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Common variants of *OPA1* conferring genetic susceptibility to leprosy in Han Chinese from Southwest China

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ABSTRACT

Background: Leprosy is an ancient chronic infection caused by *Mycobacterium leprae*. Onset of leprosy was highly affected by host nutritional condition and energy production, (partially) due to genomic loss and parasitic life style of *M. leprae*. The optic atrophy 1 (*OPA1*) gene plays an essential role in mitochondria, which function in cellular energy supply and innate immunity.
Objective: To investigate the potential involvement of *OPA1* in leprosy.
Methods: We analyzed 7 common genetic variants of *OPA1* in 1110 Han Chinese subjects with and without leprosy, followed by mRNA expression profiling and protein–protein interaction (PPI) network analysis.
Results: We observed positive associations between *OPA1* variants rs9838374 ($P_{genotypic} = 0.003$) and rs414237 ($P_{genotypic} = 0.002$) with lepromatous leprosy. expression quantitative trait loci (eQTL) analysis showed that the leprosy-related risk allele C of rs414237 is correlated with lower *OPA1* mRNA expression level. Indeed, we identified a decrease of *OPA1* mRNA expression in both with patients and cellular model of leprosy. In addition, the PPI analysis showed that OPA1 protein was actively involved in the interaction network of *M. leprae* induced differentially expressed genes.
Conclusion: Our results indicated that *OPA1* variants confer risk of leprosy and may affect *OPA1* expression, mitochondrial function and antimicrobial pathways.

1. Introduction

Leprosy, an ancient disease caused by infection of *Mycobacterium leprae*, remains and will continue to be a public health problem in some tropic and poor regions [1]. It mainly affects the skin, peripheral nerves, eyes and limbs and may lead to severe disability [1]. According to World Health Organization (WHO) guidelines, leprosy is classified into multibacillary (MB) and paucibacillary (PB) leprosy on the basis of clinical manifestations and the number of skin lesions [2]. There are two polar forms of leprosy as determined by host immune response: lepromatous leprosy (LL, belongs to MB) and tuberculoid leprosy (TT, belongs to PB), together with three immunologically unstable borderline forms (borderline tuberculoid leprosy [BT], mid-borderline leprosy [BB], borderline lepromatous leprosy [BL]) [2,3]. The TT is characterized by strong cellular immunity with type I reaction and the LL is the result of defect in cellular immune responses with type II leprosy reaction [2].

M. leprae lost about 50% coding genes of its genome, many of which are predicted to be actively involved in metabolic pathways, making it a stringent host-dependent intracellular parasite [4,5]. During the long evolutionary erosion of its genome, *M. leprae* has developed a dependence on host energy production and nutritional products and as a result the parasitic life and adaptation of this species might have shaped host genetic susceptibility to leprosy. Differences in host energy supply system may lead to different susceptibility to leprosy.

Mitochondria, the energy-producing centers within cells, play a key role in cellular energy supply, generation of reactive oxygen species (ROS), regulation of apoptosis and aging, and participation in innate immunity [6,7]. Accumulating evidence has suggested that mitochondria play an essential role in the innate immunity

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response to microbial infection [6,7]. Given the fact that *M. leprae* has developed a dependence on host energy production [1,5] and the important role of mitochondria in the cell, one would naturally speculate that mitochondrial gene may be involved in the process of *M. leprae* infection. Indeed, a recent study of gene expression profiling of Schwann cells infected with live *M. leprae* highlighted mitochondrial signatures in leprosy [8], indicating a key role of host mitochondria in *M. leprae* infection.

Mitochondria are morphologically dynamic structures with processes called fission (splitting into smaller pieces) and fusion (combining pieces) [9]. These morphological changes are essential for mitochondrial function and dysfunctions of fission and fusion are related to a wide variety of diseases [9,10]. The optic atrophy 1 (OPA1), an inner membrane protein of mitochondrion, regulates the morphology of mitochondria in the fusion process [10–12]. It is also involved in oxidative phosphorylation in energy production [13,14]. Mutations in the *OPA1* gene would cause mitochondrial dysfunction and damage of optic nerve in eyes [11,13], and more than 200 *OPA1* mutations have been found to cause optic atrophy type 1 [13]. In addition, the OPA1 protein plays a role in the maintenance of mitochondrial DNA (mtDNA) [13]. In our previous study, we found that LL patients had a significantly higher mtDNA content than controls [15]. The altered mtDNA copy number alterations in LL patients may be related to OPA1 function.

