ORIGINAL ARTICLE

LRRK2 N551K and R1398H variants are protective in Malays and Chinese in Malaysia: A case–control association study for Parkinson’s disease

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Abstract
Background: The LRRK2 gene is associated with Parkinson's disease (PD) as a number of mutations within the gene have been shown to be susceptibility factors. Studies on various global populations have determined that mutations such as G2019S, G2385R, and R1628P in LRRK2 increase the risk of developing PD while the N551K-R1398H haplotype is associated with conferring protection against developing PD. Here we report a study looking at the N551K and R1398H variants for the first time in the Malaysian population.

Methods: Cases (523) which conformed to the United Kingdom PD Brain Bank Criteria for PD were recruited through trained neurologists and age- and ethnically matched controls (491) were individuals free of any neurological disorder. The N551K and R1398H mutations were genotyped using the Taqman SNP genotyping assay.

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1 | INTRODUCTION

Parkinson’s disease (PD) is an age-related neurodegenerative disease, caused by the loss of dopaminergic neurons in the substantia nigra pars compacta of the brain. The loss of the dopaminergic neurons leads to a range of movement problems including rigidity, bradykinesia, and tremors.

The leucine-rich repeat kinase 2 (LRRK2) gene has been extensively studied in relation to both familial and sporadic forms of Parkinson’s disease. The PD mutation database reports 127 mutations in this gene, with the G2019S mutation accounting for 40% of North African Arab and 20% of Ashkenazi Jewish PD cases (Ozelius et al., 2006). The G2019S mutation has been consistently shown to result in hyperactivation of the LRRK2 kinase, which has been associated with defects in protein synthesis and degradation, apoptosis, inflammatory responses, and oxidative damage (Rui et al., 2018; Smith et al., 2006). However, this mutation is almost completely absent in Asian PD populations studied thus far (Japanese, Chinese, and Koreans) (Bekris, Mata, & Zabetian, 2010; Guedes et al., 2010; Lesage et al., 2006). In contrast, the G2385R and R1628P mutations are relatively common in Asian PD patients, suggesting differing ethnic-specific patterns of inheritance for LRRK2 mutations (Gopalai et al., 2014; Zhang et al., 2017).

The N551K and R1398H variants were first described in a PD study looking at linkage disequilibrium within LRRK2 (Paisan-Ruiz et al., 2006). A multicenter case–control study suggested that individuals carrying the N551K (c.1653C > G, rs7308720) and R1398H (c.4193G > A, rs7133914) variants had a 20% reduced risk of developing PD (Ross et al., 2011). This was replicated in two Asian studies in Singapore and Taiwan (Tan et al., 2010; Wu et al., 2013). These variants have not previously been screened in a Malaysian PD population and constitutes a significant gap in our understanding of the associated genetic factors in this population. With the advent of targeted therapies (Chan & Tan, 2017), an improved understanding of the genetic and mechanistic factors underlying PD is becoming increasingly important. Therefore, this study aimed to investigate the association between these protective alleles with the risk of PD in a multi-ethnic Malaysian population.

2 | MATERIALS AND METHODS

2.1 | Sample recruitment and genetic analysis

A total of 523 PD cases and 491 controls were screened. The patients were recruited from neurology clinics throughout Peninsular Malaysia. PD patients were diagnosed based on the United Kingdom PD Brain Bank Criteria by movement disorder specialists or neurologists with an interest in PD. Control subjects were recruited from spouses and from outpatient clinics. The controls were age and gender-matched and were not suffering from any neurological disorder. Institutional ethical approval was obtained and all participants provided written informed consent.

The N551K and R1398H variants were genotyped using TaqMan® SNP genotyping assays (Applied Biosystems) on a 7,500 Fast Real-Time PCR machine. Genotypes were confirmed by sequencing in a subset of 20 individuals to confirm the genotypes and determine the error rate.

Statistical analysis was performed using open-source software (OpenEpi) while Review Manager 5 (RevMan 5) (Collaboration, 2014) was used to conduct the meta-analysis among the Chinese cohort. Heterogeneity among the studies was assessed with the I² statistics (Higgins & Thompson, 2002).

3 | RESULTS AND DISCUSSION

The mean age at PD diagnosis was 57.4 ± 11.8 years and the mean age of controls was 59.3 ± 9.4 years (p = 0.0048). Sixty per cent of PD patients and 51% of controls were male. The cohort consisted of 168 Malay PD cases, 133 Malay controls, 279 Chinese PD cases, 269 Chinese controls, 76 Indian PD cases, and 89 Indian controls.
Linkage analysis (Haploview 4.2) indicated that N551K and R1398H are in linkage disequilibrium ($D' = 0.959, r^2 = 0.906$), similar to what has been reported by Tan et al., 2010 (Tan et al., 2010). Genotypes for both variants were in Hardy–Weinberg equilibrium. The error rate of the assay was 0%.

The mutant alleles for both variants were more frequent in the controls by almost twofold. An odds ratio (OR) of 0.623 (95% CI 0.44–0.88, $p = 0.007$) was obtained for N551K, and an OR of 0.699 (95% CI 0.50–0.98, $p = 0.036$) was obtained for R1398H, suggesting a reduced risk of developing PD in carriers (Table 1).

