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ORIGINAL ARTICLE

Association of the *LRRK2* genetic polymorphisms with leprosy in Han Chinese from Southwest China

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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 disease[22] 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 system[26] 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 (ΔWD40). As shown in Figure 2, after 12 h treatment with LPS (1 μg ml^{-1}), the reported Parkinson's disease (PD)-associated pathogenic mutation p.G2019S and the truncated LRRK2 mutant ΔWD40 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 μM), 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 ΔWD40 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*	*Control*	*All cases vs controls*			*MB vs control*			*PB vs control*		
		No. (%)	*No. (%)*	*OR (95%CI)*[a]	P-*value*[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)	1.394 (1.068–1.820)	**0.015**	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)	1.403 (1.041–1.892)	**0.026**	73 (24.7)	1.242 (0.846–1.825)	0.269	82 (32.5)	1.585 (1.074–2.338)	**0.020**
	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)	1.179 (1.016–1.366)	**0.030**	310 (52.4)	1.103 (0.917–1.326)	0.300	282 (56.0)	1.274 (1.046–1.553)	**0.016**
rs3761863	AA	319 (29.6)	178 (32.5)	Reference	—	86 (29.1)	Reference	—	92 (36.5)	Reference	—
	AG	547 (50.7)	288 (52.6)	0.939 (0.742–1.189)	0.603	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)	0.713 (0.519–0.981)	**0.038**	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)	Reference	—	335 (56.6)	Reference	—	305 (61.0)	Reference	
	G allele	971 (45.0)	452 (41.2)	0.866 (0.745–1.005)	0.059	257 (43.4)	0.948 (0.787–1.141)	0.573	195 (39.0)	0.775 (0.634–0.947)	**0.013**
rs1873613	AA	508 (47.1)	268 (48.9)	Reference	—	131 (44.3)	Reference	—	137 (54.4)	Reference	—
	AG	454 (42.1)	235 (42.9)	0.995 (0.799–1.240)	0.967	138 (46.6)	1.187 (0.903–1.561)	0.220	97 (38.5)	0.792 (0.591–1.061)	0.119
	GG	116 (10.8)	45 (8.2)	0.741 (0.507–1.084)	0.123	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)	0.909 (0.774–1.067)	0.243	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)	0.829 (0.663–1.037)	0.100	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)	0.651 (0.453–0.936)	**0.020**	28 (9.7)	0.715 (0.455–1.122)	0.145	21 (8.4)	0.592 (0.358–0.978)	**0.041**
	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)	0.804 (0.683–0.945)	**0.008**	169 (29.1)	0.843 (0.688–1.032)	0.098	136 (27.2)	0.767 (0.616–0.955)	**0.018**
rs7307310	CC	489 (46.1)	231 (42.9)	Reference	—	131 (45.3)	Reference	—	100 (40.0)	Reference	—
	CT	461 (43.4)	238 (44.2)	1.067 (0.852–1.336)	0.573	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)	1.283 (0.910–1.808)	0.155	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	—
	T allele	683 (32.2)	378 (35.1)	1.113 (0.951–1.303)	0.181	195 (33.7)	1.053 (0.865–1.283)	0.608	183 (36.6)	1.181 (0.961–1.451)	0.114
rs7298930	AA	308 (28.8)	179 (33.1)	Reference	—	87 (29.9)	Reference	—	92 (36.8)	Reference	—
	AC	498 (46.5)	267 (49.4)	0.916 (0.721–1.166)	0.477	151 (51.9)	1.071 (0.791–1.450)	0.658	116 (46.4)	0.763 (0.558–1.042)	0.089
	CC	264 (24.7)	95 (17.6)	0.647 (0.478–0.876)	**0.005**	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)	0.811 (0.698–0.942)	**0.006**	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)	1.070 (0.856–1.339)	0.552	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)	1.163 (0.823–1.644)	0.392	34 (11.7)	1.085 (0.703–1.674)	0.713	32 (12.8)	1.273 (0.811–1.999)	0.295
	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)	1.076 (0.919–1.259)	0.358	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. [a]All 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
	AA/AG/GG	497/1799/1999	**0.026**	**0.020**	—	0.218	0.249	—	0.056	0.060	—
rs4473003	C/T	2873/5711	0.241	0.447	1.053	0.689	0.992	0.999	0.150	0.256	1.115
	CC/CT/TT	484/1905/1903	0.506	0.740	—	0.896	0.943	—	0.354	0.524	—
rs7298930	C/A	3981/4609	**0.002**	**0.010**	0.846	0.105	0.305	0.916	**0.001**	**0.005**	0.770
	CC/AC/AA	944/2093/1258	**0.004**	**0.034**	—	0.062	0.306	—	**0.008**	**0.020**	—
rs7307310	T/C	2820/5746	0.110	0.159	1.100	0.498	0.682	1.037	0.067	0.095	1.174
	TT/CT/CC	462/1896/1925	0.248	0.277	—	0.527	504	—	0.187	0.246	—
rs3761863	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. [a]SNPs rs1873613 and 1427267 were not genotyped in Sichuan, Hunan and Shanghai populations and were excluded from the analysis. [b]Pooled Han Chinese without leprosy (Pooled) included reported data from Sichuan Province ($N = 776$), Hunan Province ($N = 966$) and Shanghai ($N = 1483$)[28,29] and the control samples from this study (Yuxi). [c]*P*-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 disease[22] 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. [a]Detailed information for these pathogenic mutations was listed in our previous study[28] and references therein). [b]Han Chinese without leprosy from Sichuan ($N = 776$), Hunan ($N = 966$) and Shanghai ($N = 1483$) reported in our recent studies.[28,29] [c]No significant differences were observed ($P > 0.05$; Fisher's exact test).