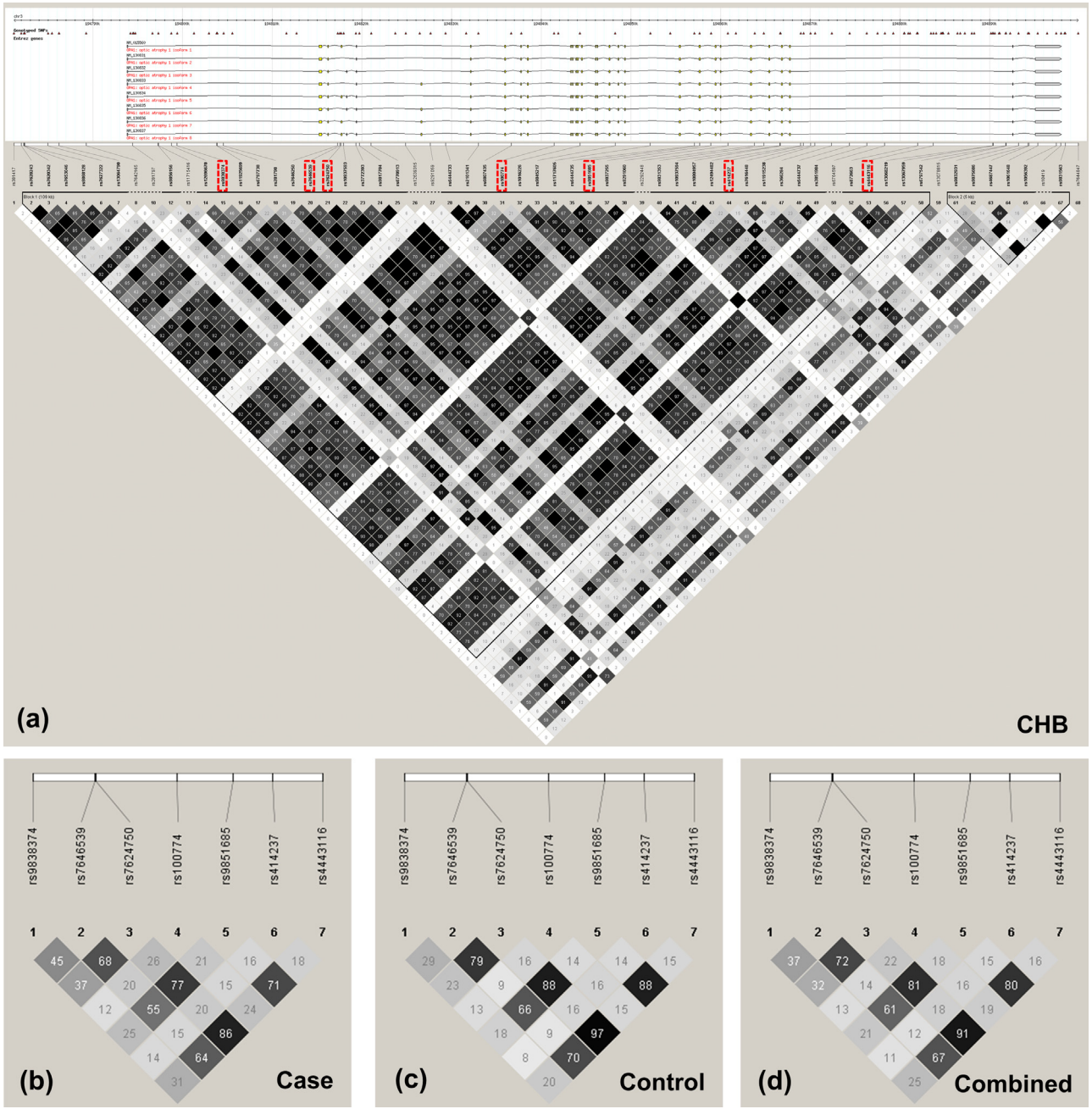

Fig. 1. Linkage disequilibrium (LD) pattern of the *OPA1* gene in CHB (a), leprosy patient (b), controls (c) and pooled samples from Yunnan, China (d). Results were performed by Haploview 4.2 based on the HapMap and our case-control data. r^2 was used for the LD color scheme. Black squares represent high LD as measured by r^2, gradually coloring down to white squares of low LD. The individual square shows the $100 \times r^2$ value for each SNP pair. For the CHB population, three blocks are observed. SNPs marked by red frames (a) were chosen in the analyses (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Table 1
Association of *OPA1* variants with leprosy *per se* and its subtypes at the allelic levels.

SNP	Allele	*P* (HWE)	CN	Leprosy vs. control			MB vs. control			PB vs. control			LL vs. control		
			MAF	MAF	OR (95% CI)	*P*	MAF	OR (95% CI)	*P*	MAF	OR (95% CI)	*P*	MAF	OR (95% CI)	*P*
rs9838374	T/C	0.386	0.22	0.26	1.211 (0.992–1.479)	0.059	0.27	1.265 (0.999–1.603)	0.051	0.249	1.151 (0.895–1.479)	0.273	0.27	1.304 (0.936–1.817)	0.116
rs7646539	G/A	0.04	0.32	0.35	1.132 (0.944–1.358)	0.181	0.35	1.111 (0.893–1.383)	0.345	0.354	1.157 (0.920–1.455)	0.213	0.37	1.232 (0.904–1.680)	0.186
rs7624750	A/G	0.029	0.36	0.41	1.225 (1.004–1.493)	0.045	0.4	1.159 (0.920–1.460)	0.21	0.428	1.305 (1.026–1.659)	0.03	0.41	1.201 (0.874–1.649)	0.258
rs100774	C/T	0.012	0.32	0.29	0.876 (0.731–1.051)	0.155	0.29	0.874 (0.701–1.090)	0.232	0.292	0.879 (0.698–1.107)	0.273	0.30	0.920 (0.670–1.262)	0.604
rs9851685	C/T	0.033	0.36	0.36	1.004 (0.839–1.200)	0.968	0.33	0.879 (0.706–1.093)	0.245	0.391	1.165 (0.931–1.458)	0.181	0.34	0.933 (0.682–1.276)	0.663
rs414237	G/A	0.07	0.27	0.31	1.230 (1.022–1.480)	0.028	0.31	1.247 (0.999–1.556)	0.051	0.306	1.211 (0.961–1.527)	0.105	0.32	1.292 (0.943–1.769)	0.110
rs4443116	G/A	0.028	0.37	0.39	1.092 (0.915–1.302)	0.33	0.38	1.025 (0.829–1.269)	0.818	0.407	1.173 (0.939–1.464)	0.16	0.37	1.020 (0.750–1.387)	0.899

HWE, Hardy–Weinberg equilibrium; CN, controls population from Yunnan; MAF, manor allele frequency; OR, odds ratios; CI, confidence interval.

Considering all these facts and a parallel damage of peripheral nerves and eyes in leprosy patients [1], it is reasonable to speculate that *OPA1* may be involved in leprosy.

In this study, we analyzed 7 *OPA1* common genetic variants in 1110 Han Chinese with and without leprosy, together with (re-) analysis of mRNA expression changes in leprosy skin lesions and a reconstruction of protein–protein interaction network with OPA1, to characterize the potential involvement of OPA1 in leprosy. We found solid evidence to indicate *OPA1* as a susceptibility gene for leprosy.

2. Materials and methods

2.1. Subjects

Patients analyzed in this study were described in our previous studies [15–20]. In brief, a total of 527 leprosy patients (mean onset age 24.7 ± 12.3 years) were recruited form the Yuxi Prefecture, Yunnan Province, Southwest China. Among them, 279 patients could be grouped into multibacillary leprosy (MB; including 109 lepromatous leprosy [LL], 145 borderline lepromatous leprosy

Table 2
Association of *OPA1* variants with leprosy *per se* and its subtypes at the genotypic levels.

