Infection, Genetics and Evolution 45 (2016) 105–110

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Genetic variants of the *MAVS*, *MITA* and *MFN2* genes are not associated with leprosy in Han Chinese from Southwest China

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ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form 19 August 2016
Accepted 19 August 2016
Available online 21 August 2016

Keywords:
Leprosy
MAVS
MITA
MFN2
SNP
Susceptibility

ABSTRACT

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* (*M. leprae*), which has massive genomic decay and dependence on host metabolism. Accumulating evidence showed a crucial role of mitochondria in metabolism and innate immunity. We hypothesized that the mitochondrial-related antimicrobial/antiviral immune genes *MAVS* (*mitochondrial antiviral signaling protein*), *MITA* (*mediator of IRF3 activation*) and *MFN2* (*mitofusin 2*) would confer a risk to leprosy. In this study, we performed a case-control study to analyze 11 tag and/or non-synonymous SNPs of the *MAVS*, *MITA* and *MFN2* genes in 527 leprosy patients and 583 healthy individuals, and directly sequenced the three genes in 80 leprosy patients with a family history from Yunnan, Southwest China. We found no association between these SNPs and leprosy (including its subtypes) based on the frequencies of alleles, genotypes and haplotypes between the cases and controls. There was also no enrichment of potential pathogenic variants of the three genes in leprosy patients. Our results suggested that genetic variants of the *MAVS*, *MITA* and *MFN2* genes might not affect the susceptibility to leprosy.

1. Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* (*M. leprae*) and has a long history. The pathogen *M. leprae* mainly affects human skin and peripheral nerve system, with consequent nerve damage and/or severe disabilities (Britton and Lockwood, 2004). Susceptibility to leprosy and its clinical manifestations were affected by human genetic background and immune response (Alter et al., 2011; Misch et al., 2010; Pinheiro et al., 2011). Although recent studies had reported many risk genes, including the innate and adaptive immune system genes, such as *TLR1*, *NOD2*, *VDR*, *MRC1*, *CFH*, *TNF*, and *IFNG* (Alter et al., 2011; Misch et al., 2010; Wang et al., 2012a; Zhang et al., 2013, 2016a), the exact mechanism of leprosy onset and development remains unclear.

The mitochondria can play a key role in cellular host-microbial interactions (Arnoult et al., 2009). Increasing evidence showed that mitochondria become an important host target for some bacterial pathogens (Escoll et al., 2016; Lobet et al., 2015), including *Escherichia coli* (Rudel et al., 2010; Suliman et al., 2005), *Listeria monocytogenes* (Stavru et al., 2011), *Vibrio cholerae* (Suzuki et al., 2014), *Chlamydia trachomatis* (Abdul-Sater et al., 2010), *Anaplasma phagocytophilum* (Niu et al., 2010), especially for *M. tuberculosis* (Shin et al., 2010), *M. bovis* (Carrithers et al., 2011). In our recent studies, we found that the mitochondrial genes *OPA1* (Xiang et al., 2015) and *LRRK2* (Wang et al., 2015) were associated with leprosy in Han Chinese. It is tentatively believed that the mitochondrial related antimicrobial genes would have a role in *M. leprae* infection and affect the genetic susceptibility to leprosy.

Many mitochondrial-mediated antimicrobial/antiviral immune genes had been identified and well characterized in previous studies (Cloonan and Choi, 2012; West et al., 2011). Among the list, the MAVS (mitochondrial antiviral signaling protein, also named VISA/Cardif/IPS-1) is a mitochondrial outer membrane adaptor protein and is primarily involved in antiviral response (Xu et al., 2005). MAVS was reported to be involved in bacterial-induced type I interferons (IFNs) production in response to *Legionella pneumophila* infection (Monroe et al., 2009). The MITA (mediator of IRF3 activation, also named STING/TMEM173/MPYS/ERIS) is a transmembrane protein that is mainly localized in endoplasmic reticulum and mitochondrial-associated endoplasmic reticulum membrane (MAM) (Horner et al., 2011). MITA can induce the NF-κB and IRF3 signaling, as well as the type I IFNs expression upon viral