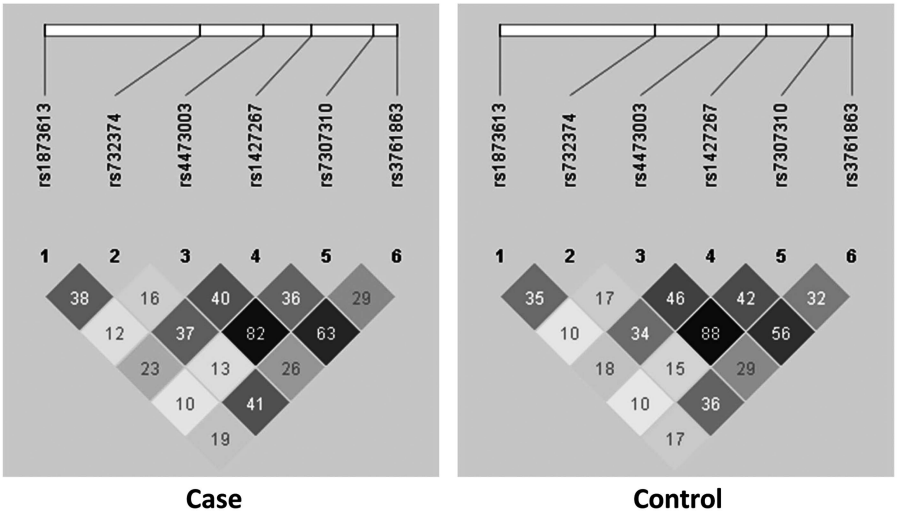

Figure 1. The linkage disequilibrium (LD) structures of six *LRRK2* SNPs in leprosy patients and healthy controls. Black squares represent high LD as measured by r^2, gradually coloring down to white squares of low LD. The individual square showed the r^2 value for each SNP pair (r^2 value is multiplied 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 ΔWD40-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*		*MB vs control*			*PB vs control*		
	No. (%)	*No. (%)*	*OR (95% CI)*[b]	*P-value*[b]	*No. (%)*	*OR (95% CI)*	*P-value*	*No. (%)*	*OR (95% CI)*	*P-value*
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**			0.221			**0.033**	