SNP	TEST	Control	Leprosy vs. control		MB vs. control		PB vs. control		LL vs. control	
		Counts	Counts	*P*	Counts	*P*	Counts	*P*	Counts	*P*
rs9838374	GENO	23/196/322	22/224/272	0.061	8/132/137	**0.006**	14/92/135	0.5025	1/57/50	**0.003**
	TREND	242/840	268/768	0.049	148/406	0.039	120/362	0.2647	59/157	0.098
	DOM	219/322	246/272	0.022	140/137	**0.006**	106/135	0.3587	58/50	0.011
	REC	23/518	22/496	0.997	8/269	0.334	14/227	0.3434	1/107	0.095
rs7646539	GENO	46/260/241	59/230/209	0.180	29/127/112	0.509	30/103/97	0.1388	12/52/39	0.377
	TREND	352/742	348/648	0.170	185/351	0.326	163/297	0.2001	76/130	0.167
	DOM	306/241	289/209	0.495	156/112	0.539	133/97	0.6286	64/39	0.244
	REC	46/501	59/439	0.065	29/239	0.263	30/200	0.04715	12/91	0.290
rs7624750	GENO	37/184/133	83/244/170	0.035	40/134/94	0.241	43/110/76	0.0166	15/54/34	0.440
	TREND	258/450	410/584	0.040	214/322	0.190	196/262	0.02496	84/122	0.230
	DOM	221/133	327/170	0.312	174/94	0.522	153/76	0.2812	69/34	0.398
	REC	37/317	83/414	0.010	40/228	0.093	43/186	0.004329	15/88	0.248
rs100774	GENO	45/276/253	29/248/249	0.235	10/142/127	0.059	19/106/122	0.3562	4/57/47	0.275
	TREND	366/782	306/746	0.129	162/396	0.196	144/350	0.251	65/151	0.578
	DOM	321/253	277/249	0.278	152/127	0.691	125/122	0.1608	61/47	0.915
	REC	45/529	29/497	0.124	10/269	0.018	19/228	0.9424	4/104	0.127
rs9851685	GENO	58/278/218	66/223/209	0.152	31/113/124	0.094	35/110/85	0.1728	13/44/46	0.374
	TREND	394/714	355/641	0.968	175/361	0.233	180/280	0.1666	70/136	0.651
	DOM	336/218	289/209	0.388	144/124	0.059	145/85	0.5309	57/46	0.313
	REC	58/496	66/432	0.162	31/237	0.635	35/195	0.0612	13/90	0.518
rs414237	GENO	32/242/300	25/275/226	**0.004**	13/148/118	0.011	12/127/108	0.04998	2/65/41	**0.002**
	TREND	306/842	325/727	0.017	174/384	0.036	151/343	0.08399	69/147	0.087
	DOM	274/300	300/226	**0.002**	161/118	0.006	139/108	0.0248	67/41	**0.006**
	REC	32/542	25/501	0.539	13/266	0.575	12/235	0.6758	2/106	0.103
rs4443116	GENO	63/283/208	82/225/192	0.032	39/123/106	0.266	43/102/86	0.01894	14/49/40	0.735
	TREND	409/699	389/609	0.323	201/335	0.812	188/274	0.1512	77/129	0.894
	DOM	346/208	307/192	0.756	162/106	0.579	145/86	0.9336	63/40	0.804
	REC	63/491	82/417	0.017	39/229	0.195	43/188	0.006811	14/89	0.520

GENO, genotypic (2 df) test; TREND, Cochran–Armitage trend test; DOM, dominant gene action (1 df) test; REC, recessive gene action (1 df) test. *P* values less than 0.007 (Bonferroni corrected) were marked in bold.

[BL] and 25 borderline leprosy [BB]) and 248 into paucibacillary leprosy (PB; including 175 tuberculoid leprosy [TT] and 73 borderline tuberculoid leprosy [BT]). These patients were diagnosed based on clinical manifestations and histopathological features and/or bacteriological index (if available). 583 healthy individuals (mean age 36.0 ± 15.5 years) without any history of leprosy infection, HIV, and tuberculosis were collected from the same area as a control group. Informed consents conforming to the tenets of the Declaration of Helsinki were obtained prior to this study. The institutional review of the Kunming Institute of Zoology, Chinese Academy of Sciences board approved this study.

2.2. SNP selection and genotyping

We selected 7 *OPA1* SNPs using the following criteria: (1) SNPs with a high tagging capability according to the linkage disequilibrium (LD) pattern of the *OPA1* gene in HapMap CHB data set (http://hapmap.ncbi.nlm.nih.gov) (Fig. 1); (2) SNPs with a minor allele frequency (MAF) > 5% in HapMap CHB data set and dbSNP (http://www.ncbi.nlm.nih.gov/SNP); (3) SNPs located in coding regions (exons and untranslated regions [UTR]). Among these SNPs, five (rs4443116, rs7646539, rs9838374, rs100774, rs414237) were tag SNPs, two SNPs (rs7624750, rs9851685) were located in coding regions (exons and 3′ UTR). The 7 SNPs were detected by the SNaPshot assay (SNaPshot® Multiplex System, Life Technologies, Carlsbad, California, USA) following the procedure described in our previous study [17]. Primers for genotyping were shown in Supplementary Table S1. The genotyping results were double-blind checked by two people (Y.-L.X., D.-F.Z.).

2.3. Gene expression and expression quantitative trait loci (eQTL) analysis

Tissue-specific expression pattern of *OPA1* mRNA was retrieved from the Genotype-Tissue Expression project (GTEx) [21], which provides a comprehensive atlas of gene expression and regulation across multiple human tissues (http://www.gtexportal.org/home/gene/OPA1). To test the effect of leprosy-related SNPs on *OPA1* mRNA expression, we performed an eQTL analysis. A large, exon-specific eQTL database [22] (http://caprica.genetics.kcl.ac.uk/BRAINEAC/) containing 10 brain regions of 134 neuropathologically normal individuals was used, as *OPA1* was highly expressed in brain tissues (revealed by tissue expression pattern). All the samples were genotyped on the Illumina Infinium Omni1-Quad BeadChip and on the Immunochip, and the expression levels were measured using Affymetrix Exon 1.0 ST arrays. More details were given in the original paper [22]. Effect of leprosy-risk genotype on *OPA1* mRNA expression difference were detected for all *OPA1*-targeting probes. Because there are 10 tissues, a P value < 0.005 (0.05/10) was set as significant after Bonferroni correction.

The *OPA1* mRNA expression data in skin lesions of 6 LL patients and 5 BT patients was retrieved from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/sites/GDSbrowser). Detailed information was described in the original paper [23] and the GEO webpage (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE443). In addition, we checked *OPA1* mRNA expression level in a leprosy cellular model: Teles et al. [24] performed analysis of healthy peripheral blood mononuclear cells (PBMCs) stimulated with IL-10 integrated with leprosy transcriptional profiles. They found that the type II interferon IFN-γ and its downstream vitamin D-dependent antimicrobial genes were preferentially expressed in

