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Negative results

Case-control analysis of leucine-rich repeat kinase 2 protective variants in Alzheimer's disease

Adeline S.L. Ng [a,*], Ebonne Y.L. Ng [b], Yi Jayne Tan [a], Nagaendran Kandiah [a], Juan Zhou [c], Shahul Hameed [b], Simon K.S. Ting [b], Eng-King Tan [b,c,**]

[a] Department of Neurology, National Neuroscience Institute, Tan Tock Seng Hospital, Singapore
[b] Department of Neurology, National Neuroscience Institute, Singapore General Hospital, Singapore
[c] Neuroscience and Behavioural Disorders Program, Duke-NUS Medical School, Singapore

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ABSTRACT

Amyloid is the main pathological substrate of Alzheimer's disease (AD) and has been described in leucine-rich repeat kinase 2 (LRRK2) carriers with Parkinson's disease. LRRK2 has been linked with amyloid precursor protein pathways in neurodegeneration. Two common LRRK2 variants, R1398H and N551K, have been shown to be protective in multiple Parkinson's disease cohorts. We hypothesized that R1398H and N551K may be protective in AD. In a case-control study involving 1390 subjects (719 controls and 671 AD cases), R1398H was demonstrated in 16.8% of AD cases compared to 16.7% in controls (odds ratio = 1.01, 95% confidence interval = 0.76–1.34, $p = 0.94$), whereas N551K was demonstrated in 17.3% of AD cases compared to 17.2% of controls (odds ratio = 1.00, 95% confidence interval = 0.76–1.32, $p = 0.98$). Overall, these results suggest that LRRK2 R1398H or N551K variants do not appear to modulate the risk of AD.

1. Introduction

Leucine-rich repeat kinase 2 (LRRK2) mutations account for between 5% and 13% of familial Parkinson's disease (PD) and <5% of sporadic PD (Tan and Skipper, 2007). Amyloid is the main pathological hallmark of Alzheimer's disease (AD) and has been reported to be present in both symptomatic LRRK2 mutation carriers (Zimprich et al., 2004) and asymptomatic LRRK2 carriers, correlating with lower striatal dopaminergic function (Aasly et al., 2012). Although G2019S remains the most common LRRK2 mutation (Kachergus et al., 2005), it is rarely reported in Asian cohorts (Cho et al., 2009; Tan et al., 2005). Two polymorphic LRRK2 variants rs34778348 (G2385R) and rs33949390 (R1628P), however, were previously reported to confer a relative risk of 1.9 for developing PD in Chinese patients (Di Fonzo et al., 2006; Tan et al., 2007). This twofold risk was reduced to 1.5–1.6 if carriers also possessed the variants rs7133914 (R1398H) or rs7308720 (N551K), both in linkage disequilibrium (Tan et al., 2010). The N551K-R1398H-K1423K protective haplotype has a reported frequency of >5% in a large Caucasian and Asian series (Ross et al., 2011), with R1398H being the most likely functional variant and its protective effect appearing independent of other *SNCA* and microtubule-associated tau variants (Heckman et al., 2014). The protective effect of N551K and R1398H has been replicated in other Asian cohorts (Chen et al., 2011; Tan et al., 2010), with multivariate regression analysis revealing that R1398H and N551K confer a 20% reduction in PD risk, independent of the Asian risk variants G2385R and R1628P (Tan et al., 2010). In non-PD cohorts, a nonsignificant protective trend was observed for the N551K-R1398H-K1423K haplotype in a clinical Lewy body dementia series (OR 0.76, $p = 0.061$) (Heckman et al., 2016). A possible mechanistic link between LRRK2 and dementia was revealed when recent studies using LRRK2 G2019S mouse models, patient-derived neurons, and postmortem brain tissue showed that mutant LRRK2 phosphorylates amyloid precursor protein at its intracellular domain, with resultant increase in its nuclear translocation and transcriptional activity, leading to neuronal loss (Chen et al., 2017). Given the evidence for a link between LRRK2 and amyloid precursor protein, we carried out a case-control study to investigate if the apparent protective effect of R1398H or N551K would be seen in AD.