Abbreviations: CI, confidence interval; MB, multibacillary leprosy; OR, odds ratio; PB, paucibacillary leprosy; SNP, single-nucleotide polymorphism. [a]The 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. [b]All data were calculated by using the Fisher's exact test. [c]Haplotypes with a frequency $< 3\%$ in the case or control group were aggregated together. [d]Global *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 leprosy[6] (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 $MgCl_2$ and 0.5 U Taq DNA polymerase (TaKaRa Biotechnology Co. Ltd, Dalian, China). PCR products were digested by specific restriction enzymes *Nmu*CI, *Taa*I and *Swa*I (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 34778348) 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

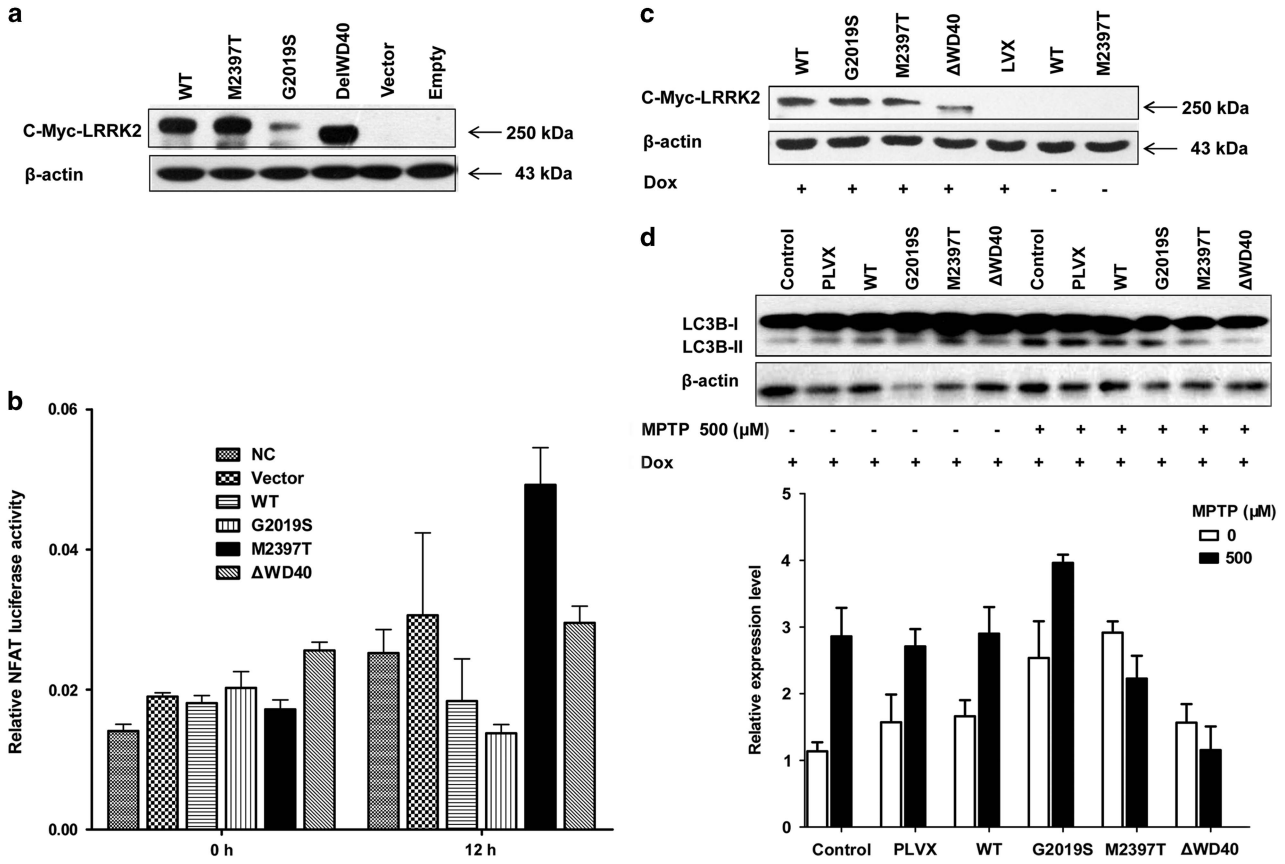

Figure 2. Functional assays of LRRK2 allele p.Thr2397. (**a**) Immunoblot analysis of total cell lysates of transfected HEK293 cells showing overexpressed LRRK2 protein. (**b**) NFAT luciferase assay of HEK293 cells transfected with equal amount of empty vector or mutant LRRK2 and stimulated with and without LPS (1 μg ml^{-1}). (**c**) Immunoblot analysis of total U251cell lysates with stable expression of mutant LRRK2 induced by doxycycline (1 μg ml^{-1}) treatment. (**d**) Immunoblot analysis of total U251 cell lysates with stable expression of mutant LRRK2 treated with or without MPTP (500 μM) 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 *Xho*I and *Not*I, 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 (*Not*I and *Mlu*I) 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 μg ml^{-1} Polybrene (Sigma) and were selected by puromycin (1 μg ml^{-1}) and G418 (500 μg 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 μg 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 μM) 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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Supplementary Information accompanies this paper on Genes and Immunity website (http://www.nature.com/gene)

Online Supplementary Data and Method

Association of the *LRRK2* genetic polymorphisms with leprosy in Han Chinese from Southwest China

