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SHORT REPORT

No association between the SNPs (rs3749446 and rs1402000) in the *PARL* gene and LHON in Chinese patients with m.11778G>A

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Abstract According to a recent genome-wide linkage scan and association study of families with m.11778G>A in Thailand, two single nucleotide polymorphisms (SNPs) (rs3749446 and rs1402000) in the presenilins-associated rhomboid-like (*PARL*) gene were found to be associated with Leber hereditary optic neuropathy (LHON). In order to verify this association in Chinese LHON patients, we genotyped three *PARL* gene variants (rs3749446, rs953419, and rs1402000) in 179 patients with m.11778G>A and 170 patients with suspected LHON, and compared them to a control population containing the HapMap Chinese and 58 normal individuals analyzed in this study. We identified no association between these *PARL* gene SNPs and LHON in Chinese patients with m.11778G>A ($P > 0.05$). Haplotype analysis also showed no statistical difference among the three Chinese populations.

Introduction

The presenilins-associated rhomboid-like (*PARL*) gene encodes a mitochondrial integral membrane protein and has been reported to be associated with diabetes, apoptosis, and survival of lymphocytes and neurons in previous studies (Chao et al. 2008; Cipolat et al. 2006; Walder et al. 2005). Recently, based on a genome-wide linkage scan and association study of families with m.11778G>A in Thailand, Phasukkijwatana et al. (2010) reported an association between two *PARL* gene SNPs (rs3749446 and rs1402000) and Leber hereditary optic neuropathy (LHON) in Thailand families with m.11778G>A. We genotyped three SNPs of the *PARL* gene (rs3749446, rs953419, and rs1402000) in 179 Chinese LHON patients with m.11778G>A, 170 samples with suspected LHON, and 58 normal individuals to confirm the association of the *PARL* gene SNPs with LHON in Chinese patients.

Materials and methods

In total, 179 LHON patients with m.11778G>A, 170 samples with suspected LHON (who had similar clinical features of LHON, but carrying no LHON primary mutation), and 58 normal Han Chinese individuals from different provinces of China were analyzed in this study. The patients were collected during our recent survey for mtDNA mutations in Chinese patients with LHON or suspected LHON (Ji et al. 2008; Jia et al. 2006; Yu et al. 2010). Patients were diagnosed by the ophthalmologists at the Zhongshan Ophthalmic Center and/or local medical centers, and were matched by gender, age, geographic location, and ethnic origin (Han Chinese) with the normal controls. Informed consents conforming to the tenets of the Declaration of Helsinki were obtained from all individuals. Allele-specific PCR and PCR-SSCP were used to detect the existence of the three known LHON primary mutations

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(m.3460G>A, m.11778G>A, and m.14484T>C) in each patient (Jia et al. 2006). The three SNPs (rs3749446, rs953419, and rs1402000) in the *PARL* gene were genotyped by direct sequencing using primer pair 16688U: 5′-AAATTATTTTGTTGACTGATAGA-3′/17261L: 5′-TCTATAATTAGAATTCATGAGC-3′. We compared the alleles, genotypes, and haplotypes of these SNPs among the three populations: sample #1, LHON patients with m.11778G>A; sample #2, patients with suspected LHON; and sample #3, Han Chinese samples (including Han Chinese from HapMap dataset [http://www.hapmap.org]) from the general populations. Fisher exact test (two tailed) was performed to quantify the statistical difference. A *P* value (with Bonferroni correction) less than 0.05 was regarded as statistically significant. Haplotype was constructed by using PHASE 2.0 (http://www.bioinf.manchester.ac.uk/resources/phase/).

Results

The three SNPs (rs3749446, rs953419, and rs1402000) in the *PARL* gene were located in a 465-bp fragment and could be conveniently genotyped by sequencing. None of these SNPs in the three populations was deviated from the Hardy–Weinberg Equilibrium test ($P > 0.05$). We first compared the allele and genotype frequencies of SNPs rs3749446 and rs1402000 between the suspected LHON patients and the control population, which contains Chinese from HapMap and 58 normal individuals genotyped in this study. We observed no difference between these two populations (Table 1). Therefore, we treated the suspected LHON patients as another control population for LHON patients with m.11778G>A in the subsequent analyses.

Unexpectedly, the allele and genotype frequencies of the two SNPs (rs3749446 and rs1402000) showed no significant

Table 1 Genotype and allele frequencies of the three SNPs in the *PARL* gene

Genotype and allele	LHON patients with m.11778G>A (sample #1; $n = 179$) (%)	Suspected LHON patients (sample #2; $n = 170$) (%)	Control sample (58 healthy individuals and Chinese from HapMap, sample #3[a]) (%)	*P* value[b]		
				#1 vs. #2	#1 vs. #3	#2 vs. #3
rs3749446						
Genotype						
AA	132 (73.7)	133 (78.2)	230 (75.7)	0.381	0.664	0.573
AG	44 (24.6)	37 (21.8)	72 (23.7)	0.612	0.826	0.651
GG	3 (1.7)	0 (0)	2 (0.7)	0.248	0.365	0.539
Allele						
A	308 (86.0)	303 (89.1)	532 (87.5)	0.252	0.553	0.531
G	50 (14.0)	37 (10.9)	76 (12.5)			
rs953419						
Genotype						
CC	32 (17.9)	41 (24.1)	61 (20.3)	0.188	0.552	0.353
CT	100 (55.9)	86 (50.6)	137 (45.7)	0.336	0.038	0.337
TT	47 (26.3)	43 (25.3)	102 (34.0)	0.903	0.083	0.061
Allele						
C	164 (45.8)	168 (49.4)	259 (43.2)	0.363	0.460	0.066
T	194 (54.2)	172 (50.6)	341 (56.8)			
rs1402000						
Genotype						
AA	5 (2.8)	0 (0)	1 (1.0)	0.061	0.422	0.375
AG	46 (25.7)	43 (25.3)	36 (35.3)	1.000	0.102	0.098
GG	128 (71.5)	127 (74.7)	65 (63.7)	0.547	0.184	0.074
Allele						
A	56 (15.6)	43 (12.6)	38 (18.6)	0.279	0.411	0.063
G	302 (84.4)	297 (87.4)	166 (81.4)			

