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ORIGINAL INVESTIGATION

Case–control association study of polymorphisms in the voltage-gated sodium channel genes *SCN1A*, *SCN2A*, *SCN3A*, *SCN1B*, and *SCN2B* and epilepsy

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Abstract High-frequency action potentials are mediated by voltage-gated sodium channels, composed of one large α subunit and two small β subunits, encoded mainly by *SCN1A*, *SCN2A*, *SCN3A*, *SCN1B*, and *SCN2B* genes in the brain. These play a key role in epilepsy, with the most commonly mutated gene in epilepsy being *SCN1A*. We examined whether polymorphisms in the above genes affect epilepsy risk in 1,529 epilepsy patients and 1,935 controls from four ethnicities or locations: Malay, Indian, and Chinese, all from Malaysia, and Chinese from Hong Kong. Of patients, 19 % were idiopathic, 42 % symptomatic, and 40 % cryptogenic. We genotyped 43 polymorphisms: 27 in Hong Kong, 28 in Malaysia, and 12 in both locations. The strongest association with epilepsy was rs3812718, or *SCN1A* IVS5N+5G>A: odds ratio (OR) = 0.85 for allele G ($p = 0.0009$) and 0.73 for genotype GG versus AA ($p = 0.003$). The OR was between 0.76 and 0.87 for all ethnicities. Meta-analysis confirmed the association (OR = 0.81 and $p = 0.002$ for G, and OR = 0.67 and $p = 0.007$ for GG versus AA), which appeared particularly strong for Indians and for febrile seizures. Allele G affects splicing and speeds recovery from inactivation. Since *SCN1A* is preferentially expressed in inhibitory neurons, G may decrease epilepsy risk. *SCN1A* rs10188577 displayed OR = 1.20 for allele C ($p = 0.003$); *SCN2A* rs12467383 had OR = 1.16 for allele A ($p = 0.01$), and displayed

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linkage disequilibrium with rs2082366 ($r^2 = 0.67$), whose genotypes tended toward association with *SCN2A* brain expression ($p = 0.10$). *SCN1A* rs2298771 was associated in Indians (OR = 0.56, $p = 0.005$) and *SCN2B* rs602594 with idiopathic epilepsy (OR = 0.62, $p = 0.002$). Therefore, sodium channel polymorphisms are associated with epilepsy.

Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent seizures, which involve high-frequency neuronal action potentials requiring voltage-gated sodium channels. These channels consist of one large subunit (α) and two small subunits (β) (Ferraro and Buono 2005). The genes *SCN1A*, *2A*, *3A*, and *8A* encode the α subunit isoforms (Nav1.1, Nav1.2, Nav1.3, Nav1.6, respectively) that are most abundant in the brain (Catterall et al. 2005), with the first three genes exhibiting similarities to each other in sequence, properties, and physical location, being clustered in one location on chromosome 2 (Yu and Catterall 2003). Mutations in *SCN1A*, *2A*, and *3A* can cause epilepsy (Meisler et al. 2010). Of β subunit genes, only *SCN1B* has so far been found to possess mutations contributing to epilepsy; however, *SCN2B* knockout mice are more susceptible to induction of seizures (Brackenbury and Isom 2011). Because mutation or deletion of *SCN1A*, *2A*, *3A*, *1B*, or *2B* can lead to seizures, we hypothesized that polymorphisms in these genes may alter epilepsy risk. To examine this hypothesis, we genotyped 43 tag SNPs in epilepsy patients and healthy participants from Hong Kong and Malaysia. In addition, we performed meta-analysis of previously reported studies for association of the *SCN1A* rs3812718 polymorphism with epilepsy.