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Supplementary Method

Positive selection analysis for the LRRK2 gene

Sixteen mammalian *LRRK2* gene sequences (Human, Chimpanzee, Gibbon, Baboon, Red-bellied titi, Marmoset, Galago, Tree shrew, Rat, Mouse, Pig, Cattle, Panda, Dog, Bat, Armadillo) were retrieved from the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) and Ensembl (http://asia.ensembl.org/index.html), to test potentially selective pressure by using the CODEML program implemented in PAML4 package (http://abacus.gene.ucl.ac.uk/software/paml.html) (Yang, 2007). The branch model test was used to detect whether there are significant changes in selective pressure between the human, hominidae (including human and chimpanzee) and primate.

Supplementary References

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Table S1. Analysis of selective pressure on the *LRRK2* gene by branch model test

Model	Parameters	ln L[a]	np[b]	Model compared	2ΔlnL[c] and *P*-value
A: All branches have the same ω_0	ω_0=0.14397	-34265.8	32	-	-
B: The human branch has ω_2, other branches have ω_1	ω_1=0.14352; ω_2=0.31459	-34264.6	33	A vs B	2ΔlnL = 2.4 *P* = 0.111
C: The hominidae branch has ω_2, other branches have ω1	ω_1=0.14124; ω_2=0.4362	-34254.7	33	A vs C	2ΔlnL = 22.2 *P* = **2.339e-06**
D: The primate branch has ω_2, other branches have ω1	ω_1=0.13714; ω_2=0.16648	-34262.2	33	A vs D	2ΔlnL = 7.2 *P* = **0.007**

Note: Sixteen sequences (Human [NCBI, NM_198578.3], Chimpanzee [NCBI, XM_001168494], Gibbon [Ensembl, ENSNLET00000022916], Baboon [NCBI, ACG64314], Red-bellied titi [NCBI, NM_001168919], Marmoset [NCBI, ACA57870], Galago [NCBI, XM_002752367.1], Tree shrew [Ensembl, ENSTBEP00000002600], Rat [NCBI, ACC62107], Mouse [NCBI, NM_001113437], Pig [NCBI, XM_615760], Cattle[NCBI, XM_002925834], Panda [Ensembl, ENSCAFT00000015801], Dog [NCBI, ACO06225], Bat [NCBI, NM_001191789.1], Armadillo [NCBI, NM_025730.3]) were analyzed here. ω is the ratio of nonsynonymous to synonymous substitution rate. Likelihood ratio test (LRT) was performed to test whether the foreground branch ω ratio has a significant difference.

[a] ln(likelihood) value.

[b] Number of parameters.

