Modeling Gene-Environment Interactions in Parkinson's Disease Using Patient-Derived Induced Pluripotent Stem Cells

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Parkinson's disease (PD) is a highly prevalent, progressive neurodegenerative disease characterized by loss of the dopaminergic neurons of the substantia nigra (SN) pars compacta. Although there are several proposed mechanisms for the pathophysiology of this debilitating illness, efforts to develop disease-modifying therapies have been hampered by the inability of existing model systems to completely reproduce the characteristic molecular and pathological features of PD. Given the potential of both environmental toxicants and genetic risk factors to modulate the onset and severity of PD, a model system that accounts for both would serve as a valuable tool in the study of PD-related environmental neurotoxicants. The advent of induced pluripotent stem cell (iPSC) technology has created the opportunity to evaluate personalized, toxicological susceptibility to specific environmental agents. Using this system, it is now possible to analyze cellular physiological pathways in human neurons, both developing and mature, and glial cells that play key roles in handling neurotoxicants. Furthermore, the utilization of living human cells with identical genetic determinants as the resource subjects, with or without PD, is a powerful resource for the development of therapeutics that modulate patient susceptibility to environmental toxicants.

Keywords: *Parkinson's disease, manganese, induced pluripotent stem cells*

Epidemiology of PD

Parkinson's disease (PD) is the second most common neurodegenerative disease. The prevalence of PD in the industrialized world is estimated at 0.3% of the general population and approximately 1% in individuals over the age of 60[1]. Thus, PD is considered an age-related disease, with prevalence rising mainly after the age of 50[2-12]. As the population ages, there is an increasing socioeconomic burden on society[2]. The incidence of PD is 8 to 18 per 100,000 person-years[2]. It has been noted that there is higher prevalence of PD in men than women, a finding hypothesized to be mediated by a neuroprotective role of estrogens[2, 3, 6-8].

PD diagnosis is contingent on presentation with at least two of the four cardinal symptoms: resting tremor, bradykinesia, rigidity, and postural instability[2]. The clinical suspicion is further supported by the patients' responsiveness to levodopa, asymmetry of symptoms, or SPECT imaging with DaTSCAN, although the latter is seldom used as a primary diagnostic procedure[13, 14]. Furthermore, secondary causes of parkinsonism, such as drug-induced parkinsonism, must be excluded. Interestingly, the course of PD is highly variable; studies analyzing PD progression suggest that functional deterioration is accelerated both early in the disease course and among patients presenting with postural instability gait difficulty[15-18]. The majority (90%) of PD cases are sporadic in etiology, with the remaining 10% of cases having known genetic causes. Furthermore, there is profound heterogeneity in age of onset, neuropathological findings, and characteristic symptoms even among the genetic forms of PD.

Pathophysiology of PD

Although a complete understanding of the

Bradykinsia:
A slowness in the execution of movement. It is one of the three key symptoms of parkinsonism, which are bradykinesia, tremor and rigidity.

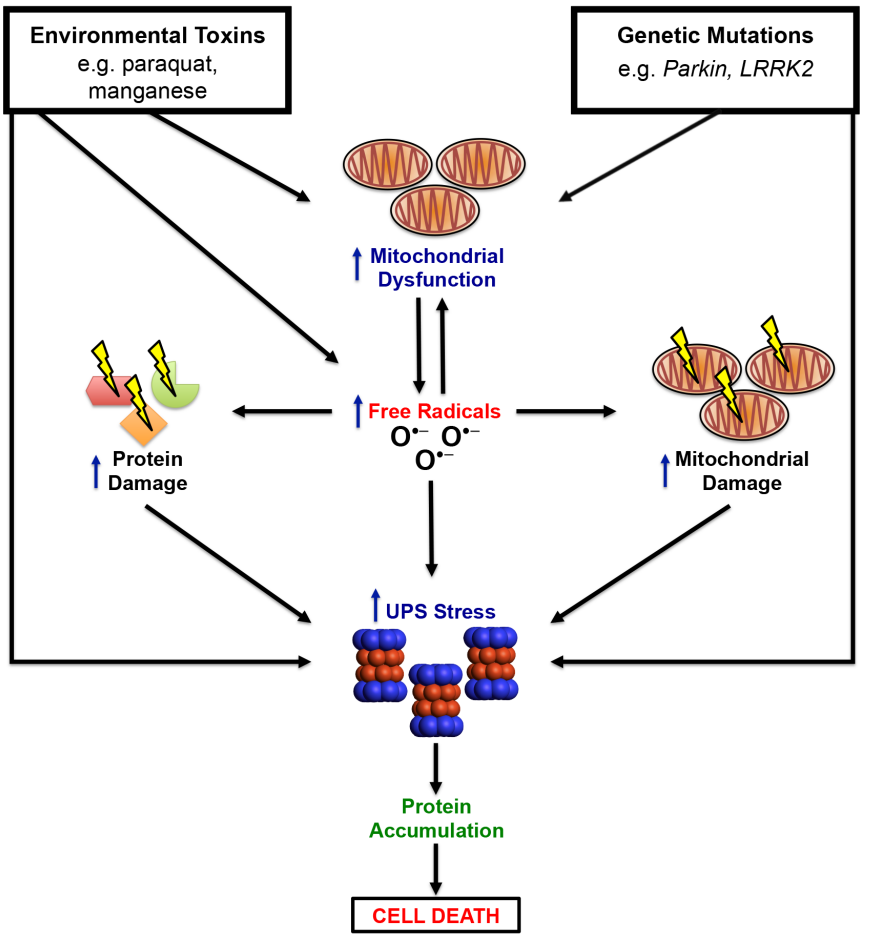

Figure 1: *Gene-environment interactions in PD.* Environmental and genetic factors result in the mitochondrial dysfunction, generation of free radicals, mitochondrial/protein damage, increased UPS stress, protein aggregation, and dopaminergic cell death in PD.

