Timed essay answering the unseen question:

Discuss the role of genetics in understanding the pathogenesis of Parkinson's disease

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease, and the most prevalent form of PD is the sporadic form. An individual's susceptibility to sporadic PD depends on interactions between a large number of genes and the environment, with age being the greatest risk factor. PD can also be caused by mendelian mutations that lead to a more severe, early-onset form of the disease, but these cases are very rare. Clinically, PD is characterised by bradykinesia, tremor, rigidity, and postural instability. Pathologically, it is characterised by the accumulation and aggregation of alpha-synuclein (aSyn) protein within neurons, forming Lewy body (LB) inclusions. These LBs can spread throughout the brain, but the primary type of nerve cell lost in PD are the dopaminergic neurons of the substantia nigra pars compacta (SNpc). This essay will discuss the role of genetics in understanding the pathogenesis of PD.

The genetics of PD

While there are no mutations that *cause* the sporadic form of PD, there are a number of genes in which genetic variation increases your risk for developing sporadic PD. In contrast, there are a number of mutations that can cause mendelian PD. The mendelian form of PD, however, has a much earlier age of onset, and differences in pathology between the two forms mean it is often debated as to whether the sporadic and mendelian forms can really be considered as the same disease. This essay will analyse the genetics of PD one gene at a time, highlighting how genetic identification of genes that cause mendelian PD or increase the risk of sporadic PD have informed us as to the possible pathogenic mechanisms of PD.

SNCA

Initial genetic evidence for a causal role of the *SNCA* gene comes from mendelian forms of PD. Sequencing the DNA of patients with early-onset PD identified a number of rare mutations in the *SNCA* gene. Genetic analysis of families with these mutations identified that the mutation segregates with the disease phenotype in the family. These mutations *cause* a highly penetrant form of PD, and hence genetics helped us to identify the disruption of the *SNCA* gene as a cause of PD. Investigating the physiological role of this gene, and how this is disrupted by mutations, may shed light on potential pathogenic mechanisms of PD.

The *SNCA* gene codes for the aSyn protein. The aSyn protein is found at the neuronal presynapse and can interact with curved membranes. It is unfolded within the cytosol but has a membrane-binding N-terminus that can adopt an alpha-helical conformation to facilitate membrane binding. It's presynaptic localisation and the fact it interacts with curved membranes implicates it as a possible regulatory protein that may control the fusion and recycling of synaptic vesicles. The accumulation of aSyn into LBs is the main pathological hallmark of PD, implicating aSyn aggregates as a potential causative factor in the pathogenesis of PD. Perhaps aSyn aggregation means that aSyn is no longer available to carry out its physiological role, leading to dysfunction of synapses. Understanding how the mutations alter the function or levels of the protein should provide further evidence for a pathogenic role of aSyn.

Some of the *SNCA* mutations are copy number variants (CNVs), implying that increased levels of alpha-synuclein protein could cause the disease. However, others are missense mutations. *In vitro* studies have been carried out to investigate the effect of some of these missense mutations. The genetic mutations can be created in the alpha-synuclein protein, and the mutated protein placed in a test tube and shaken. The same can be done with a wild-type (WT) version of the protein, and the aggregation capacity of the proteins compared. After doing this, it was found that a number of the alpha-synuclein mutations increased the aggregation capacity of the protein. Furthermore, a similar assay can be carried out in the presence of lipid membranes. Such experiments have revealed that

some mutations increase oligomerisation of the protein when bound to membranes. Additionally, the majority of missense mutations are found within the N-terminal membrane binding domain, providing further evidence that aSyns membrane binding capacity may be enhanced in PD. These studies suggest that increased aggregation of aSyn to itself or to membranes could be a causal pathogenic mechanism in PD. Such a mechanism is also in line with CNVs as being a cause of PD, because CNVs would lead to greater aSyn production, and higher levels of aSyn would lead to more aSyn aggregation. However, the studies mentioned above were carried out *in vitro*. There are a number of pathways *in vivo* that act to degrade misfolded or aggregated proteins, therefore the generalisability of these studies to the human condition is limited. Additionally, this evidence is for the mendelian forms of PD, which make up a very small percentage of cases. It is important to identify whether the *SNCA* gene has a role in sporadic PD. Once again, genetic analysis has provided this information.

Evidence implicating the *SNCA* gene in sporadic PD comes from genome-wide association studies (GWAS). Simon-Sanchez *et al.* (2009) carried out a GWAS on 1713 PD patients, and 3978 controls. They identified common variation in the *SNCA* gene as being a risk factor (odds ratio (OR) = 1.23) for PD. This therefore implicates the *SNCA* gene in the pathogenesis of the sporadic form of PD. However, it is important to note that GWAS only identifies risk factors, and there will be a number of people containing the *SNCA* risk variants who will never go on to develop PD. However, as genetic studies have implicated the *SNCA* gene in both the sporadic and mendelian forms of PD, it suggests that aSyn and LBs may have a causative role in the disease pathogenesis.