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http://dx.doi.org/10.1016/j.meegid.2016.08.021
1567-1348/

or microbe infection (Ishikawa and Barber, 2008; Ishikawa et al., 2009; Zhong et al., 2008). Activation of the MITA signaling was required for 2′–5′ oligoadenylate synthetase-like (*OASL*) production during *M. leprae* infection (de Toledo-Pinto et al., 2016). In addition, the MITA signaling pathways are required for type I IFNs induction in response to infections of *Streptococcus pneumoniae* (Koppe et al., 2012), *Listeria monocytogenes* (Archer et al., 2014; Hansen et al., 2014; Ishikawa et al., 2009) and *Brucella abortus* (de Almeida et al., 2011), and directly mediated the ubiquitin-selective autophagy during *M. tuberculosis* infection (Collins et al., 2015; Watson et al., 2012). MITA can directly interact with MAVS, RIG-I and TBK1, and activates IRF3 and type I IFNs expression (Zhong et al., 2008). Another mitochondrial protein - MFN2 (mitofusin 2), a mediator of mitochondrial fusion, can directly interact with the MAVS-mediated type I IFNs induction (Yasukawa et al., 2009) and activates the NLRP3 inflammasomes in macrophages after viral infection (Ichinohe et al., 2013). Taken together, we hypothesized that these three mitochondrial-related and/or interacted genes *MITA*, *MAVS* and *MFN2* as possible leprosy susceptibility genes.

In this study, we analyzed 11 tag and/or non-synonymous SNPs of the *MAVS*, *MITA* and *MFN2* genes in 1110 individuals with and without leprosy from Yunnan, Southwest China. We observed no association of any SNPs with leprosy *per se* and its subtypes. Direct sequencing the exons of the three genes in 80 unrelated leprosy patients from families with a high risk of leprosy identified several potentially pathogenic (rare) variants based on program-affiliated prediction, but none of these variants were enriched in patients.

2. Materials and methods

2.1. Study subjects

This study was carried out in 1110 individuals collected from the Yuxi Prefecture, Yunnan Province of Southwest China. Among these subjects, 527 leprosy patients (onset age from 2 to 67 years, mean age: 24.7 ± 12.3 years; male/female ratio = 387/140; multibacillary (MB)/paucibacillary (PB) = 279/248) and 583 healthy controls (age from 4 to 88 years, mean age: 36.0 ± 15.5 years; male/female ratio = 365/218). These patients and controls had been described in our previous studies (Wang et al., 2012a; Xiang et al., 2015; Zhang et al., 2013). A total of 80 unrelated leprosy patients (38 lepromatous leprosy [LL] patients and 42 tuberculoid leprosy [TT] patients) with a family history of disease (each family has at least two leprosy patients) were enrolled in the Wenshan Prefecture, Yunnan Province. Written informed consents conforming to the tenets of the Declaration of Helsinki were obtained from each participant prior to the study. The institutional review board of the Kunming Institute of Zoology (KIZ) approved this study.