Ubiquitin proteasome system (UPS): A multicomponent system that identifies and degrades unwanted proteins in the cytoplasm of all cells; involved in cell growth and differentiation, DNA replication and repair, apoptosis, and stress and immune responses.

pathogenesis of PD remains elusive, current evidence suggests that PD results from a multitude of factors, including: oxidative stress, protein aggregation, and mitochondrial dysfunction[19]. PD is characterized by loss of dopaminergic neurons of the substantia nigra and the presence of intraneuronal α-synuclein protein aggregates composed of α-synuclein known as Lewy bodies[20]. Loss of dopamine levels in the striatum leads to downstream dysregulation of basal ganglia motor circuitry, resulting in the motor symptoms observed in PD. Studies exploring the genetic forms of PD have offered insight regarding central mechanisms in PD pathogenesis. Defective proteins in familial PD result from mutations in genes that function in critical cellular processes, such as the ubiquitin proteasome system (UPS), vesicle trafficking, mitochondrial function, and oxidative stress responses[21-23]. These findings suggest that while there is a common endpoint of decreased striatal dopamine levels, multiple pathways can influence an individual's pattern of neuronal cell death and the mechanism by which it occurs. For example, dysfunction in mitochondrial complex I results in upregulated free radical production causing protein damage. The damaged protein burden increases the stress on the UPS, leading to protein aggregation and subsequent neuronal death[24]. However, this simple pathway could be influenced at any level by multiple inputs, such as environmental toxins, genetic risk, and enhanced oxidative stress **(Figure 1)**. Thus, an individual patient's history of environmental exposure and genetic risk are critical to their clinical manifestation of PD.

Dystonia: A neurological movement disorder, in which sustained muscle contractions cause twisting and repetitive movements or abnormal postures.

Environmental Influences in PD

Epidemiological and laboratory research has revealed a number of environmental exposures and toxicants that contribute to PD. One of the most notable of PD-causing environmental toxicants is 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), a contaminant of a synthetic heroin analogue, which was identified as the culprit in the dramatic onset of parkinsonian symptoms in four individuals after self-administration of the drug[25]. MPTP administration to both mice and non-human primates revealed a buildup of α-synuclein within nigral dopaminergic cell bodies[26, 27]. These MPTP studies produced both a valuable model system of parkinsonism and launched a wave of investigation focusing on environmental exposures that contribute to PD.

Environmental Exposures: Pesticides, Heavy Metals, and Beyond

One of the most well-studied environmental associations with PD is that of pesticides and herbicides. Case control studies have revealed an association between exposure to herbicides, insecticides, and farming as an occupation and PD[28, 29]. Individuals exposed to pesticides have a 70% higher incidence of PD compared to those not exposed[30]. The timing of exposure has a profound impact on susceptibility; exposure at a younger age increases PD risk relative to those exposed over the age of 60[31]. However, these studies are limited to self-reporting by subjects; , a variable that is highly dependent on the awareness of the individual and subject to recall bias. In addition, subjects recruited for these studies would most likely be unable to report prenatal or early-childhood exposures, further limiting the interpretation of epidemiological data. Despite these limitations, several major pesticides have been associated with PD, including: dieldrin, maneb, paraquat and rotenone[32, 33]. Although it is outside the scope of this article to discuss the mechanism of these toxicants individually, paraquat is representative of how such exposures produce neurodegeneration resulting in parkinsonism, and will be given a brief discussion here.

The toxic properties of paraquat are caused by its redox cycle in which it is reduced by NAPDH-CYP450 reductase, NAPDH-cytochrome *c* reductase, and mitochondrial complex I[34-37]. This process generates a paraquat monocation free radical that is rapidly re-oxidized, producing the superoxide radical (O_2^{2-}). This process sets off a cascade of reactions in which more reactive oxygen species are generated, leading to cellular stress and, when the cellular antioxidant capacity is overwhelmed, eventual degeneration. Furthermore, paraquat is known to generate selective neurodegeneration of SN dopaminergic neurons when administered to rodents in a manner similar to that of MPTP.[38] In addition to its acute effects, paraquat exposure during critical developmental periods generates progressive and permanent lesions of the SN dopaminergic system, rendering it hypersusceptible to adult neurotoxicant exposures[39].

In addition to pesticides, exposure to heavy metals is associated with PD. In particular, exposure to iron, manganese, lead, copper, zinc, amalgam, and aluminum have each been demonstrated to increase risk of PD[40]. This is of direct clinical relevance, as studies have shown that high manganese exposure produces a secondary form of parkinsonism, known as manganism, that is clinically indistinguishable from idiopathic PD aside from age of onset.[41]. The mechanism of neurodegeneration in heavy metal toxicity is hypothesized to be due to deposition of the metals in the SN and increased oxidative stress.[40].

Important to note, not all environmental exposures are neurotoxic, several agents have a demonstrated neuroprotective role such as coffee drinking, smoking, and statin use.[42, 43]. This suggests that environmental impact on PD is bidirectional, and recommendations regarding neuroprotective strategies may be of utility in those at high risk for developing the disease.

