Parallel PARKing: Parkinson's Genes Function in Common Pathway

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Parkinson's disease (PD) is associated with diverse genetic and environmental susceptibilities. Functional connections between PD genes have remained elusive. In this issue of *Neuron*, MacLeod et al. (2013) link three PD susceptibility genes, LRRK2, PARK16, and VSP35, to a common cellular pathway and show how these deficits contribute to dysfunction.

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting seven to ten million people worldwide. Classic motor features of PD consist of resting tremor, bradykinesia, rigidity, and postural instability that are caused by the selective degeneration of nigral-striatal dopaminergic neurons. A pathological hallmark of PD is the presence of Lewy bodies, which are protein aggregates that accumulate in affected brain regions (Goedert et al., 2012). PD is a mostly sporadic disease, but rare inherited forms of PD offer clues to possible underlying genetic factors and mechanisms that might also be relevant to sporadic PD. The discovery in 1997 of mutations in the alpha-synuclein gene (*SNCA*) as a cause of PD was significant not only because it was the first gene associated with PD, but also because alpha synuclein protein was found to be the main building block of Lewy bodies (Lee and Trojanowski, 2006).

After alpha-synuclein, mutations in several additional genes (DJ-1, LRRK2, PARKIN, PINK1, ATP13A2, VPS35, and EIF4G1) have been linked to familial PD (Kumar et al., 2012). However, monogenic causes account for only ~3% of all PD cases. Moreover, incomplete penetrance within these families suggests additional genetic risk factors and interactions with the environment are crucial for developing disease. Genome-wide association studies (GWASs) are a powerful tool for defining common genetic variants that are associated with increased risk of disease. Several recent PD GWASs and meta-analyses have facilitated the identification of further genetic risk factors for sporadic disease. Each successive GWAS presents a list of risk loci, some of which overlap with prior studies and others of which fail to replicate (Lill et al., 2012).

The identification of key Mendelian genes in familial PD and an explosion of new susceptibility loci associated with sporadic PD presents a critical question: do the genes associated with PD interact with each other or rather do each of them function independently but ultimately converge in a common pathological outcome—dopaminergic neuron loss and resulting parkinsonism? Accruing evidence points to genetic interactions between some of the rare familial PD genes (e.g., *parkin* and *pink1*; Clark et al., 2006; Park et al., 2006) and potential functional associations between some other PD genetic contributors (e.g., *PARK9* and alpha-synuclein; Gitler et al., 2009; LRRK2 and parkin; Smith et al., 2005; and glucocerebrosidase and alpha-synuclein; Mazzulli et al., 2011). However, interactions between genes associated with the more common sporadic PD remain unclear.

The study by MacLeod et al. (2013) starts out by tackling this problem in an elegant way. They begin their analysis on common variants at seven genetic loci that have been associated by GWAS with increased PD risk in diverse patient populations: SNCA, LRRK2, MAPT, HLA-DRA, PARK16, LAMP3, and STK39. They used as a tractable readout changes in brain gene expression profiles correlated with the presence or absence of a given PD risk allele. Importantly, they focused their analysis on brain tissue from unaffected individuals, in order to avoid confounding effects on gene expression owing to disease progression and neuron loss. They scoured publically available transcriptome data sets for common effects on gene expression signatures associated with the presence or absence of PD risk alleles. Of the seven PD risk loci analyzed, the effects on gene expression signatures by variants at the PARK16 and LRRK2 loci were most similar. With this clue in hand, they proceed to reanalyze existing GWAS data sets and uncover remarkably strong evidence for a genetic interaction between LRRK2 and PARK16. They find that the effect of an LRRK2 variant on PD risk strongly modifies the effects of a risk variant at the PARK16 locus and vice versa. Thus, two common variants associated with sporadic PD seem to interact genetically.

