BRAIN Scientific Commentary

Mutations and mechanism: how PINK1 may contribute to risk of sporadic Parkinson's disease

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The pathogenesis of Parkinson's disease (PD), in keeping with other common neurodegenerative diseases, is a complex interplay of genetic and environmental factors. Significant progress has been made in dissecting the genetic architecture of both rare monogenic and sporadic PD. Mutations in three genes, SNCA, LRRK2 and VPS35 cause autosomal dominant PD. Mutations in six genes cause autosomal recessive PD (AR-PD)/parkinsonism: PINK1, DJ-1, PARKIN, PLA2G6, ATP13A2, and FBOX7. Taken together, the monogenic forms only account for 30% familial PD and 3-5% of sporadic PD. Nonetheless much of our knowledge of the pathogenic pathways that lead to neuronal dysfunction and death have emerged from the identification of these genes. The discovery of the monogenic forms of PD has naturally posed the question of whether they also influence the genetic risk of sporadic PD. If the same genes that can harbour highly penetrant mutations in mendelian PD also contain risk variants for sporadic disease, this would provide a genetic and pathophysiological link between mendelian and sporadic PD [Lesage and Brice 2012]. Several genome wide association studies (GWAS) have independently identified SNCA and LRRK2 as risk loci for sporadic disease, thus linking the risk of sporadic PD to the autosomal dominant forms of PD [Nalls *et al.* 2014]. However the functional basis, or the mechanism, by which a risk variant alters biological function and confers susceptibility to disease remains an essential missing piece of the puzzle. This study by Puschmann *et al.* 2016 addresses whether a genetic variant in a gene known to cause AR-PD contributes to the risk of sporadic PD, and explores the biological mechanism by which it confers risk.

Homozygous (hom) and compound heterozygous (het) mutations in PINK1 are the second most frequent cause of AR-PD. PINK1 associated PD resembles sporadic PD with an akinetic rigid syndrome with good response to levodopa, levodopa induced dyskinesias, and occasionally dystonia. Many het putative pathogenic mutations in the PINK1 gene have been observed in sporadic and familial PD, which has led to the hypothesis that they may act as a susceptibility factor in the development of sporadic PD. This controversy has been challenged by the finding of het PINK1 mutations in apparently healthy controls, and the lack of parkinsonism in relatives who are het carriers in pedigrees with PINK1 hom mutations [Klein *et al.* 2007]. If this hypothesis is true, one would expect het mutations to be more common in patients than in control; the mean age of onset would be predicted to be intermediate between the state of the hom mutations and no mutation; there may be preclinical manifestations of disease in neuroimaging of het mutation carriers; and there should be evidence of how the het mutation affects the biological function of PINK1, either through haploinsufficiency, dominant negative, or dominant effects.

This study focusses on one particular PINK1 variant p.G411S which has occasionally shown dominant inheritance, and therefore the authors propose that a single risk allele may present sizeable risk, and may have a pathological effect distinct from other variants. They performed a case control study on five different Caucasian series, studying a total of 2560 PD patients and 2145 controls. Het PINK1 p.G411S variants were present in 19 cases (0.74%) and 5 controls (0.23%), confirming a significant association of the het p.G411S variant with risk of PD with an odds ratio of 2.92 (p=0.032), which remained significant in a meta-analysis incorporating previous published studies. Interestingly the median age of disease onset was significantly earlier in the PINK1 hets, compared to non-carrier

status (59yrs vs 64 yrs, p=0.012). These findings are in contrast to the previous largest meta-analysis [Marongiu *et al.* 2008] that studied 1100 PD patients and 400 controls for het variants in the PINK1 gene and reported no significant difference in frequencies (1.8% vs 1.5%) between cases and controls. However this was a smaller study and considered all PINK1 variants rather than an association with only the p.G411S variant. Variation at the PINK1 locus has also not been previously identified in the meta analyses of GWAS, although this may be due to the low frequency of the p.G411S variant in non-Scandinavian populations.

The study then addresses how the p.G411S variant exerts its effect on PINK1. PINK1 is a ubiquitously expressed serine/threonine kinase with a mitochondrial targeting sequence. Relevant to this study is the emergence of a critical role for PINK1 in the regulation of mitochondrial quality control through the selective elimination of damaged mitochondria via mitophagy [Pickrell and Youle 2015]. Mitochondrial depolarisation causes accumulation of a PINK1 dimer on the outer mitochondrial membrane of damaged mitochondria. Crucially PINK1 has been found to phosphorylate residue Ser65 of ubiquitin (p-ser65-ub) and ubiquitin chains conjugated to mitochondrial proteins [Durcan and Fon 2015]increasing the level of p-ser65-ub in the mitochondria from undetectable levels to 20% of total ubiquitin in mitochondria [Ordureau *et al.* 2014]. A function of p-ser65-ub is to serve as a receptor for binding and recruitment of autophagy receptors and parkin [Lazarou *et al.* 2015]. Together with the PINK1 dependent ser65 phosphorylation of the Ubl domain in parkin, binding of p-ser65-ub to parkin converts it from inactive conformation to fully active conformation, resulting in parkin's E3 ligase ubiquitinating many outer mitochondrial membrane proteins, and the triggering of mitophagy.