Materials and methods

Case control study

Participants

The relevant institutional research ethics committees approved this multicenter study. All patients or their parents/legal guardians gave written informed consent to participate in genetic research. Epilepsy patients and healthy participants were recruited from Hong Kong and from Kuala Lumpur, Malaysia. Unrelated epilepsy patients of Han Chinese ethnicity were recruited by neurologists from neurology clinics of five regional hospitals in Hong Kong, and patients of Chinese, Indian, and Malay ethnicity were recruited from four epilepsy clinics of two regional hospitals in Malaysia. The diagnosis of epilepsy was largely based on clinical history and semiology and supported by imaging and EEGs, which were ordered as clinically indicated. All patients had neuroimaging; MRIs were obtained on 1.5T or 3T scanners. MRI parameters were as follows: 3 mm cut; T1, T2, Flair, inversion recovery T1, coronal and the cut is perpendicular to the temporal lobe; T2W, T2 flair coronal, both of 3 mm slice thickness; T2W axial; IR (inversion recovery); coronal; T1 MPRAGE, reconstructed to axial, coronal and sag; GRE (1.5T) or SWI (3T); Kiv T1 post contrast (if any mass is detected), MRA TOF (if infarcts seen). Epilepsy was classified by International League Against Epilepsy criteria (Berg et al. 2010). A standardized form was used to collect information including demographic details, seizure types and frequency, epilepsy type, and relevant family history.

Hong Kong controls were obtained from Red Cross blood donors and clinical genetic studies. Malaysia controls were obtained from blood donors in the Transfusion Medicine Department and the Department of Trauma of University Malaya Medical Centre.

Ethnicity was assessed because genetic variants associated with diseases may vary in frequency among ethnic groups. Subjects self-identified their ethnicity from options defined by the investigator: Chinese or non-Chinese in Hong Kong; and Malay, Chinese, or Indian in Malaysia.

Genotyping

Genomic DNA was extracted from either whole blood or buccal swabs by standard methods. SNPs were chosen independently in Malaysia and Hong Kong. A total of 43 tag single-nucleotide polymorphisms (tSNPs) from *SCN1A* ($N = 15$), *SCN2A* ($N = 16$), *SCN3A* ($N = 8$), *SCN1B* ($N = 2$), and *SCN2B* ($N = 2$) were genotyped: 27 in Hong Kong subjects, 28 in Malaysia subjects, and 12 in all subjects. The strategy of tSNPs selection in Hong Kong and Malaysia was previously reported (Kwan et al. 2008; Baum et al. 2009; Haerian et al. 2013). Subjects from Malaysia were genotyped using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MassArray, Sequenom, San Diego, CA, USA) at the Sequenom facility in Brisbane, Australia. For quality control, about 20 % of samples were genotyped again by MassArray at The University of Hong Kong Centre for Genomic Sciences.

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The Hong Kong samples were all genotyped by MassArray at The University of Hong Kong Centre for Genomic Sciences.

Statistical analysis

Subject data other than genotypes were analyzed by SPSS software package (ver. 15.0; SPSS, Chicago, IL, USA). All values are presented either as mean ± SD for continuous data or as frequency for categorical data. Ages at study entry were compared between patients and controls by *t* test or, when Levene's test indicated that variances were significantly different, Mann–Whitney test (Table 1). Sex distributions were compared between patients and controls by Fisher's exact test.

The study outcome was risk of epilepsy. Power was estimated using Epi6 software (Epi6, Centers for Disease Control, Atlanta, GA, USA). For each SNP and each ethnic group and location, as well as by summing genotype counts for all Chinese and for all subjects, odds ratios (ORs) and *p* values were calculated for association of alleles with epilepsy. When pooling data from all ethno-geographic groups, the contribution of each group was monitored using adjusted binomial logistic regression, with group as a covariate. A goodness-of-fit χ^2 test with one degree of freedom was applied to test Hardy–Weinberg equilibrium (HWE). Linkage disequilibrium (LD) was calculated using Haploview software (http://www.broad.mit.edu/mpg/haploview).

