

## LETTER TO THE EDITOR

### Reply: Parkinson's disease in GTP cyclohydrolase I mutation carriers

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Sir,

Thank you for the opportunity to reply to the correspondence concerning our recent publication in *Brain*, 'Parkinson's disease in GTP cyclohydrolase 1 mutation carriers' (Mencacci *et al.*, 2014). We read with great interest these letters and we thank the authors for their insights.

Guella *et al.* (2014) report the screening of *GCH1* in 528 Canadian cases with Parkinson's disease and atypical parkinsonism and 290 matched controls. They identified two variants, the known pathogenic p.K224R ( $\times 2$ ) and the novel variant p.A99D (likely pathogenic according to *in silico* prediction tools and interspecies conservation) in three unrelated cases with Parkinson's disease and the two benign variants p.P23L and p.P69L in one single control individual. The mutational frequency, excluding the aforementioned benign variants, was 0.56% (3/528) in cases versus 0% (0/290) in controls, consistent with the frequency we observed in our study (0.75% in cases versus 0.1% in controls).

This result is relevant as it represents the first independent confirmation that rare deleterious *GCH1* variants are enriched in patients with Parkinson's disease compared to control subjects. Furthermore, they describe the post-mortem findings of one of the mutated patients, who presented at the age of 82 with DOPA-responsive asymmetric

rest tremor. This showed a combination of brainstem Lewy body pathology together with the presence of tau-immunoreactive neurofibrillary tangles. To date, the only available brain pathology analysis of a *GCH1*-associated neurodegenerative parkinsonism case showed severe nigral neurodegeneration and Lewy bodies in surviving nigral cells and in the locus coeruleus (Gibb *et al.*, 1991; Segawa *et al.*, 2004). Further studies are needed to establish if the tauopathy described by Guella *et al.* (2014) in their case represents simply an incidental finding.

The finding that *GCH1* loss-of-function variants are not only responsible for childhood-onset DOPA-responsive dystonia, but are also associated with adult-onset neurodegenerative parkinsonism, is strengthened by the recent identification, through the meta-analysis of genome-wide association studies (GWAS) data deriving from ~13 000 cases and 95 000 controls, that *GCH1* is also a low-risk susceptibility locus for Parkinson's disease (Nalls *et al.*, 2014). This finding potentially extends the role of GTP cyclohydrolase 1 (*GCH1*) deficiency in the pathogenesis of Parkinson's disease beyond carriers of rare deleterious coding mutations.

The causal link between *GCH1* and Parkinson's disease remains a matter of speculation. Ryan *et al.* (2014) expand the discussion of our manuscript and add insight

into the possible pathogenic mechanisms that predispose *GCH1* loss-of-function mutation carriers to nigrostriatal degeneration.

In our paper we proposed various hypotheses whereby *GCH1* and BH<sub>4</sub> deficiency and consequent chronic reduction of dopamine levels may predispose carriers of *GCH1* mutations to nigral cell degeneration.

Ryan *et al.* (2014) point out that different cellular mechanisms secondary to BH<sub>4</sub> deficiency, other than reduced dopamine levels, could contribute to the death of nigral dopaminergic neurons. BH<sub>4</sub> acts as an antioxidant itself and is an essential cofactor for nitric oxide synthases (NOS) activity. Furthermore decreased BH<sub>4</sub> levels have been demonstrated to lead to NOS uncoupling, which results in increased oxidative and nitrative stress (Chen *et al.*, 2014). The authors previously described that a haplotype of three SNPs (rs8007267, rs3783641 and rs10483639) at the *GCH1* genomic locus influences plasma *GCH1* activity and BH<sub>4</sub> levels (Antoniades *et al.*, 2008) and identified BH<sub>4</sub> as a vascular defence mechanism against inflammation-induced endothelial dysfunction (Antoniades *et al.*, 2011). Consequently the authors propose that a link between BH<sub>4</sub> levels, oxidative stress, and neuroinflammation could represent the mechanism underlying *GCH1*-associated Parkinson's disease.