Rasagiline:
An irrever-sible inhibitor of monoamine oxidase used as a monotherapy in early Parkinson's disease or as an adjunct therapy in more advanced cases.

Genetic Influences in PD

Several loci and genes have been identified as causative of the genetic forms of PD. These include the autosomal dominant *PARK1* and *PARK4* (*SNCA*/α-synuclein), *PARK5* (*UCHL1*), *PARK8* (*LRRK2*), *PARK11* (*GIGYF2*), *PARK13* (*Omi/Htra2*) and the autosomal recessive *PARK2* (*Parkin*), *PARK6* (*PINK1*), *PARK7* (*DJ-1*), and *PARK9* (*ATP13A2*)[44]. These genetic forms vary significantly between each other and are different from the sporadic forms of PD in their age of onset, clinical course, and response to treatment. One example of how a genetic mutation can produce PD is illustrated by *PARK2* loss-of-function mutations, the most common cause of autosomal recessive juvenile parkinsonism (ARJP). In addition to the cardinal symptoms of PD, ARJP is distinguished by prominent lower limb dystonia, severe levodopa-induced dyskinesias, and early age of onset, usually before the age of 40[45]. Notably, patients with ARJP caused by a mutation in *PARK2,* have loss of dopaminergic neurons without the presence of Lewy bodies[20]. Parkin (the protein product of *PARK2*) functions as an E2-dependent E3 ubiquitin ligase that functions as a substrate-recognition molecule within the UPS[46]. It is hypothesized that loss of Parkin function of Parkin results in reduced ubiquitination of its substrates and subsequent protein accumulation and toxicity to dopaminergic neurons (**Figure 1**)[47, 48]. There is evidence that Parkin plays a critical role in engulfment of mitochondria with low membrane potential, leading to the hypothesis that failure to eliminate dysfunctional mitochondria contributes to PD pathogenesis.[49]

In addition to these monogenic forms, sporadic forms of PD are likely influenced by an individual's global genetic variation. A number of genome-wide association studies (GWAS) have identified particular PD-associated loci. Susceptibility loci include regions within the monogenic causative genes such as SNCA (4q22) and LRRK2 (12q12), as well as newly identified loci such as PARK16 (1q32), BST1 (4p15), and *HLA-DRA*[50-53]. Given that the previously discussed environmental toxicants act on related processes, genetic findings have generated interest in the study of gene-environment interactions that may underlie the heterogeneity in presentation among PD patients.

Gene-environment interactions in PD – Modeling neurotoxicological risk

Examination of neurotoxicological risk in PD model systems has emerged as an area of active research given the strong evidence for the influence of both environmental and genetic factors on PD onset. The concept of mutations in individual genes or single nucleotide polymorphisms across multiple loci that alter the susceptibility of an individual to a given toxicant has been validated in many studies. For example, mutations in the gene *MDR1* predispose individuals to the injurious effects of pesticides and other P-glycoprotein transported xenobiotics, resulting in PD[54]. Similarly, polymorphisms in metabolic enzymes such as MAOB, CYP2D6, and GSTT1 have been associated with PD[55]. On the other hand, experiments in yeast and animal model systems have revealed a protective role of certain genes, such as *PARK9* (ATP13A2) that protects against manganese toxicity and dopaminergic cell death due to α-synuclein overexpression[56]. Despite these advances, there is a knowledge gap between clinical data from PD patients and laboratory data generated using model systems. It is assumed, however, that by modulating individual response to neurotoxicants, the clinical course of PD can be manipulated. This concept has been demonstrated in clinical trials demonstrating the beneficial effects of levodopa, tai chi, and rasagiline on PD symptoms and progression.[57-59]. Thus, the need to test different interactions between subject-specific genetic background and environmental exposures makes patient-specific iPSCs a powerful tool to predict clinical outcomes and guide clinical investigations and intervention.

The utility of iPSC technology for neurotoxicology

Background of iPSCs

In 2007, Takahashi *et al.* reported for the first time the possibility of reprogramming adult human-derived fibroblasts to pluripotent stem cells using four defined transcription factors, *OCT4*, *SOX2*, *c-MYC*, and *KLF4*[60]. iPSCs exhibit the typical characteristic of inner cell mass-derived human embryonic stem cells, including self-renewal and the ability to differentiate into cell types of all three germ layers. This landmark study launched a new field of research focused on improving the efficiency of reprogramming and deriving cells from various patient types. Initial experiments utilized retroviruses for transduction, which introduced a set of drawbacks including mutagenesis at insertion sites and persistent expression of reprogramming factors. In efforts to overcome these obstacles, several alternative reprogramming strategies have been developed, including doxycycline-inducible expression, the use of *loxP* sites, *PiggyBac* transposons, adenovirus transduction, plasmid transfections, and episomal vectors[61-67]. In addition, other groups are investigating the use of compounds that permit iPSC induction without the use of genetic material[68]. These improvements in reprogramming enhance the utility of this system for the study of gene-environment interactions, as they minimize the contribution of the reprogramming process to genetic heterogeneity among iPSCs derived from different individuals. After reprogramming patient fibroblasts to iPSCs, the cells can be differentiated into a variety of neuronal and glial subtypes including functional midbrain dopaminergic neurons[69, 70].

iPSCs as a model for gene-environment interactions in PD

One of the major advantages of utilizing patient-specific iPSCs to study neurotoxicological interaction is that an individual may be evaluated for environmental risk without *a priori* knowledge of their genetic risk factors[71]. The use of iPSCs for toxicological risk assessment is dependent on the assumption that cells derived from patients serve as a model system for understanding the influence of human genetic factors and their ability to modulate the vulnerability of differentiated cells to a given toxicant[71]. Although efforts to validate these assumptions are underway, iPSC technology remains an exciting opportunity to examine changes in the development and maintenance of neuronal function after genetic and toxicant perturbation.