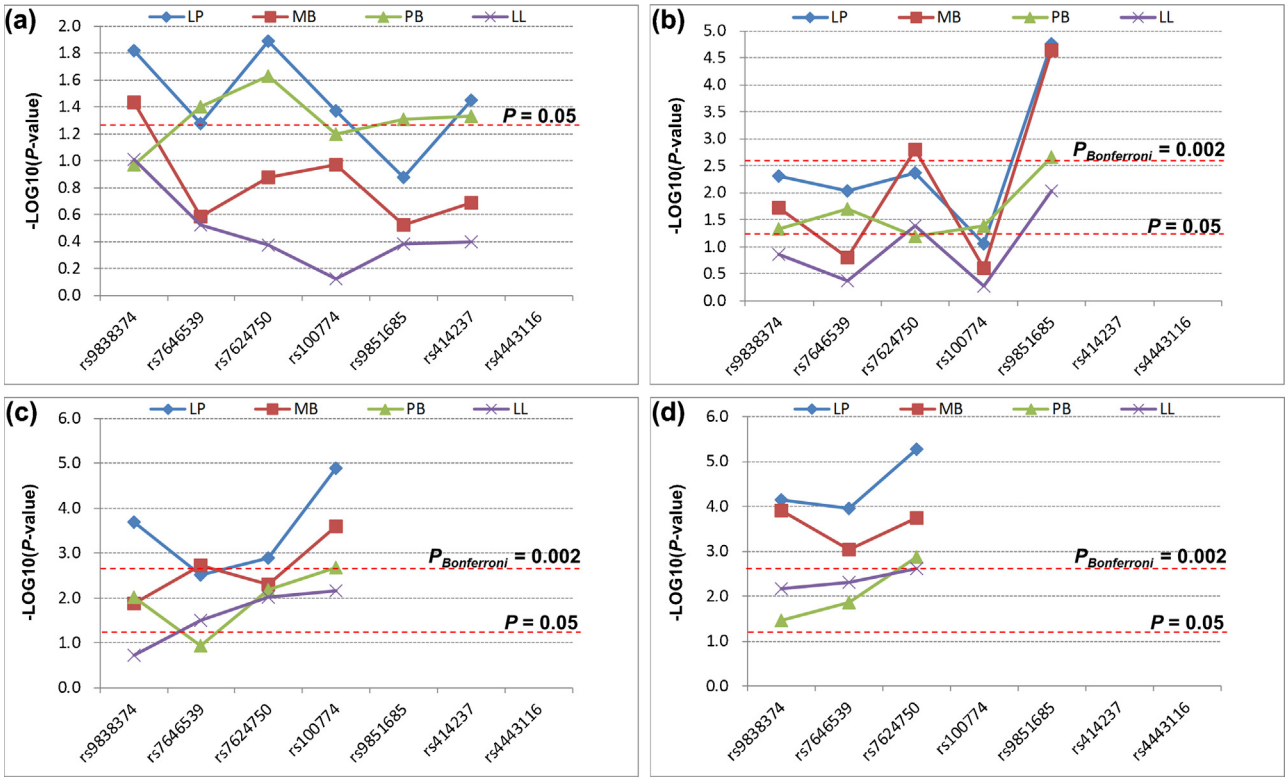

Fig. 2. Sliding window haplotype analysis. A total of 21 windows were found for the 7 SNPs with 2–6 SNPs width (sliding by 1 SNP). Results of 2-SNPs (a) 3-SNPs (b), 4-SNPs (c), and 5-SNPs (d) windows were shown. A P value < 0.0024 [0.05/21 windows] was regarded as significant after Bonferroni correction. LP, leprosy *per se*; MB, multibacillary leprosy; PB, paucibacillary leprosy; LL, lepromatous leprosy. In this haplotype analysis, stronger associations were found for leprosy *per se* while weaker associations were observed for lepromatous leprosy.

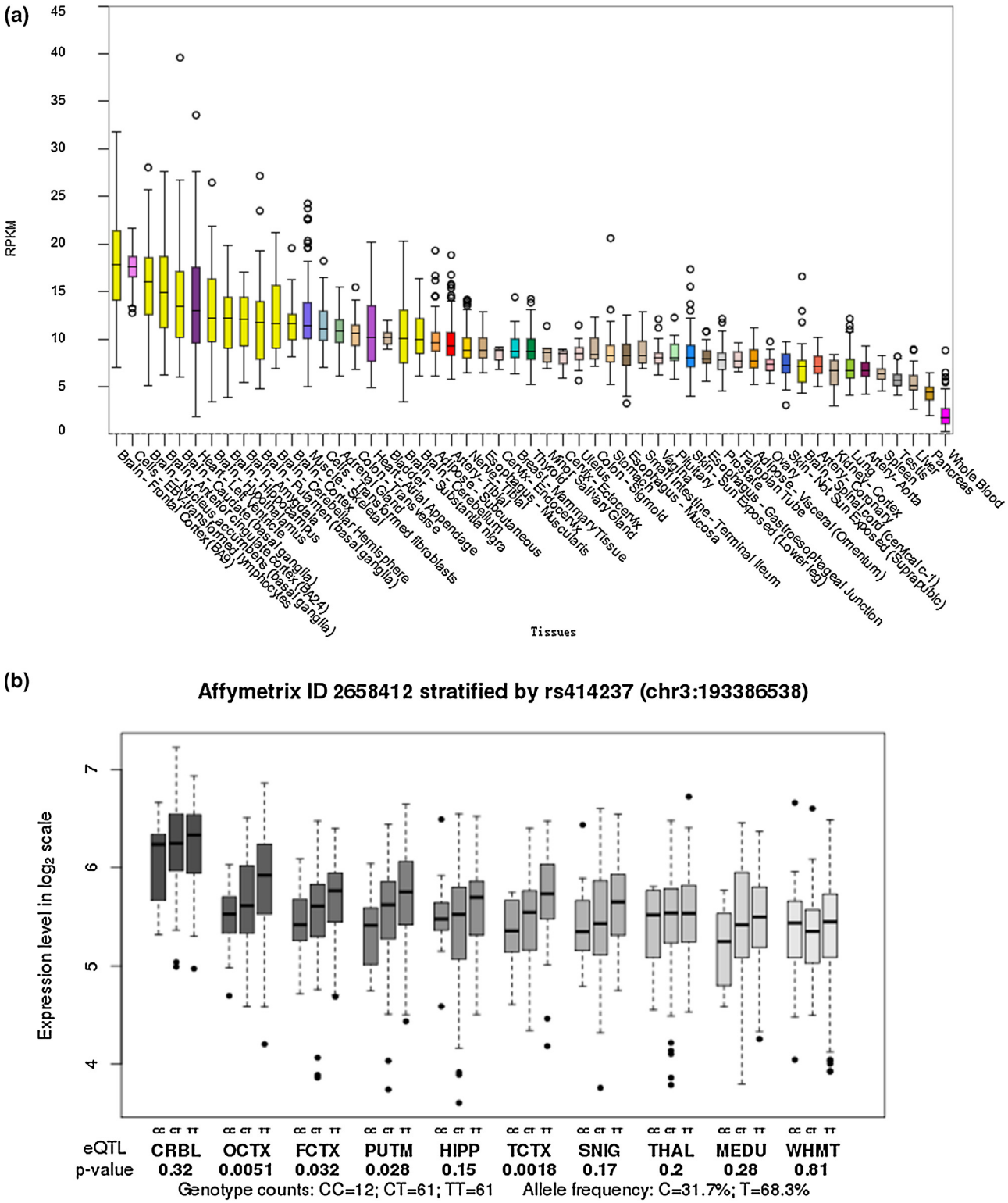

Fig. 3. Tissue-specific expression pattern and effects of leprosy-related SNPs on *OPA1* expression. Tissue-specific expression pattern of *OPA1* mRNA (a) was retrieved from the Genotype-Tissue Expression project (GTEx, http://www.gtexportal.org/home/gene/OPA1). The highest *OPA1* expression level was observed in most brain tissues, such as frontal cortex, nucleus accumbens, caudate, and hippocampus. Expression quantitative trait loci (eQTL) analysis (b) was conducted in a brain eQTL database (http://caprica.genetics.kcl.ac.uk/BRAINEAC/), because *OPA1* was highly expressed in brain tissues. A P value < 0.005 (0.05/10) was set as Bonferroni corrected significance. SNIG, substantia nigra; PUTM, putamen (at the level of the anterior commissure); MEDU, the inferior olivary nucleus (sub-dissected from the medulla); THAL, thalamus (at the level of the lateral geniculate nucleus); OCTX, occipital cortex; HIPP, hippocampus; FCTX, frontal cortex; TCTX, temporal cortex; WHMT, intralobular white matter; CRBL, cerebellar cortex. Effect of leprosy-risk genotype on *OPA1* mRNA expression difference were detected for all *OPA1*-targeting probes. An exon-specific (Affymetrix probe ID 2658412) eQTL effect of rs414237 on *OPA1* mRNA level was observed in temporal cortex (TCTX, $P = 0.0018$) after Bonferroni correction.