[c] Twice the difference of ln(likelihood) values (2ΔlnL) between the two models compared

Table S2 Primers for genotyping 13 SNPs by using PCR-RFLP and SNaPshot assay

SNPs	Primer (5'-3')	Tm (^{o}C)	Restriction enzyme
For PCR-RFLP			
rs1427267	Forward: TTGGGTTCCACAAAGTACAAGT	50	*Nmu*CI
	Reverse: GCTTCCCGTGGTTTATGAT		
rs3761863	Forward: AAGGAAAGAAACTTGCCTAGC	50	*Taa*I
	Reverse: GAAGGGCTATTGTTATCTTTG		
rs1873613	Forward: AGATGACAGCCAGTTGACTCCT	50	*Swa*I
	Reverse: GTTCCCTCCCTTGTTGCATTA		
For SNaPshot			
rs732374	Forward: CTATTTACATCAATAATTTTGAATGGTG	55	-
	Reverse: ATTGTCTAAGTAAGATAATTAAAGGACAGC		
	Probe: $(GACT)_1$AATTAAAATAGCTTAATATGATTGAGTAAA		
rs7307310	Forward: AAAGAGTATCAAGCACAGTTTAAAAATAC	55	-
	Reverse: TTAAACTGGAACCCAACTAGATCA		
	Probe: ACT$(GACT)_2$ATACATATTTGTCTTTTCATGTAATTTTAT		
rs7298930	Forward: AAATTAGGTGTATCCTTCTAAAAACATT	55	-
	Reverse: AAATGGAAAGAGGGAAAATTG		
	Probe: $(GACT)_4$CAGGTAGCGACTCCAGCATCTTTATATTAG		
rs4473003	Forward: TTAATTATTCAGGATCACTAGTGTAAGG	55	-
	Reverse: AAAATAAGAGATACACCAGCAACTTAG		
	Probe: T$(GACT)_5$GACTTTGAAAGGAAAAATAGAAATATTCTC		
rs34594498	Forward: TCCATGCTGATGCATTCTT	55	-
	Reverse: CCACTGCTTACCATTTTGTTCT		
	Probe: T$(GACT)_7$CTTCATCAAAGGAAGTTTTCCAGGCATCTG		
rs33939927	Forward: CAGTTTGAAAGCAAACACAAGA	55	-
	Reverse: TTGCTTCTCATCAGAAACATCC		
	Probe: T$(GACT)_8$AAGAGGGTTTTGTGTCTTTCCCTCCAGGCT		
rs35801418	Forward: TTCCCCATTGTGAGAACTCT	55	-
	Reverse: TAATAAGTCTTCATTAGATACTTACCTCTCC		
	Probe: ACT$(GACT)_9$AAATTATCATCCGACTATATGAAATGCCTT		
rs34637584	Forward: same with rs35801418	55	-
	Reverse: same with rs35801418		
	Probe: $(GACT)_{11}$GCTGCCATCATTGCAAAGATTGCTGACTAC		
rs35870237	Forward: TTTTCACACTGTATCCCAATGC	55	-
	Reverse: ATAGAATTATGAGACAGACCTGATCAC		
	Probe: T$(GACT)_{12}$TTTATCCCCATTCTACAGCAGTACTGAGCA		
rs34778348	Forward: TAGCCCTGTTGTGGAAGTGT	55	-
	Reverse: AACCACAGAATTTACCTTAAAAAGTG		
	Probe: ACT$(GACT)_{13}$GTGTGGGATAAGAAAACTGAAAAACTCTGT		

Note: (GACT)n, n repeats of "GACT"; the primer pairs for SNaPshot assay were reported in our recent study (Bi et al. 2014).