To explore association of SNPs with gene expression, we used Matrix eQTL (see http://www.bios.unc.edu/research/genomic_software/Matrix_eQTL/manual.html) (Xia et al. 2012). We downloaded genotype and expression data from http://www.bios.unc.edu/research/genomic_software/seeQTL/data_source and used matrix_mdata_eqtl_Myers.txt as the SNP file and snp2loci_Myers_Affy500k_hg19.txt as the SNP location file from a genotype-expression study of human cortical brain samples from neuropathologically normal ethnic Europeans (Myers et al. 2007). Expression file data were SCN gene data copied from matrix_edata_eqtl_Myers.txt, and gene location data were from geneid2loci_hapmap_hg19.txt, with the last column (strand) deleted. The contents of the file Matrix_eQTL_cis.r (see Online Resource 1) were copied and pasted into R for execution, generating the file eQTL_results_R_cis.txt. In HapMap Data Release 24/phaseII, each gene was zoomed out to include enough region 5′ and 3′ of the gene to include SNPs in LD of at least 0.5 (r^2) with SNPs of interest. In Haploview 4.2, we performed a HapMap Download of HapMap ver 2 rel 24 analysis panel CHB+JPT of each gene and neighboring region (see above). In eQTL_results_R_cis.txt, we examined each SNP starting from the lowest *p* value. Expression and genotype data were copied into SPSS, and expression was compared

Table 1 Characteristics of epilepsy patients and controls

	Malaysia: Indian	Malaysia: Malay	Malaysia: Chinese	Hong Kong: Chinese	All
N					
Patients	162	251	288	828	1,529
Controls	244	359	484	848	1,935
Sex					
Patients: male *N* (%)	87 (53.7)	134 (53.4)	160 (55.6)	418 (50.5)	799 (52.3)
Controls: male *N* (%)	135 (55.3)	201 (56.0)	299 (61.8)	428 (50.5)	1,063 (54.9)
p	0.76	0.56	0.10	1.00	0.12
Onset age					
Patients: mean ± SD, Y (*N*)	16 ± 14 (139)	12 ± 13 (213)	17 ± 17 (250)	22 ± 18 (801)	19 ± 17 (1,403)
Recruitment age					
Patients: mean ± SD, Y	31 ± 16	29 ± 15	33 ± 18	38 ± 15	35 ± 16
Controls: mean ± SD, Y	29 ± 15	27 ± 14	32 ± 15	37 ± 17	34 ± 16
p	0.40	0.08	0.59	0.63	0.15
Type of epilepsy					
Idiopathic: *N* (%)	60 (38)	61 (25)	57 (20)	101 (12)	279 (19)
Symptomatic: *N* (%)	45 (28)	80 (33)	104 (37)	393 (48)	622 (42)
Cryptogenic: *N* (%)	55 (34)	102 (42)	122 (43)	318 (39)	597 (40)

p values for differences between patients and controls within each source were calculated using Chi-square for sex and *t* test for recruitment age. The number of patients for which onset age was available is shown for each source

SD Standard deviation, *Y* year

among genotypes by ANOVA for the set of 193 brain samples.

Meta-analysis

Meta-analysis was performed based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement (Swartz 2011). Articles that determined the distribution of the *SCN1A* rs3812718 genotype in unrelated epilepsy patients compared with healthy subjects were sought using the terms "epilepsy," "*SCN1A*," and "polymorphism" in PubMed without language limitation. The last search was conducted on May 9, 2013. Inclusion criteria for each study were as follows: (a) the study compared epilepsy patients and controls, either without epilepsy or from the general population; (b) allele frequency data were available for both cases and controls; (c) the study was of meaningful size (at least 50 patients); (d) data did not duplicate those of previous publications. The per-allele OR of the rare allele (A) as well as the corresponding 95 % confidence intervals (CI) and p values were calculated to compare epilepsy patients and controls. Co-dominant (G/G versus A/A and G/A versus A/A), recessive (G/G+G/A versus A/A), and dominant (G/G versus G/A+A/A) models were also tested. Subsidiary meta-analyses were performed to evaluate the above models on epilepsy with or without FS or all epilepsies in each ethnic group. To measure variation across studies, the I^2 test was used for assessing the proportion of statistical heterogeneity, and the Q-statistic test with $p < 0.1$ was used to define a significant degree of heterogeneity. The fixed-effects summary measures were calculated as inverse-variance-weighted averages of the log OR if there was no heterogeneity ($p > 0.1$) and random-effects where substantial heterogeneity ($p \leq 0.1$) existed. Sensitivity analyses were performed to assess the stability of the results of the meta-analysis. All probability values are two sided, and values of $p < 0.05$ were considered statistically significant. Statistical analyses were performed using validated meta-analysis made easy (MIX) version 1.7 (Bax et al. 2006).