The authors first analysed skin fibroblasts, and induced neurons, from two patients with het p.G411S, and compared them to controls, and furthermore to cells with the Q456X hom (affected PD patients) and Q456X het mutation (unaffected carriers). The Q456X mutation provides a useful comparison as in the hom state the PINK1 protein is absent and thus kinase function is absent, while in the het state, intermediate levels of PINK1 protein are found that, if functionally significant, would be due to haploinsufficiency. Of note, the het p.G411S state is associated with the same level of PINK1 protein as controls. Next as a measure of the kinase function of PINK1, they tested phosphorylation of ser65-ubiquitin using two methods: an ELISA based approach and an automated high content imaging approach. Using prolonged treatment with valinomycin to induce mitochondrial depolarisation, and subsequent mitophagy, they demonstrate that the p-ser65-ub signal, and hence PINK1 kinase activity, is significantly reduced in cells with the p.G411S het compared to controls or the Q456X het state. This confirms that the p.G411S het state does indeed result in impaired PINK1 function in neurons, but that the effect is not recapitulated in a model of haploinsufficiency.

To dissect the mechanism of the p.G411S variant , the authors performed theoretical molecular modelling of the PINK1 protein, and molecular dynamics simulation of the effect of the p.G411S variant. In the PINK1 monomer, the G411S induces mispositioning of ubiquitin in the active site which results in increased distance between ser65 residue on ubiquitin and ATP that would reduce kinase activity. However, the modelling of the heterodimer formed between wildtype PINK1 and PINK1 p.G411S suggests that the p.G411S mutant PINK1 poisons the wildtype PINK1 so that it adopts the same misalignment of ubiquitin and ATP at the active site, impairing kinase function. Whilst the conclusions from the 3D modelling may be interpreted cautiously without experimental structural

data, this nonetheless represents key evidence that through dimerisation with wildtype PINK1, the G411S variant may exert a dominant negative effect.

Finally the authors confirmed that wildtype and p.G411S can dimerise when over-expressed in Hela cells, and that the heterodimer displays reduced kinase activity with respect to WT homodimers. Overexpression of wildtype PINK1 in this model is able to activate parkin translocation to the mitochondria. However co-expression of p.G411S (but not Q456X) with wildtype PINK1 significantly reduces mitochondrial translocation of parkin, confirming that the dominant negative effect of p.G411S variant in reducing PINK1 kinase function also translates into downstream impairment of parkin recruitment.

In summary, this study proposes that a het variant in the PINK1 gene is a genetic risk factor in certain populations for sporadic PD, and describes an interesting putative dominant negative mechanism by which it leads to reduction in PINK1 kinase function impairing mitophagy, and contributing to neuronal loss. In this study, the major challenge of dissecting the mechanism underpinning genetic variants has been overcome by recent successful discoveries in PINK1 biology, notably, the role of PINK1 as a ubiquitin kinase. This crucially yielded a target to measure PINK1 activity, enabling the effect of the variant to be ascertained directly, rather than relying on indirect downstream assays of mitochondrial dysfunction or neuronal loss. Future work will confirm whether PINK1 does indeed need to dimerise for activation, whether wildtype PINK1 and p.G411S PINK1 dimerise at the endogenous level, and whether such an endogenous heterodimer also exhibits impaired kinase function. Overall this study further supports the hypothesis that variability in the genes that cause mendelian PD may act as genetic risk factors for sporadic PD, and strengthens the proposition that pathways affected in AR-PD such as mitochondrial quality control, are of significance in the development of sporadic PD.

GLOSSARY

Mitophagy: the selective targeting and elimination of defective mitochondria by autophagy in order to avoid the accumulation of toxic mitochondria

PINK1: PTEN-induced kinase 1

Ubiquitin kinase: An enzyme with the ability to transfer phosphate groups to the protein ubiquitin

Dominant negative: A single mutant allele exerts a negative effect on the normal DNA, RNA or protein resulting in impaired protein function

Haploinsufficiency: A single mutant allele results in 50% activity of the encoded protein, which is insufficient to maintain normal function

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