2.2. SNP selection and genotyping

Genomic DNA was extracted from whole blood by using the AxyPrep ™ Blood Genomic DNA Miniprep Kit (Axygen, USA). Eight tag SNPs (*MAVS*: rs6084497, rs3746660; *MITA*: rs13153461, rs7380824 [p.R293Q]; *MFN2*: rs4240897, rs2103876, rs2295281, rs4845892) were selected according to the linkage disequilibrium (LD) pattern of each gene in the international HapMap project data set (www.hapmap.ncbi.nlm.nih.gov/, Phase 3, CHB), and were genotyped in the Yuxi cohort. Non-synonymous SNP rs11554776 [p.R71H] of the *MITA* gene was reported to be associated with viral infection (Jin et al., 2011), and two non-synonymous SNPs rs7262903 [p.Q198K] and rs7269320 [p.S409F] of the *MAVS* gene were also considered. Nine of these eleven SNPs are *cis* expression quantitative trait loci (eQTLs) in leprosy-related human blood, skin or nerve tissues ($P < 1.200 \times 10^{-6}$, Table S1) according to the Genotype-Tissue Expression project data (GTEx, http://www.gtexportal.org/home/ (GTEx Consortium, 2013)). All SNPs were genotyped by the SNaPshot assay following the procedure described in our previous studies (Wang et al., 2012a; Xiang et al., 2015) (the primers were listed in Table S2) at the Kunming Biological Diversity Regional Center of Instruments, KIZ.

The 80 leprosy patients from Wenshan were sequenced for the three genes by retrieving the related data collected by the NimbleGene SeqCap EZ Human Exome v3.0 (Roche). For the exome sequencing, captured DNA libraries (2 × 150 base pairs) were constructed following the protocol of manufacture and were sequenced using the Illumina HiSeq 4000 Genome Analyzer. The alignment and variant calling were performed following the same procedure in our previous study (Zhang et al., 2016b). The potential roles of SNPs, *e.g.* affecting transcription factor binding sites or enacting other regulatory factor/mechanism, were estimated by referring to the RegulomeDB dataset (http://www.regulomedb.org/) (Boyle et al., 2012).

2.3. Interaction network analysis

To further characterize the potential involvement of the *MAVS*, *MITA* and *MFN2* genes in leprosy, we constructed the interaction network to show the potential interactions between these three genes and other related proteins by using the high-confidence protein interaction databases GeneMANIA (http://www.genemania.org/; (Warde-Farley et al., 2010)).

2.4. Statistical analysis

Power calculations were estimated by using the Quanto software (Gauderman, 2002). Cases and controls were compared according to the frequencies of genotypes and alleles. Linkage disequilibrium (LD) structure was determined by using the Haploview 4.2 (Barrett et al., 2005). Deviation from the Hardy-Weinberg equilibrium (HWE), haplotype comparisons were performed by using the PLINK v1.07 (Purcell et al., 2007). The potential pathogenicity of variants in the three genes as identified by sequencing was predicted by using an *in silico* program affiliated prediction (SIFT (Kumar et al., 2009; Ng and Henikoff, 2003), PolyPhen2 HumDiv, PolyPhen2 HumVar (Adzhubei et al., 2010), LRT (Chun and Fay, 2009) and MutationTaster (Schwarz et al., 2014)). Bonferroni corrected *P*-value was adopted for multiple comparisons. A *P*-value < 0.05 was considered as statistically significant.

3. Results

The minor allele frequency (MAF) for the 11 SNPs of the *MAVS*, *MITA* and *MFN2* genes in the 527 leprosy patients and 583 healthy subjects ranged from 7.2 to 46.7% (Table 1). The power to detect an odds ratio (OR) value as 1.6 for risk allele was expected to be from 83.4% to 91.7% (Fig. S1). SNP rs2103876 was not in Hardy–Weinberg equilibrium in controls ($P = 0.005$) and was excluded in the following analysis. None of the analyzed variants showed a positive association with leprosy *per se* or leprosy subtypes (Table 1 and Table S3). The linkage disequilibrium (LD) map of the tested SNPs in each gene was similar in the leprosy cases and controls (Fig. 1). Note that rs7380824 and rs11554776 of *MITA*, rs7262903 and rs7269320 of *MAVS* were linked together ($r^2 > 0.8$ in case and control populations), and we excluded rs7380824 and rs7269320 from the haplotype analysis. We observed no significant difference of haplotype distribution frequencies between the cases and controls from the Yuxi Prefecture (Table S4).