Results

Case–control study

The study included 1,529 epilepsy patients and 1,935 control subjects from four ethnic groups or locations: Malay, Indian, and Chinese, all from Malaysia, and Chinese from Hong Kong (Table 1). Power was estimated using the minimum number of patients (701) and controls (848) genotyped for each SNP: 81 % for a SNP with minor allele frequency of 30 % in controls and odds ratio of 1.25 for association with epilepsy. Patients and controls were age- and sex-matched within each of the four sources, as well as overall. The proportion of patients with each type of epilepsy varied among sources, and to ensure that this did not affect the conclusions, genetic association analysis was conducted by both source and type.

SNPs were chosen and genotyped independently in Malaysia and Hong Kong, and data were then analyzed together. All the SNPs and their LD patterns are shown in Online Resource 2. Hong Kong results have been reported in abstract form (Baum et al. 2009). Genotyping was performed for 43 tSNPs of *SCN1A*, *SCN2A*, *SCN3A*, *SCN1B*, and *SCN2B*: 28 in Malaysia and 27 in Hong Kong, including 12 in both locations. For these 12 SNPs, allele frequencies in control subjects did not differ ($p > 0.01$) between Chinese in Malaysia and Hong Kong. Hardy–Weinberg equilibrium testing showed $p > 0.01$ for all SNPs in all groups except for rs4145346 in *SCN3A* in all controls (this SNP displayed a very different G allele frequency in Indians [21 %] than in other groups [52–54 %]).

In all subjects, three SNPs displayed $p < 0.01$ for association with epilepsy in all subjects combined: two in *SCN1A* and one in *SCN2A* (Table 2). The strongest association was rs3812718, or *SCN1A* IVS5N + 5G > A, with an odds ratio (OR) of 0.85 for the G allele ($p = 0.0009$) and 0.73 for the GG versus AA genotype ($p = 0.003$). The allele association would remain significant after Bonferroni correction for the 43 SNPs tested: $p < 0.05/43 = 0.0012$. ORs for the association of this SNP with epilepsy were within the range of 0.76–0.87 across ethnic groups (Table 2). Stratifying by epilepsy syndrome (Table 3) suggested a

Table 2 Association of selected polymorphisms with epilepsy in all subjects

Gene	SNP	Odds ratio (p value)				
		Malaysia: Indian	Malaysia: Malay	Malaysia: Chinese	Hong Kong: Chinese	All
SCN1A	rs10188577	1.14 (0.42)	1.22 (0.14)	1.21 (0.16)	1.26 (0.01)	1.20 (0.003)
SCN1A	rs3812718	0.76 (0.07)	0.87 (0.23)	0.87 (0.20)	0.84 (0.02)	0.85 (<0.001)
SCN2A	rs12467383	0.96 (0.86)	1.11 (0.52)	1.10 (0.50)	1.21 (0.008)	1.16 (0.010)

SNPs are shown if they are associated with epilepsy at $p < 0.01$ for all subjects. Odds ratio is for association of minor allele with epilepsy

Table 3 Association of selected polymorphisms with types of epilepsy in all subjects or in any ethnicity or location