the self-healing TT form and mediated antimicrobial activity against the pathogen. In contrast, the type I interferon IFN-β and its downstream gene IL-10 were induced in monocytes by *M. leprae* in vitro, and were preferentially expressed in the lesions of disseminated and progressive LL form. The IFN-γ induced macrophage antimicrobial response was inhibited by IFN-β/IL-10. Detailed information of this data source was given in the original paper [24] and the webpage (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE43700). OPA1 expression changes were investigated in the IL-10 treated PMBCs to see whether OPA1 was involved in macrophage antimicrobial response.

2.4. Protein–protein interaction (PPI) network analysis

To further explore whether OPA1 is involved in leprosy, we investigated the PPI network between OPA1 and those recognized leprosy risk genes (c.f. Zhang et al. [25] and references therein), as well as differentially expressed genes (detailed gene list was described in Table S2 of Guerreiro et al. [8]) in *M. leprae*-infected Schwann cells [8]. PPI network analysis was performed by Disease Association Protein–Protein Link Evaluator (DAPPLE, http://www.broadinstitute.org/mpg/dapple/dapple.php) [26]. DAPPLE looks for significant physical connectivity (protein–protein interactions) among proteins encoded by genes in disease-associated loci. In the PPI analysis, *OPA1* was regarded as a leprosy susceptibility gene. The list of leprosy related genes were then set as input seed to generate direct and indirect interaction network.

2.5. Statistical analyses

The *OPA1* mRNA expression difference between two groups (LL and BT) was measured by Student's *t* test using software GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, USA). We estimated deviation from the Hardy–Weinberg equilibrium (HWE), individual SNP association and haplotype analysis using the software PLINK [27]. In genetic association analysis for complex diseases, the underlying genetic model is often unknown. The weights are selected according to the suspected mode of inheritance. Basic allelic test (which compares frequencies of alleles in cases vs. controls) and different genotype-based test (dominant, recessive and general genotypic model) were performed in the current analyses. In detail, allelic test compares frequencies of alleles in cases vs. controls, the genotypic (2 df) test provides a general test of association in the 2-by-3 table of disease-by-genotype. The dominant (1 df) and recessive (1 df) models are tests for the minor allele. That is, if D is the minor allele (and d is the major allele): allelic means D vs. d, dominant means (DD, Dd) vs. dd, recessive means DD vs. (Dd, dd), genotypic means DD vs. Dd vs. dd. The Cochran–Armitage test does not assume HWE, as the individual, not the allele, is the unit of analysis. Associations of *OPA1* SNPs with leprosy *per se*, MB and PB were detected. As we previously found that mtDNA copy number was altered in LL patients [15], we also investigated the associations of *OPA1* SNPs with LL subtype. To control for possible false positive in multiple testing, Bonferroni correction were used. A *P*-value < 0.007 (0.05/7 SNPs) was set as statistical significance considering 7 independent individual SNP association. Statistical power was calculated by Quanto [28].

3. Results

3.1. Association of OPA1 SNPs with leprosy susceptibility

The minor allele frequency (MAF) of analyzed SNPs in our control population ranged from 0.22 to 0.37. The power to detect the odds ratio (OR) value as 1.5 for risk allele was expected to be 97% in our samples assuming a type 1 error rate of 0.05 and a MAF of 0.2. When considered an OR value of 1.3 as observed in our study for rs414237 in LL patients (MAF = 0.27), the power to detect the genetic risk was expected to be 78%. All SNPs had no deviation from HWE in control sample ($P < 0.001$; Table 1).

Two SNPs (rs7624750 and rs414237) showed a marginal association with leprosy *per se* ($P = 0.045$ for rs7624750 and $P = 0.028$ for rs414237) or its subtypes ($P_{PB} = 0.03$ for rs7624750 and $P_{MB} = 0.051$ for rs414237) at the allelic level (Table 1), but the associations disappeared after Bonferroni correction ($P > 0.007$). SNP rs414237 showed a significant genotypic association with leprosy *per se* ($P = 0.004$), especially with LL subtype ($P = 0.002$) (Table 2). SNP rs9838374 showed a positive association ($P = 0.006$) with MB at the genotypic level, and the association is even stronger in LL patients ($P = 0.003$).

We performed linkage disequilibrium (LD) analysis of these *OPA1* SNPs in the case and control populations and found a similar LD structure in both populations (Fig. 1). In the sliding window haplotype analysis (Fig. 2), we observed several significant haplotypic associations in 3-SNPs, 4-SNPs, and 5-SNPs windows after Bonferroni correction ($P < 0.0024$ [0.05/21 windows]). Further fine mapping analyses are needed to determine whether there are causal variants within these leprosy-related haplotype blocks.

3.2. Tissue-specific expression pattern and effects of leprosy-related SNPs on OPA1 expression

As shown in Fig. 3, *OPA1* mRNA expression presented a tissue-specific pattern based on the data of the Genotype-Tissue Expression project (GTEx) [21]. These tissues with high energy consumption had a relatively higher *OPA1* mRNA expression level. The highest *OPA1* expression level was observed in the brain tissues, such as frontal cortex, nucleus accumbens, caudate, and hippocampus. This mitochondrial protein was also highly expressed in heart (left ventricle). The leprosy-related tissue—the skin, have a moderate or average *OPA1* mRNA expression level.