Once MacLeod et al. (2013) established genetic interaction between LRRK2 and PARK16, they next set out to define whether and how LRRK2 and PARK16 might functionally interact. LRRK2 has been extensively studied and its cellular functions and the effects of disease-associated mutations are being unraveled (Tsika and Moore, 2012). But much less is known about PARK16. Moreover, the PARK16 locus encompasses five candidate genes (SLC45A3, NUCKS, RAB7L1, SLC41A1, and PM20D1). Which of these is the key gene and how does it interact with LRRK2? To answer this question, MacLeod et al. (2013) systematically

tested each of the five genes for the ability to rescue a phenotype caused by LRRK2 mutation. In primary rat neurons, expressing a PD-linked mutant form of LRRK2 (G2019S) causes a dramatic reduction in neurite length. Overexpression of RAB7L1, but not the other four PARK16 locus genes, was able to suppress the mutant LRRK2-induced neurite length phenotype. Next, MacLeod et al. (2013) powerfully extend their findings to an animal model and show that upregulation of RAB7L1 rescues dopaminergic neuron loss and reduced lifespan associated with LRRK2 mutation in *Drosophila*, whereas dopamine neuron-specific knockdown of RAB7L1 in flies causes dopaminergic neuron degeneration. Taken together, these data strongly suggest that RAB7L1 functions in a pathway with LRRK2.

RAB7L1 belongs to a family of small GTPases that function in diverse aspects of cell biology, including essential roles as regulators of vesicular trafficking. Beyond the compelling genetic and cellular evidence by MacLeod et al. (2013) implicating RAB7L1, this gene was a good candidate to consider because of previous connections between Rabs, vesicle trafficking, and PD (Gitler et al., 2008). Having provided evidence that alteration in RAB7L1 function is the likely culprit responsible for the PARK16 locus association with PD risk, MacLeod et al. (2013) next tried to figure out how at the molecular level. In other words, could they zoom in on the RAB7L1 gene and try and find a SNP associated with PD risk and then figure out the consequence of this SNP on RAB7L1 function? This type of analysis is exceedingly challenging because of linkage disequilibrium—for any given chromosomal region, many variants are often closely associated. In the quagmire of variants, how does one identify the "causal" SNP?

A breakthrough for MacLeod et al. (2013) came when they explored a recently compiled treasure trove of genome-wide splicing data from human lymphoblasts (Montgomery et al., 2010). Remarkably, they found that the same exact haplotype (a collection of variants that are located closely together) at PARK16 associated with PD risk was also associated with alternative splicing of RAB7L1. The PD risk allele was characterized by the skipping of RAB7L1 exons 2 and 3 and the protective PARK16 allele was associated with increased exon 2 inclusion in RAB7L1 mRNA. MacLeod et al. (2013) report that skipping of these exons is predicted to lead to a truncated RAB7L1 protein lacking a critical GTP-binding domain. Importantly, they test this hypothesis in primary neurons and in *Drosophila* and show that this truncated protein, unlike wild-type RAB7L1, is unable to protect against the LRRK2 mutant phenotypes. Furthermore, analysis of human brain tissue revealed a significant reduction in full-length RAB7L1 protein in individuals harboring the PARK16 risk allele compared to individuals with the protective allele. Thus, using a battery of genomic analyses coupled with functional studies in cell cultures and animal models, MacLeod et al. (2013) provide us with a mechanistic explanation for how variants in PARK16 increase risk for PD: by affecting splicing of RAB7L1 mRNA, levels of fully functional RAB7L1 protein decrease.

MacLeod et al. (2013) turn their attention to elucidating first how LRRK2 and RAB7L1 work together and, second, the cellular consequences of defects in this pathway. Previous data implicated LRRK2 mutations in abnormal lysosomal morphology and delivery of proteins to the lysosome. Moreover, RAB7L1 localizes prominently to the Golgi and the retromer complex plays a critical role in protein sorting between lysosomes and Golgi. Therefore, MacLeod et al. (2013) hypothesized that the cellular defects caused by mutant LRRK2 could be caused, at least in part, by defects in the retromer machinery. The retromer complex was also probably on MacLeod et al. (2013)'s radar because of the recent identification by exome sequencing of mutations in VPS35, a component of the retromer complex, in some rare familial forms of PD (Kumar et al., 2012). Again, turning to mechanistic studies in primary neurons and *Drosophila*, MacLeod et al. (2013) provide further evidence linking LRRK2, RAB7L1, and VPS35 in a functional pathway. They show that VPS35 overexpression is able to rescue the neurite length defects and dopaminergic neuron loss caused by either expression of mutant LRRK2 or knockdown of RAB7L1. Moreover, expressing mutant LRRK2 or knockdown of RAB7L1 in neuronal cell lines or in mouse brain caused a marked reduction in protein levels of two components of the retromer complex, VPS35 and VPS29. Finally, in human PD brain, MacLeod et al. (2013) observed a significant decrease in VPS35 mRNA. Thus, it appears that deficits in the LRRK2/RAB7L1 pathway may lead to retromer complex dysfunction. These deficits can be rescued by suppressing retromer defects (for example, by upregulation of VPS35).