There are a variety of exposure paradigms where iPSC-derived neurons and neural progenitors are of value. Through this experimental system, environmental insults or protectants can be screened across different temporal delivery patterns to understand response to acute and chronic exposures. The pattern of exposure is of interest, since toxicants such as methylmercury exhibit acute and latent effects with variable sensitivity based on developmental time point[72-74]. *In vitro* neuronal differentiation of hiPSCs permits assessment of interactions between early exposure and subsequent risk of neurodegenerative phenotypes in acute, multi-hit, and chronic exposure paradigms. Prior to iPSCs, such studies could only be performed utilizing primary cell culture or embryonic stem cells, which are high-cost alternatives that lack patient specificity.

Furthermore, developing neural progenitors can be exposed to chronic low concentrations of the agent to mimic the effect of cumulative toxicity across the lifetime of a neuron. The pluripotent nature of iPSCs allows the assessment of a diverse set of neuronal subtypes to a given exposure. For example, one could investigate if midbrain dopaminergic neurons have a heightened susceptibility to manganese during development compared to forebrain dopaminergic neurons derived from the same patient. Alternatively, polymorphisms at different loci between patients could heighten developmental sensitivity to a toxicant between PD patients and controls. Such findings from iPSCs can inform downstream *in vivo* vertebrate studies that account for endogenous processes such as detoxification, neuronal regeneration, and immune response.

Current challenges in modeling PD with iPSCs

Perhaps the greatest challenge utilizing iPSCs is assuring the coherence of genotype and phenotype. Many groups have identified methylation pattern and gene expression differences between iPSC lines from the same patient[75-78]. This could be due to a multitude of factors, including expression of reprogramming vectors, point mutations, and copy number variants generated in the reprogramming process[79]. Any induced genetic or epigenetic abnormalities are of concern in the study of gene-environment interactions in PD since they may mask the effect of a patient's individual genetic variation. In order to account for these effects, karyotyping should be performed at minimum, and whole genome and bisulphite sequencing should also be considered. Furthermore, it should be noted that *in vitro* studies utilizing iPSC-derived cells are limited in interpretation because they lack complex extracellular environments, neuronal architecture, and glial interactions. However, there have been efforts to address these concerns through development of mixed neuronal-glial cultures, but results have been inconsistent thus far[80]. Finally, since PD

is an age-related disease, newly differentiated neurons from iPSCs fail to simulate long-term simulate age-related phenotypes.

The promise of PD patient-derived iPSCs for personalized medicine and risk assessment

PD has the potential to benefit tremendously from the wide utilization of iPSC technology. This system permits the study of gene-environment interactions utilizing cellular subtypes derived from patients afflicted with PD. iPSC technology provides a critical link between epidemiological studies and animal, cellular, and computational models. This feature has potential for direct clinical application, as iPSC-derived neurons can be used to design customized neuroprotective strategies and recommendations for patients at the preclinical or early clinical stages of PD. In a parallel fashion, drug development can be accelerated by the development of high-throughput assays utilizing iPSC-derived neurons. Although PD-derived iPSCs share the limitations of other *in vitro* model systems, the fact that they are derived from patients with a clinical diagnosis offers the ability to explore processes such as oxidative stress, protein aggregation, and mitochondrial dysfunction in response to toxicants without a full understanding of the genetic factors underlying PD pathogenesis.

References

1. Nussbaum, R.L. & Ellis, C.E. Alzheimer's disease and Parkinson's disease. *N Engl J Med* **348**, 1356-64 (2003).

2. de Lau, L.M. & Breteler, M.M. Epidemiology of Parkinson's disease. *Lancet Neurol* **5**, 525-35 (2006).

3. de Rijk, M.C., Breteler, M.M., Graveland, G.A., Ott, A., Grobbee, D.E., van der Meche, F.G. & Hofman, A. Prevalence of Parkinson's disease in the elderly: the Rotterdam Study. *Neurology* **45**, 2143-6 (1995).

4. Benito-Leon, J., Bermejo-Pareja, F., Morales, J.M., Vega, S. & Molina, J.A. Prevalence of essential tremor in three elderly populations of central Spain. *Mov Disord* **18**, 389-94 (2003).

5. Schoenberg, B.S., Osuntokun, B.O., Adeuja, A.O., Bademosi, O., Nottidge, V., Anderson, D.W. & Haerer, A.F. Comparison of the prevalence of Parkinson's disease in black populations in the rural United States and in rural Nigeria: door-to-door community studies. *Neurology* **38**, 645-6 (1988).

6. Tison, F., Dartigues, J.F., Dubes, L., Zuber, M., Alperovitch, A. & Henry, P. Prevalence of Parkinson's disease in the elderly: a population study in Gironde, France. *Acta Neurol Scand* **90**, 111-5 (1994).

