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LETTER TO THE EDITOR

Parkinson's disease in GTP cyclohydrolase 1 mutation carriers

Brent J. Ryan,[1] Mark J. Crabtree,[2] Keith M. Channon[2,3] and Richard Wade-Martins[1]

1 Oxford Parkinson's Disease Centre, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3QX, UK
2 British Heart Foundation Centre of Research Excellence, Division of Cardiovascular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK
3 National Institute for Health Research (NIHR) Oxford Biomedical Research Centre, University of Oxford, UK

Correspondence to: Richard Wade-Martins
Oxford Parkinson's Disease Centre,
Department of Physiology,
Anatomy and Genetics,
University of Oxford,
Oxford OX1 3QX, UK
E-mail: richard.wade-martins@dpag.ox.ac.uk

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Sir,

We read with great interest the study titled 'Parkinson's disease in GTP cyclohydrolase 1 mutation carriers' in the September edition of *Brain* (Mencacci *et al.*, 2014). The study demonstrates loss-of-function variants in the *GCH1* gene are not only a major cause of DOPA-responsive dystonia but are also enriched in relatives with adult-onset parkinsonism. Furthermore, the authors identify, through exome re-sequencing, a number of *GCH1* variants that are enriched in patients with Parkinson's disease compared to control subjects. This elegant study demonstrates the power of exome re-sequencing and highlights the potential role for rare variants in genes such as *GCH1* as susceptibility factors in Parkinson's disease. A genetic role for the *GCH1* locus is reinforced by the recent discovery that a SNP at the *GCH1* locus is associated with Parkinson's disease in a large-scale genome-wide association study (GWAS) meta-analysis (Nalls *et al.*, 2014).

The coding variants identified in the exome re-sequencing study increased the risk of Parkinson's disease by 7.5-fold (2.4–25.3, 95% confidence intervals) and the authors note that this is likely to be an underestimation of the true odds ratio, because of the method of implementing prediction scores. Therefore, these variants seem to significantly impact Parkinson's disease aetiology. The authors propose a number of mechanisms by which loss-of-function *GCH1* variants may lead to increased nigral degeneration and Parkinson's disease including that dopamine exerts a protective, anti-apoptotic role through dopamine receptors, that variants in *GCH1* result in compensatory mechanisms that stave off DOPA-responsive dystonia, but increase the vulnerability of the neurons to ageing, and finally, that the lower striatal dopamine levels observed in *GCH1* mutation carriers mean a lower threshold of nigral cell loss is sufficient to induce clinical symptoms. In addition to these logical hypotheses put forward in the manuscript, it may be significant to note that tetrahydrobiopterin (BH4) has a number of other cellular roles, which may contribute to nigral cell loss in individuals carrying *GCH1* variants. These mechanisms may include the role of BH4 as a cofactor for nitric oxide synthases (NOS), alkylglycerol monooxygenase (AGMO) or other amino acid hydroxylases, in addition to the role of BH4 as an antioxidant.

BH4 levels have been demonstrated to be decreased in patients with Parkinson's disease but high doses of oral BH4 for 5 days have no immediate therapeutic benefits in a short-term study of two patients with Parkinson's disease (Nagatsu *et al.*, 1981; Dissing *et al.*, 1989). Patients with DOPA-responsive dystonia typically have 10% residual GCH1 activity whereas carriers have 35% activity (Ichinose *et al.*, 1994). It may be postulated that many of

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the heterozygous variants identified by Mencacci *et al.* (2014) would result in a less severe GCH1 impairment. BH4 acts as a cofactor for all amino acid hydroxylases including phenylalanine, tryptophan and tyrosine hydroxylases and, therefore, potentially provides a link both to the motor and non-motor symptoms of Parkinson's disease. In addition, BH4 acts as a general antioxidant and may be oxidized by various reactive oxygen species (Fisher and Kaufman, 1973; Milstien and Katusic, 1999; Kohnen *et al.*, 2001). Increased levels of oxidants can lead to decreased cellular BH4 levels and oxidative stress.

BH4 acts as a cofactor for the three isoforms of nitric oxide synthase, and a loss of BH4 synthesis or a decreased BH4:BH2 ratio in cells results in 'NOS uncoupling' and production of superoxide from NOS instead of nitric oxide (Vasquez-Vivar *et al.*, 2003; Crabtree *et al.*, 2009*a*). NOS uncoupling is recognized as a major factor in endothelial dysfunction and cardiovascular disease (Landmesser *et al.*, 2003; McNeill and Channon, 2012). This concept of NOS uncoupling, potentially influenced by (amongst other factors) BH4 levels via *GCH1* variants or by inflammation, fits with recent data demonstrating a major role of nitrative stress in Parkinson's disease (Giasson *et al.*, 2000; Murray *et al.*, 2003; Ryan *et al.*, 2013). Dihydrofolate reductase (DHFR) is important in maintenance of BH4 levels and NOS coupling and may serve as an important therapeutic target in cases of decreased BH4 availability due to oxidative stress or decreased GCH1 activity (Crabtree *et al.*, 2008, 2009*b*; Henchcliffe and Beal, 2008). We have previously demonstrated that three SNPs present in the *GCH1* genomic locus represent a haplotype that alters plasma BH4 levels, *GCH1* expression and vascular superoxide production (Antoniades *et al.*, 2008). Furthermore, these haplotypes influence the ability of patients to produce BH4 in response to inflammation (Antoniades *et al.*, 2011). These observations suggest a link between BH4 availability, oxidative stress and neuroinflammation in patients with Parkinson's disease with SNPs or variants in *GCH1*.

In addition to these mechanisms, we have recently demonstrated that the Parkinson's-associated protein α-synuclein modulates cellular BH4 levels by regulating GCH1 activity (Ryan *et al.*, 2014). We found that reduction of cellular α-synuclein protein levels resulted in increased GCH1 activity and increased cellular BH4 levels. However, whether this is a direct or indirect modulation, by pathways such as the PI3K-Akt or GCH1 feedback regulatory protein (GFRP), remains to be elucidated (He *et al.*, 2011). Furthermore, we have also demonstrated that α-synuclein knockdown reduced NOS activity in SH-SY5Y cells (Fountaine *et al.*, 2008) and our recent data suggest that this may be by regulation of GCH1 by α-synuclein.

Rare variants in genes identified by exome re-sequencing such as *GCH1*, as identified by Mencacci *et al.* (2014), offer a novel insight into Parkinson's disease risk and disease-modifying genes and identify novel therapeutic targets. Such recent progress in the identification of causative Parkinson's disease genes in the disease population benefits our understanding of how rare variants impact on sporadic disease.

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