

ORIGINAL ARTICLE

Association of the *LRRK2* genetic polymorphisms with leprosy in Han Chinese from Southwest ChinaD Wang¹, L Xu^{1,2}, L Lv^{1,2}, L-Y Su^{1,2}, Y Fan^{1,2}, D-F Zhang^{1,2}, R Bi^{1,2}, D Yu¹, W Zhang¹, X-A Li³, Y-Y Li⁴ and Y-G Yao¹

Leprosy is a chronic infectious and neurological disease that is caused by infection of *Mycobacterium leprae* (*M. leprae*). A recent genome-wide association study indicated a suggestive association of *LRRK2* genetic variant rs1873613 with leprosy in Chinese population. To validate this association and further identify potential causal variants of *LRRK2* with leprosy, we genotyped 13 *LRRK2* variants in 548 leprosy patients and 1078 healthy individuals from Yunnan Province and (re-)analyzed 3225 Han Chinese across China. Variants rs1427267, rs3761863, rs1873613, rs732374 and rs7298930 were significantly associated with leprosy *per se* and/or paucibacillary leprosy (PB). Haplotype A-G-A-C-A was significantly associated with leprosy *per se* ($P=0.018$) and PB ($P=0.020$). Overexpression of the protective allele (Thr2397) of rs3761863 in HEK293 cells led to a significantly increased nuclear factor of activated T-cells' activity compared with allele Met2397 after lipopolysaccharides stimulation. Allele Thr2397 could attenuate 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced autophagic activity in U251 cells. These data suggest that the protective effect of *LRRK2* variant p.M2397T on leprosy might be mediated by increasing immune response and decreasing neurotoxicity after *M. leprae* loading. Our findings confirm that *LRRK2* is a susceptible gene to leprosy in Han Chinese population.

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INTRODUCTION

Leprosy is a chronic infectious and neurological disease that is caused by *Mycobacterium leprae* (*M. leprae*) infection. The bacterium can affect human peripheral nerve and skin with consequent nerve damage and/or disabilities.¹ Over decades, the prevalence of leprosy has been reduced gradually; however, it still affects approximately 200 000 people annually according to the World Health Organization report.² Up to now, leprosy remains a public health problem, and the molecular underpinnings of *M. leprae* infection and leprosy onset have not been fully elucidated.

Development of leprosy after *M. leprae* infection depends on host genetic background. Among those people who were potentially exposed to *M. leprae*, <5% of them develop disease.^{3,4} In addition, most of leprosy patients living in endemic areas always have poor nutrition conditions, suggesting that environment and host genetic background have key roles in leprosy susceptibility. During the past years, accumulating evidence showed that host genetic background can influence the infection of *M. leprae* and its clinical manifestation, such as *TLR1*,⁵ *HLA-DRB1*,⁶ *NOD2*,^{6,7} *ILs*,^{8,9} *TNF*,¹⁰ *VDR*,¹¹ *MRC1*,¹² *IFNG*,^{13,14} *FCN2*, *MBL2* and *CFH*.^{15,16} These innate and adaptive immune response relevant genes had a complex interplay with pathogen and host during disease onset.

Human *leucine-rich repeat kinase 2* (*LRRK2*, OMIM 609007) gene encodes dardarin (PARK8), which is involved in the interferon-gamma response and host response to pathogens.¹⁷ This gene is located in the 12q12 chromosomal region and contains 51 exons. *LRRK2* is a multi-domain protein with 2527 amino acids, includes

enzymatic activity domains (a GTPase and a kinase domain), a leucine-rich repeat (LRR) domain and a C-terminal WD40 repeat domain.¹⁸ Recently, single-nucleotide polymorphism (SNP) rs1873613 of the *LRRK2* gene was identified as a risk factor for leprosy in Han Chinese in a genome-wide association study (GWAS).⁶ In previous studies, *LRRK2* mutations were recognized as the most common cause of hereditary Parkinsonism,^{19,20} which is a neurodegenerative movement disorder characterized by the presence of intracytoplasmic lewy bodies and lewy neuritis.²¹ Genetic polymorphisms of *LRRK2* were also reported to be associated with susceptibility to Crohn's disease²² and cancer.²³ In addition, *LRRK2* might have a key role in autophagy,²⁴ and overexpression of mutant *LRRK2* in human neuroblastoma cells caused autophagy activation and significantly decreased neurite length.²⁵ *LRRK2* could act as a regulator to control the nuclear factor of activated T-cells (NFAT) activity in the immune system²⁶ and regulated microglial inflammatory responses.²⁷

In this study, we genotyped 13 SNPs of the *LRRK2* gene (including the GWAS hit rs1873613)⁶ in 527 Han Chinese with leprosy and 1078 healthy subjects from Southwest China to investigate whether this gene is involved in leprosy and to identify the potential causal variant(s). We found that five variants (rs1427267, rs3761863 (p.M2397T), rs1873613, rs732374 and rs7298930) were significantly associated with leprosy *per se* and/or paucibacillary leprosy (PB) patients. Functional characterization showed that polymorphism p.M2397T (rs3761863) can increase the NFAT activity after lipopolysaccharides (LPS) stimulation in HEK293 cells and attenuate 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced autophagic activity in

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U251 cells. Our results confirmed that *LRRK2* is a susceptibility gene to leprosy in Han Chinese population.

RESULTS

Association of *LRRK2* SNPs and haplotypes with leprosy *per se* and PB patients

The minor allele frequency (MAF) for SNPs (except for six rare/pathogenic SNPs, which had a frequency of 0.0–0.04) analyzed in this study ranged from 28.2% to 49.4% (Tables 1 and 2). Considering a MAF of 0.282 as observed in our samples, the power to detect an odds ratio (OR) value as low as 1.6 for risk allele was expected to be >97% while the power for MAF of 0.494 was expected to be >95%.

With the exception of rs732374 ($P=0.010$) and rs7298930 ($P=0.027$), none of the analyzed tag SNPs and the reported GWAS hit SNP had a deviation from Hardy–Weinberg equilibrium (HWE) in the control group. Note that we also observed a marginally significant HWE P -value for rs732374 ($P=0.05$) in Han Chinese from Eastern China in our previous study.²⁸ The deviation from HWE was unlikely caused by genotyping errors, as we found no problem during the double check of the original genotyping data, and we could consistently validate the genotyping results by sequencing 2% randomly selected individuals. There was no significant difference ($P>0.05$) when we compared genotype frequency of these two SNPs in the control population with HapMap CHB data (82 Han Chinese) or with 3225 Han Chinese reported in our recent studies.^{28,29} The reported pathogenic LRRK2 mutations were also found in some subjects, albeit with a very low frequency (Table 3).