7. Morgante, L., Rocca, W.A., Di Rosa, A.E., De Domenico, P., Grigoletto, F., Meneghini, F., Reggio, A., Savettieri, G., Castiglione, M.G., Patti, F. & et al. Prevalence of Parkinson's disease and other types of parkinsonism: a door-to-door survey in three Sicilian municipalities. The Sicilian Neuro-Epidemiologic Study (SNES) Group. *Neurology* **42**, 1901-7 (1992).

8. de Rijk, M.C., Tzourio, C., Breteler, M.M., Dartigues, J.F., Amaducci, L., Lopez-Pousa, S., Manubens-Bertran, J.M., Alperovitch, A. & Rocca, W.A. Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease. *J Neurol Neurosurg Psychiatry* **62**, 10-5 (1997).

9. Claveria, L.E., Duarte, J., Sevillano, M.D., Perez-Sempere, A., Cabezas, C., Rodriguez, F. & de Pedro-Cuesta, J. Prevalence of Parkinson's disease in Cantalejo, Spain: a door-to-door survey. *Mov Disord* **17**, 242-9 (2002).

10. Mayeux, R., Marder, K., Cote, L.J., Denaro, J., Hemenegildo, N., Mejia, H., Tang, M.X., Lantigua, R., Wilder, D., Gurland, B. & et al. The frequency of idiopathic Parkinson's disease by age, ethnic group, and sex in northern Manhattan, 1988-1993. *Am J Epidemiol* **142**, 820-7 (1995).

11. Li, S.C., Schoenberg, B.S., Wang, C.C., Cheng, X.M., Rui, D.Y., Bolis, C.L. & Schoenberg, D.G. A prevalence survey of Parkinson's disease and other movement disorders in the People's Republic of China. *Arch Neurol* **42**, 655-7 (1985).

12. Fall, P.A., Axelson, O., Fredriksson, M., Hansson, G., Lindvall, B., Olsson, J.E. & Granerus, A.K. Age-standardized incidence and prevalence of Parkinson's disease in a Swedish community. *J Clin Epidemiol* **49**, 637-41 (1996).

13. Litvan, I., Bhatia, K.P., Burn, D.J., Goetz, C.G., Lang, A.E., McKeith, I., Quinn, N., Sethi, K.D., Shults, C. & Wenning, G.K. Movement Disorders Society Scientific Issues Committee report: SIC Task Force appraisal of clinical diagnostic criteria for Parkinsonian disorders. *Mov Disord* **18**, 467-86 (2003).

14. Tolosa, E., Borght, T.V. & Moreno, E. Accuracy of DaTSCAN (123I-Ioflupane) SPECT in diagnosis of patients with clinically uncertain parkinsonism: 2-year follow-up of an open-label study. *Mov Disord* **22**, 2346-51 (2007).

15. Jankovic, J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry* **79**, 368-76 (2008).

16. Jankovic, J. & Kapadia, A.S. Functional decline in Parkinson disease. *Arch Neurol* **58**, 1611-5 (2001).

17. Lang, A.E. The progression of Parkinson disease: a hypothesis. *Neurology* **68**, 948-52 (2007).

18. Post, B., Merkus, M.P., de Haan, R.J. & Speelman, J.D. Prognostic factors for the progression of Parkinson's disease: a systematic review. *Mov Disord* **22**, 1839-51; quiz 1988 (2007).

19. Greenamyre, J.T. & Hastings, T.G. Biomedicine. Parkinson's--divergent causes, convergent mechanisms. *Science* **304**, 1120-2 (2004).

20. Wood-Kaczmar, A., Gandhi, S. & Wood, N.W. Understanding the molecular causes of Parkinson's disease. *Trends Mol Med* **12**, 521-8 (2006).

21. Jakes, R., Spillantini, M.G. & Goedert, M. Identification of

two distinct synucleins from human brain. *FEBS Lett* **345**, 27-32 (1994).

22. Mori, F., Tanji, K., Yoshimoto, M., Takahashi, H. & Wakabayashi, K. Demonstration of alpha-synuclein immunoreactivity in neuronal and glial cytoplasm in normal human brain tissue using proteinase K and formic acid pretreatment. *Exp Neurol* **176**, 98-104 (2002).

23. Nuytemans, K., Theuns, J., Cruts, M. & Van Broeckhoven, C. Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: a mutation update. *Hum Mutat* **31**, 763-80 (2010).

24. Ali, S.F., Binienda, Z.K. & Imam, S.Z. Molecular aspects of dopaminergic neurodegeneration: gene-environment interaction in parkin dysfunction. *Int J Environ Res Public Health* **8**, 4702-13 (2011).

25. Langston, J.W., Ballard, P., Tetrud, J.W. & Irwin, I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* **219**, 979-80 (1983).

26. Vila, M., Vukosavic, S., Jackson-Lewis, V., Neystat, M., Jakowec, M. & Przedborski, S. Alpha-synuclein up-regulation in substantia nigra dopaminergic neurons following administration of the parkinsonian toxin MPTP. *J Neurochem* **74**, 721-9 (2000).

27. McCormack, A.L., Mak, S.K., Shenasa, M., Langston, W.J., Forno, L.S. & Di Monte, D.A. Pathologic modifications of alpha-synuclein in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated squirrel monkeys. *J Neuropathol Exp Neurol* **67**, 793-802 (2008).

28. Gorell, J.M., Johnson, C.C., Rybicki, B.A., Peterson, E.L. & Richardson, R.J. The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. *Neurology* **50**, 1346-50 (1998).

29. Ritz, B. & Yu, F. Parkinson's disease mortality and pesticide exposure in California 1984-1994. *Int J Epidemiol* **29**, 323-9 (2000).