Gene	SNP	Odds ratio (p value)				
		Malaysia: Indian	Malaysia: Malay	Malaysia: Chinese	Hong Kong: Chinese	All
Symptomatic						
SCN1A	rs10188577	1.48 (0.11)	1.31 (0.19)	1.01 (0.97)	1.04 (0.68)	1.04 (0.68)
SCN1A	rs3812718	0.66 (0.09)	0.87 (0.45)	0.90 (0.50)	0.91 (0.26)	0.89 (0.08)
SCN1A	rs2298771	0.52 (0.04)	0.87 (0.60)	0.97 (0.90)	0.84 (0.13)	0.73 (<0.001)
SCN2A	rs12467383	1.03 (0.92)	0.98 (0.92)	0.95 (0.77)	1.27 (0.008)	1.18 (0.03)
SCN2B	rs602594	1.48 (0.48)	0.33 (0.007)	0.73 (0.22)	1.35 (0.006)	1.14 (0.17)
Cryptogenic						
SCN1A	rs10188577	0.93 (0.77)	1.34 (0.12)	1.33 (0.10)	1.38 (0.006)	1.27 (0.004)
SCN1A	rs3812718	0.89 (0.61)	0.79 (0.15)	0.85 (0.26)	0.77 (0.008)	0.81 (0.002)
SCN1A	rs2298771	0.66 (0.14)	1.21 (0.41)	0.85 (0.54)	0.83 (0.23)	0.90 (0.32)
SCN2A	rs12467383	0.85 (0.87)	1.04 (0.85)	1.06 (0.74)	1.21 (0.05)	1.15 (0.07)
Idiopathic						
SCN1A	rs10188577	1.12 (0.64)	1.04 (0.86)	1.11 (0.68)	0.94 (0.78)	1.10 (0.40)
SCN1A	rs3812718	0.70 (0.11)	0.94 (0.76)	0.93 (0.71)	0.87 (0.35)	0.86 (0.10)
SCN1A	rs2298771	0.49 (0.01)	0.75 (0.34)	1.17 (0.64)	0.64 (0.11)	0.85 (0.28)
SCN2A	rs12467383	0.89 (0.68)	1.44 (0.11)	1.43 (0.11)	1.06 (0.72)	1.13 (0.24)
SCN2B	rs602594	0.70 (0.59)	0.44 (0.05)	0.72 (0.31)	0.86 (0.46)	0.62 (0.002)

SNPs are shown if they are associated with symptomatic, cryptogenic, or idiopathic epilepsy at $p < 0.01$ for all subjects or in any ethnicity or location, or if they appeared in Table 2. In addition, rs2298771 is shown

somewhat stronger association of the G allele with cryptogenic (OR = 0.81, $p = 0.002$) than either symptomatic (OR = 0.89, $p = 0.08$) or idiopathic (OR = 0.86, $p = 0.10$) epilepsy. Stratifying by sex revealed that the association was stronger in males (OR = 0.80, $p = 0.001$) than in females (OR = 0.90, $p = 0.15$), but trying to remove the possibility of ethnic heterogeneity by examining only Chinese subjects (in both Hong Kong and Malaysia combined) eliminated the sexual dichotomy.

Two other SNPs exhibited p values <0.01: rs10188577, in *SCN1A*, with OR = 1.20 for the C allele and $p = 0.003$; and rs12467383, in *SCN2A*, with OR = 1.16 for the A allele and $p = 0.0097$ (Table 2). The associations remained when re-analyzed in only Chinese (in Hong Kong and Malaysia combined): rs10188577 had OR = 1.23 and $p = 0.005$; and rs12467383 had OR = 1.19 and $p = 0.007$. For rs10188577 in Chinese, associations with epilepsy were similar for males (OR = 1.21, $p = 0.07$) and females (OR = 1.26, $p = 0.03$) but stronger for cryptogenic (OR = 1.36, $p = 0.002$) than either symptomatic (OR = 1.14, $p = 0.17$) or idiopathic (OR = 1.01, $p = 0.97$) epilepsy. For rs12467383 in Chinese, associations with epilepsy were weaker for males (OR = 1.11, $p = 0.25$) than females (OR = 1.29, $p = 0.007$) and similar for all epilepsy types: cryptogenic (OR = 1.17, $p = 0.06$), symptomatic (OR = 1.20, $p = 0.03$), and idiopathic (OR = 1.20, $p = 0.16$).