To test the effect of the above leprosy-related SNPs on *OPA1* expression, we performed an eQTL analysis for rs9838374 and rs414237 in tissues with a high *OPA1* expression level by using the brain eQTL database (http://caprica.genetics.kcl.ac.uk/BRAINEAC/) [22]. We observed a significant exon-specific (Affymetrix probe ID 2658412) eQTL effect of rs414237 on *OPA1* mRNA level in temporal

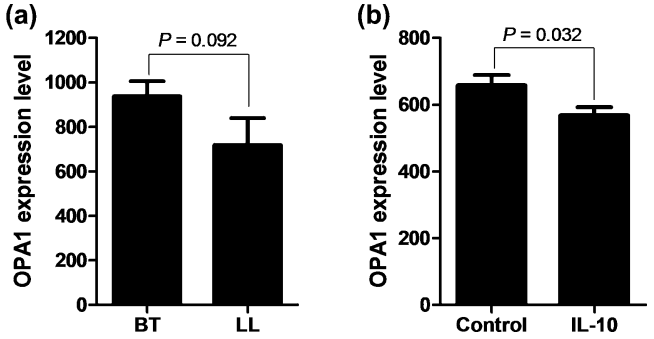

Fig. 4. *OPA1* mRNA expression alteration in leprosy. *OPA1* mRNA expression data in skin lesions (a) of 6 lepromatous leprosy (LL) patients and 5 borderline tuberculoid (BT) patients was retrieved from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/sites/GDSbrowser). Detailed information was described in the original paper (Bleharski et al. [23]) and the GEO webpage (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE443). Healthy peripheral blood monuclear cells (PBMCs) were stimulated with IL-10 and detailed information was given in the original paper [24] and webpage (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE43700). *OPA1* mRNA expression changes were investigated in IL-10 treated PMBCs (b) to see whether OPA1 was involved in macrophage antimicrobial response. Comparison between two groups was measured by one-tailed unpaired *t* test. Bars represent mean ± SEM.

cortex (TCTX, $P = 0.0018$) after Bonferroni correction (Fig. 3b). Notably, the leprosy risk allele C of rs414237 was correlated with lower *OPA1* level. This trend was validated by other brain tissues such as occipital cortex (OCTX, $P = 0.0051$), frontal cortex (FCTX, $P = 0.032$), and putamen (PUTM, $P = 0.028$). For SNP rs9838374, no significant eQTL effect was observed after Bonferroni correction ($P > 0.005$).

3.3. OPA1 mRNA expression changes in leprosy

Consistent with the above eQTL finding that risk carriers had a lower *OPA1* mRNA expression level, we observed a marginally significant decrease of *OPA1* mRNA expression in skin lesions of 6 LL patients compared with 5 BT patients based on the data-mining of the reported mRNA expression data [23] (Fig. 4). However, the difference did not reach a statistical significance ($P = 0.092$), partially due to the relatively small sample size of analyzed patients. Further analysis of the mRNA expression reported by Teles et al. [24] showed that the *OPA1* mRNA expression level was significantly decreased in IL-10 stimulated PBMCs ($P = 0.032$), suggesting that *OPA1* may be down-regulated by type I interferon IFN-β and IL-10. Invasion of *M. leprae* might cause an up-regulation of IL-10, leading to inhibition of macrophage antimicrobial response [24]. Nevertheless, it is unclear whether OPA1 functions in this progress. The exact role of *OPA1* expression alterations in leprosy remains to be clarified.

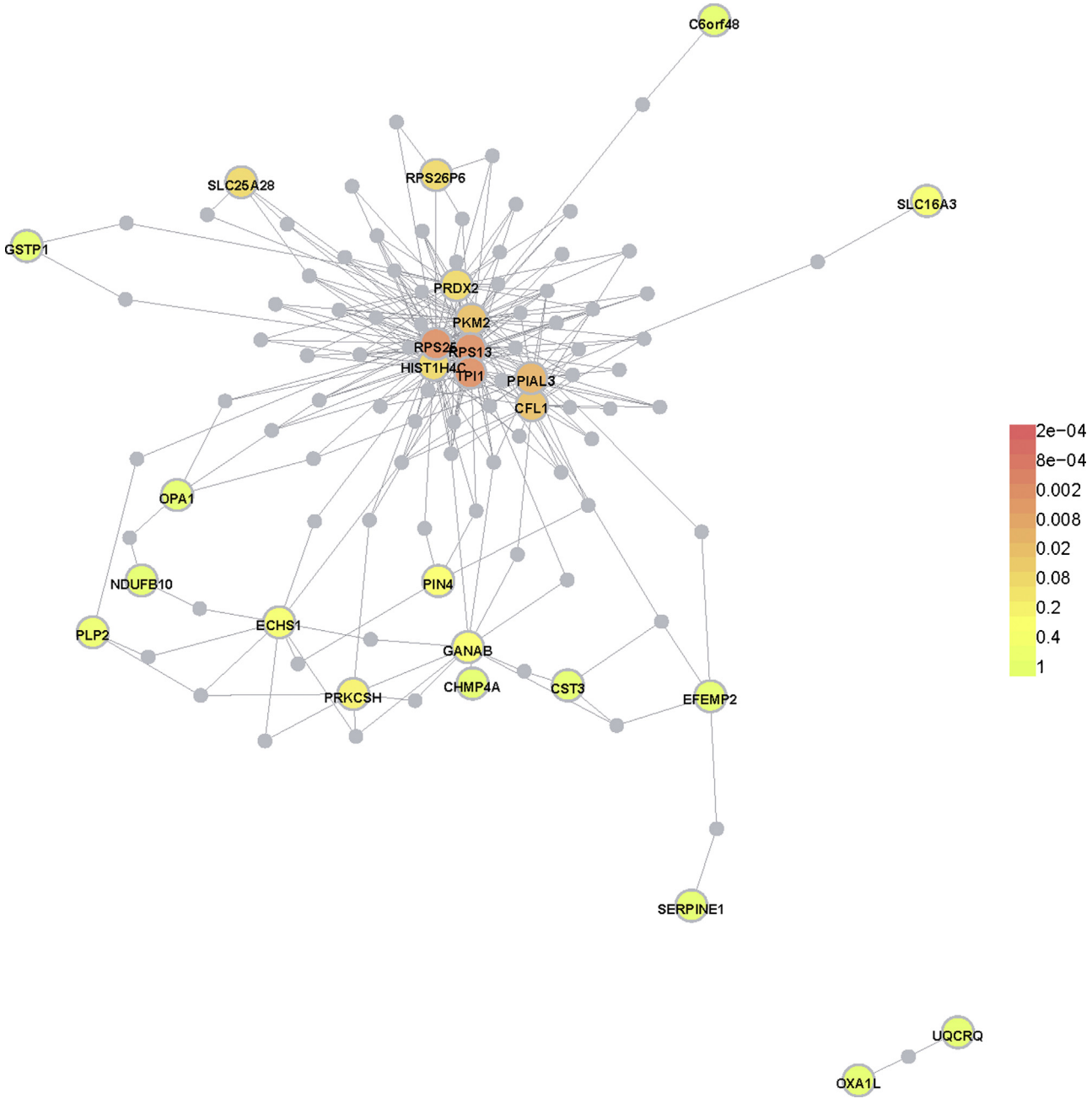

Fig. 5. Protein–protein interaction (PPI) network between OPA1 and *M. leprae* induced differentially expressed genes. We investigated the protein–protein interaction (PPI) network between OPA1 and differentially expressed genes in *M. leprae*-infected Schwann cells (detailed gene list was described in Table S2 of Guerreiro et al. [8]). The list of leprosy related genes were then set as input seed to generate direct and indirect interaction networks.