Similarly, we did not find any rare (allele frequency < 1%) or common variants that would confer risk to leprosy by targeted gene sequencing of 80 leprosy patients from the Wenshan Prefecture and compared to the CHB data in 1000 Genomes dataset (1000 Genomes Project Consortium et al., 2015). One missense variant in *MAVS* (rs7269320 [p.R293Q]) and two missense variants in *MITA* (rs117897081 [p.R375L] and rs7380824 [p.R293Q]) were predicted to be pathogenic according to *in silico* program affiliated prediction (Table 2). However, these variants were also present in the CHB data

Table 1
Comparison of allele frequencies of 11 SNPs of the *MAVS*, *MITA* and *MFN2* genes in 527 leprosy patients and 583 healthy controls from the Yuxi Prefecture, Yunnan, Southwest China.

SNP	Allele	HWE P (control)	MAF (control)	Leprosy *vs.* controls			MB *vs.* controls			PB *vs.* controls		
				MAF	*P*[a]	OR (95%CI)	MAF	*P*[a]	OR (95%CI)	MAF	*P*[a]	OR (95%CI)
MAVS												
rs6084497	T/C	0.855	0.345	0.384	0.056	1.184 (0.995–1.408)	0.392	0.056	1.226 (0.995–1.510)	0.375	0.247	1.138 (0.914–1.416)
rs7262903	T/G	1.000	0.075	0.071	0.716	0.942 (0.684–1.298)	0.063	0.346	0.823 (0.549–1.234)	0.081	0.701	1.079 (0.731–1.593)
rs7269320	A/G	1.000	0.072	0.066	0.558	0.906 (0.651–1.260)	0.058	0.262	0.787 (0.517–1.198)	0.075	0.838	1.043 (0.698–1.559)
rs3746660	T/C	0.363	0.251	0.233	0.363	0.907 (0.736–1.119)	0.257	0.812	1.031 (0.801–1.329)	0.208	0.080	0.783 (0.595–1.030)
MITA												
rs13153461	T/C	1.000	0.385	0.371	0.508	0.944 (0.795–1.121)	0.367	0.467	0.926 (0.751–1.141)	0.377	0.743	0.964 (0.776–1.198)
rs7380824	T/C	1.000	0.397	0.395	0.909	0.990 (0.835–1.175)	0.390	0.766	0.969 (0.788–1.192)	0.401	0.897	1.014 (0.818–1.257)
rs11554776	T/C	0.796	0.401	0.396	0.804	0.979 (0.825–1.160)	0.390	0.660	0.955 (0.776–1.174)	0.403	0.956	1.006 (0.812–1.247)
MFN2												
rs4240897	G/A	0.803	0.467	0.464	0.874	0.987 (0.835–1.166)	0.451	0.539	0.938 (0.766–1.150)	0.478	0.693	1.043 (0.845–1.288)
rs2103876	C/T	0.005	0.339	0.301	0.051	0.836 (0.699–1.001)	0.309	0.206	0.869 (0.699–1.080)	0.292	0.057	0.801 (0.637–1.007)
rs2295281	A/G	0.479	0.397	0.412	0.464	1.066 (0.899–1.263)	0.444	0.063	1.214 (0.989–1.489)	0.377	0.432	0.917 (0.738–1.139)
rs4845892	C/A	0.322	0.360	0.346	0.481	0.939 (0.789–1.118)	0.340	0.408	0.914 (0.739–1.131)	0.353	0.770	0.968 (0.777–1.206)

HWE: Hardy–Weinberg equilibrium; MAF: minor allele frequency; MB: multibacillary leprosy; PB: paucibacillary leprosy; OR: odds ratio; 95%CI: 95% confidence interval.
[a] *P* values were calculated by using the Chi-square test.