When analyzed according to ethnicities, a protective association for N551K was detected in Malays (OR 0.446, 95% CI 0.22–0.90, $p = 0.025$). No significant difference was found between the Chinese case and controls (OR 0.700, 95% CI 0.46–1.07, $p = 0.096$). The Indian subgroup showed a similar, albeit nonsignificant trend (OR 0.574, 95% CI 0.17–1.95, $p = 0.373$), likely due to the small sample size. When we performed a meta-analysis for N551K on the combined Chinese datasets from this study,
and those by Tan et al. and Wu et al., the analysis showed a significant protective effect with an OR of 0.79 (95% CI 0.67–0.92, \( p = 0.003 \) (Table 2, Figures 1a, 2a). No heterogeneity was detected amongst the studies included in the meta-analysis for N551K (\( p_{\text{heterogeneity}} = 0.57, I^2 = 0% \)). Meta-analysis on Malay and Indian samples was not performed as there are no other published studies on these populations for N551K.

When analyzing R1398H according to ethnicities, the protective association was detectable with borderline significance in Malays (OR 0.495, 95% CI 0.24–1.01, \( p = 0.055 \)), but the results were not significant in the Chinese (OR 0.808, 95% CI 0.54–1.22 \( p = 0.306 \)) and Indians (OR 0.571, 95% CI 0.19–1.71 \( p = 0.317 \)). We performed a meta-analysis of R1398H (combined Chinese datasets from this study, and those by Tan et al., Wu et al., and Wu-Chou et al.) and this showed an OR of 0.81 (95% CI 0.70–0.93, \( p = 0.002 \), (Table 2, Figures 1b, 2b). Similar to N551K, meta-analysis on Malays and Indians was not possible as this is the first report on these populations for N551K.

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## 4 | CONCLUSION

We have previously reported an association between PD in our Malaysian cohort with the risk alleles G2385R and R1628P within the \( \text{LRRK2} \) gene (Gopalai et al., 2014). However, the association with the R1398H and N551K protective alleles have not previously been determined in this population. Here we report that N551K variant is associated in a protective manner in the Malay population, with the R1398H variant also showing a similar protective trend. N551K and R1398H showed a significant protective association when we pooled our Chinese cohort with other reported studies. The N551K-R1398H association was unable to be detected in the Malaysian Indian cohort, likely due to the relatively small sample size.

The exact mechanism(s) underlying the protective effects of the N551K-R1398H haplotype are not clear at present. The N551K variant is not within any domain of the \( \text{LRRK2} \) protein, but is in linkage disequilibrium with the R1398H variant, which lies within the Ras-of-complex (ROC) GTPase domain, and enables the binding of guanine nucleotides via a phosphate-binding motif.

The kinase activity of \( \text{LRRK2} \) is modulated by GTPase activity, GTP hydrolysis, and GTP binding (Cookson, 2010). Pathogenic mutations in \( \text{LRRK2} \) such as G2019S elevate the level of kinase activity (West et al., 2005), which in turn has been shown to cause neuronal toxicity (Smith et al., 2006). Lower levels of GTPase activity lead to a lower level of \( \text{LRRK2} \) kinase activity (Biosa et al., 2012). Studies on R1398H have indicated that it plays a role in decreasing GTP-bound \( \text{LRRK2} \), in addition to positive effects on axon outgrowth and activation of associated Wnt signaling pathways (Nixon-Abell et al., 2016). This may be one

| N551K (c.1653C>G), rs7308720 | PD cases | Controls |
| --- | --- | --- |
| Malay | Chinese | Indian | Malay | Chinese | Indian |
| Wild type (C/C) | 155 | 239 | 72 | 113 | 214 | 81 |
| Heterozygous mutant (C/G) | 13 | 38 | 4 | 18 | 54 | 8 |
| Homozygous mutant (G/G) | – | 2 | – | 2 | 1 | – |

| Allelic frequency (%) | Malay | Chinese | Indian |
| --- | --- | --- | --- |
| Wild type (C) | 96.1 | 92.5 | 97.4 |
| Mutant (G) | 3.9 | 7.5 | 2.6 |

| R1398H (c.4193G>A), rs73313914 | PD cases | Controls |
| --- | --- | --- |
| Malay | Chinese | Indian | Malay | Chinese | Indian |
| Wild type (G/G) | 155 | 233 | 71 | 115 | 215 | 80 |
| Heterozygous mutant (G/A) | 13 | 45 | 5 | 16 | 53 | 8 |
| Homozygous mutant (A/A) | – | 1 | – | 2 | 1 | 1 |

| Allelic frequency (%) | Malay | Chinese | Indian |
| --- | --- | --- | --- |
| Wild type (G) | 95.6 | 91.5 | 96.7 |
| Mutant (A) | 4.4 | 8.6 | 3.3 |
mechanism through which it may be conferring a protective effect on the cell.

Apart from having a protective effect in PD, another study investigated a possible link with rapid eye movement-sleep behavior disorder (RBD), a condition now regarded as a prodromal symptom of synucleinopathies, most commonly PD. In a case–control study involving 350 RBD patients and 869 controls, the N551K-R1398H haplotype was significantly associated with a reduced risk of developing RBD (Bencheikh et al., 2018). No association was found in studies of Alzheimer's disease patients (Ng, Ng, Tan, Kandiah et al., 2018) or essential tremor (Ng, Ng, Tan, Prakash et al., 2018).

In conclusion, we show that consistent with other published reports on the protective effect of N551K and R1398H, these variants are also protective in the Malaysian Malay and Chinese ethnicities. Further studies will need to be done to determine the cellular mechanism of how this protective effect is mediated.

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**CONFLICT OF INTEREST**

The authors have no conflicts of interest to declare.
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