The *SCN1A* SNP rs2298771 was associated with a particular ethnic group. In all Indians, OR = 0.56 and $p = 0.005$, while in other groups, OR ranged from 0.84 ($p = 0.13$) to 0.98 ($p = 0.91$). Across all types of epilepsy, the association in Indians tended to be consistently stronger than in other groups (Table 3). This SNP causes the protein alteration Ala1067Thr. Polyphen-2 gives this alteration a score of 0.0, predicting no effect on protein function (Adzhubei et al. 2010). Several SNPs displayed associations with particular types of epilepsy. The *SCN1A* SNP rs2298771 was associated with symptomatic epilepsy (OR = 0.73, $p = 0.0006$) (Table 3) though the association was weaker for cryptogenic (OR = 0.90, $p = 0.32$) or idiopathic (OR = 0.85, $p = 0.28$), or for all epilepsy (OR = 0.86, $p = 0.06$). The *SCN2B* SNP rs602594 was associated with idiopathic epilepsy (OR = 0.62, $p = 0.002$) (Table 3), but the association was inconsistent for cryptogenic (OR = 0.91, $p = 0.35$) or symptomatic (OR = 1.14, $p = 0.17$), or for all epilepsy (OR = 0.96, $p = 0.58$). The association of rs602594 with symptomatic epilepsy was highly heterogeneous by ethnic group or location, varying from OR = 0.33 ($p = 0.007$) in Malays to OR = 1.35 ($p = 0.006$) in Hong Kong Chinese and OR = 1.48 ($p = 0.48$) in Indians.

To examine the effect of age, patients were stratified by epilepsy onset age younger or older than the median of 16.2 years in Hong Kong patients and were compared

Table 4 Meta-analysis of the association of *SCN1A* rs3812718 with epilepsy patients vs. controls

Meta-analysis was performed using a random effects model. Data are from Online Resource 4

	G vs. A		GG vs. AA	
	OR (95 % CI)	*p*	OR (95 % CI)	*p*
Epilepsy with febrile seizures	0.71 (0.60–0.85)	0.0001	0.51 (0.35–0.73)	0.0003
Epilepsy without febrile seizures	0.90 (0.82–1.00)	0.04	0.84 (0.69–1.02)	0.08
All epilepsy (with or without febrile seizures)	0.81 (0.71–0.93)	0.002	0.67 (0.50–0.89)	0.007
Asian	0.78 (0.65–0.94)	0.009	0.62 (0.42–0.92)	0.02
Indian	0.70 (0.53–0.91)	0.009	0.48 (0.27–0.85)	0.01

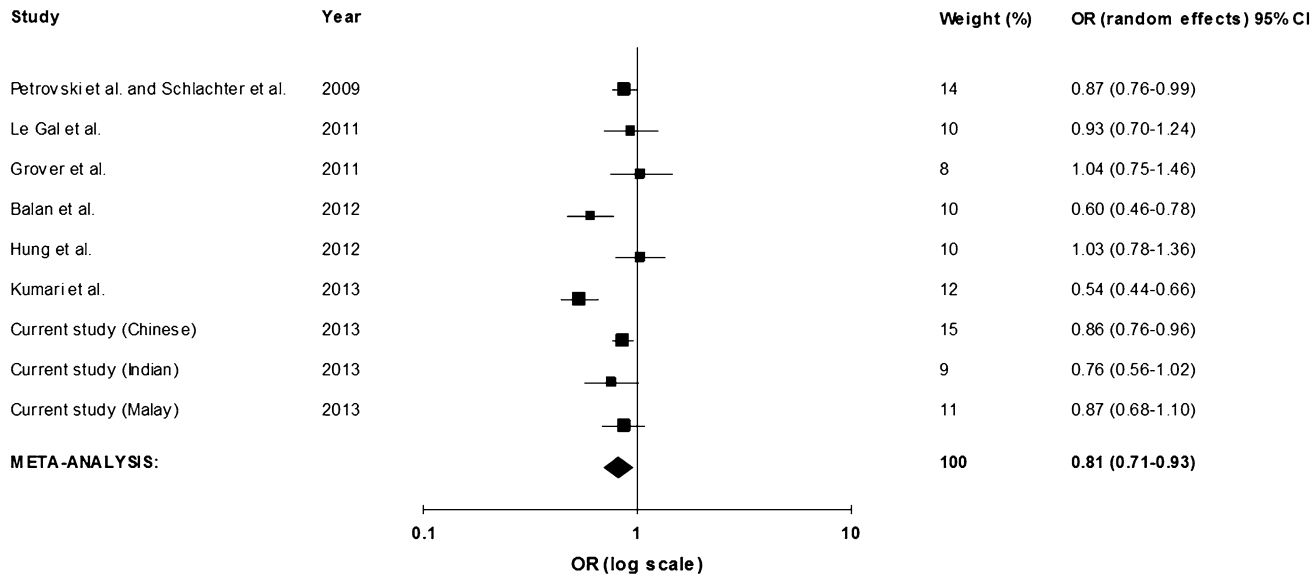

Fig. 1 Forest plot of the association of *SCN1A* rs3812718 with epilepsy (with or without febrile seizures) vs. controls in all ethnic groups, using a random effects model. The studies of Petrovski et al. and Schlachter et al. were combined because they used the same controls