We failed to validate the association between the GWAS-reported SNP rs1873613 with leprosy *per se*. However, when we subdivided leprosy into PB and multibacillary leprosy (MB) populations according to their clinical expression, genotype GG of rs1873613 had a marginal significant difference ($P=0.045$) between the PB group and control group, and allele G had a lower frequency in the PB group (26.4%) compared with the control group (31.8%; OR=0.769, $P=0.020$). Among the six tag SNPs, three of them appeared to have a protective role against leprosy *per se* (rs3761863, genotype GG: OR=0.713, $P=0.038$; rs732374, genotype AA: OR=0.651, $P=0.020$; and rs7298930, genotype CC: OR=0.647, $P=0.005$) and the PB group (rs3761863, genotype GG: OR=0.584, $P=0.014$; rs732374, genotype AA: OR=0.592, $P=0.026$; and rs7298930, genotype CC: OR=0.558, $P=0.005$) at the genotypic level. However, tag SNP rs1427267 conferred a risk to leprosy *per se* (genotype AA: OR=1.403, $P=0.026$; genotype AG: OR=1.394, $P=0.015$), MB group (genotype AG: OR=1.512, $P=0.015$) and the PB group (genotype AA: OR=1.585, $P=0.020$) at the genotypic level. This pattern was robust when these SNPs were analyzed at the allelic level. SNPs rs732374 and rs7298930 appeared to have a protective role against leprosy *per se* (A allele of rs732374: OR=0.804, $P=0.008$; and C allele of rs7298930: OR=0.811, $P=0.006$) and the PB group (A allele of rs732374: OR=0.767, $P=0.018$; and C allele of rs7298930: OR=0.739, $P=0.003$). SNP rs3761863 was associated with the PB group (G allele: OR=0.775, $P=0.013$). Moreover, SNP rs1427267 conferred a risk to leprosy *per se* (A allele: OR=1.179, $P=0.030$) and the PB group (A allele: OR=1.274, $P=0.016$). When we aggregated the Han Chinese samples that were reported in our previous studies^{28,29} and this study ($n=4303$) together to increase the statistical power, the above associations with leprosy *per se* were further confirmed at the genotypic level (Table 2).

We performed linkage disequilibrium analysis to test whether these tag SNPs in the case and control groups were linked together. As shown in Figure 1, both populations had a similar linkage disequilibrium structure, and this result was consistent with the observation in our recent studies of Han Chinese from

other parts of China.^{28,29} Note that rs1427267 was linked with rs7298930 ($r^2=0.81$ in case population and $r^2=0.83$ in control population), so we excluded SNP rs7298930 in the following analysis.

A total of 22 haplotypes in the cases and 23 haplotypes in the controls (order of SNPs: rs1873613–rs732374–rs1427267–rs7307310–rs3761863) were reconstructed based on five tag SNPs. Among them, seven haplotypes were observed as the most common haplotype. The overall haplotype test was performed to show the global difference in haplotype frequencies between the case (also grouped into PB and MB) and control populations. There were significant differences for the two groups (Chi-square test: case vs control, $P=0.031$; MB vs control, $P=0.221$; PB vs control, $P=0.033$). In particular, haplotype A-G-A-C-A (consists of non-protective allele of each SNPs) posed a risk effect on leprosy *per se* (OR=1.282, $P=0.018$) and the PB group (OR=1.369, $P=0.020$). Haplotype A-G-G-C-A had a marginally significant protective effect on leprosy *per se* (OR=0.685, $P=0.046$; Table 4).

We also measured the serological difference of LRRK2 between cases and controls. However, we found no significant difference of serum LRRK2 concentrations between the cases and controls (t -test, $P>0.05$; Supplementary Figure S1).

LRRK2 allele Thr2397 increases NFAT activity in HEK-293 cell stimulated by LPS

To understand the underpinning of the protective role of SNP rs3761863 (p.Met2397Thr) on PB leprosy, we performed an evolutionary comparison to evaluate the conservation of this position in vertebrates and characterized different alleles of variant p.M2397T on NFAT activity in HEK293 cells treated with and without LPS. LRRK2 variant p.M2397T was relatively conserved among the primates and rodentia (Supplementary Figure S2). We made a variety of constructs, including full-length LRRK2 (wild type (WT)) and variants bearing pathogenic mutations p.G2019S, p.M2397T and deletion of WD40 domain (Δ WD40). As shown in Figure 2, after 12 h treatment with LPS ($1 \mu\text{g ml}^{-1}$), the reported Parkinson's disease (PD)-associated pathogenic mutation p.G2019S and the truncated LRRK2 mutant Δ WD40 had no observed effect on regulating NFAT activity compared with the WT LRRK2. In contrast, variant p.M2397T significantly increased NFAT activity (t -test, $P<0.05$) upon LPS treatment compared with the WT.

Stable expression of LRRK2 allele Thr2397 attenuates MPTP-induced autophagy in U251 cells

MPTP is a neurotoxic drug that induces autophagy in neuroblastoma cells.³⁰ We tested whether the stable expression of LRRK2 p.M2397T variant could regulate autophagy in U251 cells with and without MPTP treatment. Consistent with previous study that LRRK2 p.G2019S variant could cause a high-level basal autophagy in cultured fibroblasts,³¹ we found that p.G2019S and p.M2397T of LRRK2 had a similar level of basal autophagy based on the quantification of LC3B-I to LC3B-II conversion. However, after treatment with MPTP ($500 \mu\text{M}$), the control (without transfection), PLVX (vector), WT LRRK2 and p.G2019S variant groups had a higher autophagic activity than those without MPTP treatment. Variants Thr2397 and Δ WD40 of LRRK2 salvaged autophagy in cells after MPTP treatment (Figure 2).

DISCUSSION

Recently, Zhang *et al.*⁶ carried out the first leprosy GWAS in Han Chinese population, and rs1873613 of the *LRRK2* gene was identified to be associated with leprosy. The association between this variant and leprosy was said to be validated in Indian patients.³² Previous investigations also showed that overexpression of mutant LRRK2 (for example, p.G2019S, p.R1441C, p.I2020T, p.Y1699C) in cultured cells caused neuron damage,³³ autophagy,³⁴

Table 1. Genotype and allele frequencies of 7 LRRK2 SNPs in 548 leprosy patients and 1078 healthy controls from Yunnan, China

