 5′ proregion of the human BDNF protein [18, 19]

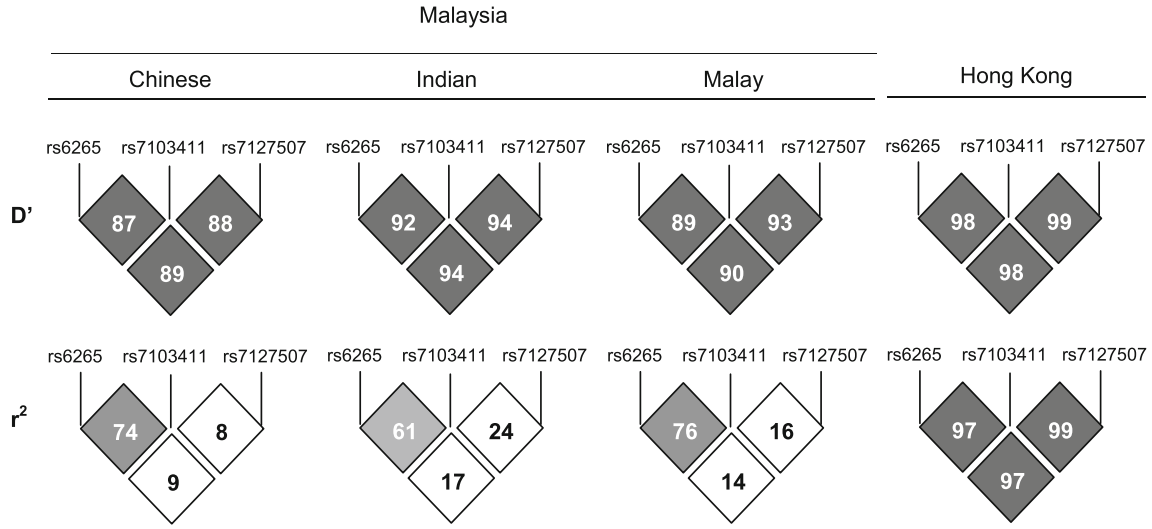

Fig. 2 Patterns of linkage disequilibrium (D′ and r^2) among the epilepsy and control subjects from Malaysia (Chinese, Indians, and Malays) and from Hong Kong

this study. Other polymorphisms that may affect gene function or expression might likewise vary in LD among ethnic groups. Figure 3 shows wild-type allele frequencies of *BDNF* polymorphisms amongst different ethnogeographic groups. For most polymorphisms, there is a high similarity among Chinese from China, Hong Kong, and Malaysia as well as between Europeans and Malaysian Indians. As a consequence of environmental effects, such as differences in climate, diet, infectious diseases, and cultures, genetic variation and disease prevalence vary considerably among populations [34, 35]. In several studies, rs6265 was not associated with seizures in European Brazilians and Americans with TLE [10, 11], fragile X with epilepsy in Spanish [9], febrile seizure in Taiwanese [12], and benign epilepsy with centrotemporal spikes in Greeks [36]. There was neither any report from India for the role of this polymorphism in epilepsy nor any study for rs11030104, rs7103411, and rs7127507 and their haplotypes in epilepsy.

V66M is associated with risk for some other neurological and mental disorders, including memory impairment [20], anxiety disorder [31], suicidal behavior [37], obesity [38], obsessive-compulsive disorder (OCD) [39], anorexia bulimia nervosa (bulimia nervosa) and late age at onset of weight loss [40], bipolar disorder [41–43], schizophrenia with or without alcohol dependence [44–46], and methamphetamine dependence [47]. In mice, BDNF seems to inhibit depression but increase anxiety [48]. Mice homozygous for Met66 showed an increased anxiety behavior against stress, which could not be controlled by antidepressant treatment, and *BDNF* overexpression decreased depressive behavior and eradicated stress-induced hippocampal atrophy [31].

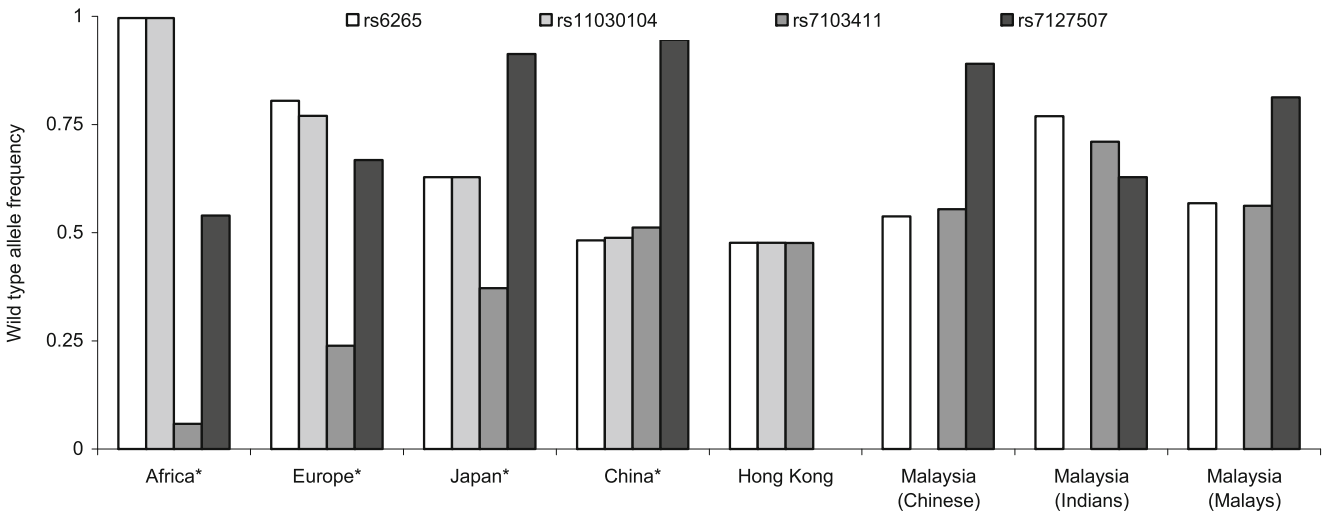

Fig. 3 Pattern of wild-type allele frequencies amongst African, European, Japanese, and Beijing Chinese populations (*data from hapmap.org) as well as in the control subjects in this study recruited from Hong Kong and Malaysia (Chinese, Indians, and Malays). Genotypes of rs7127507 and rs11030104 were not determined in the subjects from Hong Kong and Malaysia, respectively

In this study, we acknowledge two main limitations. Firstly, small sample sizes of Malaysian Chinese, Indians, and Malays resulted from subanalysis by ethnicity, which was necessitated by the second limitation, the heterogeneous study population in Malaysia. However, this restriction resulted in multiple cohorts, allowing confirmation of some of the associations. The final limitation was the absence of data on febrile seizures, which prevented comparison of the present and previous studies. In conclusion, *BDNF* polymorphisms might affect susceptibility to epilepsy in Malaysian Indians or Chinese. Confirmation of these associations in a larger sample size is suggested.

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Compliance with Ethical Standards

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Conflict of Interest The authors declare that they have no conflict of interest.

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