with all Hong Kong controls. Only Hong Kong subjects were analyzed because other groups had limited size to allow subgroup analysis. This analysis revealed no SNPs associated with epilepsy at $p < 0.01$ in older patients, but two SNPs demonstrating associations in younger patients: rs3812718 (OR = 0.80, $p = 0.009$) and rs12467383 (OR = 1.27, $p = 0.006$).

To investigate the extent of LD among SNPs in *SCN1A* found to be associated with epilepsy, LD (r^2) was calculated for pairs of SNPs in each of the four ethnic groups or locations (see Online Resource 2). For rs3812718 and rs10188577, r^2 ranged from 0.12 to 0.21. For rs3812718 and rs2298771, r^2 increased from East to West, from low values in Chinese (0.16 in Hong Kong and 0.20 in Malaysia) to 0.33 in Malays to 0.43 in Indians. For rs10188577 and rs2298771, r^2 ranged from 0.02 to 0.06.

To explore the association of SNPs with gene expression, we used HapMap to examine LD of SNPs in our study with SNPs from a study of 193 human cortical brain samples analyzed on a 500K SNP genotyping array and a chip measuring expression of all genes (Myers et al. 2007). *SCN1A* rs3812718 was in partial LD ($r^2 = 0.50$) with rs4667869, whose genotypes tended toward association with *SCN1A* expression ($p = 0.15$). *SCN2A* rs12467383 was in partial LD with rs2082366 ($r^2 = 0.67$), whose genotypes tended toward association with *SCN2A* expression ($p = 0.10$, Online Resource 3).

Meta-analysis

To review the association of rs3812718 with epilepsy, a search of published studies revealed 58 publications, of which 8 case–control studies met our eligibility criteria (see Online Resource 4). Adding our current study brought the total number of non-overlapping subjects to 7,751 (4,088 epilepsy patients and 3,663 controls). The range of G allele frequency in controls among the studies was 38–55 %. Table 4 shows significant allele and genotype association with all epilepsy. Significant association with epilepsy appeared particularly strong in the subset of

studies conducted in Indians and in epilepsy with febrile seizures. In all epilepsy patients and in all ethnic groups, the association of the G allele with epilepsy was significant (OR = 0.81, $p = 0.002$), as shown in Fig. 1.

Discussion

Of the genes examined in this study, *SCN1A* is the most commonly mutated as a cause of epilepsy (Lossin 2009; Meisler et al. 2010). Interestingly, a SNP in this gene displayed the strongest association with epilepsy in our study. IVS5N+5G>A, or rs3812718, was associated with epilepsy, with a similar OR in all ethnic groups examined. A protective effect of the G allele on epilepsy risk emerged from meta-analysis of our current results and published reports (Petrovski et al. 2009; Le Gal et al. 2011; Zhang et al. 2010; Grover et al. 2010; Hung et al. 2012; Schlachter et al. 2009; Balan et al. 2012; Kumari et al. 2013) despite the quite heterogeneous epilepsy phenotypes in these studies. The SNP may display a somewhat stronger association with epilepsy with febrile seizures than with epilepsy without febrile seizures. IVS5N+5G>A affects alternative splicing, with the G allele increasing the inclusion in Nav1.1 of exon 5N instead of 5A (Heinzen et al. 2007). Transfecting cells with both forms of the channel revealed no differences in their electrophysiological properties at room temperature (Thompson et al. 2011), but at 37 °C the 5N form of Nav1.1 recovered faster from inactivation than did the 5A form (Fletcher et al. 2011). Nav1.1 is preferentially expressed in inhibitory (primarily GABAergic) neurons; thus faster recovery from inactivation would enhance inhibitory activity and may decrease the probability of seizures (Oliva et al. 2012). By this mechanism, rs3812718 may affect the risk of epilepsy.

