Research Article

LRRK2 G2385R and R1628P Mutations Are Associated with an Increased Risk of Parkinson’s Disease in the Malaysian Population

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The LRRK2 gene has been associated with both familial and sporadic forms of Parkinson’s disease (PD). The G2019S variant is commonly found in North African Arab and Caucasian PD patients, but this locus is monomorphic in Asians. The G2385R and R1628P variants are associated with a higher risk of developing PD in certain Asian populations but have not been studied in the Malaysian population. Therefore, we screened the G2385R and R1628P variants in 1,202 Malaysian subjects consisting of 695 cases and 507 controls. The G2385R and R1628P variants were associated with a 2.2-fold \((P = 0.019)\) and 1.2-fold \((P = 0.054)\) increased risk of PD, respectively. Our data concur with other reported findings in Chinese, Taiwanese, Singaporean, and Korean studies.

1. Introduction

Parkinson’s disease (PD) is an age-related illness, and, as populations age, the proportion of people with this neurodegenerative disease will continue to rise. It is projected that, by the year 2030, 9.3 million individuals above the age of 50 will suffer from PD and these cases will be concentrated outside the western world [1]. Studies have implicated exposure to environmental toxins and trauma as aetiological factors for PD [2], Genetic variations also play a role, especially in cases where there is a family history of PD, which account for around 10–20% of all PD cases [3]. However, studies have shown that even late-onset sporadic PD may also have a genetic contribution [4].

One of the genes commonly implicated in both familial and sporadic PD is the leucine-rich repeat kinase 2 (LRRK2) gene. Several variants of LRRK2 such as R1441C, G2019S, and I2020T have been well established as risk factors for PD [3]. Interestingly, there appear to be population-specific variants in LRRK2; for example, the G2019S variant is prevalent among the Ashkenazi Jews and North African Arabs...
Table 1: Summary of the genotyping data.

| SNP | PD (MAF) | Controls (MAF) | OR (95% confidence interval) |
|-----|----------|----------------|-----------------------------|
| Wild type (G) | 1354 (0.974) | 1002 (0.999) | OR 2.22 (1.15–4.29) |
| Variant (A) | 36 (0.026) | 12 (0.001) | P = 0.019 |
| Wild type (G) | 1347 (0.969) | 966 (0.982) | OR 1.23 (1.039–1.448) |
| Variant (C) | 43 (0.031) | 10 (0.001) | P = 0.054 |

Table 2: Summary of published Asian data on G2385R and R1628P.

| Study | Asian country | Sample size | Results |
|-------|---------------|-------------|---------|
| Di Fonzo et al., 2006 [9] | Taiwan | 608 PD, 373 controls | OR 2.24 (P = 0.004) |
| Fung et al., 2006 [20] | Taiwan | 305 PD, 176 controls | OR 1.70 (P = 0.0002) |
| Farrer et al., 2007 [21] | Taiwan | 410 PD, 335 controls | OR 2.24 (P = 0.014) |
| Tan et al., 2007 [14] | Singapore | 495 PD, 494 controls | OR 2.14 (P = 0.014) |
| Tan et al., 2007 [16] | Non-Chinese Asian (Malays and Indians) | 98 PD, 173 controls | OR 1.78 (P = 0.3) |
| An et al., 2008 [11] | Mainland China | 600 PD, 334 controls | OR 3.94 (P < 0.01) |
| Funayama et al., 2007 [10] | Japan | 448 PD, 457 controls | OR 2.60 (P = 1.24 × 10^{-4}) |
| Zabetian et al., 2009 [7] | Japan | 601 PD, 1,628 controls | OR 1.96 (P < 0.001) |
| Miyake et al., 2010 [22] | Japan | 229 PD, 358 controls | OR 2.06 |
| Kim et al., 2010 [12] | Korea | 923 PD, 422 controls | OR 1.83 (P = 0.017) |
| | | 119 YOPD | OR 2.28 (P = 0.098) |
| | | 814 LOPD | OR 1.81 (P = 0.022) |
| Ross et al., 2011 [19] | Asian | 369 PD, 300 controls | OR 1.62 |
| | | 844 PD, 587 controls | * P value not stated |
| | | 173 PD, 95 controls | OR 1.87 |
| | | 1,386 PD, 982 controls | * P value not stated |
| Current study | Malaysia | 695 PD, 507 controls | OR 1.87 (P value not stated) |