30. Ascherio, A., Chen, H., Weisskopf, M.G., O'Reilly, E., McCullough, M.L., Calle, E.E., Schwarzschild, M.A. & Thun, M.J. Pesticide exposure and risk for Parkinson's disease. *Ann Neurol* **60**, 197-203 (2006).

31. Costello, S., Cockburn, M., Bronstein, J., Zhang, X. & Ritz, B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. *Am J Epidemiol* **169**, 919-26 (2009).

32. Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V. & Greenamyre, J.T. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* **3**, 1301-6 (2000).

33. Kamel, F., Tanner, C., Umbach, D., Hoppin, J., Alavanja, M., Blair, A., Comyns, K., Goldman, S., Korell, M., Langston, J., Ross, G. & Sandler, D. Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. *Am J Epidemiol* **165**, 364-74 (2007).

34. Dinis-Oliveira, R.J., Remiao, F., Carmo, H., Duarte, J.A., Navarro, A.S., Bastos, M.L. & Carvalho, F. Paraquat exposure as an etiological factor of Parkinson's disease. *Neurotoxicology* **27**, 1110-22 (2006).

35. Clejan, L. & Cederbaum, A.I. Synergistic interactions between NADPH-cytochrome P-450 reductase, paraquat, and iron in the generation of active oxygen radicals. *Biochem Pharmacol* **38**, 1779-86 (1989).

36. Fernandez, Y., Subirade, I., Anglade, F., Periquet, A. & Mitjavila, S. Microsomal membrane peroxidation by an Fe3+/paraquat system. Consequences of phenobarbital induction. *Biol Trace Elem Res* **47**, 9-15 (1995).

37. Fukushima, T., Yamada, K., Isobe, A., Shiwaku, K. & Yamane, Y. Mechanism of cytotoxicity of paraquat. I. NADH oxidation and paraquat radical formation via complex I. *Exp Toxicol Pathol* **45**, 345-9 (1993).

38. McCormack, A.L., Thiruchelvam, M., Manning-Bog, A.B., Thiffault, C., Langston, J.W., Cory-Slechta, D.A. & Di Monte, D.A. Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. *Neurobiol Dis* **10**, 119-27 (2002).

39. Thiruchelvam, M., Richfield, E.K., Goodman, B.M., Baggs, R.B. & Cory-Slechta, D.A. Developmental exposure to the pesticides paraquat and maneb and the Parkinson's disease phenotype. *Neurotoxicology* **23**, 621-33 (2002).

40. Lai, B.C., Marion, S.A., Teschke, K. & Tsui, J.K. Occupational and environmental risk factors for Parkinson's disease. *Parkinsonism Relat Disord* **8**, 297-309 (2002).

41. Racette, B.A., McGee-Minnich, L., Moerlein, S.M., Mink, J.W., Videen, T.O. & Perlmutter, J.S. Welding-related parkinsonism: clinical features, treatment, and pathophysiology. *Neurology* **56**, 8-13 (2001).

42. Hernan, M.A., Takkouche, B., Caamano-Isorna, F. & Gestal-Otero, J.J. A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. *Ann Neurol* **52**, 276-84 (2002).

43. Gao, X., Simon, K.C., Schwarzschild, M.A. & Ascherio, A. Prospective study of statin use and risk of Parkinson disease. *Arch Neurol* **69**, 380-4 (2012).

44. Lesage, S. & Brice, A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Hum Mol Genet* **18**, R48-59 (2009).

45. Lucking, C.B., Abbas, N., Durr, A., Bonifati, V., Bonnet, A.M., de Broucker, T., De Michele, G., Wood, N.W., Agid, Y. & Brice, A. Homozygous deletions in parkin gene in European and North African families with autosomal recessive juvenile parkinsonism. The European Consortium on Genetic Susceptibility in Parkinson's Disease and the French Parkinson's Disease Genetics Study Group. *Lancet* **352**, 1355-6 (1998).

46. Shimura, H., Hattori, N., Kubo, S., Mizuno, Y., Asakawa, S., Minoshima, S., Shimizu, N., Iwai, K., Chiba, T., Tanaka, K. & Suzuki, T. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat Genet* **25**, 302-5 (2000).

47. Sriram, S.R., Li, X., Ko, H.S., Chung, K.K., Wong, E., Lim, K.L., Dawson, V.L. & Dawson, T.M. Familial-associated mutations differentially disrupt the solubility, localization, binding and ubiquitination properties of parkin. *Hum Mol Genet* **14**, 2571-86 (2005).

48. von Coelln, R., Dawson, V.L. & Dawson, T.M. Parkin-associated Parkinson's disease. *Cell Tissue Res* **318**, 175-84 (2004).

49. **Narendra, D., Tanaka, A., Suen, D.F. & Youle, R.J. Parkin is recruited selectively to impaired mitochondria and promotes**

their autophagy. J Cell Biol 183, 795-803 (2008).

This study helps establish the function of PARK2 (Parkin) in the elimination of impaired mitochondria. The authors conclude that failure of Parkin to promote autophagy of damaged mitochrondria as a factor in the pathogenesis of PD. This article utilizes a number of molecular biology approaches to explore mitochondrial phenotypes in response to environmental and genetic insults. This is an example of study examining specific gene-environment interactions in PD.