In our study, epilepsy was also associated with other *SCN1A* SNPs, perhaps due to LD with rs3812718, although r^2 with the other SNPs was generally low. Maybe these SNPs are in LD with variants affecting expression or function of Nav1.1. For example, a SNP tending toward association with *SCN1A* expression ($p = 0.15$) was in LD ($r^2 = 0.50$) with rs3812718. We found an association of rs2298771 with epilepsy particularly in Indians, the ethnic group displaying the highest LD of rs2298771 with rs3812718 (Online Resource 2). Another paper has reported an association of rs2298771 with epilepsy, and the direction of association was the same as in our study, with the minor allele, G, decreased in patients (Makoff et al. 2010). That report examined only idiopathic generalized epilepsy, while we found a trend in all types of epilepsy, with a possibly stronger association with symptomatic. Why was the association largely restricted to Indians in our study? The study by Makoff et al. (2010) was conducted in Europeans, who perhaps share more similarity with Indians than with the geographically more distant Malays and Chinese, at least in regard to this association. In support of this hypothesis, the G allele frequency in control subjects was closer to the European value (34 %) in Indians (28 %) than in either Malays (18 %), Chinese in Malaysia (12 %), or Chinese in Hong Kong (11 %). A possible mechanism for association with epilepsy is LD, in Indians and Europeans but not Malays or Chinese, with other variants, either common or rare, that affect risk of epilepsy.

A SNP in *SCN2A*, rs12467383, was associated with epilepsy. This association might be due to the fact that rs12467383 is in partial LD with a SNP tending toward association with *SCN2A* expression. Alternatively, as for rs2298771, rs12467383 may be in LD with other variants that affect epilepsy risk.

Both rs3812718 and rs12467383 exhibited stronger associations with epilepsy in younger than older patients. Although idiopathic epilepsy syndromes have young age of onset, this age effect was not due to stronger associations of these SNPs with idiopathic epilepsy than with other types (Table 3). In general, genetic effects on disease risk may lower age of onset, thus increasing the strength of genetic associations in younger patients.

The *SCN2B* SNP rs602594 was associated with epilepsy, but with opposite directions of association among ethnic groups or types of epilepsy. The minor allele frequency varied from 4 % in Indians to 18 % in Chinese (either in Malaysia or Hong Kong), corresponding to HapMap frequencies of 6 % in Europeans to 15 % in Chinese. The cause of the heterogeneity in association with types of epilepsy is puzzling and perhaps is due to complex effects of this gene product in response to different conditions. In idiopathic epilepsy, the association tended to be consistent across ethnic groups. Mutations in other ion channel genes have been identified as causing idiopathic epilepsies (Helbig et al. 2008), and although mutations in SCN2B have not previously been reported to cause epilepsy, it is possible that polymorphisms which might subtly affect expression could alter the risk of epilepsy and that previous studies were not large enough to detect an effect of modest size. Although our study was large, with over 3,000 subjects, it still suffered from inadequate number of patients in subtypes of idiopathic (or other types of) epilepsy to analyze associations that may be specific to particular subtypes. Another limitation of this study was that the SNPs genotyped in Malaysia and Hong Kong were not all the same.

Association studies may suffer weaknesses in design, methods of analysis, and interpretation of results. A statistically significant finding in an underpowered study might be a false-positive result due to chance or be attributable to systematic bias like occult stratification. Therefore, we used a large sample with adequate power and adjustment

for multiple comparisons, and we confirmed the association of rs3812718 with epilepsy using meta-analysis of previous reports to reduce the possibility of such artifacts as occult stratification.

This report has suggested several associations of SCN genes with epilepsy. Confirmation of the data in independent sets of samples will shed further light on these findings and would allow us to start collecting a list of common genetic variants that could be genotyped to determine the vulnerability of each individual to develop epilepsy.

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Ethical standards The experiments comply with the current laws of Hong Kong and Malaysia.

Conflict of interest None declared.

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