SNP	Genotype	All cases vs controls			MB vs control			PB vs control			
		No. (%)	No. (%)	P-value ^a	OR (95%CI) ^a	No. (%)	OR (95%CI) ^a	P-value ^a	No. (%)	OR (95%CI) ^a	P-value ^a
rs1427267	GG	291 (27.0)	111 (20.3)	—	Reference	59 (19.9)	Reference	—	52 (20.6)	Reference	—
	AG	510 (47.3)	282 (51.5)	0.015	1.394 (1.068–1.820)	164 (55.4)	1.512 (1.083–2.113)	0.015	118 (46.8)	1.233 (0.860–1.768)	0.255
	AA	277 (25.7)	155 (28.3)	0.026	1.403 (1.041–1.892)	73 (24.7)	1.242 (0.846–1.825)	0.269	82 (32.5)	1.585 (1.074–2.338)	0.020
rs3761863	G allele	1092 (50.6)	504 (46.0)	—	Reference	282 (47.6)	Reference	—	222 (44.0)	Reference	—
	A allele	1064 (49.4)	592 (54.0)	0.030	1.179 (1.016–1.366)	310 (52.4)	1.103 (0.917–1.326)	0.300	282 (56.0)	1.274 (1.046–1.553)	0.016
	AA	319 (29.6)	178 (32.5)	—	Reference	86 (29.1)	Reference	—	92 (36.5)	Reference	—
rs1873613	AG	547 (50.7)	288 (52.6)	0.603	0.939 (0.742–1.189)	163 (55.1)	1.085 (0.804–1.463)	0.594	125 (49.6)	0.780 (0.574–1.060)	0.112
	GG	212 (19.7)	82 (15.0)	0.038	0.713 (0.519–0.981)	47 (15.9)	0.852 (0.571–1.271)	0.431	35 (13.9)	0.584 (0.380–0.899)	0.014
	A allele	1185 (55.0)	644 (58.8)	0.059	0.866 (0.745–1.005)	335 (56.6)	Reference	—	305 (61.0)	Reference	—
rs1873613	G allele	971 (45.0)	452 (41.2)	—	Reference	257 (43.4)	0.948 (0.787–1.141)	0.573	195 (39.0)	0.775 (0.634–0.947)	0.013
	AA	508 (47.1)	268 (48.9)	—	Reference	131 (44.3)	Reference	—	137 (54.4)	Reference	—
	AG	454 (42.1)	235 (42.9)	0.967	0.995 (0.799–1.240)	138 (46.6)	1.187 (0.903–1.561)	0.220	97 (38.5)	0.792 (0.591–1.061)	0.119
rs732374	GG	116 (10.8)	45 (8.2)	0.123	0.741 (0.507–1.084)	27 (9.1)	0.920 (0.574–1.465)	0.725	18 (7.1)	0.585 (0.338–0.988)	0.045
	A allele	1470 (68.2)	771 (70.3)	—	Reference	400 (67.6)	Reference	—	371 (73.6)	Reference	—
	G allele	686 (31.8)	325 (29.7)	0.243	0.909 (0.774–1.067)	192 (32.4)	1.037 (0.852–1.263)	0.714	133 (26.4)	0.769 (0.617–0.959)	0.020
rs732374	GG	499 (46.5)	284 (52.6)	—	Reference	149 (51.4)	Reference	—	135 (54.0)	Reference	—
	AG	437 (40.8)	207 (38.3)	0.100	0.829 (0.663–1.037)	113 (39.0)	0.864 (0.653–1.142)	0.305	94 (37.6)	0.796 (0.592–1.071)	0.132
	AA	136 (12.7)	49 (9.1)	0.020	0.651 (0.453–0.936)	28 (9.7)	0.715 (0.455–1.122)	0.145	21 (8.4)	0.592 (0.358–0.978)	0.041
rs7307310	G allele	1435 (66.9)	775 (71.8)	—	Reference	411 (70.9)	Reference	—	364 (72.8)	Reference	—
	A allele	709 (33.1)	305 (28.2)	0.008	0.804 (0.683–0.945)	169 (29.1)	0.843 (0.688–1.032)	0.098	136 (27.2)	0.767 (0.616–0.955)	0.018
	CC	489 (46.1)	231 (42.9)	—	Reference	131 (45.3)	Reference	—	100 (40.0)	Reference	—
rs7298930	CT	461 (43.4)	238 (44.2)	0.573	1.067 (0.852–1.336)	121 (41.9)	0.958 (0.723–1.269)	0.763	117 (46.8)	1.191 (0.883–1.607)	0.252
	TT	111 (10.5)	70 (13.0)	0.155	1.283 (0.910–1.808)	37 (12.8)	0.219 (0.797–1.863)	0.361	33 (13.2)	0.384 (0.883–2.169)	0.156
	C allele	1439 (67.8)	700 (64.9)	—	Reference	383 (66.3)	Reference	—	317 (63.4)	Reference	—
rs7298930	T allele	683 (32.2)	378 (35.1)	0.181	1.113 (0.951–1.303)	195 (33.7)	1.053 (0.865–1.283)	0.608	183 (36.6)	1.181 (0.961–1.451)	0.114
	AA	308 (28.8)	179 (33.1)	—	Reference	87 (29.9)	Reference	—	92 (36.8)	Reference	—
	AC	498 (46.5)	267 (49.4)	0.477	0.916 (0.721–1.166)	151 (51.9)	1.071 (0.791–1.450)	0.658	116 (46.4)	0.763 (0.558–1.042)	0.089
rs4473003	CC	264 (24.7)	95 (17.6)	0.005	0.647 (0.478–0.876)	53 (18.2)	0.746 (0.508–1.094)	0.133	42 (16.8)	0.558 (0.373–0.837)	0.005
	A allele	1114 (52.1)	625 (57.8)	—	Reference	325 (55.8)	Reference	—	300 (60.0)	Reference	—
	C allele	1026 (47.9)	457 (42.2)	0.006	0.811 (0.698–0.942)	257 (44.2)	0.879 (0.730–1.060)	0.176	200 (40.0)	0.739 (0.605–0.903)	0.003
rs4473003	TT	487 (45.6)	232 (43.0)	—	Reference	130 (44.8)	Reference	—	102 (40.8)	Reference	—
	CT	465 (43.6)	242 (44.8)	0.552	1.070 (0.856–1.339)	126 (43.4)	1.001 (0.757–1.324)	0.993	116 (46.4)	1.146 (0.850–1.545)	0.370
	CC	115 (10.8)	66 (12.2)	0.392	1.163 (0.823–1.644)	34 (11.7)	1.085 (0.703–1.674)	0.713	32 (12.8)	1.273 (0.811–1.999)	0.295
rs4473003	T allele	1439 (67.4)	706 (65.4)	—	Reference	386 (66.6)	Reference	—	320 (64.0)	Reference	—
	C allele	695 (32.6)	374 (34.6)	0.358	1.076 (0.919–1.259)	194 (33.4)	1.030 (0.845–1.253)	0.772	180 (36.0)	1.134 (0.923–1.395)	0.233

Abbreviations: CI, confidence interval; MB, multibacillary leprosy; OR, odds ratio; PB, paucibacillary leprosy; SNP, single-nucleotide polymorphism. ^aAll data were calculated by using the unconditional logistic regression, with an adjustment for sex. P-values < 0.05 were marked in bold.

Table 2. Comparison of allele and genotype frequencies of 5 LRRK2 SNPs in 548 leprosy patients and 1078 healthy controls from Yuxi Prefecture of Yunnan Province and in 4303 pooled Han Chinese across China

SNP ID ^a	Allele/genotype	No. of pooled samples ^b	Leprosy per se			MB			PB		
			P-value ^c (Yuxi)	P-value ^c (pooled)	OR (pooled)	P-value ^c (Yuxi)	P-value ^c (pooled)	OR (pooled)	P-value ^c (Yuxi)	P-value ^c (pooled)	OR (pooled)
rs732374	A/G	2793/5797	0.005	0.004	0.817	0.072	0.092	0.853	0.013	0.016	0.780
rs4473003	AA/AG/GG	497/1799/1999	0.026	0.020	—	0.218	0.249	—	0.056	0.060	—
	C/T	2873/5711	0.241	0.447	1.053	0.689	0.992	0.999	0.150	0.256	1.115
rs7298930	CC/CT/TT	484/1905/1903	0.506	0.740	—	0.896	0.943	—	0.354	0.524	—
	C/A	3981/4609	0.002	0.010	0.846	0.105	0.305	0.916	0.001	0.005	0.770
rs7307310	CC/AC/AA	944/2093/1258	0.004	0.034	—	0.062	0.306	—	0.008	0.020	—
	T/C	2820/5746	0.110	0.159	1.100	0.498	0.682	1.037	0.067	0.095	1.174
rs3761863	TT/CT/CC	462/1896/1925	0.248	0.277	—	0.527	0.504	—	0.187	0.246	—
	G/A	3861/4745	0.039	0.023	0.863	0.476	0.486	0.942	0.010	0.007	0.776
	GG/AG/AA	857/2147/1299	0.059	0.021	—	0.285	0.156	—	0.032	0.023	—

