PARKINSON DISEASE

Pathogenetic insights into young onset Parkinson disease

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An understanding of the biochemical processes underlying Parkinson disease (PD) will be essential for the development of disease-modifying therapies. In a recent study, a novel biochemical phenotype of the disease was identified from analysis of inducible pluripotent stem cell-derived dopaminergic neurons from individuals with young-onset PD.

Refers to Laperle, A.H. *et al.* iPSC modeling of young-onset Parkinson's disease reveals a molecular signature of disease and novel therapeutic candidates. *Nature Medicine* **26**, 289–299 (2020)

Parkinson disease (PD) is a neurodegenerative disease that typically presents with asymmetric bradykinesia, rigidity and resting tremor, caused by the death of dopamine-secreting neurons in the substantia nigra of the brain. The pathological hallmark of the disease is the accumulation of α-synuclein in Lewy bodies and neurites in the brain. The lifetime risk of developing PD is close to 4% and the number of individuals with the disease in the USA is expected to reach 1.2 million by 2030[1]. Although the motor symptoms of PD can be managed with dopaminergic replacement therapy, we do not have interventions that can prevent disease onset or slow progression. The development of such disease-modifying therapies has been limited by our incomplete understanding of the causes of PD and their downstream biochemical consequences.

Several genetic causes of PD have been identified, and familial forms of the disease often have a younger age of onset than the sporadic form. Individuals are usually diagnosed with PD in their

seventies, so those who are diagnosed with the disease before they reach 40 years of age are referred to as having young-onset PD. Although an environmental cause of PD has not yet been identified, non-genetic factors might influence genetically determined disease penetrance. Despite the clinical and aetiological diversity of PD, genetic and biochemical studies have identified some common pathogenic pathways, including mitochondrial and lysosomal dysfunction, inflammation, and altered α-synuclein metabolism[2].

Young-onset PD can result from mutations in a single high-risk gene, such as *PARKIN* or *PINK1*, or from a high burden of low-risk variants[3,4]. The age of onset of PD is also earlier in individuals with single mutations in the gene encoding the lysosomal enzyme glucocerebrosidase (*GBA*)[5]. However, many individuals with young-onset PD do not have known PD-associated mutations and detailed study of this group of individuals is likely to identify further genetic causes of the disease and provide information on the pathogenic pathways involved. In a series of elegant and comprehensive experiments, the results of which were recently published in *Nature Medicine*, Laperle et al.[6] analysed inducible pluripotent stem cell (iPSC)-derived dopaminergic neurons from individuals with young-onset PD to investigate the cause and pathophysiology of the disease.

In the study by Laperle et al.[6], the participants with young-onset PD did not have known monogenic causes of PD and seemed to have typical disease presentation. In the iPSC-derived dopaminergic neurons from these individuals, α-synuclein deposition was prominent, whereas deposition was not seen in neurons derived from healthy individuals. This finding shows that iPSC-derived neurons from individuals with young-onset PD can recapitulate a key feature of PD pathology, even in the absence of any known PD-associated mutations.

Proteomic and transcriptomic analysis of young-onset PD cell lines identified increased levels of α-synuclein RNA and protein, and decreased levels of endoplasmic-reticulum lumen proteins and

lysosomal proteins in these cells when compared with control cell lines. However, the mRNA and protein signature was not uniform across the different young-onset PD cell lines, cells from one particular individual showed changes in the levels of mitochondrial-related mRNA and protein. This suggests this individual may have an underlying cause affecting the mitochondrial pathway, and is consistent with the notion that young-onset PD is heterogeneous in aetiological terms.

