

## Identification of a Common Genetic Risk Variant (*LRRK2* Gly2385Arg) in Parkinson's Disease

Eng-King Tan,<sup>1</sup>MD, FRCP, FAMS

### Abstract

The recent identification of a common genetic variant (*LRRK2* G2385R) which is associated with a two-fold increased risk of sporadic Parkinson's Disease (PD) in two independent Chinese populations in Singapore and Taiwan has generated considerable excitement. Thus far, this variant appears specific for the Asian population, emphasising further that ethnic-specific effects should be considered in genetic association studies. Cautious optimism is advised as we await more scientific studies and clarification if this risk variant is specific to ethnic Chinese race. Our in-vitro studies suggest the Gly2385Arg variant is biologically relevant and it might act through pro-apoptotic mechanisms, especially under cellular stresses. This may provide a partial explanation why some carriers develop the disease while others do not. The presence of other epigenetic factors, gene-gene and gene-environmental interaction could modulate the phenotype expression. Further validation of these findings would be needed to confirm this variant as the single most important common genetic risk factor in ethnic Chinese and/or Asian PD patients. The identification of the *LRRK2* Gly2385Arg variant could potentially facilitate the development of clinical, bioimaging, genetic and biological biomarkers, useful in the monitoring and neuroprotective therapy in asymptomatic individuals.

Ann Acad Med Singapore 2006;35:840-2

**Key words:** Chinese, Gene, Mutation

For diseases with complex inheritance, the age-old debate regarding the relative contribution of gene-environment interaction never fails to generate interest, discussion and hypothesis within the scientific community. The unraveling of the human genome project brings hope and great optimism that a verdict on such debates may be in the near horizon. However, hope and reality are frequent distant lovers who might require spirit and persistence to bring their supposedly destined marriage into fruition. The twists and turns of medical science research and discovery never fail to amaze, and the recent discovery that a common genetic variant (*LRRK2* G2385R) increases the risk of Parkinson's Disease (PD) makes one good illustrative example. I will take you through a quick journey of the roller coaster ride in this field and share our personal experience and our contribution in uncovering this genetic variant that is beginning to attract worldwide attention.

Parkinson's disease (PD) is the second most common neurodegenerative disease globally and is characterised clinically by rest tremor, rigidity, bradykinesia and postural

instability.<sup>1-2</sup> It is a significant cause of morbidity amongst the elderly population and while medical and surgical treatment is effective, no cure is currently available.<sup>3-8</sup> Since the description by James Parkinson more than two centuries ago, it has always been a widely held view that PD is of "idiopathic" in origin. The first twist accompanies the discovery of MPTP (methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced Parkinsonism in intravenous drug users in 1983 which led many to initially speculate that environmental factors could be the primary aetiology.<sup>9</sup> However, numerous epidemiologic studies evaluating potential environmental causative agents have not conclusively identified any specific environmental agent. Then came an unexpected genetic turn in 1997 when a missense mutation in the alpha-synuclein gene was found to be associated with the disease in some families with autosomal dominant (AD) mode of inheritance of parkinsonism.<sup>10</sup> This "genetic revolution" continues unabated in recent years, largely due to the enterprising spirit of investigators worldwide who have a determined

<sup>1</sup> Department of Neurology

Singapore General Hospital, National Neuroscience Institute, SingHealth, Singapore

Address for Correspondence: Dr Eng-King Tan, Department of Neurology, Singapore General Hospital, National Neuroscience Institute, SingHealth, Outram Road, Singapore 169608.

Email: gnrtk@sgh.com.sg

effort to map and identify putative pathogenic gene variants in different ethnic populations. Supporters of PD as a genetic disease must now be pleasantly delighted as currently 13 genetic loci have been ascribed and 6 disease-causing genes have been uncovered.<sup>11</sup> Two significant genes, *Parkin* (*PARK2*) and *LRRK2* are worth mentioning as the former accounts for up to 50% and 15% to 20% of autosomal recessive and young onset sporadic PD.<sup>11</sup> Mutations in the leucine-rich repeat kinase 2 (*LRRK2*, *PARK8*) are the most frequent known cause of familial autosomal dominant PD.<sup>12-16</sup> The common G2019S mutation accounts for 3% to 7% of familial PD and 1% to 3% in sporadic PD in several ethnic populations, with the highest prevalence (up to 40%) in North Africans and Ashkanezi Jews.<sup>11</sup> The striking absence of the common G2019S mutations in three independent Chinese populations involving 2000 study subjects suggests the possibility that ethnicity specific differences may exist for other *LRRK2* mutations.<sup>17-20</sup> The lack of differentiating features between carriers and non-carriers of these gene mutations and the alarmingly high frequency of mutations in sporadic cases in certain populations certainly challenge a previously held view by some that the genetic forms are different from the common garden variety and should be termed separately as “familial parkinsonism” and not PD. The nosology of “idiopathic” PD is being questioned as a clear defined genetic cause has been found even amongst the typical PD cases.

