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RORA gene rs12912233 and rs880626 polymorphisms and their interaction with SCN1A rs3812718 in the risk of epilepsy: A case–control study in Malaysia

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ABSTRACT

RAR-related orphan receptors A (*RORA*) and B (*RORB*) and voltage-gated sodium channel type 1 (*SCN1A*) genes play critical roles in the regulation of the circadian clock. Evidence has shown an association of *RORA* and *RORB* polymorphisms with susceptibility to autism and depression. Hence, we tested the association of *RORA* rs12912233, rs16943429, rs880626, rs2290430, and rs12900948; *RORB* rs1157358, rs7022435, rs3750420, and rs3903529; and *SCN1A* rs3812718 with epilepsy risk in the Malaysians. DNA was genotyped in 1789 subjects (39% epilepsy patients) by using MassARRAY (Sequenom). Significant association was obtained for rs12912233 in Malaysian Chinese ($p = 0.003$). Interaction between rs12912233–rs880626 and rs3812718 was associated with the epilepsy risk in the subjects overall ($p = 0.001$). Results show that *RORA* rs12912233 alone might be a possible risk variant for epilepsy in Malaysian Chinese, but that, together with *RORA* rs880626 and *SCN1A* rs3812718, this polymorphism may have a synergistic effect in the epilepsy risk in Malaysians.

1. Introduction

The hypothalamus suprachiasmatic nucleus (SCN) organizes circadian rhythms, which are critical in mammals for efficient physiological functioning in synchronization with the light–dark cycle [1]. The master circadian pacemaker located in the SCN receives environmental stimuli, converting them into neuronal and hormonal signals in the independent peripheral circadian oscillators that reside in several organs, including the liver, skeletal muscle, and testis [2]. Disruption of the circadian system is implicated in the development of disorders such as cancer, schizophrenia, depression, and epilepsy [3,4]. Epileptic seizures can modulate the circadian system, depending on the epilepsy syndrome and location of seizure foci. The daytime peak pattern is more significant in complex partial seizures than other types of seizures, originating from extra-temporal and temporal regions in children and adults, respectively. In contrast, myoclonic seizures in juvenile myoclonic epilepsy mostly occur following awakening in the morning [5]. Despite the influence of epilepsy on the circadian system, little data have been reported on the molecular basis of the circadian rhythm in this disorder.

RAR-related orphan receptors (RORs) and voltage-gated sodium channel type 1 (NaV1.1) have critical roles in the regulation of circadian clock machinery. The NaV1.1 channels are vital to SCN interneuronal communication, whereas RORs are involved in the intermediate coupling of circadian oscillators with the cyclic control of all physiological functions and behaviour. More than 90% of neurons in the SCN region are GABAergic and express NaV1.1 channels. These channels play a primary role in the action potential firing of GABAergic interneurons [6,7]. In vivo and in vitro studies of the $Scn1a^{+/-}$ mutant mice model showed loss of firing of GABAergic neurons, which caused severe myoclonic epilepsy of infancy, as well as neuronal desynchronization and altered circadian periods. Sodium-dependent action potentials are associated with numerous coupling signalling pathways of clock genes, such as RORs, of relevance to SCN synchronization [8–10]. ROR genes comprise various isoforms (α–γ) belonging to nuclear hormone receptors. They modulate circadian rhythms by regulating the expression of several genes in the pathways, including those related to neurogenesis, stress response, energy homeostasis, and thymopoiesis [11,12].

The NaV1.1, RORα, and RORβ proteins are highly expressed in the brain [9,13]. Our recent report identified a strong association of the common splice-site SCN1A rs3812718 polymorphism with susceptibility to epilepsy in the Hong Kong cohort and in the pooled data of Hong Kong

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and Malaysia, but not in the Malaysian cohort [14]. This variant is associated with electrophysiologic properties of the NaV1.1 channel [15]. Several studies have provided evidence for the contribution of RORA rs12912233 to the risk of autism and bipolar disorder. Furthermore, a study of 18 core circadian genes in the Swedish population suggested that RORA polymorphisms may contribute to vulnerability to depression [16]. A meta-analysis of two genome-wide association studies from 3972 genetically homogeneous Sardinian and 839 European American subjects also suggested a strong association between *RORA* rs12912233 and the risk of depression ($p = 6 \times 10^{-7}$) [17]. A study from China, however, reported no association of RORA rs880626, rs4281667, rs8037155, or rs6500271 with symptomatic epilepsy [18]. Finally, studies investigating the role of RORB polymorphisms in mental disorders identified rs10491929, rs7022435, rs7022435, rs3750420, rs1157358, and rs3903529 risk variants that were associated with bipolar disorder, anxiety, and schizophrenia in Caucasians [19]. No data were available for RORA or RORB gene polymorphisms or their interaction with SCN1A in the Malaysian epilepsy population. In the present study, we evaluated the association of RORA rs12912233, rs16943429, rs880626, rs2290430, and rs12900948; RORB rs1157358, rs7022435, rs3750420, and rs3903529; and SCN1A rs3812718 gene polymorphisms, along with their haplotypes and gene–gene interaction, with the risk of epilepsy in the Malaysian population.