[a] The sample size of Han Chinese from HapMap with information for each of the three SNPs varied. We aggregated the normal individuals analyzed in this study with the HapMap data in the analysis: rs3749446, $n = 304$; rs953419, $n = 300$; rs1402000, $n = 102$

[b] The *P* values were not adjusted by the Bonferroni correction

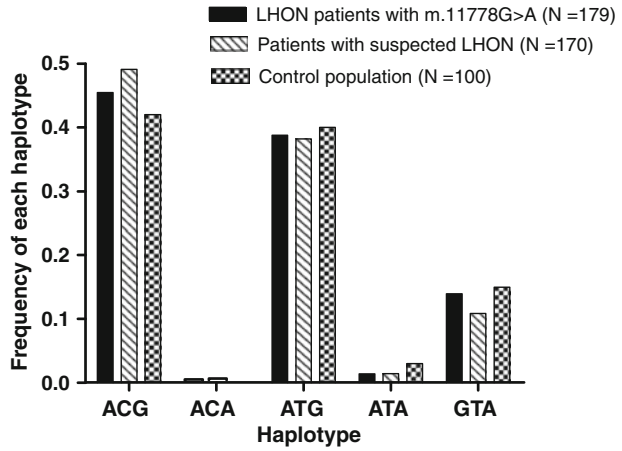

Fig. 1 Haplotype frequency of three *PARL* gene SNPs (rs3749446–rs953419–rs1402000) in Chinese LHON patients with m.11778G>A, patients with suspected LHON and the control population. Because one or two of the three SNPs were missing in some Han Chinese samples from HapMap, we only used 42 CHB samples (with genotyping information for all three SNPs) and aggregated it with the 58 normal individuals analyzed in this study as the control population. Same result was obtained when we excluded the HapMap Chinese from the control population

difference between the LHON patients with m.11778G>A and the suspected LHON patients or between the LHON patients with m.11778G>A and the control population (Table 1), though the frequencies of genotype GG of rs3749446 and genotype AA of rs1402000 were slightly higher in LHON patient population than the other two populations. However, the frequency of genotype CT of rs953419 (this SNP was not significant after multiple testing correction in Phasukkijwatana et al. (2010)) was higher ($P = 0.038$) in LHON patients (55.9%) than in the control population (45.7%; Table 1), but the P value changed to 0.114 after the Bonferroni correction.

Five haplotypes were constructed based on the three SNPs (rs3749446–rs953419–rs1402000). Three main haplotypes (A–C–G, A–T–G, and G–T–A) were prevalent and account for 98% of observations. However, we found no statistical difference regarding the haplotype distribution in the three populations (Fig. 1).

Discussion

As the classical and most extensively studied mitochondrial disorder, knowledge about LHON has been updated during the past decades. We know that about 95% of LHON cases are caused by one of the three primary mutations; many factors, such as heteroplasmy of the primary mutation, mtDNA background, nuclear genes, and environmental factors affect the clinical expression of LHON (Yu-Wai-Man et al. 2009). However, no nuclear gene or SNP has been identified to explain the riddle of LHON. Recently, by using a genome-wide linkage scan and association study, Phasukkijwatana et al. (2010) first reported that two SNPs (rs3749446 and rs1402000) in the *PARL* gene were associated with the expression of LHON in patients from Thailand. Because of potential limits in that study, these authors called for more studies to confirm their observation (Phasukkijwatana et al. 2010).

In this study, we genotyped three SNPs (rs3749446, rs953419, and rs1402000) in the *PARL* gene in 179 Chinese LHON patients with m.11778G>A, 170 suspected LHON patients and 58 normal individuals. We found no statistical difference for rs3749446 and rs1402000 regarding the allele frequency, genotype frequency, and haplotype frequency among the LHON population, suspected LHON population and the control population. The frequency difference of genotype CT of rs953419 between the LHON patients with m.11778G>A and the control population, but not between the LHON patients with m.11778G>A and the suspected LHON patients, might be caused by potential population stratification, as we aggregated the normal individuals analyzed in this study with the Chinese from HapMap to increase the sample size of the control population. Note that we observed a similar frequency of genotype CT of rs953419 in our normal Han Chinese individuals (33/58; 56.9%) compared to the LHON patients (100/179; 55.9%). The lack of association between the two reported SNPs in the *PARL* gene and LHON in the current study is not totally surprising when we consider the complex pathogenesis of this disease. In fact, the LHON susceptibility loci identified by linkage analyses seem to restrict to the studied populations/families only (Ji et al. 2010). More studies with large sample size and populations from different regions should be carried out to further elucidate the genetic basis of clinical expression of LHON.

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