Abbreviations: MB, multibacillary leprosy; OR, odds ratio; PB, paucibacillary leprosy; SNP, single-nucleotide polymorphism. ^aSNPs rs1873613 and 1427267 were not genotyped in Sichuan, Hunan and Shanghai populations and were excluded from the analysis. ^bPooled Han Chinese without leprosy (Pooled) included reported data from Sichuan Province (N = 776), Hunan Province (N = 966) and Shanghai (N = 1483)^{26,29} and the control samples from this study (Yuxi). ^cP-values were calculated by the Pearson's Chi-square test. P-values < 0.05 were marked in bold.

inflammation^{26,27} and mitochondrial dysfunction.³⁵ All these observations suggested that LRRK2 had important roles in cellular activity and might explain why LRRK2 was involved in the pathogenesis of PD, Crohn's disease, cancer and/or leprosy.³⁶ Intriguingly, we found that the LRRK2 gene had experienced positive selection both in hominidae and primate lineages during the evolution ($\omega_2 > \omega_1$ and P -value < 0.05; Supplementary Method and Supplementary Table S1) by branch model test, which might account for the adaptation to its biological functions and/or host diseases in primates. Moreover, these vertebrate species that were reported to be infected by *M. leprae*, including primates (human, chimpanzee, gorilla and monkey), rodentia (guinea pig, mouse and rat), hedgehog, tree shrew and nine-banded armadillo,^{4,37,38} were clustered together in their respective clades in the neighbor-joining tree of LRRK2 amino-acid sequences (Supplementary Figure S2). Based on the clustering pattern of the tree, we speculated that those species which were marked with a '?' might be infected by *M. leprae*. Taken all these lines of evidence together, it seemed that LRRK2 gene is likely to be involved in leprosy.

In this study, we aimed to define the potential role of LRRK2 in leprosy. We first attempted to validate and identify LRRK2 genetic variants that were associated with leprosy, followed by functional characterization of risk allele at the cellular level. Besides the previously reported GWAS-hit SNP rs1873613,⁶ additional SNPs were genotyped to have a better characterization of the association of LRRK2 variants with leprosy. The analyzed case and control populations had no potential population stratification and sampling bias based on our previous analysis of matrilineal genetic components,³⁹ which could serve as a good basis for genetic association analysis. In contrast with the GWAS report,⁶ we failed to replicate the reported association of rs1873613 with leprosy *per se*, but we discerned an association of this SNP with PB leprosy. This result might be explained by the weak linkage disequilibrium of rs1873613 with potential causal variant and/or a large chromosomal distance from the LRRK2 gene. The complexity of leprosy clinical outcomes and different regional samples might also account for the incomplete consistency between our study and the GWAS report.⁶ Note that rs1873613 had a positive signal as expression quantitative trait loci for the nearby SLC2A13 gene according to the information provided by the expression quantitative trait loci database Genevar (www.sanger.ac.uk/resources/software/genevar/). Further analysis of the SLC2A13 gene would be worthwhile to clarify the role of rs1873613 in leprosy.

Intriguingly, four LRRK2 SNPs (rs1427267, rs3761863, rs732374 and rs7298930) were identified to be strongly associated with leprosy *per se* and PB at both the genotypic and allelic levels. Similarly, we observed significant associations of LRRK2 haplotypes that were composed of SNPs rs1873613-rs732374-rs1427267-rs7307310-rs3761863 with leprosy *per se* and/or PB (Table 4). These results reinforced the notion that LRRK2 genetic variants confer susceptibility to leprosy.

Among these leprosy-associated SNPs, the non-synonymous SNP rs3761863 (p.Met2397Thr) was previously identified to be associated with Crohn's disease²² and was reported to affect the amount and stability of LRRK2 protein *in vitro*.²⁶ However, we observed no effect of this variant on the serum LRRK2 level between the case and control populations (Supplementary Figure S1). We next characterized the putative role of p.M2397T compared with the PD-associated pathogenic mutation p.G2019S. We demonstrated that overexpression of allele p.T2397 had a significantly increased NFAT activity after LPS stimulation in HEK293 cells, whereas mutant p.S2019 had no effect on NFAT activity (Figure 2). This result was reasonable as the kinase function of LRRK2 did not participate in NFAT regulation.²⁶

There is increasing evidence that demonstrates the active role of LRRK2 in regulating macroautophagy, chaperone-mediated

Table 3. Distribution of six rare variants of the *LRRK2* gene in Han Chinese with and without leprosy

Pathogenic mutation ^a	No. of Yuxi individuals				No. of pooled Han Chinese ^b (N = 3225)
	Control (N = 1078)	Case (N = 548)	MB (N = 295)	PB (N = 252)	
rs34594498 (p.A419V) ^c	13	6	2	4	64
rs33939927 (p.R1441C)	0	1	1	0	1
rs35801418 (p.Y1699C)	0	0	0	0	0
rs34637584 (p.G2019S)	0	0	0	0	0
rs35870237 (p.I2020T)	0	0	0	0	0
rs34778348 (p.G2385R) ^c	41	25	18	7	193

Abbreviations: MB, multibacillary leprosy; PB, paucibacillary leprosy. ^aDetailed information for these pathogenic mutations was listed in our previous study²⁸ and references therein. ^bHan Chinese without leprosy from Sichuan (N = 776), Hunan (N = 966) and Shanghai (N = 1483) reported in our recent studies.^{28,29} ^cNo significant differences were observed ($P > 0.05$; Fisher's exact test).

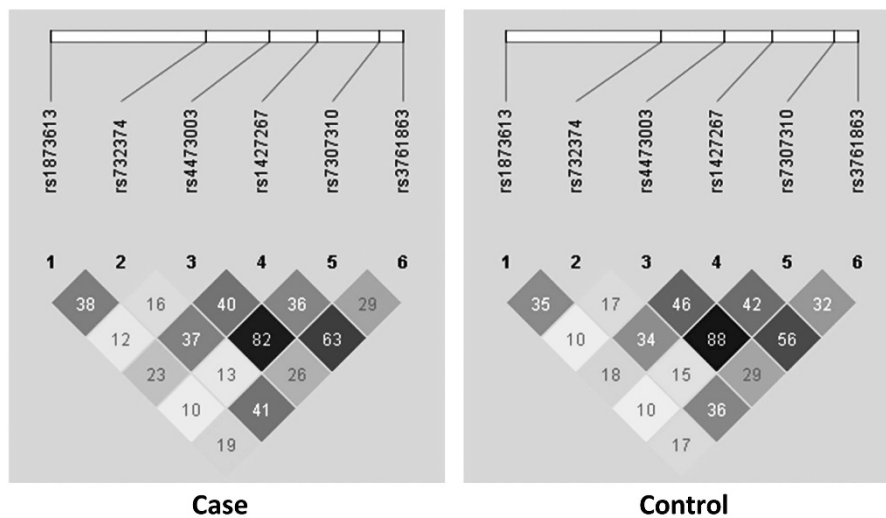


Figure 1. The linkage disequilibrium (LD) structures of six *LRRK2* SNPs in leprosy patients and healthy controls. Black squares represent high LD as measured by r^2 , gradually coloring down to white squares of low LD. The individual square showed the r^2 value for each SNP pair (r^2 value is multiplied by 100).