The association of a disease with genetic mutations is frequently clear-cut in cases where co-segregation of the genotype and phenotype could be demonstrated in family and case control studies and a disruption of biological function from the mutation is evident. However, the role of genetic risk factors in disease is more debatable. For a long time, research into genetic susceptibility risk factors to diseases has been a common focus that cuts across the entire realm of medical illnesses. The rationale for genetic association studies is based on the hypothesis that there may be an association of a defined disease trait and specific genetic variants. The relationship between genetic variants of the candidate gene and disease status, and the pattern of linkage disequilibrium in the population and genomic region under study will influence the variability and validity of the findings. However, unlike investigations into clinical risk factors, such as hypertension and diabetes mellitus, association studies of genetic risk factors are limited by various methodological problems. Genetic association studies in PD have frequently given conflicting findings, likely because of inadequate sample size, bias in selecting only individual polymorphic loci of susceptibility genes so that background genetic variations are not systematically studied and population stratification.<sup>21</sup> Various investigators have tried to partly overcome some of these problems

through studies using sib-pairs, genetic isolates, whole genome amplification and multi-centre meta-analysis of pooled data.<sup>22</sup> However, no single genetic risk variant has yet to be consistently replicated in PD.

It is in this back-drop that when we undertook the onerous task of conducting a detailed case control haplotype tagging analysis of the *LRRK2* gene in our Chinese population in early 2005 shortly after the genetics mutations of this gene were reported, few would believe any useful data could be generated. We identified a haplotype that dramatically increases disease risk when present in two copies (OR = 5.5, 95% CI = 2.1-14.0,  $P = 0.0001$ ).<sup>15</sup> At the same time, we also found an association of a few individual polymorphic variants, including the *LRRK2* G2385R variant with an increased risk of PD. Conventional wisdom and many historic publications told us then that the chance of a false positive association with these individual variants is high, particularly if the frequency of these variants is low. We decided to publish only our haplotype data instead of these individual variants. At around the same time, three studies suggest little role of common *LRRK2* variants in Caucasian PD populations,<sup>23-25</sup> echoing a familiar tune in the futility of genetic association studies. A twist came in early 2006 when our Dutch collaborators informed us that they have found an association of the *LRRK2* Gly2385Arg variant, (which was originally described in a PD family from Taiwan<sup>26</sup>) with an increased risk of PD in Taiwanese Chinese.<sup>27</sup> Interestingly, this variant has yet to be detected in Caucasians. We went back to dig out our old *LRRK2* G2385G data which we have shelved and were surprised to find a similar magnitude of risk in our Chinese patients. Our further in-vitro studies seemed to suggest that this variant is more toxic under stressful cellular conditions, reinforcing the clinical observation.<sup>28</sup> The extent of the importance of this finding was subsequently highlighted at a recent conference (10<sup>th</sup> International Congress of Parkinson's Disease and Movement Disorders, Kyoto, Japan) where poster presentations from other groups showed that the clinical association could be replicated in other Asians populations and the risk seems to be even higher in familial cases.

Cautious optimism is advised while we await more scientific studies and further clarification if this risk variant is specific to the ethnic Chinese race only. Our recent in-vitro studies suggest the Gly2385Arg variant is biologically relevant and it might act through pro-apoptotic mechanisms, especially under cellular stresses. In our initial experiments, the variant is associated with increased cell death compared to the wildtype only when exposed to an oxidative stress environment.<sup>28</sup> This observation may provide a partial explanation why some carriers develop the disease while other do not. The presence of other epigenetic factors,

gene-gene and gene-environmental interaction could modulate the phenotype expression.

If indeed this risk variant turns out to be the most important common genetic risk factor specific to the Chinese populations or other Asian races globally, credit should be given to those investigators in the field who persisted and pursued optimistically their hopes in finding a biomarker in genetic association studies. The courage of the journals to publish these “non-Rocket Science” association studies should be gratefully acknowledged. The identification of the *LRRK2* Gly2385Arg variant could potentially facilitate the development of clinical, bioimaging, genetic and biological biomarkers, useful in the monitoring and neuroprotective therapy in asymptomatic individuals.

While there could be more surprises ahead for investigators in this field,<sup>29</sup> we learn from this experience that conventional wisdom does not always apply in medical science and sometimes a single innocent observation not in the most robust scientific sense could bring about important clinical discovery.

#### Acknowledgement

The author would like to thank Zhao Yi, Bonifati V, JJ Liu, L Skipper, and all collaborators. The work is supported by National Medical Research Council, Biomedical Research Council, Singapore General Hospital and SingHealth.