Supplementary References

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. *Neurobiology of aging* 2014; **35:** 444.e5-e9

Table S3 Primers for cloning and site mutagenesis of the *LRRK2* gene

Item	Primer (5'-3')	Applications
For plasmid construct:		
*Xho*I-1F	CCG***CTCGAG***CCATGGCTAGTGGCAGCTGTCAGG	Forward primer for vector construction of WT and ΔWD40
*Not*I-7563R	ATAAGAAT***GCGGCCGC***TTACTCAACAGATGTTCGTCTC	Reverse primer for vector construction of WT
*Not*I- delWD40-6278R	ATAAGAAT***GCGGCCGC***TTAACCATATTCTTTAACTGGAT	Reverse primer for vector construction of ΔWD40
For lentivirus construct:		
*Not*I-LVX-F	ATAAGAAT***GCGGCCGC***ATGGCATCAATGCAGAAG	Forward primer for vector construction of WT and ΔWD40
*Mlu*I-LVX-R	CG***ACGCGT***TTACTCAACAGATGTTCGTCTC	Reverse primer for vector construction of WT
*Mlu*I-LVX-WD40-R	CG***ACGCGT***TTATAATAAAATGCGTCTCGTCA	Reverse primer for vector construction of ΔWD40
For site mutation:		
G2019S-F	TGCAAAGATTGCTGACTA***C***AGCATTGCTCAGT	Mutant G2019S-LRRK2
G2019S-R	GTACTGAGCAATGC***T***GTAGTCAGCAATCTT	
M2397T-F	TTTAAGGGAGGTAA***C***GGTAAAAGAAAACAAG	Mutant M2397T-LRRK2
M2397T-R	TTCCTTGTTTTCTTTTACC***G***TTACCTCCCTTAA	

Note: Restriction recognition sites or directed mutagenesis sites are marked with italic, bold and underlining, respectively. F - forward primer; R - reverse primer. WT - wild type LRRK2 protein; ΔWD40 - LRRK2 mutant with a deletion of residues 2010-2527 that represent the WD40 domain.