autophagy and mitophagy.^{40–42} Therefore, we investigated whether *LRRK2* mutations could affect the progress of autophagy in the presence of MPTP. MPTP caused a destruction of dopaminergic neurons in the nigrostriatal pathway,⁴³ and this degeneration of neurons was associated with MPTP-induced autophagy.^{30,44} We observed that Thr2397-*LRRK2* and Δ WD40-*LRRK2* could inhibit the process of MPTP-induced autophagy. In contrast, the PD-pathogenic mutation p.G2019S could increase the highest ratio of the LC3-II conversion. This result suggested that variant p.M2397T might have an opposite role with p.G2019S in regulating autophagy. Variant p.M2397T is located within the WD40 domain of the *LRRK2* protein. Deletion of WD40 domain could eliminate *LRRK2* dimer complex formation and kinase activity.⁴⁵ Moreover, *in vivo* loss-of-function study of *LRRK2* in zebrafish showed that deletion of WD40 domain caused brain dopaminergic neurons loss and axon tract disorganization.⁴⁶ We found that the WD40 domain had a protective role in MPTP-induced neurotoxicity. In addition, p.M2397T had a similar role as the WD40 domain during this process, suggesting that individuals with allele T2397 might have different susceptibility to autophagy. Collectively, we speculate that the protective effect of allele T2397 on leprosy might be mediated by an increasing immune response and decreasing neurotoxicity after *M. leprae* loading.

A limitation of this study is the lack of direct relevance between autophagy and *M. leprae* infection affected by *LRRK2* genetic polymorphisms. It may provide more evidence to work on the

early onset leprosy patients with Met2397 or Thr2397, and assess the autophagy and bacteria index at the lesion, because the role of *LRRK2* and autophagy in bacterial clearance and disease clinical manifestations may be most important early in infection. Unfortunately, we do not have access to these samples at the moment. Another limitation of the study is that there is a possibility of rare *LRRK2* mutations in leprosy even though we analyzed several reported pathogenic mutations.

In summary, we showed that the GWAS top hit SNP rs1873613 of the *LRRK2* gene was not associated with leprosy *per se* but was associated with PB in our samples. We identified additional tag and functional SNPs that were associated with leprosy *per se* and its subtypes. Functional assays showed that *LRRK2* variant p.M2397T affected NFAT activity in immune response and MPTP-induced autophagy. Further experiments are needed to explore this association and to understand the *LRRK2* functional change in leprosy.

MATERIALS AND METHODS

Ethics statement

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 approved this study.

Table 4. Association of the LRRK2 haplotypes with leprosy in Han Chinese

Haplotype ^a	Control		Case		Case vs control		Case vs control		Case vs control		MB vs control		PB vs control	
	No. (%)	No. (%)	No. (%)	No. (%)	OR (95% CI) ^b	P-value ^b	No. (%)	OR (95% CI)	P-value	No. (%)	OR (95% CI)	P-value	No. (%)	OR (95% CI)
A-G-A-T-A	604 (28.0)	337 (30.8)	1.141 (0.976–1.341)	0.102	174 (29.5)	1.075 (0.880–1.313)	0.503	163 (32.3)	1.228 (0.997–1.514)	0.056				
A-G-A-C-A	292 (13.5)	183 (16.7)	1.282 (1.049–1.568)	0.018	94 (15.9)	1.210 (0.940–1.558)	0.142	89 (17.7)	1.369 (1.055–1.776)	0.020				
A-G-G-C-G	196 (9.1)	104 (9.5)	1.051 (0.818–1.349)	0.701	56 (9.5)	1.049 (0.768–1.433)	0.748	48 (9.5)	1.053 (0.755–1.467)	0.733				
A-G-G-C-A	116 (5.4)	41 (3.7)	0.685 (0.476–0.985)	0.046	20 (3.4)	0.617 (0.380–1.001)	0.053	21 (4.2)	0.765 (0.475–1.230)	0.314				
A-A-G-C-G	158 (7.3)	66 (6.0)	0.812 (0.603–1.093)	0.187	32 (5.4)	0.725 (0.490–1.073)	0.119	34 (6.7)	0.915 (0.623–1.343)	0.703				
G-A-G-C-G	476 (22.1)	216 (19.7)	0.868 (0.725–1.040)	0.135	124 (21.0)	0.939 (0.752–1.173)	0.613	92 (18.3)	0.788 (0.615–1.010)	0.061				
G-G-G-C-G	68 (3.2)	33 (3.0)	0.955 (0.626–1.457)	0.915	23 (3.9)	1.246 (0.769–2.017)	0.365	10 (2.0)	0.622 (0.318–1.216)	0.187				
Other ^c	246 (11.4)	114 (10.4)	0.903 (0.714–1.142)	0.408	67 (11.4)	0.995 (0.747–1.325)	1.000	47 (9.3)	0.799 (0.575–1.109)	0.206				
Global P-value ^d			0.031			0.021			0.033					

Abbreviations: CI, confidence interval; MB, multibacillary leprosy; OR, odds ratio; PB, paucibacillary leprosy; SNP, single-nucleotide polymorphism. ^aThe order of LRRK2 SNPs in each haplotype is rs1873613–rs732374–rs1427267–rs7307310–rs3761863. We excluded rs4473003 and rs7298930 that were in linkage disequilibrium ($r^2 > 0.8$) with other SNPs. ^bAll data were calculated by using the Fisher's exact test. ^cHaplotypes with a frequency < 3% in the case or control group were aggregated together. ^dGlobal P-value calculated by Chi-square test. P-values < 0.05 were marked in bold.

Study subjects

We performed a case–control study in a population from Yuxi Prefecture, Yunnan Province of Southwest China. This study was carried out in 1626 samples, including 548 leprosy patients (onset age from 2 to 67 years, mean age: 24.9 ± 12.5 years; male/female ratio = 399/149; multibacillary: paucibacillary = 295: 252, one individual without a clear clinical classification) and 1078 healthy control subjects from the same geographic area (age from 4 to 91 years, mean age: 39.9 ± 17.6 years; male/female ratio = 590: 488). The diagnosis of leprosy patients was based on clinical and histopathological features, as well as the bacteriological index if available, as described in our recent epidemiological study for leprosy in this region.⁴⁷ Additional 3225 Han Chinese (776 samples from Sichuan Province, 966 samples from Hunan Province and 1483 samples from Shanghai) reported in our recent studies were included in this study for comparison.^{28,29} All healthy individuals had no history of leprosy, HIV infection and tuberculosis. These samples had been analyzed for other susceptibility genes to leprosy in our previous studies.^{14–16,39}

LRRK2 SNP selection and genotyping

Genomic DNA was extracted from whole blood by using the AxyPrep Blood Genomic DNA Miniprep Kit (AP-MN-BL-GDNA-250, Axygen, Union City, CA, USA). Thirteen SNPs of the LRRK2 gene were selected and analyzed in this study, following a similar strategy described in our previous studies.^{28,29} Among them, 6 tag SNPs (rs732374, rs4473003, rs1427267, rs7298930, rs7307310, rs3761863) captured 57 SNPs (totally 67 SNPs) of the LRRK2 gene according to the international HapMap project database of CHB (<http://hapmap.ncbi.nlm.nih.gov/>, Phase 3, CHB). Six rare variants (MAF < 0.05; rs34594498 (p.A419V), rs33939927 (p.R1441C), rs35801418 (p.Y1699C), rs34637584 (p.G2019S), rs35870237 (p.I2020T), rs34778348 (p.G2385R)) were chosen, because these pathogenic mutations led to aberrant LRRK2 function and were regarded as the genetic cause of PD.¹⁸ We included these reported (rare) pathogenic LRRK2 mutations in the analysis to discern whether these mutations may have a role in leprosy. Finally, the GWAS-hit SNP rs1873613 of leprosy⁵ (which is located at 66.4 Kb upstream of the LRRK2 gene) was also included in the analysis.