3.4. Indirect interactions between *OPA1* and M. leprae induced differentially expressed genes

The protein–protein interaction (PPI) analysis showed that there is no interaction between OPA1 and those recognized leprosy risk genes (c.f. Zhang et al. [25] and reference therein). Intriguingly, we observed indirect interactions between OPA1 protein and *M. leprae* induced differentially expressed genes (Fig. 5). The closest indirect interaction node with OPA1 was NDUFB10 (NADH dehydrogenase (ubiquinone) 1 beta subcomplex), which plays a role in mitochondrial energy production. Consistent with the decreased *OPA1* mRNA expression level in leprosy patients, the *NDUFB10* mRNA expression was also down-regulated in the *M. leprae*-infected cells [8]. However, the original expression profiling did not indicate an alteration of *OPA1* expression level in *M. leprae* infected Schwann cells, which might be explained by cell-specific effect.

4. Discussion

Epidemiological studies have shown that nutritional conditions and environment play essential role in development of leprosy [1]. At the individual level, onset and progress of the disease was highly determined by host energy production and nutritional products, because of massive gene loss and parasitic life style of *M. leprae* [5,29]. This dependence would shape host genetic susceptibility to leprosy, and there was a proposal of treating leprosy as a genetic disease [30,31]. Mitochondria play multiple roles in cellular energy supply and innate immunity [7,12]. We speculated that OP*A1*, a mitochondrial gene, may play a key role in leprosy development and progress through its involvement in energy production, mitochondrial dynamics and mtDNA maintenance.

Consistent with our expectation, we found that *OPA1* common variants conferred genetic susceptibility to leprosy, and the risk SNP rs414237 was an eQTL for *OPA1* mRNA expression. In particular, *OPA1* common variants were strongly associated with LL subtype. In our recent study, we characterized another mitochondrial gene, *LRRK2*, and confirmed its association with leprosy [20]. The involvement of *LRRK2* and *OPA1* in conferring risk for leprosy indicated the mitochondria should be well considered when we interpreted the pathogenesis of leprosy, and indicated an active role of mitochondria in antimicrobial infection. Indeed, in *M. leprae*-infected Schwann cells, mitochondria related genes were among the top list of differentially expressed signals [8]. Although we observed no significant *OPA1* expression alteration in *M. leprae*-infected Schwann cells, the PPI network analysis showed that OPA1 had indirect interactions with these *M. leprae* induced differentially expressed genes (Fig. 5). This result was further enhanced by an observation that the *OPA1* mRNA expression decreased in the IL10 treated cells [24] (Fig. 4). Concordantly, we found that the leprosy-related risk allele C of rs414237 was associated with lower *OPA1* level (Fig. 3). All these results were in good agreement with the finding that *M. leprae* infection could induce type I interferon IFN-β and IL-10, which inhibited the type II interferon IFN-γ and its downstream antimicrobial genes in LL patients [24]. Taken together, all these observations suggested that decreased *OPA1* expression level would confer risk for leprosy, especially for LL subtype.

It should be mentioned that we observed no protein–protein interaction between OPA1 and those recognized leprosy risk genes (c.f. Zhang et al. [25] and references therein). This result was not unexpected, as OPA1 might contribute to leprosy in a different pathway. The indirect interactions between OPA1 and other mitochondrial genes that were differentially expressed in Schwann's cells upon *M. leprae* infection [24] indicated an active role of mitochondria in antibacterial action.

There are limitations of the current study. First, we were unable to fresh leprosy skin lesions to explore and validate the alteration of *OPA1* mRNA and protein expression levels in leprosy. Second, it would be perfect to replicate the genetic associations of *OPA1* SNPs with leprosy in independent populations, though we had sufficient statistical power in this analysis. Third, only 7 common variants were analyzed in this study and these variants might have limited coverage for the *OPA1* gene. In addition, rare variants or non-synonymous mutations in the *OPA1* gene may be more important for leprosy susceptibility.

In short, we found that *OPA1* common variants confer risk to leprosy. This genetic effect might be enacted by the involvement of OPA1 in mitochondrial function, maintenance of mtDNA content and mitochondrial dynamics, as well as mitochondrial antimicrobial activity. Further study will be essential to confirm the results and solidify our speculation in the future.

Conflict of interest

The authors declare that they have no conflicts of interest.

Authors' contributions

Y.-G. Y., Y.-Y.L. and D.-F.Z. designed the study; Y.-L.X., D.-F.Z. and D.W. performed the experiments and read the genotyping results; Y.-Y.L. collected patient samples; D.-F.Z. and Y.-G. Y. analyzed the data and wrote the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2015.09.001.

References

[1] W.J. Britton, D.N. Lockwood, Leprosy, Lancet 363 (2004) 1209–1219.
[2] K. Eichelmann, S.E. González González, J.C. Salas-Alanis, J. Ocampo-Candiani, Leprosy. An update: definition, pathogenesis, classification, diagnosis, and treatment, Actas Dermosifiliogr. 104 (2013) 554–563.
[3] O. Parkash, Classification of leprosy into multibacillary and paucibacillary groups: an analysis, FEMS Immunol. Med. Microbiol. 55 (1) (2009) 1–5.
[4] L. Gomez-Valero, E.P. Rocha, A. Latorre, F.J. Silva, Reconstructing the ancestor of *Mycobacterium leprae*: the dynamics of gene loss and genome reduction, Genome Res. 17 (2007) 1178–1185.
[5] M. Monot, N. Honore, T. Garnier, N. Zidane, D. Sherafi, A. Paniz-Mondolfi, et al., Comparative genomic and phylogeographic analysis of *Mycobacterium leprae*, Nat. Genet. 41 (2009) 1282–1289.
[6] S.M. McWhirter, B.R. Tenoever, T. Maniatis, Connecting mitochondria and innate immunity, Cell 122 (2005) 645–647.
[7] S.E. Weinberg, L.A. Sena, N.S. Chandel, Mitochondria in the regulation of innate and adaptive immunity, Immunity 42 (2015) 406–417.
[8] L.T. Guerreiro, A.B. Robottom-Ferreira, M. Ribeiro-Alves, T.G. Toledo-Pinto, T. Rosa Brito, P.S. Rosa, et al., Gene expression profiling specifies chemokine, mitochondrial and lipid metabolism signatures in leprosy, PLoS One 8 (2013) e64748.
[9] R.J. Youle, A.M. van der Bliek, Mitochondrial fission, fusion, and stress, Science 337 (2012) 1062–1065.
[10] H. Chen, D.C. Chan, Mitochondrial dynamics – fusion, fission, movement, and mitophagy – in neurodegenerative diseases, Hum. Mol. Genet. 18 (2009) R169–R176.