Fitting well with this model, and possibly supporting Ryan *et al.*'s view, we found that the *GCH1* SNP (rs11158026), recently identified as a risk variant for Parkinson's disease (Nalls *et al.*, 2014), is in moderate linkage disequilibrium ( $r^2$  0.457;  $D'$  0.932) with the SNPs constituting the functional haplotype. This possibly suggests a potential functional basis for the association of this variant to Parkinson's disease.

The authors have also demonstrated the existence of an interaction between  $\alpha$ -synuclein, mitochondrial function and *GCH1* activity. Their work may support the compelling hypothesis that a pathogenic cascade occurs in nigral neurons, whereby increased levels of  $\alpha$ -synuclein and mitochondrial dysfunction lead to decreased *GCH1* activity and BH<sub>4</sub> levels, which in turn may result in increased oxidative stress and cell death (Ryan *et al.*, 2014).

We believe that one of the outstanding questions is whether patients with DOPA-responsive dystonia eventually develop nigral neurodegeneration, or whether neurodegeneration can be avoided by dopaminergic replacement therapy. Answering this question will help to understand to what extent low dopamine levels play a role in nigral cell death, with obvious therapeutic implications for asymptomatic carriers of pathogenic variants.

Dopaminergic imaging studies performed in a few cases with classic DOPA-responsive dystonia (mostly genetically not confirmed) have shown no evidence of reduced nigrostriatal innervation (Snow *et al.*, 1993; Jeon *et al.*, 1998). This is consistent with post-mortem analysis of four extra cases showing normal nigral cell count (Furukawa *et al.*, 1999; Grotzsch *et al.*, 2002; Segawa *et al.*, 2013). However, Sawle *et al.* (1991) report that six cases with

DOPA-responsive dystonia displayed modest but significant reduction in the uptake of <sup>18</sup>F-fluorodopa into both caudates and putamen. Furthermore, Tadic *et al.* (2012) report that in DOPA-responsive dystonia cases parkinsonian signs are a relatively common residual motor sign following treatment, possibly suggesting underlying neurodegeneration.

With regards to this, the case report of Terbeek *et al.* (2014) is of great interest. They describe a 41-year-old patient carrying a known pathogenic *GCH1* variant (p.Y75S) with onset of classic DOPA-responsive dystonia at age 11. He was treated with L-DOPA (300 mg/day) from the age of 20 with good and sustained response. At age 41, because of rapid recurrence of dystonia after skipping a L-DOPA dose, dopaminergic imaging (<sup>123</sup>I-FP-CIT SPECT) was performed and showed severe bilateral and asymmetric reduction of putaminal tracer uptake, a pattern typical of idiopathic Parkinson's disease. However, clinical examination, performed after withdrawing L-DOPA, revealed purely dystonic features without any obvious sign of parkinsonism.

In agreement with the interpretation of Terbeek *et al.* (2014), we believe that this case may indeed represent a case with overlapping DOPA-responsive dystonia and asymptomatic, as yet, nigrostriatal degeneration, possibly arguing against a neuroprotective role of dopamine replacement in *GCH1* mutation carriers.

Lastly, with regards to the letter by Furukawa and Kish (2014), we agree it is not easy to reconcile the evidence of nigral neurodegeneration that we and others have demonstrated in several individuals with *GCH1*-related parkinsonism and the intact dopaminergic innervation showed in some other cases (Nygaard *et al.*, 1992; Kang *et al.*, 2004).

However, the phenotype of these latter cases, characterized by excellent and prolonged response to very small doses of L-DOPA and no motor fluctuations or dyskinesias in spite of decades of treatment, is very different from what we observed in the 'neurodegenerative' cases. It is therefore possible that there may exist two different types of adult-onset parkinsonism associated with *GCH1* mutations; on one side, a benign non-degenerative form, part of the phenotypic spectrum of metabolic *GCH1*-related striatal dopamine deficiency; on the other, a progressive form of parkinsonism with underlying nigral degeneration.

In conclusion, we anticipate that post-mortem analysis and longitudinal clinical, neuroimaging, and metabolic studies of larger series of *GCH1* mutation carriers—including asymptomatic carriers, individuals with classic DOPA-responsive dystonia and cases with adult-onset parkinsonism—will give way to important understandings of the pathogenesis of *GCH1*-associated Parkinson's disease.

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