Two genotyping methods were employed in this study. SNPs rs1427267, rs3761863 and rs1873613 were detected by PCR restriction fragment length polymorphism. Briefly, PCR was performed in a 20-μl reaction volume with 50 ng of genomic DNA, 10 pmol of each specific primer (Supplementary Table S2), 0.2 mM of each dNTP, 2 mM of MgCl₂ and 0.5 U Taq DNA polymerase (TaKaRa Biotechnology Co. Ltd, Dalian, China). PCR products were digested by specific restriction enzymes *Nmu*CI, *Tal*I and *Sw*al (New England Biolabs, Beverly, MA, USA), respectively. Each SNP and genotypic profiles were determined by 2% agarose gel electrophoresis. The other 10 SNPs (rs732374, rs7307310, rs7298930, rs4473003, rs34594498, rs33939927, rs35801418, rs34637584, rs35870237 and rs34778348) were detected using multiplex PCR and the SNaPshot technique (ABI PRISM SNaPshot Multiplex System, Foster City, CA, USA) as described in our recent study.²⁸ All SNPs had a call rate of > 98%. Further analysis of SNPs rs1427267, rs3761863 and rs1873613 that were initially genotyped by restriction fragment length polymorphism using SNaPshot confirmed the genotyping results. Direct sequencing of 2% of samples validated 100% correctness of genotyping.

Statistical analysis for LRRK2 genetic association

Deviation from HWE was assessed for each SNP by Chi-square tests. Cases and controls were compared according to the frequencies of genotypes and alleles. Linkage disequilibrium structure was determined by using Haploview.⁴⁸ Haplotype construction and frequency were estimated by using the Phase software (Seattle, WA, USA).⁴⁹ The global difference in haplotype frequency between cases and controls was estimated by Chi-square test. Potential association of SNP with leprosy (including leprosy subtype) was estimated using unconditional logistic regression model, with an adjustment for sex. All analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Power calculations were performed using the Quanto software (Los Angeles, CA, USA).⁵⁰

Quantification of plasma LRRK2 level

We randomly chose 229 leprosy samples (including 112 MB patients and 117 PB patients) and 57 healthy controls to measure plasma LRRK2 level by using the Enzyme-Linked Immunosorbent Assay Kit (R&D

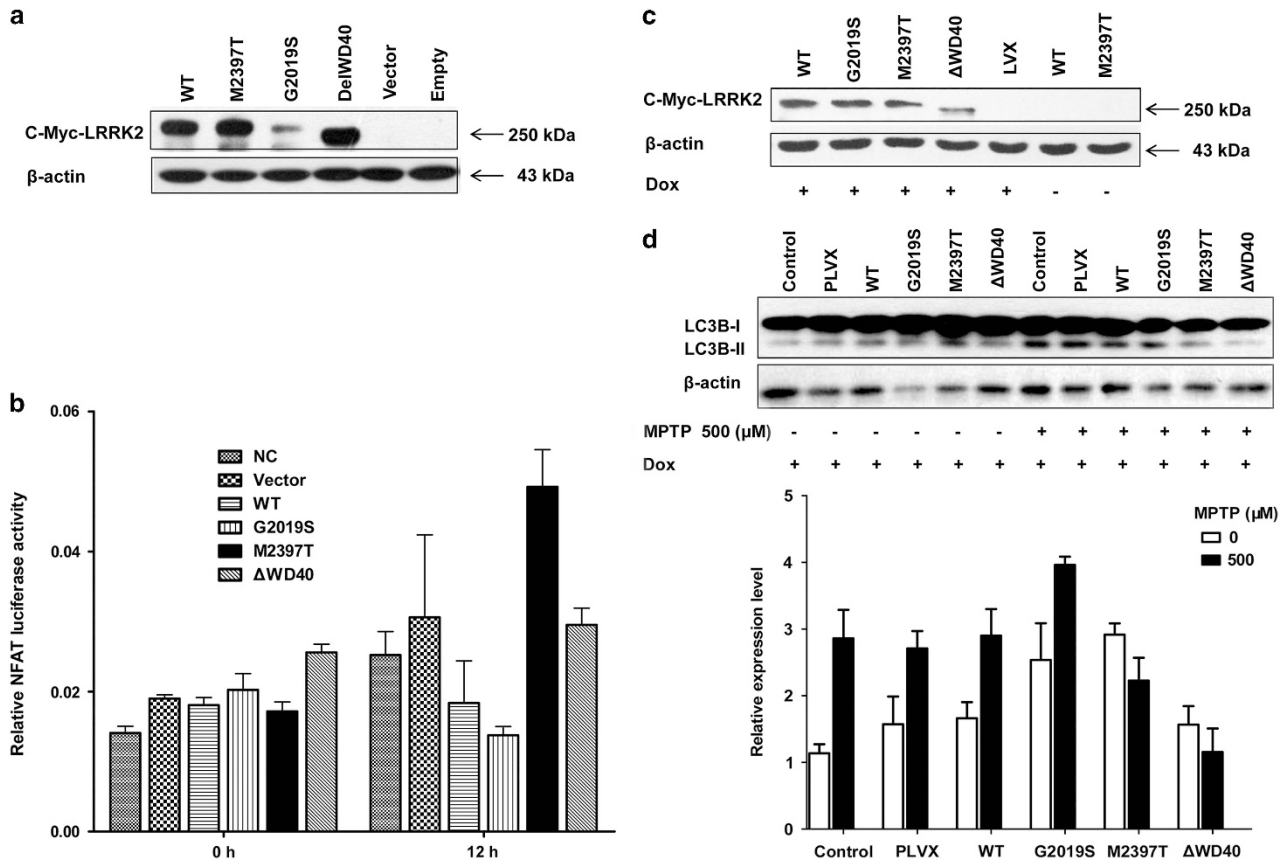


Figure 2. Functional assays of LRRK2 allele p.Thr2397. (a) Immunoblot analysis of total cell lysates of transfected HEK293 cells showing overexpressed LRRK2 protein. (b) NFAT luciferase assay of HEK293 cells transfected with equal amount of empty vector or mutant LRRK2 and stimulated with and without LPS ($1 \mu\text{g ml}^{-1}$). (c) Immunoblot analysis of total U251 cell lysates with stable expression of mutant LRRK2 induced by doxycycline ($1 \mu\text{g ml}^{-1}$) treatment. (d) Immunoblot analysis of total U251 cell lysates with stable expression of mutant LRRK2 treated with or without MPTP ($500 \mu\text{M}$) in the presence of doxycycline (top), and densitometry of the results presented as the ratio of LCB-II/LCB-I (below). Data were representative of three independent experiments.

systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Functional assay for LRRK2 variant p.M2397T (rs3761863)

We performed cellular assay to characterize the potential effect of the LRRK2 variant p.M2397T (rs3761863). Human *LRRK2* cDNA and a truncated mutant, LRRK2-delWD40 (ΔWD40 , deletion of residues 2010–2527 of LRRK2, which represents the WD40 domain), were amplified by two sets of primers (Supplementary Table S3) to introduce restriction endonuclease sites for *XhoI* and *NotI*, and PCR products were cloned into pCMV-c-Myc vector (Clontech, Mountain View, CA, USA), respectively. We generated two mutants (p.G2019S and p.M2397T) based on the WT LRRK2 by using site-directed mutagenesis PCR methods. All mutants were verified by sequencing.