| Study | Asian country | Sample size | Results |
|-------|---------------|-------------|---------|
| Mata et al., 2005 [15] | Europe, Asia, and North America | 100 PD probands with family history of parkinsonism, 300 controls | MAF 0.01 |
| Lu et al., 2008 [18] | Taiwan | 834 PD, 543 controls | OR 2.13 (P = 0.004) |
| Tan et al., 2008 [16] | Singapore | 246 PD, 243 controls | OR 2.5 (P = 0.046) |
| Tan et al., 2008 [23] | Non-Chinese Asian (Malays and Indians) | 132 PD, 160 controls | OR 0.61 (P = 0.600) |
| | | 60 PD, 105 controls | Indians-monomorphic |
| Ross et al., 2008 [13] | Taiwan, Singapore | 484 PD, 341 controls | OR 2.15 (P = 0.025) |
| | | 345 PD, 316 controls | OR 1.39 (P = 0.179) |
| | | 250 PD, 250 controls | OR 2.20 (P = 0.163) |
| | | 1,079 PD, 907 controls | OR 1.84 (P = 0.006) |
| Zabetian et al., 2009 [7] | Japanese | 631 PD, 320 controls | Monomorphic |
| Yu et al., 2009 [24] | Mainland China | 328 PD, 300 controls | OR 2.68 (P < 0.05) |
| Zhang et al., 2009 [25] | Mainland China | 600 PD, 459 controls | OR 3.14 (P < 0.01) |
| Kim et al., 2010 [12] | Korea | 384 PD, 384 controls | OR 2.98 (P = 0.32) |
| Pulkes et al., 2011 [17] | Thai | 154 PD, 156 controls | OR 3.25 (P = 0.021) |
A subset of 20 individuals was sequenced for the G2385R variant in Asian PD populations (Chinese, Taiwanese, and Singaporean) [13]. The R1628P variant is another common risk factor. Interestingly, the G2385R mutation was present in control subjects as well (MAF = 0.001), although it was less frequently present than in the PD cohort (MAF = 0.026).

Given the lack of data regarding how these variants contribute to PD in Malaysian patients, we sought to investigate the prevalence of G2385R and R1628P in a Malaysian PD cohort. We found that G2385R was significantly associated with PD and R1628P showed a trend towards being a risk factor.

### 2. Methodology

A total of 1,202 subjects participated in this study. Six hundred and ninety-five PD patients were diagnosed by neurologists based on the United Kingdom PD Brain Bank Criteria and 507 controls who did not suffer from any neurological or movement disorders were recruited. Ethics approval and written consent from subjects were obtained. DNA was extracted from lymphocytes that were obtained from venous blood using the phenol-chloroform method. The G2385R (rs34778348) and R1628P (rs33949390) genotyping was done by Taqman allelic discrimination assay on a 7500 Fast Real-Time PCR machine. A subset of 20 individuals was sequenced to determine the error rate. The allele and genotype frequencies in PD cases and controls were compared with Fisher’s exact test. Statistical analyses were performed using an open-source software (OpenEpi).

### 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. Sixty percent of PD patients and 51% of controls were male. Results of the G2385R and R1628P genotyping are summarised in Table 1. The error rate of the assay was 0% in the subset of 20 individuals. Fifty-five patients (7.9%) had early-onset PD (onset < 40 years). Four patients were compound heterozygous for G2385R and R1628P; two of these patients had a family history of PD and developed PD before the age of 50, while the other two patients had no family history and had a later age of onset (>55).

The G2385R variant was associated with PD, with an odds ratio (OR) of 2.22 (P = 0.019), while the R1628P variant had an OR of 1.23 with a trend towards significance (P = 0.054). Interestingly, the G2385R mutation was present in control subjects as well (MAF = 0.001), although it was less frequently present than in the PD cohort (MAF = 0.026).

Our findings are in keeping with other published reports on G2385R, where this variant is associated with an increased risk of developing PD by approximately twofold (Chinese, Taiwanese, Singaporean, and Japanese populations) (Table 2). The G2385R variant is located within the WD40 domain of LRRK2, which is responsible for a variety of functions including signal transduction, pre-mRNA processing, and cytoskeleton assembly, and cells carrying the G2385R variant are more susceptible to oxidative stress and apoptosis [14].