2. Results

2.1. Demographic characteristics

A total of 1789 subjects (39% epilepsy patients) were enrolled in this study (Table 1). Of the patients, 58% were diagnosed as having generalized epilepsy. The ratio of Chinese, Indian, and Malay subjects was 43, 23, and 34%, respectively. The distribution of ages at study entry did not significantly differ between patients and controls in Chinese, Indians, and Malays ($p = 0.15$, $p = 0.46$, and $p = 0.22$, respectively). The distribution of the age of onset of epilepsy differed among the three ethnicities ($p = 0.01$). The lowest and highest onset ages were >observed in Malay and Chinese subgroups as compared with Indians (12 ± 13, 17 ± 17 and 16 ± 14, respectively). Despite the significant over-representation of males ($p < 0.05$), gender distribution was not significantly different among Chinese, Indian, and Malay patients ($p = 0.10$, $p = 0.76$, and $p = 0.51$, respectively). Compared with focal epilepsy, generalized epilepsy was significantly more predominant in Chinese, Indian, and Malay patients ($p < 0.05$). Cryptogenic epilepsy was more often diagnosed in Chinese and Malay patients (43 and 42%, respectively) than in Indians (34%).

2.2. Association study

Table 2 lists the allele and genotype frequencies of the *RORA*, *RORB*, and *SCN1A* gene polymorphisms in the Malaysian epilepsy patients and controls. The association study data are summarized in Table 3. Distributions of Chinese and Malay genotypes were consistent with Hardy–Weinberg equilibrium ($p > 0.05$). No statistically significant differences were found in allele or genotype frequencies between epilepsy patients and controls for all polymorphisms in the Malaysian Malays. The *RORA* rs12912233 CT genotype was associated with the risk of epilepsy in the Malaysian Indians (odds ratio [OR], 0.53, 95% confidence interval [CI] 0.31–0.90, $p = 0.02$). Significant associations were observed between *RORA* rs12912233, rs880626, rs2290430, and rs12900948 and *RORB* rs3903529 polymorphisms and the risk of epilepsy in the Malaysian Chinese. After we applied age- and gender-adjusted analysis and the Bonferroni test for all ethnic subgroups, the results remained significant for *RORA* rs12912233 in the Malaysian Chinese. In this ethnicity, the CC genotype in patients was remarkably less frequent than in controls (49% and 61%, respectively). Hence, the *RORA* rs12912233 CC genotype might protect Malaysian Chinese carriers against epilepsy.

Haplotype-based analysis identified no significant association between the haplotypes from *RORA* gene rs12912233, rs16943429, rs880626, rs2290430, and rs12900948 polymorphisms and from *RORB* gene rs1157358, rs7022435, rs3750420, and rs3903529 polymorphisms in epilepsy patients and controls. Pairwise linkage disequilibrium (LD) was conducted for all loci of the *RORA* and *RORB* genes in each ethnicity. The D′ for RORArs880626-rs2290430 pairwise LD in Indians was remarkably higher than in either Chinese or Malays (Fig. 1).

2.3. Analysis of gene–gene interaction

The cross-validation consistency (CVC) and the prediction error obtained from multifactor dimensionality reduction (MDR) analysis for the best model for each number of loci are shown in Table 4. Significant gene–gene interaction was obtained for the two-locus model RORA rs12912233–SCN1A rs3812718 and the three-locus model RORA (rs12912233–rs880626)–SCN1A rs3812718, with maximum testing accuracy and CVC, after empirical determination by a 1000 permutation test (55.45%, 10/10, $p = 0.023$ and 57.13%, 10/10, $p = 0.001$, respectively). A variety of high- and low-risk genotype combinations were observed in the two- and three-locus models associated with epilepsy (Fig. 2). Genotypes of the RORA (rs12912233–rs880626)–SCN1A rs3812718 exerted their risk for susceptibility to epilepsy in combination. There was a high-synergy redundancy interaction between RORA rs12912233 and SCN1A rs3812718 (interaction entropy

Table 1
Demographic characteristics of the patients and controls.

Characteristics	Chinese			Indian			Malay		
	Epilepsy	Control	*p*	Epilepsy	Control	*p*	Epilepsy	Control	*p*
Age (years), mean (SD)	34 (18)	32 (16)	0.15	31 (17)	29 (15)	0.26	29 (15)	27 (14)	0.11
Onset age (years), mean (SD)	17 (17)	–	–	16 (14)	–	–	12 (13)	–	–
Sex, *N* (%)									
Females	128 (44)	185 (38)	0.10	75 (46)	109 (45)	0.76	117 (47)	158 (44)	0.51
Males	160 (56)	209 (52)		87 (54)	135 (55)		134 (53)	202 (56)	
Seizure type, *N* (%)									
Generalized	140 (55)	–	–	94 (63)	–	–	129 (59)	–	–
Partial	116 (45)	–	–	55 (37)	–	–	90 (41)	–	–
Aetiology, *N* (%)									
Cryptogenic	122 (43)	–	–	55 (34)	–	–	102 (42)	–	–
Idiopathic	57 (20)	–	–	60 (38)	–	–	61 (25)	–	–
Symptomatic	104 (37)	–	–	45 (28)	–	–	80 (33)	–	–