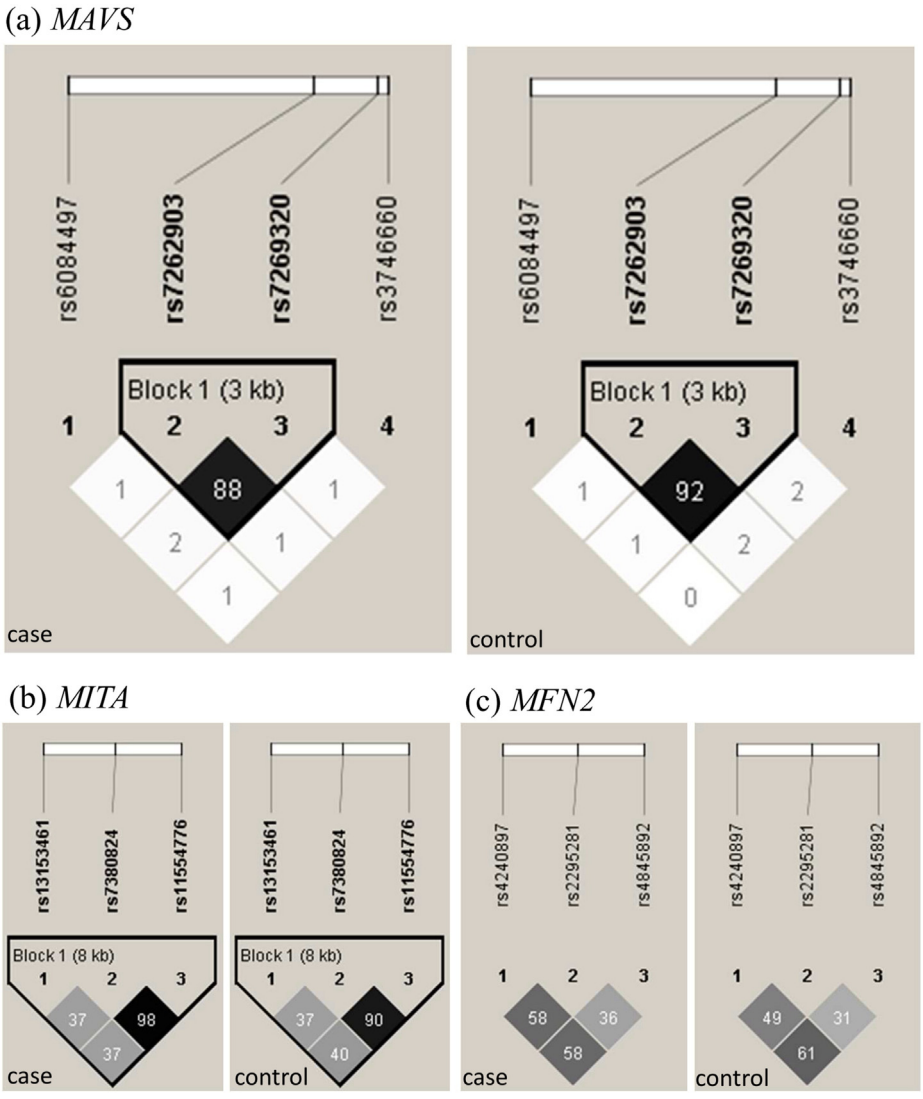

Fig. 1. The linkage disequilibrium (LD) maps of the analyzed SNPs in the *MAVS* (a), *MITA* (b) and *MFN2* (c) genes. Black squares represent high LD as measured by r^2, gradually coloring down to white squares of low LD. The individual square showed the r^2 value for each SNP pair (r^2 value is multiplied by 100).

Table 2
The list of SNPs in the exon and flank regions in the *MITA*, *MAVS* and *MFN2* genes in 80 leprosy patients as revealed by using the next-generation sequencing technology.