The R1628P variant was first identified by Mata et al. [15]. Subsequently, Ross et al. reported this variant to be the second common genetic risk factor for PD in the ethnic Chinese (Taiwanese and Singaporean) population, with an OR of 1.84 (P = 0.006) [13]. Other independent studies carried out by Tan et al., Pulkes et al., and Lu et al. in Singapore, Thailand, and China showed a similar trend with OR values of 2.5, 3.3, and 2.1, respectively [16–18]. However, this was not observed in a Japanese cohort where the locus was found to be monomorphic [7]. This mutation alters a highly conserved amino residue within the “COR” domain of the LRRK2 protein [18]. The substitution of a highly basic polar arginine (R) with a neutral nonpolar proline (P) is likely to cause a conformational change in the protein secondary structure, thus altering the function of the protein. We note however that a recent multicentre study by Ross et al. involving 1386 Asian PD cases and 982 Asian controls did not find an association with R1628P (OR 0.62, 95% CI 0.36–1.07, P = 0.087) [19]. Whilst the findings in their Japanese and Korean subsets were consistent with previously published data, their Taiwanese cohort did not show a risk association, but rather a trend in the opposite direction (i.e., protective, with an OR of 0.56, 95% CI 0.32–1.01, P = 0.054).

In conclusion, our data concur with other reports in the Chinese, Taiwanese, Singaporean, and Korean populations. The G2385R variant is significantly associated with an increased risk of developing PD, while the R1628P variant is predicted to have a more modest effect. These data together with others can lead to a better understanding of the
pathogenetic pathways leading to cell dysfunction and death in PD, with the ultimate hope that more specific drugs can be developed to treat this disabling disease.

**Conflict of Interests**

The authors declare that they have no conflict of interests regarding the publication of this paper.

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**References**

[1] E. R. Dorsey, R. Constantinescu, J. P. Thompson et al., “Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030,” Neurology, vol. 68, no. 5, pp. 384–386, 2007.

[2] P. Lee, Y. Bordelon, J. Bronstein, and B. Ritz, “Traumatic brain injury, paraquat exposure, and their relationship to Parkinson disease,” Neurology, vol. 79, no. 20, pp. 2061–2066, 2012.

[3] L. M. Bekris, F. M. Ignacio, and C. P. Zabetian, “The genetics of Parkinson disease,” Journal of Geriatric Psychiatry and Neurology, vol. 23, no. 4, pp. 228–242, 2010.

[4] W. Satake, Y. Nakabayashi, I. Mizuta et al., “Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson’s disease,” Nature Genetics, vol. 41, no. 12, pp. 1303–1307, 2009.

[5] L. J. Ozcelik, G. Senthil, R. Saunders-Pullman et al., “LRRK2 G2019S as a cause of Parkinson’s disease in Ashkenazi Jews,” New England Journal of Medicine, vol. 354, no. 4, pp. 424–425, 2006.

[6] E. K. Tan, H. Shen, L. C. S. Tan et al., “The G2019S LRRK2 mutation is uncommon in an Asian cohort of Parkinson’s disease patients,” Neuroscience Letters, vol. 384, no. 3, pp. 327–329, 2005.

[7] C. P. Zabetian, M. Yamamoto, A. N. Lopez et al., “LRRK2 mutations and risk variants in Japanese patients with Parkinson’s disease,” Movement Disorders, vol. 24, no. 7, pp. 1034–1041, 2009.

[8] M. Toft, K. Haugarvoll, O. A. Ross, M. J. Farrer, and J. O. Aasly, “LRRK2 and Parkinson’s disease in Norway,” Acta Neurologica Scandinavica, vol. 115, no. 187, pp. 72–75, 2007.

[9] A. Di Fonzo, Y. H. Wu-Chou, C. S. Lu et al., “A common missense variant in the LRRK2 gene, Gly2385Arg, associated with Parkinson’s disease risk in Taiwan,” Neurogenetics, vol. 7, no. 3, pp. 133–138, 2006.