This paper is an elegant example of pursuing a PD genetic pathway using a combination of state-of-the-art computational and genomics approaches with tried and true cell biological and genetic model system approaches. In one fell swoop, MacLeod et al. (2013) have (1) provided compelling evidence for RAB7L1 to be the key gene in the PARK16 locus; (2) discovered a novel functional interaction between RAB7L1 and the PD disease gene LRRK2; (3) connected LRRK2 and RAB7L1 functionally to another PD disease gene, VPS35; (4) defined how SNPs in the PARK16 locus associated with PD risk cause alterations in RAB7L1 mRNA splicing, leading to lower levels of RAB7L1 protein—providing a mechanism for how PARK16 variants can increase risk for disease; and (5) showed that the high-risk common variants at the PARK16 and LRRK2 loci are dependent on one another. These findings promise to not only have a profound impact on our understanding of PD mechanisms but also on how we think about PD genetics and how we interpret genetic studies and utilize genomic data sets in the future.

Technological innovations have spurred the study of PD genetics with evolution from classic linkage analysis for identifying Mendelian genes to GWAS for defining common genetic variants associated with increased risk of sporadic disease. Next generation approaches such as exome sequencing and even whole-genome sequencing are increasing in prevalence and promise to help unravel even more of the PD genetic landscape. While GWAS is a powerful approach for identifying new genes and loci associated with a diverse collection of human diseases, a major challenge

with GWAS has been its inability to translate potential causal SNPs to understanding mechanistically how these variants confer risk. The approach taken by MacLeod et al. (2013) offers us a lesson on how functional studies in model systems can be combined with unbiased human genetics and genomics studies to help elucidate novel genetic contributors to PD and many other human diseases.

In parallel to the tremendous genetic advances, there has been a recent seismic shift in understanding of mechanisms of PD initiation and progression. Neuropathologists have long appreciated that PD is characterized by widespread changes, involving both the peripheral and central nervous system (Braak et al., 2003). Remarkably, alpha-synuclein, the pathological substrate of Lewy bodies, seems to be able to spread from neuron to neuron and to propagate throughout anatomically interconnected brain regions. Indeed, a single injection of a preparation of alpha-synuclein aggregates into the mouse striatum is sufficient to kick off an inexorable spread of PD-like pathology and progressive loss of dopaminergic neurons, decreased dopamine levels, and eventual motor impairments (Luk et al., 2012). It is now clear that alpha-synuclein spread is a critical aspect in PD pathogenesis (Goedert et al., 2012).

A challenge will now be to integrate the genetic and pathological breakthroughs in order to define how the genetic susceptibility factors interface with alpha-synuclein spread. Do genetic loci linked to PD increase risk of disease, at least in part, by enhancing the initiation or accelerating the spread of alpha-synuclein pathology? Although this study focused on LRRK2 and PARK16, similar approaches can be launched to analyze potential interactions between other genetic susceptibility factors, including alpha-synuclein. One day, these parallel approaches may converge on a common pathway.

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In Vitro Human Corticogenesis

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Whether neurons generated in vitro from human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) have in vivo-like properties is unknown. In this issue of *Neuron*, Espuny-Camacho et al. (2013) show that ESC-/iPSC-derived cortical neurons make specific projections and functional synapses when transplanted into a neonatal mouse brain.

Efforts to study the development of the human cerebral cortex have been complicated by the difficulty of obtaining human fetal brain tissue. An attractive solution to this problem is to use human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) to recapitulate human brain development in vitro or in experimental animals. Over the last decade,