Chr.	Position	SNP ID[a]	Gene	Function	Ref.	Alt.	Residue change	Damaging predication[c]	Allele counts in 80 leprosy patients	Allele counts in the CHB	*P*-value[d]	OR	RegulomeDB Score[b]
chr20	3838237	rs8122961	*MAVS*	Intron	A	G	–	–	1/160	2/206	1.000	0.642	5
chr20	3838441	rs17857295	*MAVS*	Missense	C	G	p.Q93E	Tolerated	83/160	112/206	0.673	0.905	5
chr20	3838505	rs200848451	*MAVS*	Intron	C	T	–	–	1/160	1/206	1.000	1.289	5
chr20	3841980	.	*MAVS*	Synonymous	G	T	p.R98	–	1/160	NA	NA	NA	–
chr20	3842984	rs2326369	*MAVS*	Synonymous	C	T	p.D183	–	39/160	41/206	0.311	1.297	5
chr20	3843027	**rs7262903**	*MAVS*	Missense	C	A	p.Q198K	Tolerated	8/160	18/206	0.219	0.550	5
chr20	3845179	rs138598490	*MAVS*	Missense	C	T	p.T301I	Tolerated	5/160	4/206	0.512	1.629	5
chr20	3846397	**rs7269320**	*MAVS*	Missense	C	T	p.S409F	Damaging	9/160	16/206	0.532	0.708	5
chr20	3846479	rs200985651	*MAVS*	Synonymous	C	T	p.F436	–	4/160	1/206	0.172	5.256	5
chr20	3846753	rs201222623	*MAVS*	Missense	G	C	p.V528L	Tolerated	2/160	1/206	0.583	2.595	5
chr20	3847325	rs3746662	*MAVS*	3′UTR	A	C	–	–	9/160	16/206	0.532	0.708	2b
chr20	3847635	rs76557664	*MAVS*	3′UTR	G	A	–	–	3/160	2/206	0.657	1.949	3a
chr20	3851951	rs6515831	*MAVS*	3′UTR	T	C	–	–	49/160	52/206	0.289	1.307	5
chr5	138855862	rs117897081	*MITA*	Missense	C	A	p.R375L	Damaging	2/160	3/206	1.000	0.857	5
chr5	138856982	**rs7380824**	*MITA*	Missense	C	T	p.R293Q	Damaging	67/160	76/206	0.388	1.232	5
chr5	138857919	rs1131769	*MITA*	Missense	T	C	p.H232R	Tolerated	136/160	186/206	0.145	0.609	5
chr5	138857925	rs78233829	*MITA*	Missense	C	G	p.G230A	Tolerated	69/160	76/206	0.238	1.297	5
chr5	138861078	**rs11554776**	*MITA*	Missense	C	T	p.R71H	Tolerated	68/160	73/206	0.194	1.347	4
chr5	138861146	rs7447927	*MITA*	Synonymous	C	G	p.V48	–	54/160	89/206	0.068	0.670	4
chr1	12049375	rs78841746	*MFN2*	Synonymous	C	A	p.I50	–	7/160	10/206	1.000	0.897	5
chr1	12049390	rs77458527	*MFN2*	Synonymous	C	T	p.T55	–	7/160	12/206	0.638	0.740	5
chr1	12056,309	rs78814413	*MFN2*	Synonymous	A	T	p.V136	–	7/160	12/206	0.638	0.740	4
chr1	12057321	rs76051569	*MFN2*	Intron	C	T	–	–	8/160	12/206	0.819	0.851	4
chr1	12058802	rs41278626	*MFN2*	Intron	T	C	–	–	29/160	31/206	0.478	1.250	5
chr1	12062017	rs6680984	*MFN2*	Intron	T	C	–	–	21/160	19/206	0.242	1.487	2b
chr1	12062205	rs2236057	*MFN2*	Intron	A	G	–	–	113/160	126/206	0.061	1.527	4
chr1	12064217	rs74453521	*MFN2*	Intron	C	T	–	–	1/160	1/206	1.000	1.289	5
chr1	12064817	rs78503576	*MFN2*	Intron	G	A	–	–	2/160	5/206	0.475	0.509	2b
chr1	12065841	rs1042837	*MFN2*	Synonymous	C	T	p.S523	–	28/160	31/206	0.568	1.197	4
chr1	12066660	.	*MFN2*	Synonymous	C	T	p.L594	–	1/160	NA	NA	NA	–
chr1	12069798	rs77262016	*MFN2*	Intron	T	C	–	–	27/160	31/206	0.667	1.146	5
chr1	12071680	rs1042842	*MFN2*	3′UTR	A	G	–	–	111/160	129/206	0.185	1.352	5