[11] M.V. Alavi, N. Fuhrmann, Dominant optic atrophy, OPA1, and mitochondrial quality control: understanding mitochondrial network dynamics, Mol. Neurodegener. 8 (2013) 32.
[12] A. Olichon, E. Guillou, C. Delettre, T. Landes, L. Arnaune-Pelloquin, L.J. Emorine, et al., Mitochondrial dynamics and disease, OPA1, Biochim. Biophys. Acta 1763 (2006) 500–509.
[13] P. Amati-Bonneau, D. Milea, D. Bonneau, A. Chevrollier, M. Ferre, V. Guillet, et al., OPA1-associated disorders: phenotypes and pathophysiology, Int. J. Biochem. Cell Biol. 41 (2009) 1855–1865.
[14] G. Lenaers, P. Reynier, G. Elachouri, C. Soukkarieh, A. Olichon, P. Belenguer, et al., OPA1 functions in mitochondria and dysfunctions in optic nerve, Int. J. Biochem. Cell Biol. 41 (2009) 1866–1874.
[15] D. Wang, L.Y. Su, A.M. Zhang, Y.Y. Li, X.A. Li, L.L. Chen, et al., Mitochondrial DNA copy number, but not haplogroup, confers a genetic susceptibility to leprosy in Han Chinese from Southwest China, PLoS One 7 (2012) e38848.
[16] Y.Y. Li, X.A. Li, L. He, D. Wang, W.Y. Chen, L. Chen, et al., Trends in new leprosy case detection over 57 years (1952–2008) in Yuxi, Yunnan Province of Southwest China, Lepr. Rev. 82 (2011) 6–16.
[17] D. Wang, J.Q. Feng, Y.Y. Li, D.F. Zhang, X.A. Li, Q.W. Li, et al., Genetic variants of the MRC1 gene and the IFNG gene are associated with leprosy in Han Chinese from Southwest China, Hum. Genet. 131 (2012) 1251–1260.
[18] D.F. Zhang, X.Q. Huang, D. Wang, Y.Y. Li, Y.G. Yao, Genetic variants of complement genes ficolin-2, mannose-binding lectin and complement factor H are associated with leprosy in Han Chinese from Southwest China, Hum. Genet. 132 (2013) 629–640.
[19] D.F. Zhang, D. Wang, Y.Y. Li, Y.G. Yao, Mapping genetic variants in the CFH gene for association with leprosy in Han Chinese, Genes Immun. 15 (2014) 506–510.
[20] D. Wang, L. Xu, L. Lv, L.Y. Su, Y. Fan, D.F. Zhang, et al., Association of the LRRK2 genetic polymorphisms with leprosy in Han Chinese from Southwest China, Genes Immun. 16 (2015) 112–119.
[21] The GTEx Consortium, The Genotype-Tissue Expression (GTEx) project, Nat. Genet. 45 (2013) 580–585.
[22] A. Ramasamy, D. Trabzuni, S. Guelfi, V. Varghese, C. Smith, R. Walker, et al., Genetic variability in the regulation of gene expression in ten regions of the human brain, Nat. Neurosci. 17 (2014) 1418–1428.
[23] J.R. Bleharski, H. Li, C. Meinken, T.G. Graeber, M.T. Ochoa, M. Yamamura, et al., Use of genetic profiling in leprosy to discriminate clinical forms of the disease, Science 301 (2003) 1527–1530.
[24] R.M. Teles, T.G. Graeber, S.R. Krutzik, D. Montoya, M. Schenk, D.J. Lee, et al., Type I interferon suppresses type II interferon-triggered human anti-mycobacterial responses, Science 339 (2013) 1448–1453.
[25] D.-F. Zhang, D. Wang, Y.-Y. Li, Y.-G. Yao, Integrated analyses of leprosy susceptibility genes indicate a common autoimmune profile. Unpublished, 2015, (submitted to J. Dermatol. Sci., MS# JDS-15-399)
[26] E.J. Rossin, K. Lage, S. Raychaudhuri, R.J. Xavier, D. Tatar, Y. Benita, et al., Proteins encoded in genomic regions associated with immune-mediated disease physically interact and suggest underlying biology, PLoS Genet. 7 (2011) e1001273.
[27] S. Purcell, B. Neale, K. Todd-Brown, L. Thomas, M.A. Ferreira, D. Bender, et al., PLINK: a tool set for whole-genome association and population-based linkage analyses, Am. J. Hum. Genet. 81 (2007) 559–575.
[28] W.J. Gauderman, Sample size requirements for matched case-control studies of gene-environment interaction, Stat. Med. 21 (2002) 35–50.
[29] E. Fischer, S. De Vlas, A. Meima, D. Habbema, J. Richardus, Different mechanisms for heterogeneity in leprosy susceptibility can explain disease clustering within households, PLoS One 5 (2010) e14061.
[30] E.A. Misch, W.R. Berrington, J.C. Vary Jr., T.R. Hawn, Leprosy and the human genome, Microbiol. Mol. Biol. Rev. 74 (2010) 589–620.
[31] A. Alter, A. Grant, L. Abel, A. Alcais, E. Schurr, Leprosy as a genetic disease, Mamm. Genome 22 (2011) 19–31.

Supplementary Table S1. SNaPshot primers for genotyping7 SNPs of *OPA1*.

SNPID	Chr	Position	Primer	Sequence (5'-3')
rs9838374	3	193316705	Upper primer	AGCTAGTAAGCAGTGGAAATGTG
			Lower primer	TTATCACTTACAGAGAGGGATATCAAC
			SNP primer-Tail	GTAGTTGAAAGAAGGGTGAGCTAAA-t(gact)4
rs7646539	3	193334846	Upper primer	CACTTCCTTGCTGTGTACCTTAG
			Lower primer	TAAAGTATTAACTTGTTTAATCCTCATGAC
			SNP primer-Tail	TTCTCATCTGTAAATGGTGTTGATA-(gact)8
rs7624750	3	193334991	Upper primer	TAATACTTTAGCCCTTTATAAGAATAGTATCTG
			Lower primer	AATCTTGTCAAAGTCTGGTGCT
			SNP primer-Tail	GAGAAAATTAGAAAAGCCCTTCCTA-(gact)2
rs100774	3	193358734	Upper primer	TCACACAGTACCACACACAGAA
			Lower primer	TATGGGCAGCCTAGTGTGAT
			SNP primer-Tail	GTAACACATAACACGATTCAATCCA-(gact)7
rs9851685	3	193374964	Upper primer	TTTATGACAGAACCGAAAGGG
			Lower primer	TATACAAACAACATACCAAGCTGTC
			SNP primer-Tail	ACATATTTGATAAACTTAAAGAGGCct-(gact)11
rs414237	3	193386538	Upper primer	CTACAAGAAACAACCAAATATCAACC
			Lower primer	ATTAACACCTGCAGTAGGCTACC
			SNP primer-Tail	TGATTATTAGTAGGGCCAAGCTAGGa-ct(gact)5
rs4443116	3	193401021	Upper primer	CACTTTCTTCAAACTGAGACTGCC
			Lower primer	GAATTAGTTCTGTTGTAATATGATAAAACTTG
			SNP primer-Tail	ATGATAAAACTTGAACAGATGAAAC-ct