To generate a stable and inducible cell line expressing WT or mutant LRRK2 upon doxycycline (Dox, Sigma, St Louis, MO, USA) induction, WT or mutant LRRK2 was subcloned into pLVX-tight-puro (Clontech) by using two sets of primers to introduce restriction endonuclease sites (*NotI* and *MluI*) and the c-Myc tag (Supplementary Table S3). HEK293 cells were transfected with LRRK2 vector, packaging plasmid psPAX2 (Addgene, Cambridge, MA, USA) and envelop plasmid PMD2.G (Addgene) with a ratio of 3:2:1, respectively. The lentivirus was collected from cell supernatant at 48 h after transfection. Human U251 cells grown in a 12-well plate were sequentially infected with 500 μl lentivirus supernatant in the presence of 1 $\mu\text{g ml}^{-1}$ Polybrene (Sigma) and were selected by puromycin (1 $\mu\text{g ml}^{-1}$) and G418 (500 $\mu\text{g ml}^{-1}$) for 2 weeks.

HEK293 cells were plated in 24-well plates at a density of 1×10^4 cells, cultured overnight and then were transfected with 0.2 μg of pNFAT-TA-Luc (Clontech) reporter vector and 0.3 μg of expression constructs (LRRK2-cMyc and its mutants) by using Lipofectamine 2000 (Life Technologies, Carlsbad, CA, USA). As an internal control, cells were also transfected with 0.02 μg

Renilla luciferase construct. The transfected cells were left untreated or treated with LPS ($1 \mu\text{g ml}^{-1}$, Sigma) for 12 h. Cells were lysed and subjected to a luciferase reporter assay (Promega, Madison, WI, USA) following the manufacturer's instructions. U251 cells with stable expression of WT and mutant LRRK2 were treated with or without MPTP ($500 \mu\text{M}$) for 24 h, and cell lysates were prepared using protein lysis buffer (Beyotime, Shanghai, China). Western blots for target protein were performed using the standard method. In brief, a total of 30 μg protein was separated by 10–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and was transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA). The membrane was blocked with 5% (w/v) skim milk for 2 h at room temperature. The membrane was incubated with primary antibodies against c-Myc (Sigma, 1:1000), LC3B (Cell Signaling Technology, Danvers, MA, USA, 1:1000) and β -actin (EnoGene Biotech Co. Ltd, New York, NY, USA, 1:10000) overnight at 4°C , and after three washes with TBST buffer, the membrane was incubated with a peroxidase-conjugated anti-rabbit immunoglobulin G (KPL, Milford, MA, USA, 1:5000) for 1 h at room temperature. The epitope was visualized using an ECL Western Blot Detection Kit (Millipore, Billerica, MA, USA).

CONFLICT OF INTEREST

The authors declare no conflict interest.