#### REFERENCES

- Lim EC. Parkinson's disease: looking back, looking forward. *Ann Acad Med Singapore* 2005;34:221-2.
- Lim E. A walk through the management of Parkinson's disease. *Ann Acad Med Singapore* 2005;34:188-95.
- Chu LW, Chi I, Chiu AY. Incidence and predictors of falls in the Chinese elderly. *Ann Acad Med Singapore* 2005;34:60-72.
- Low JA, Pang WS, Chan DK, Chye R. A palliative care approach to end-stage neurodegenerative conditions. *Ann Acad Med Singapore* 2003;32:778-84.
- Tan AK. Current and emerging treatments in Parkinson's disease. *Ann Acad Med Singapore* 2001;30:128-33.
- Tan LC, Tan AK, Tjia HT. The profile of hospitalised patients with Parkinson's disease. *Ann Acad Med Singapore* 1998;27:808-12.
- Tan AK, Yeo TT, Tjia HT, Khanna S, Nowinski WL. Stereotactic microelectrode-guided posteroventral pallidotomy and pallidal deep brain stimulation for Parkinson's disease. *Ann Acad Med Singapore* 1998;27:767-71.
- Tan EK. Parkinson's disease surgery: advances and future strategies. *Int J Clin Pract* 1999;53:623-6.
- Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 1983;219:979-80.
- Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 1997;276:2045-7.
- Tan EK, Jankovic J. Genetic testing in Parkinson disease: promises and pitfalls. *Arch Neurol* 2006;63:1232-7.
- 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;44:601-7.
- Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon 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.
- Skipper L, Shen H, Chua E, Bonnard C, Kolatkar P, Tan LC, et al. Analysis of *LRRK2* functional domains in nondominant Parkinson disease. *Neurology* 2005;65:1319-21.
- Skipper L, Li Y, Bonnard C, Pavanni R, Yih Y, Chua E, et al. Comprehensive evaluation of common genetic variation within *LRRK2* reveals evidence for association with sporadic Parkinson's disease. *Hum Mol Genet* 2005;14:3549-56.
- Tan EK, Skipper L, Chua E, Wong MC, Pavanni R, Bonnard C, et al. Analysis of 14 *LRRK2* mutations in Parkinson's plus syndromes and late-onset Parkinson's disease. *Mov Disord* 2006;21:997-1001.
- Tan EK, Shen H, Tan LC, Farrer M, Yew K, Chua E, et al. The G2019S *LRRK2* mutation is uncommon in an Asian cohort of Parkinson's disease patients. *Neurosci Lett* 2005;384:327-9.
- Tan EK, Skipper L, Tan L, Liu JJ. *LRRK2* G2019S founder haplotype in the Chinese population. *Mov Disord* 2006; [Epub ahead of print]
- Lu CS, Simons EJ, Wu-Chou YH, Fonzo AD, Chang HC, Chen RS, et al. The *LRRK2* I2012T, G2019S, and I2020T mutations are rare in Taiwanese patients with sporadic Parkinson's disease. *Parkinsonism Relat Disord* 2005;11:521-2.
- Fung HC, Chen CM, Hardy J, Hernandez D, Singleton A, Wu YR. Lack of G2019S *LRRK2* mutation in a cohort of Taiwanese with sporadic Parkinson's disease. *Mov Disord* 2006; [Epub ahead of print]
- Tan EK, Khajavi M, Thornby JJ, Nagamitsu S, Jankovic J, Ashizawa T. Variability and validity of polymorphism association studies in Parkinson's disease. *Neurology* 2000;55:533-8.
- Maraganore DM, de Andrade M, Elbaz A, Farrer MJ, Ioannidis JP, Kruger R, et al. Collaborative analysis of alpha-synuclein gene promoter variability and Parkinson disease. *JAMA* 2006;296:661-70.
- Biskup S, Mueller JC, Sharma M, Lichtner P, Zimprich A, Berg D, et al. Common variants of *LRRK2* are not associated with sporadic Parkinson's disease. *Ann Neurol* 2005;58:905-8.
- Paisan-Ruiz C, Evans EW, Jain S, Xiromerisiou G, Gibbs JR, Eerola J, et al. Testing association between *LRRK2* and Parkinson's disease and investigating linkage disequilibrium. *J Med Genet* 2006;43:e9.
- Paisan-Ruiz C, Lang AE, Kawarai T, Sato C, Salehi-Rad S, Fisman GK, et al. *LRRK2* gene in Parkinson disease: mutation analysis and case control association study. *Neurology* 2005;65:696-700.
- Mata IF, Kachergus JM, Taylor JP, Lincoln S, Aasly J, Lynch T, et al. *LRRK2* pathogenic substitutions in Parkinson's disease. *Neurogenetics* 2005;6:171-7.
- Di Fonzo A, Wu-Chou YH, Lu CS, van Doeselaar M, Simons EJ, Rohe CF, et al. A common missense variant in the *LRRK2* gene, Gly2385Arg, associated with Parkinson's disease risk in Taiwan. *Neurogenetics* 2006;7:133-8.
- Tan EK, Zhao Y, Skipper L, Tan MG, Di Fonzo A, Sun L, et al. The *LRRK2* Gly2385Arg variant is associated with Parkinson's disease: genetic and functional evidence. *Hum Genet* 2006; [Epub ahead of print]
- Tan EK. Re-defining neurological syndromes: the genotype meets the phenotype. *Ann Acad Med Singapore* 2006;35:63-5.