The reduced levels of lysosomal proteins in young-onset PD cell lines suggested that α-synuclein accumulation was a result of impaired protein degradation, which can occur via lysosomal or proteasomal pathways. Proteins other than α-synuclein did not accumulate in these cells, and proteasomal inhibitors did not further increase α-synuclein levels, indicating that dysregulation of α-synuclein breakdown by the proteasome was not involved in accumulation. The results of a previous study[ref] suggested that increased levels of oxidised dopamine in cells from individuals with PD were associated with lysosomal dysfunction and α-synuclein accumulation. However, in the study by Laperle et al. the decrease in levels of lysosomal protein LAMP1 and activity of glucocerebrosidase preceded the accumulation of oxidised dopamine in the cells, implying that the reduction in lysosomal function and accumulation of α-synuclein were not a result of free radical-mediated damage.

Laperle et al. used compounds that increase lysosomal activity to further explore the lysosomal involvement in α-synuclein accumulation. The protein kinase C (PKC) agonist PEP005 reduced levels of α-synuclein protein and increased levels of tyrosine hydroxylase (a marker of dopaminergic neurons) in control cell lines and young-onset PD cell lines. Basal levels of phosphorylated PKC-α (pPKC-α) were higher in young-onset PD cell lines than control cell-lines; PEP005 normalized the levels of pPKC-α and increased levels of LAMP1. A lower dose of PEP005 reduced α-synuclein protein levels without modifying levels of pPKC-α, indicating that the effects of PEP005 on α-synuclein and LAMP1 were probably not mediated through pPKC-α.

The effects of PEP005 were blocked by epoxomicin, suggesting that PEP005 clears α-synuclein via the activation of proteasomal degradation. These initial observations were made in cell lines from three individuals with young-onset PD and three healthy individuals. Laperle et al. then analysed cell lines from an additional nine individuals with young-onset PD and seven healthy individuals, and all but one of the young-onset PD cell lines had higher levels of α-synuclein and pPKC-α than the control cell lines. In mice, unilateral intrastriatal injection of PEP005 reduced ipsilateral α-synuclein levels, suggesting that the findings from the iPSC cell lines are also applicable in vivo.

The results of the study by Laperle et al.[6] suggest that individuals with young-onset PD have genetically determined lysosomal dysfunction, thus providing new information on the aetiology of PD and highlighting this pathway for therapeutic intervention. Indeed, mutations in several of the genes involved in the lysosomal degradation pathway are already known to cause PD or Parkinsonism[7]. Prominent amongst these genes is *GBA*, mutations in which are the most common genetic cause of PD identified to date[7]. Therefore, further study of young-onset PD genetics, particularly genes involved in the lysosomal pathway, will be important. However, Laperle et al.[6] also identified inter-individual differences in RNA and protein signatures, indicating that young-onset PD is likely to be a heterogeneous condition.

Laperle et al.[6] also identified a novel biochemical phenotype of young-onset PD, which is characterized by increased levels of α-synuclein and pPKC-α. Suprisingly, PEP005 seemed to reverse this phenotype by increasing proteasomal activity, not by reducing pPKC-α or through lysosomal stimulation. However, lysosomal stimulation, and glucocerebrosidase activation in particular, are already being investigated as potential therapeutic interventions for PD. Ambroxol, a repurposed respiratory drug, is a glucocerebrosidase small molecule chaperone and an activator of transcription factor EB. In vitro and in vivo studies have demonstrated that ambroxol

increases glucocerebrosidase activity and reduces α-synuclein levels[8]. Ambroxol has now been tested in the first clinical trial of a drug for a genetically stratified subgroup of individuals with PD — those with glucocerebrosidase mutations[9]. The results of this trial indicate that ambroxol crosses the blood–brain barrier and engages its target to increase glucocerebrosidase levels; the drug was well-tolerated and safe. Further trials of ambroxol are now planned.

The development of effective drugs to slow or prevent PD will rely on a thorough understanding of the causes and pathophysiology of the disease. The study by Laperle et al.[6] highlights the lysosomal pathway as a likely final common denominator of several aetiologies of PD and provides insights into novel routes for intervention.

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Competing interests

The authors declare no competing interests.