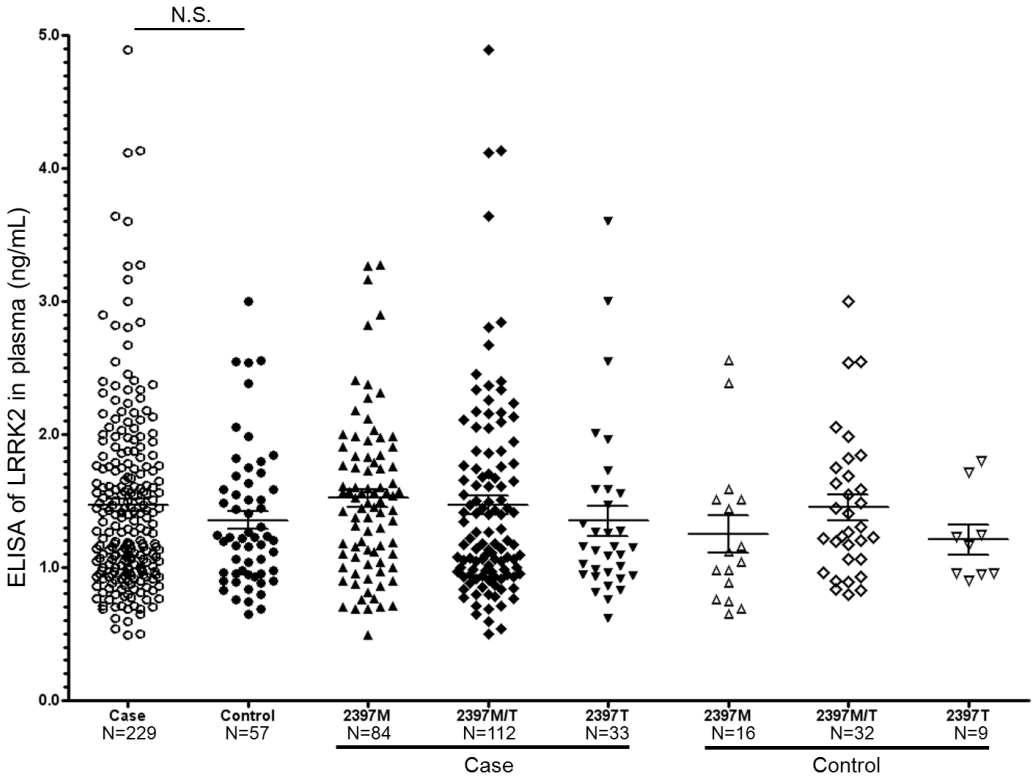
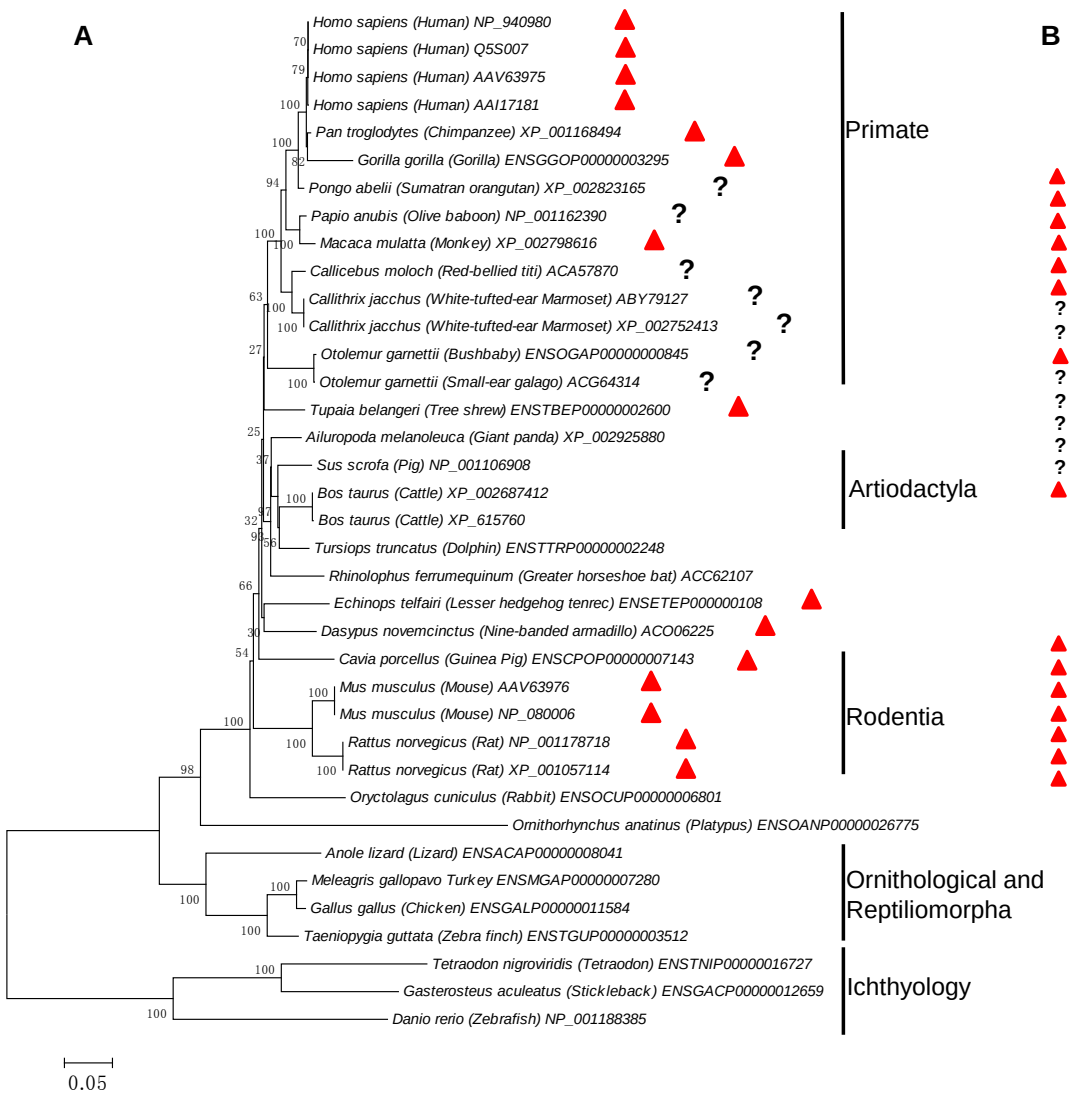


Figure S1 Measurement of serum LRRK2 in the leprosy cases and controls by ELISA. N.S., no statistical significance. We divided the analyzed individuals into different groups according to different genotypes of rs3761863 (2397M, homozygote of p. Met2397; 2397M/T, heterozygote of p.Met2397 and p.Thr2397; 2397T, homozygote of p.Thr2397), but found no statistical difference between groups.