2. Methods

Consecutive AD cases recruited from the memory clinics at the National Neuroscience Institute and age-, gender-, and race-matched controls were included this study. Some of the control

* Corresponding author at: Department of Neurology, National Neuroscience Institute, 11 Jalan Tan Tock Seng, Singapore 308433. Tel.: +65 6357 7171; fax: +65 6357 7133.
** Corresponding author at: Department of Neurology, Singapore General Hospital, Singapore 169108. Tel.: +65 6326 5003; fax: +65 6320 3321.
E-mail addresses: adeline.ng.s.l@singhealth.com.sg (A.S.L. Ng), tan.eng.king@singhealth.com.sg (E.-K. Tan).

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subjects had participated in an earlier study (Tan et al., 2010). AD was diagnosed using the revised National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria (McKhann et al., 2011). The controls did not have any known neurodegenerative diseases. The study received approval from the institutional ethics committees at both National Neuroscience Institute hospitals (Tan Tock Seng Hospital and Singapore General Hospital), and all study subjects gave their informed consent. Genotyping of the R1398H and N551K variants was carried out as previously described (Tan et al., 2010). Our study has 70%–80% power to detect a relative risk of 0.75 at a significance level of 0.05.

3. Results

DNA samples from a total of 1390 subjects comprising 671 AD subjects and 719 age- and race-matched controls were analyzed; 88.3% were ethnic Chinese while the rest were of mixed Asian ethnicity. The mean ± SD age of cases and controls was 68.0 ± 10.8 and 67.2 ± 6.5 years, respectively, comprising approximately 51.5% men and 48.5% women. The genotype frequencies of both R1398H and N551K in both AD and controls are summarized in Table 1. The genotype frequencies of heterozygous R1398H and N551K carriers were not significantly different in AD compared to controls. Three of 671 dementia cases and 3 of 719 controls were homozygous for the R1398H variant. Three of 671 dementia cases and 3 of 719 controls were homozygous for the N551K variant.

4. Discussion

Previous work by our group using dopaminergic neuronal lines revealed that both LRRK2 Asian risk variants G2385R and R1628P showed increased kinase activity consistent with dominant missense LRRK2 mutations that act via a toxic gain-of-function mechanism, while the protective R1398H variant displayed diminished extrinsic kinase activity compared to wild-type lines (Tan et al., 2010). R1398H may exert its protective effect by affecting guanosine triphosphate function, axon outgrowth, and Wnt signaling pathways opposite to pathogenic LRRK2 mutations (Nixon-Abell et al., 2016). R1398H was shown to increase guanosine triphosphate domain dimerization and GTP hydrolysis; reduce GTP binding leading to decrease in active GTP-bound LRRK2; increase axon length of primary cortical neurones in comparison to wild-type LRRK2; and enhance the stimulatory effect of LRRK2 on the canonical Wnt signaling pathway. This was in opposition to the effects seen with the G2385R risk variant, suggesting that R1398H is a bona fide protective variant (Nixon-Abell et al., 2016). As N551K is in linkage disequilibrium with R1398H, it remains possible that its protective effect is driven primarily by R1398H.

While it is disappointing that the apparent protective effect of both R1398H and N551K variants were not detected in AD subjects in this study, this was consistent with a previous study by our group that found no significant difference in frequencies of the G2385R Asian risk variant in AD compared with controls (Tan et al., 2009). Furthermore, LRRK2 has not been identified as a significant locus in any genome-wide association study of AD. While these results do not exclude a possible association of other genetic variants within the *LRRK2* gene in AD, we conclude that the LRRK2 protective variants R1398H and N551K are unlikely to play a major role in modulating the risk of AD in our population.

Table 1
R1398H and N551K genotypes and frequencies in AD cases and controls

Genotype	AD	Controls	Total
R1398H			
GG (WT)	558 (83.2%)	599 (83.3%)	1157 (83.2%)
AG	110 (16.4%)	117 (16.3%)	227 (16.3%)
AA	3 (0.4%)	3 (0.4%)	6 (0.4%)
N551K			
CC (WT)	555 (82.7%)	595 (82.8%)	1150 (82.7%)
CG	113 (16.9%)	121 (16.8%)	234 (16.8%)
GG	3 (0.4%)	3 (0.4%)	6 (0.4%)
Total	671	719	1390

Key: AD, Alzheimer's disease; WT, wild type.

Disclosure statement

The authors report no conflicts of interest.

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