[10] M. Funayama, Y. Li, H. Tomiyama et al., “Leucine-rich repeat kinase 2 G2385R variant is a risk factor for Parkinson disease in Asian population,” NeuroReport, vol. 18, no. 3, pp. 273–275, 2007.

[11] X.-K. An, R. Peng, T. Li et al., “LRRK2 Gly2385Arg variant is a risk factor of Parkinson’s disease among Han-Chinese from mainland China,” European Journal of Neurology, vol. 15, no. 3, pp. 301–305, 2008.

[12] J. M. Kim, J. Y. Lee, H. J. Kim et al., “The LRRK2 G2385R variant is a risk factor for sporadic Parkinson’s disease in the Korean population,” Parkinsonism and Related Disorders, vol. 16, no. 2, pp. 85–88, 2010.

[13] O. A. Ross, Y. Wu, M. Lee et al., “Analysis of Lrrk2 R1628P as a risk factor for Parkinson’s disease,” Annals of Neurology, vol. 64, no. 1, pp. 88–92, 2008.

[14] E. K. Tan, Y. Zhao, L. Skipper et al., “The LRRK2 Gly2385Arg variant is associated with Parkinson’s disease: genetic and functional evidence,” Human Genetics, vol. 120, no. 6, pp. 857–863, 2007.

[15] I. F. Mata, J. M. Kachergus, J. P. Taylor et al., “Lrrk2 pathogenic substitutions in Parkinson’s disease,” Neurogenetics, vol. 6, no. 4, pp. 171–177, 2005.

[16] E. K. Tan, L. C. Tan, H. Q. Lim et al., “LRRK2 R1628P increases risk of Parkinson’s disease: replication evidence,” Human Genetics, vol. 124, no. 3, pp. 287–288, 2008.

[17] T. Pulkes, C. Papsing, S. Mahasirimongkol, M. Busabarata, K. Kulkantakorn, and S. Tiamkao, “Frequencies of LRRK2 variants in Thai patients with Parkinson’s disease: evidence for an R1628P founder,” Journal of Neurology, Neurosurgery and Psychiatry, vol. 82, no. 10, pp. 1179–1180, 2011.

[18] C. Lu, Y. Wu-Chou, M. Van Doeselaar et al., “The LRRK2 Arg1628Pro variant is a risk factor for Parkinson’s disease in the Chinese population,” Neurogenetics, vol. 9, no. 4, pp. 271–276, 2008.

[19] O. A. Ross, A. I. Soto-Ortolaza, M. J. Heckman et al., “Association of LRRK2 exonic variants with susceptibility to Parkinson’s disease: a casecontrol study,” Lancet Neurology, vol. 10, pp. 989–998, 2011.

[20] H. C. Fung, C. M. Chen, J. Hardy, A. B. Singleton, and Y. R. Wu, “A common genetic factor for Parkinson disease in ethnic Chinese population in Taiwan,” BMC Neurology, vol. 6, article 47, 2006.

[21] M. J. Farrer, J. T. Stone, C. Lin et al., “Lrrk2 G2385R is an ancestral risk factor for Parkinson’s disease in Asia,” Parkinsonism and Related Disorders, vol. 13, no. 2, pp. 89–92, 2007.

[22] Y. Miyake, Y. Tsuiboi, M. Koyanagi et al., “LRRK2 Gly2385Arg polymorphism, cigarette smoking, and risk of sporadic Parkinson’s disease: a case-control study in Japan,” Journal of the Neurological Sciences, vol. 297, no. 1–2, pp. 15–18, 2010.

[23] E. Tan, M. Tang, L. C. Tan et al., “Lrrk2 R1628P in non-Chinese Asian races,” Annals of Neurology, vol. 64, no. 4, pp. 472–473, 2008.

[24] L. Yu, F. Hu, X. Zou et al., “LRRK2 R1628P contributes to Parkinson’s disease susceptibility in Chinese Han populations from mainland China,” Brain Research, vol. 1296, pp. 113–116, 2009.

[25] Z. Zhang, J. Burgunder, X. An et al., “LRRK2 R1628P variant is a risk factor of Parkinson’s disease among Han-Chinese from mainland China,” Movement Disorders, vol. 24, no. 13, pp. 1902–1905, 2009.