Chr, Chromosome; Ref, Reference allele; Alt, Alternate allele; CHB, 103 Han Chinese from Beijing in the 1000 Genomes dataset; OR, Odds ratio; NA, no data available.

[a] Among 11 SNPs genotyped in this study, four missense variants were captured in the following targeted gene sequencing and were marked in bold.

[b] The RegulomeDB Score was taken from http://www.regulomedb.org/ (Boyle et al., 2012): 2b, TF binding + any motif + DNase Footprint + DNase peak; 3a, TF binding + any motif + DNase peak; 4, TF binding + DNase peak; 5, TF binding or DNase peak.

[c] Missense variants are rated as damaging when at least two of five prediction algorithms (SIFT (Kumar et al., 2009; Ng and Henikoff, 2003), PolyPhen2 HumDiv, PolyPhen2 HumVar (Adzhubei et al., 2010), LRT (Chun and Fay, 2009) and MutationTaster (Schwarz et al., 2014)) suggesting a potential deleterious effect, otherwise the variants are rated as tolerated.

[d] *P*-values were calculated by using the Fisher's exact test.

of the 1000 Genomes datasets and there was no statistically difference between these datasets. We also used the RegulomeDB database (Boyle et al., 2012) to annotate the analyzed SNPs. The three damaging SNPs (rs7269320 of *MAVS*, rs117897081 and rs7380824 of *MITA*) showed a DNaseI hypersensitivity (Table 2). Protein interaction network showed that MAVS could be physically interacted with MITA and MFN2 (Fig. 2).

4. Discussion

Mitochondria are crucial organelles for cellular energy supply, regulation of apoptotic signals and autophagy, and defenses against pathogenic microbe invasion (Mayer and Oberbauer, 2003; Okamoto and Kondo-Okamoto, 2012; Xu et al., 2005). Accumulating evidence showed that the host mitochondria might play important roles in *M. leprae* infection. First, the genome of *M. leprae* is extremely eroded, which leads to a dependence on host energy metabolites and nutritional products for survival (Cole et al., 2001; Gómez-Valero et al., 2007; Monot et al., 2009). Second, different expression profile of mitochondrial genes has been observed in nerve biopsies from patients with and without leprosy (Guerreiro et al., 2013). Third, a significantly increased mtDNA copy number was observed in lepromatous leprosy patients compared with controls (Wang et al., 2012b). Fourth, mitochondrial related genes *LRKK2* and *OPA1* conferred leprosy susceptibility according to our previous studies (Wang et al., 2015; Xiang et al., 2015).