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REFERENCES

- 1 Britton WJ, Lockwood DN. Leprosy. *Lancet* 2004; **363**: 1209–1219.
- 2 WHO. Leprosy update 2011. *Wkly Epidemiol Rec* 2011; **86**: 389–399.
- 3 Scollard DM, Adams LB, Gillis TP, Krahenbuhl JL, Truman RW, Williams DL. The continuing challenges of leprosy. *Clin Microbiol Rev* 2006; **19**: 338–381.
- 4 Worobec SM. Treatment of leprosy/Hansen's disease in the early 21st century. *Dermatol Ther* 2009; **22**: 518–537.
- 5 Johnson CM, Lyle EA, Omuetti KO, Stepensky VA, Yegin O, Alpsyoy E *et al*. Cutting edge: a common polymorphism impairs cell surface trafficking and functional responses of TLR1 but protects against leprosy. *J Immunol* 2007; **178**: 7520–7524.
- 6 Zhang FR, Huang W, Chen SM, Sun LD, Liu H, Li Y *et al*. Genomewide association study of leprosy. *N Engl J Med* 2009; **361**: 2609–2618.
- 7 Misch EA, Berrington WR, Vary JC Jr., Hawn TR. Leprosy and the human genome. *Microbiol Mol Biol Rev* 2010; **74**: 589–620.
- 8 Yang D, Song H, Xu W, Long H, Shi C, Jing Z *et al*. Interleukin 4-590T/C polymorphism and susceptibility to leprosy. *Genet Test Mol Biomarkers* 2011; **15**: 877–881.
- 9 Sousa AL, Fava VM, Sampaio LH, Martelli CM, Costa MB, Mira MT *et al*. Genetic and immunological evidence implicates interleukin 6 as a susceptibility gene for leprosy type 2 reaction. *J Infect Dis* 2012; **205**: 1417–1424.
- 10 Cardoso CC, Pereira AC, Brito-de-Souza VN, Duraes SM, Ribeiro-Alves M, Nery JA *et al*. TNF -308G>A single nucleotide polymorphism is associated with leprosy among Brazilians: a genetic epidemiology assessment, meta-analysis, and functional study. *J Infect Dis* 2011; **204**: 1256–63.
- 11 Sapkota BR, Macdonald M, Berrington WR, Misch EA, Ranjit C, Siddiqui MR *et al*. Association of TNF, MBL, and VDR polymorphisms with leprosy phenotypes. *Hum Immunol* 2010; **71**: 992–998.
- 12 Alter A, de Léséleuc L, Van Thuc N, Thai VH, Huong NT, Ba NN *et al*. Genetic and functional analysis of common MRC1 exon 7 polymorphisms in leprosy susceptibility. *Hum Genet* 2010; **127**: 337–348.
- 13 Cardoso CC, Pereira AC, Brito-de-Souza VN, Dias-Baptista IM, Maniero VC, Venturini J *et al*. IFNG +874 T>A single nucleotide polymorphism is associated with leprosy among Brazilians. *Hum Genet* 2010; **128**: 481–490.
- 14 Wang D, Feng J-Q, Li Y-Y, Zhang D-F, Li X-A, Li QW *et al*. Genetic variants of the MRC1 gene and the IFNG gene are associated with leprosy in Han Chinese from Southwest China. *Hum Genet* 2012; **131**: 1251–1260.
- 15 Zhang D-F, Huang X-Q, Wang D, Li Y-Y, Yao Y-G. 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* 2013; **132**: 629–640.
- 16 Zhang D-F, Wang D, Li Y-Y, Yao Y-G. Mapping genetic variants in the CFH gene for association with leprosy in Han Chinese. *Genes Immun* 2014; **15**: 506–510.
- 17 Gardet A, Benita Y, Li C, Sands BE, Ballester I, Stevens C *et al*. LRRK2 is involved in the IFN-gamma response and host response to pathogens. *J Immunol* 2010; **185**: 5577–5585.
- 18 Mata IF, Wedemeyer WJ, Farrer MJ, Taylor JP, Gallo KA. LRRK2 in Parkinson's disease: protein domains and functional insights. *Trends Neurosci* 2006; **29**: 286–293.
- 19 Paísán-Ruiz C, Jain S, Evans EW, Gilks WP, Simón J, van der Brug M *et al*. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* 2004; **44**: 595–600.
- 20 Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S *et al*. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* 2004; **44**: 601–607.
- 21 Recchia A, Debetto P, Negro A, Guidolin D, Skaper SD, Giusti P. Alpha-synuclein and Parkinson's disease. *FASEB J* 2004; **18**: 617–626.
- 22 Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD *et al*. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008; **40**: 955–962.
- 23 Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G *et al*. Patterns of somatic mutation in human cancer genomes. *Nature* 2007; **446**: 153–158.
- 24 Tong Y, Giaime E, Yamaguchi H, Ichimura T, Liu Y, Si H *et al*. Loss of leucine-rich repeat kinase 2 causes age-dependent bi-phasic alterations of the autophagy pathway. *Mol Neurodegener* 2012; **7**: 2.
- 25 Plowey ED, Cherra SJ 3rd, Liu YJ, Chu CT. Role of autophagy in G2019S-LRRK2-associated neurite shortening in differentiated SH-SY5Y cells. *J Neurochem* 2008; **105**: 1048–1056.
- 26 Liu Z, Lee J, Krummey S, Lu W, Cai H, Lenardo MJ. The kinase LRRK2 is a regulator of the transcription factor NFAT that modulates the severity of inflammatory bowel disease. *Nat Immunol* 2011; **12**: 1063–1070.
- 27 Moehle MS, Webber PJ, Tse T, Sukar N, Standaert DG, DeSilva TM *et al*. LRRK2 inhibition attenuates microglial inflammatory responses. *J Neurosci* 2012; **32**: 1602–1611.
- 28 Bi R, Zhao L, Zhang C, Lu W, Feng J-Q, Wang Y *et al*. No association of the LRRK2 genetic variants with Alzheimer's disease in Han Chinese individuals. *Neurobiol Aging* 2014; **35**: 444. e5–e9.
- 29 Li X, Zhang W, Zhang C, Gong W, Tang J, Yi Z *et al*. No association between genetic variants of the LRRK2 gene and schizophrenia in Han Chinese. *Neurosci Lett* 2014; **566**: 210–215.
- 30 Zhu JH, Horbinski C, Guo F, Watkins S, Uchiyama Y, Chu CT. Regulation of autophagy by extracellular signal-regulated protein kinases during 1-methyl-4-phenylpyridinium-induced cell death. *Am J Pathol* 2007; **170**: 75–86.
- 31 Bravo-San Pedro JM, Niso-Sántano M, Gómez-Sánchez R, Pizarro-Estrella E, Aiastui-Pujana A, Gorostidi A *et al*. The LRRK2 G2019S mutant exacerbates basal autophagy through activation of the MEK/ERK pathway. *Cell Mol Life Sci* 2013; **70**: 121–136.
- 32 Marcinek P, Jha AN, Shinde V, Sundaramoorthy A, Rajkumar R, Suryadevara NC *et al*. LRRK2 and RIPK2 variants in the NOD 2-mediated signaling pathway are associated with susceptibility to *Mycobacterium leprae* in Indian populations. *PLoS One* 2013; **8**: e73103.
- 33 Smith WW, Pei Z, Jiang H, Moore DJ, Liang Y, West AB *et al*. Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration. *Proc Natl Acad Sci USA* 2005; **102**: 18676–18681.
- 34 Alegre-Abarrategui J, Christian H, Lufino MM, Muthiac R, Venda LL, Ansorge O *et al*. LRRK2 regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model. *Hum Mol Genet* 2009; **18**: 4022–4034.
- 35 Saha S, Guillily MD, Ferree A, Lanceta J, Chan D, Ghosh J *et al*. LRRK2 modulates vulnerability to mitochondrial dysfunction in *Caenorhabditis elegans*. *J Neurosci* 2009; **29**: 9210–9218.
- 36 Lewis PA, Manzoni C. LRRK2 and human disease: a complicated question or a question of complexes? *Sci Signal* 2012; **5**: pe2.
- 37 Watson SR, Morrison NE, Collins FM. Delayed hypersensitivity responses in mice and guinea pigs to *Mycobacterium leprae*, *Mycobacterium vaccae*, and *Mycobacterium nonchromogenicum* cytoplasmic proteins. *Infect Immun* 1979; **25**: 229–236.
- 38 Wang HY, Liu JH, Ye SZ, Yu LC, Shi MQ, Sang HG. Preliminary observations on experimental leprosy in tupaia (*Tupaia belangeri yunalis*). *Leprosy Rev* 1990; **61**: 12–18.
- 39 Wang D, Su L-Y, Zhang A-M, Li YY, Li XA, Chen LL *et al*. Mitochondrial DNA copy number, but not haplogroup, confers a genetic susceptibility to leprosy in Han Chinese from Southwest China. *PLoS One* 2012; **7**: e38848.
- 40 Orenstein SJ, Kuo SH, Tasset I, Arias E, Koga H, Fernandez-Carasa I *et al*. Interplay of LRRK2 with chaperone-mediated autophagy. *Nat Neurosci* 2013; **16**: 394–406.
- 41 Manzoni C, Mamais A, Dihanich S, Abeti R, Soutar MP, Plun-Favreau H *et al*. Inhibition of LRRK2 kinase activity stimulates macroautophagy. *Biochim Biophys Acta* 2013; **1833**: 2900–2910.
- 42 Zhu Y, Wang C, Yu M, Cui J, Liu L, Xu Z. ULK1 and JNK are involved in mitophagy induced by LRRK2 G2019S expression. *Protein Cell* 2013; **4**: 711–721.
- 43 Bandopadhyay R, de Bellerocche J. Pathogenesis of Parkinson's disease: emerging role of molecular chaperones. *Trends Mol Med* 2010; **16**: 27–36.
- 44 Wong AS, Lee RH, Cheung AY, Yeung PK, Chung SK, Cheung ZH *et al*. Cdk5-mediated phosphorylation of endophilin B1 is required for induced autophagy in models of Parkinson's disease. *Nat Cell Biol* 2011; **13**: 568–579.
- 45 Jorgensen ND, Peng Y, Ho CC, Rideout HJ, Petrey D, Liu P *et al*. The WD40 domain is required for LRRK2 neurotoxicity. *PLoS One* 2009; **4**: e8463.
- 46 Sheng D, Qu D, Kwok KH, Ng SS, Lim AY, Aw SS *et al*. Deletion of the WD40 domain of LRRK2 in Zebrafish causes Parkinsonism-like loss of neurons and locomotive defect. *PLoS Genet* 2010; **6**: e1000914.
- 47 Li Y-Y, Li X-A, He L, Wang D, Chen WY, Chen L *et al*. Trends in new leprosy case detection over 57 years (1952–2008) in Yuxi, Yunnan Province of Southwest China. *Leprosy Rev* 2011; **82**: 6–16.
- 48 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**: 263–265.
- 49 Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001; **68**: 978–989.
- 50 Gauderman WJ. Sample size requirements for matched case-control studies of gene-environment interaction. *Stat Med* 2002; **21**: 35–50.

Supplementary Information accompanies this paper on Genes and Immunity website (<http://www.nature.com/gene>)