B

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                                    2222 2222222222 222222
                                    3333 3333333334 444444
                                    8889 9999999990 000000
                                    7890 1234567890 123456
Homo sapiens NP 940980              IDCV HFLREVMVKE NKESK-
Homo sapiens Q5S007                 .... .......... .....-
Homo sapiens AAV63975               .... .......... .....-
Homo sapiens AAI17181               .... ......T... .....-
Pan troglodytes XP 001168494        .... .L....T... .....-
Gorilla gorilla ENSGGOP00000003295  .... .L....T... .....-
Pongo abelii XP 002823165           .... ......T... .....-
Papio anubis NP 001162390           .... .........V .....-
Macaca mulatta XP 002798616         .... .........V .....-
Callicebus moloch ACA57870          .... .....LL..V .....-
Callithrix jacchus ABY79127         .... .....L...V .....-
Callithrix jacchus  XP 002752413    .... .....L...V .....-
Otolemur garnettii ENSOGAP00000000845 ???? ????.E...V S....-
Otolemur garnettii ACG64314         .... ...K.E...V S....-
Tupaia belangeri ENSTBEP00000002600 .... ...K.I.L.I .....-
Ailuropoda melanoleuca XP 002925880 .... ...K.E...V SA...-
Sus scrofa NP 001106908             .... ...K.ELA.A ...L.-
Bos taurus XP 002687412             .... ...K.EL.RV S..L.-
Bos taurus XP 615760                .... ...K.EL.RV S..L.-
Tursiops truncatus ENSTTRP00000002248 .... ...K.EL..A ...A.-
Rhinolophus ferrumequinum ACC62107  .... .....ELL.. ..D..-
Echinops telfairi ENSETEP000000108  .... ...K.E...G .....-
Dasypus novemcinctus ACO06225       .... ...K.E...A S....-
Cavia porcellus ENSCPOP00000007143  .... .L.K.....M .Q...-
Mus musculus  AAV63976              .... ...K.....L .....-
Mus musculus NP 080006              .... ...K.....L .....-
Rattus norvegicus NP 001178718      .... ...K.....I ..D..-
Rattus norvegicus XP 001057114      .... ...K.....I ..D..-
Oryctolagus cuniculus ENSOCUP00000006801 .... ...K.A.AVG KQ...-
Ornithorhynchus anatinus ENSOANP00000026775 .... ...K.E.ETL .....-
Anole lizard ENSACAP00000008041     .... ...K.EVP.L .....-
Meleagris gallopavo ENSMGAP00000007280 ...A ...KDEVE.L .Q...-
Gallus gallus ENSGALP00000011584    ...A ...KDEVE.L .....-
Taeniopygia guttata ENSTGUP00000003512 ...A ...KNKVE.L .....-
Tetraodon nigroviridis ENSTNIP00000016727 ...A .II.RDSGGT T-----
Gasterosteus aculeatus ENSGACP00000012659 V..A QII.HDSAVR SRRASE
Danio rerio NP 001188385            ...M QL.GLSSTRK P.VHSE
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Figure S2. Neighbor-joining tree based on the LRRK2 amino acid sequences (A) and evolutionary conservation analysis of p.M2397T of LRRK2 (B) in 30 vertebrate species. Numbers above branches are bootstrap values. Those species with a confirmed infection of *M. leprae* are marked by red triangle, and "?" means that the species might be infected by *M. leprae* based on evolutionary prediction. Accuracies and statistical test of phylogenetic tree were measured by bootstrap method with 1000 replications. Sequence accession number was listed after the species name.