As mitochondria play a crucial role in innate immune signaling against viral and bacterial infections (West et al., 2011), it is would be rewarding to check whether these mitochondrial related genes that are actively involved in innate immunity would affect genetic susceptibility to leprosy. Indeed, there were reports that genetic variants in the pattern recognition receptors (PRRs), such as MCR1 (belongs to C-type lectin receptors), TLR1, TLR2, TLR 4 (belong to Toll-like receptors) and NOD2 (belongs to nuclear oligomerization domain (NOD)-like receptors), were associated with leprosy (Alter et al., 2011; Misch et al., 2010). In this study, we focused on three mitochondrial-mediated antimicrobial/antiviral immune genes (*MAVS*, *MITA* and *MFN2*) that were physically interacted (Fig. 2). We speculated that genetic variants in these genes might affect host resistance to *M. leprae* infection and/or clinical presentations. By screening 11 SNPs (including 8 tag SNPs and 3 non-synonymous SNPs) of the three genes in 527 leprosy and 583 healthy individuals from Yuxi, and targeted gene sequencing for 80 leprosy patients with a family history from Wenshan, we found no evidence for an association of these variants with leprosy. Note that three non-synonymous variants in *MITA* and *MAVS* were predicted to be (potentially) damaging according to the program-affiliated prediction (Table 2). However, a comparison with the available CHB data ($n = 103$) in the 1000 Genomes data (1000 Genomes Project Consortium et al., 2015) revealed no essential difference between the leprosy population ($n = 80$) and the CHB data. Further study with a large sample size and functional characterization are needed to confirm the role of these potentially damaging variants in leprosy. Taken together, our results indicated that genetic variants in the three physically interacted *MITA*, *MAVS* and *MFN2* genes were not significantly associated with leprosy in Han Chinese from Southwest China.

The current study, however, could not exclude a possibility that other mitochondrial-related antimicrobial/antiviral innate immunity

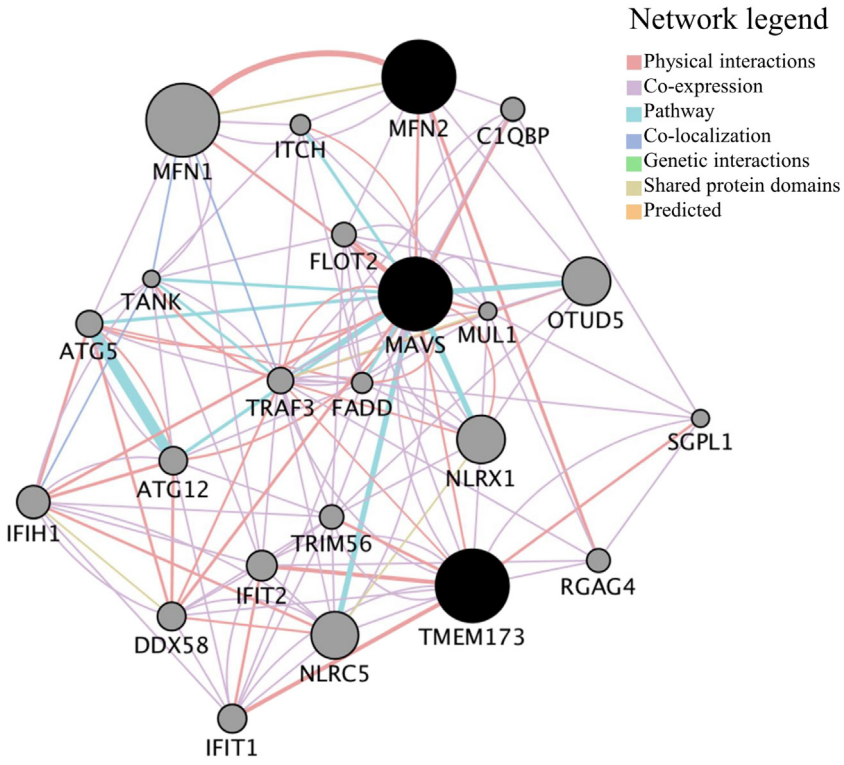

Fig. 2. Protein interaction network of MAVS, MITA (TMEM173) and NFN2 by using the GeneMANIA prediction server (http://www.genemania.org/) (Warde-Farley et al., 2010).

pathway genes might have a role in host immune reactions and clinical presentations after *M. leprae* infection. Further study with large number of samples and more SNPs in each gene might be necessary to validate the current result.

Conflict of interest

The authors declare no conflict of interests.

Acknowledgement

This study was supported by the National Natural Science Foundation of China (31271346 and 81573034) and Yunnan Province (2014FB177). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meegid.2016.08.021.

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