50. Satake, W., Nakabayashi, Y., Mizuta, I., Hirota, Y., Ito, C., Kubo, M., Kawaguchi, T., Tsunoda, T., Watanabe, M., Takeda, A., Tomiyama, H., Nakashima, K., Hasegawa, K., Obata, F., Yoshikawa, T., Kawakami, H., Sakoda, S., Yamamoto, M., Hattori, N., Murata, M., Nakamura, Y. & Toda, T. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet* **41**, 1303-7 (2009).

51. **Simon-Sanchez, J., Schulte, C., Bras, J.M., Sharma, M., Gibbs, J.R., Berg, D., Paisan-Ruiz, C., Lichtner, P., Scholz, S.W., Hernandez, D.G., Kruger, R., Federoff, M., Klein, C., Goate, A., Perlmutter, J., Bonin, M., Nalls, M.A., Illig, T., Gieger, C., Houlden, H., Steffens, M., Okun, M.S., Racette, B.A., Cookson, M.R., Foote, K.D., Fernandez, H.H., Traynor, B.J., Schreiber, S., Arepalli, S., Zonozi, R., Gwinn, K., van der Brug, M., Lopez, G., Chanock, S.J., Schatzkin, A., Park, Y., Hollenbeck, A., Gao, J., Huang, X., Wood, N.W., Lorenz, D., Deuschl, G., Chen, H., Riess, O., Hardy, J.A., Singleton, A.B. & Gasser, T. Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet 41, 1308-12 (2009).**

This article is recent work, within a population of European ancestry, suggesting the strong role of genetic variations in the etiology of Parkinson's Disease. The identification of the association between PD and MAPT was not found in a parallel study in the Japanese population suggesting that risk loci can be masked in analyses across populations. Most importantly, the study affirms the role of common genetic variants in contributing to PD risk in sporadic PD.

52. Hamza, T.H., Zabetian, C.P., Tenesa, A., Laederach, A., Montimurro, J., Yearout, D., Kay, D.M., Doheny, K.F., Paschall, J., Pugh, E., Kusel, V.I., Collura, R., Roberts, J., Griffith, A., Samii, A., Scott, W.K., Nutt, J., Factor, S.A. & Payami, H. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet* **42**, 781-5 (2010).

53. Nichols, W.C., Pankratz, N., Hernandez, D., Paisan-Ruiz, C., Jain, S., Halter, C.A., Michaels, V.E., Reed, T., Rudolph, A., Shults, C.W., Singleton, A. & Foroud, T. Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. *Lancet* **365**, 410-2 (2005).

54. Drozdzik, M., Bialecka, M., Mysliwiec, K., Honczarenko, K., Stankiewicz, J. & Sych, Z. Polymorphism in the P-glycoprotein drug transporter MDR1 gene: a possible link between environmental and genetic factors in Parkinson's disease. *Pharmacogenetics* **13**, 259-63 (2003).

55. Tan, E.K., Khajavi, M., Thornby, J.I., Nagamitsu, S., Jankovic, J. & Ashizawa, T. Variability and validity of polymorphism association studies in Parkinson's disease. *Neurology* **55**, 533-8 (2000).

56. Gitler, A.D., Chesi, A., Geddie, M.L., Strathearn, K.E., Hamamichi, S., Hill, K.J., Caldwell, K.A., Caldwell, G.A., Cooper, A.A., Rochet, J.C. & Lindquist, S. Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. *Nat Genet* **41**, 308-15 (2009).

57. Fahn, S., Oakes, D., Shoulson, I., Kieburtz, K., Rudolph, A., Lang, A., Olanow, C.W., Tanner, C. & Marek, K. Levodopa and the progression of Parkinson's disease. *N Engl J Med* **351**, 2498-508 (2004).

58. Li, F., Harmer, P., Fitzgerald, K., Eckstrom, E., Stock, R., Galver, J., Maddalozzo, G. & Batya, S.S. Tai chi and postural stability in patients with Parkinson's disease. *N Engl J Med* **366**, 511-9 (2012).

59. **Olanow, C.W., Rascol, O., Hauser, R., Feigin, P.D., Jankovic, J., Lang, A., Langston, W., Melamed, E., Poewe, W., Stocchi, F. & Tolosa, E. A double-blind, delayed-start trial of rasagiline in Parkinson's disease. N Engl J Med 361, 1268-78 (2009).**

This article is a major double-blind, delayed-start trial assessing rasagiline as a disease modifying therapy in PD. The authors establish the treatment of rasagiline at 1mg/day has a possible disease modifying effect. This study is an important example of how identification of compounds that act on protein products of genes with polymorphisms associated with PD have potentital for clinical utility.

60. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. & Yamanaka, S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861-72 (2007).

61. Brambrink, T., Foreman, R., Welstead, G.G., Lengner, C.J., Wernig, M., Suh, H. & Jaenisch, R. Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. *Cell Stem Cell* **2**, 151-9 (2008).

62. Yusa, K., Rad, R., Takeda, J. & Bradley, A. Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon. *Nat Methods* **6**, 363-9 (2009).

63. **Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T. & Yamanaka, S. Generation of mouse induced pluripotent stem cells without viral vectors. Science 322, 949-53 (2008).**

This article establishes the episomal method of reprogramming of fibroblasts into induced pluripotent stem cells (iPSCs). This is the current method we utilize in the Bowman laboratory. Using this approach, we limit the risk of integration of the reprogramming vectors into the genome. By preventing integration, phenotypes observed with iPSC-derived neurons are more likely related to the genetic background of the patient than the reprogramming process itself.

64. Stadtfeld, M., Nagaya, M., Utikal, J., Weir, G. & Hochedlinger, K. Induced pluripotent stem cells generated without viral integration. *Science* **322**, 945-9 (2008).

65. Zhou, W. & Freed, C.R. Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. *Stem Cells* **27**, 2667-74 (2009).

66. Yu, J., Hu, K., Smuga-Otto, K., Tian, S., Stewart, R., Slukvin, II & Thomson, J.A. Human induced pluripotent stem cells free of vector and transgene sequences. *Science* **324**, 797-801 (2009).

67. Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., Hong, H., Nakagawa, M., Tanabe, K., Tezuka, K., Shibata, T., Kunisada, T., Takahashi, M., Takahashi, J., Saji, H. & Yamanaka, S. A more efficient method to generate integration-free human iPS cells. *Nat Methods* **8**, 409-12 (2011).

68. Lin, T., Ambasudhan, R., Yuan, X., Li, W., Hilcove, S., Abujarour, R., Lin, X., Hahm, H.S., Hao, E., Hayek, A. & Ding, S. A chemical platform for improved induction of human iPSCs. *Nat Methods* **6**, 805-8 (2009).

69. Swistowski, A., Peng, J., Liu, Q., Mali, P., Rao, M.S., Cheng, L. & Zeng, X. Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions. *Stem Cells* **28**, 1893-904 (2010).

70. Cho, M.S., Lee, Y.E., Kim, J.Y., Chung, S., Cho, Y.H., Kim, D.S., Kang, S.M., Lee, H., Kim, M.H., Kim, J.H., Leem, J.W., Oh, S.K., Choi, Y.M., Hwang, D.Y., Chang, J.W. & Kim, D.W. Highly efficient and large-scale generation of functional dopamine neurons from human embryonic stem cells. *Proc Natl Acad Sci U S A* **105**, 3392-7 (2008).

71. Kumar, K.K., Aboud, A.A. & Bowman, A.B. The potential of induced pluripotent stem cells as a translational model for neurotoxicological risk. *Neurotoxicology* **33**, 518-29 (2012).

72. Farina, M., Rocha, J.B. & Aschner, M. Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies. *Life Sci* **89**, 555-63 (2011).

73. Ni, M., Li, X., Yin, Z., Sidoryk-Wegrzynowicz, M., Jiang, H., Farina, M., Rocha, J.B., Syversen, T. & Aschner, M. Comparative study on the response of rat primary astrocytes and microglia to methylmercury toxicity. *Glia* **59**, 810-20 (2011).

74. Weiss, B., Clarkson, T.W. & Simon, W. Silent latency periods in methylmercury poisoning and in neurodegenerative disease. *Environ Health Perspect* **110 Suppl 5**, 851-4 (2002).

75. Boulting, G.L., Kiskinis, E., Croft, G.F., Amoroso, M.W., Oakley, D.H., Wainger, B.J., Williams, D.J., Kahler, D.J., Yamaki, M., Davidow, L., Rodolfa, C.T., Dimos, J.T., Mikkilineni, S., MacDermott, A.B., Woolf, C.J., Henderson, C.E., Wichterle, H. & Eggan, K. A functionally characterized test set of human induced pluripotent stem cells. *Nat Biotechnol* **29**, 279-86 (2011).

76. Chin, M.H., Mason, M.J., Xie, W., Volinia, S., Singer, M., Peterson, C., Ambartsumyan, G., Aimiuwu, O., Richter, L., Zhang, J., Khvorostov, I., Ott, V., Grunstein, M., Lavon, N., Benvenisty, N., Croce, C.M., Clark, A.T., Baxter, T., Pyle, A.D., Teitell, M.A., Pelegrini, M., Plath, K. & Lowry, W.E. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* **5**, 111-23 (2009).

77. Doi, A., Park, I.H., Wen, B., Murakami, P., Aryee, M.J., Irizarry, R., Herb, B., Ladd-Acosta, C., Rho, J., Loewer, S., Miller, J., Schlaeger, T., Daley, G.Q. & Feinberg, A.P. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat Genet* **41**, 1350-3 (2009).

78. Stadtfeld, M., Apostolou, E., Akutsu, H., Fukuda, A., Follett, P., Natesan, S., Kono, T., Shioda, T. & Hochedlinger, K. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature* **465**, 175-81 (2010).

79. Gore, A., Li, Z., Fung, H.L., Young, J.E., Agarwal, S., Antosiewicz-Bourget, J., Canto, I., Giorgetti, A., Israel, M.A., Kiskinis, E., Lee, J.H., Loh, Y.H., Manos, P.D., Montserrat, N., Panopoulos, A.D., Ruiz, S., Wilbert, M.L., Yu, J., Kirkness, E.F., Izpisua Belmonte, J.C., Rossi, D.J., Thomson, J.A., Eggan, K., Daley, G.Q., Goldstein, L.S. & Zhang, K. Somatic coding mutations in human induced pluripotent stem cells. *Nature* **471**, 63-7 (2011).

80. Haidet-Phillips, A.M., Hester, M.E., Miranda, C.J., Meyer, K., Braun, L., Frakes, A., Song, S., Likhite, S., Murtha, M.J., Foust, K.D., Rao, M., Eagle, A., Kammesheidt, A., Christensen, A., Mendell, J.R., Burghes, A.H. & Kaspar, B.K. Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. *Nat Biotechnol* **29**, 824-8 (2011).

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