GBA

The *GBA* gene codes for glucocerebrosidase (GCase). GCase is a lysosomal enzyme that is essential for glycolipid metabolism – it hydrolyses the beta-glucosidic linkage of glucocerebroside, which is an intermediate in glycolipid metabolism. Homozygous mutations in this gene can lead to a defective GCase enzyme and cause Gaucher's disease, a lysosomal storage disorder characterised by the accumulation of glucocerebroside within cells and organs. Genetic sequencing of patients with sporadic forms of PD have identified that individuals heterozygous for *GBA* mutations do not get Gaucher's disease, but have a 5-10x increased risk of developing PD. This genetic finding identifies GBA as a significant risk factor for developing PD, suggesting that this protein may play an important role in the pathogenesis of PD. Investigating the function of this gene's protein product may illuminate potential pathogenic mechanisms.

The mutant GCase enzyme is thought to become trapped in the endoplasmic reticulum (ER), leading to ER stress and a lack of GCase within lysosomes. This impairs the enzymatic and hence functional activity of lysosomes, hindering their ability to degrade misfolded and aggregated proteins. aSyn is cleared via the lysosome, hence impaired lysosomal function should lead to the accumulation of aSyn. Additionally, it is likely that the excess aSyn impairs the transport of GCase from the ER to the Golgi, further reducing the amount of GCase in lysosomes. Therefore, the genetic finding and subsequent functional studies suggest that *GBA* mutations could lead to a self-perpetuating cycle of lysosomal dysfunction and aSyn accumulation, contributing to the pathogenesis of PD. Furthermore, besides *GBA* mutations, GWAS studies have identified that common variation in the *GBA* gene can also increase the risk of PD, further supporting the idea that this gene is important in the pathogenesis of PD.

LRRK2

DNA sequencing of patients with early-onset PD has identified mutations in the *LRRK2* gene as being the most common mendelian cause of PD. However, it is important to note that some cases of PD caused by *LRRK2* mutations lack LB pathology. It is therefore questionable as to whether this really is

causing the same disease. However, the large majority of cases do develop LB pathology, and the clinical manifestations are mostly indistinguishable between sporadic PD and *LRRK2* PD. LRRK2 is thought to have a role in vesicular dynamics. Subcellular localisation studies have shown that LRRK2 is localised within endosomes and lysosomes, and functional studies implicate LRRK2 in regulating synaptic vesicle trafficking and degradation. What happens when this gene is mutated? Genetic analysis implicated a causative role for this gene in PD, but genetic techniques are also indispensable for identifying the pathogenic mechanisms resulting from the mutations. Genetic techniques, such as gene knock-out studies, can be used to knock-out the LRRK2 gene from mice to investigate how this alters the genes' function. Indeed, this has been carried out to investigate the consequences of the LRRK2 gene mutations.

Researchers used genetic techniques to knock out the LRRK1 and LRRK2 genes of mice, generating a double knock-out mouse model for studying the effect of LRRK2 loss-of-function. They found that this led to aSyn accumulation and impairment of the autophagy-lysosomal pathway in the dopaminergic neurons of ageing mice, as well as a loss of SNpc dopaminergic neurons. This suggests that human mutations in the LRRK2 gene may also cause PD by impairing the autophagy-lysosomal pathway. However, mouse physiology differs to that of humans, and we cannot be certain that these mutations would lead to the same effect in humans. Additionally, these mice were deficient for both LRRK1 and LRRK2, hence they do not faithfully represent the genetics of the human condition. Even so, these genetic studies provide essential evidence for the pathogenic role of LRRK2. Furthermore, evidence for LRRK2 in sporadic PD comes from GWAS. GWAS have implicated common variation in the *LRRK2* gene as a significant risk factor for developing the sporadic form of PD. Extrapolating from the LRRK2 knock-out studies, perhaps these common variants could lead to mild impairments in the efficiency of the autophagy-lysosomal pathway, leading to aSyn accumulation and subsequent synaptic dysfunction.

PINK1 and Parkin

So far, only autosomal dominant and sporadic forms of PD have been discussed. Genetics has also identified recessive forms of PD. Genetic sequencing of effected individuals has identified homozygous mutations in PINK1 and Parkin genes as causing recessive forms of PD. The protein products of these genes are important for the cell's mitochondrial quality control pathway, whereby they can identify damaged mitochondria and activate their selective degradation via mitophagy. PINK1 is thought to identify and bind to damaged mitochondria. PINK1 then recruits Parkin to the outer membrane of the mitochondria, where Parkin ubiquitinates this membrane to label it for destruction. The fact mutations in these genes can cause PD implicates a failure of the mitophagy quality control pathway in the pathogenesis of PD. However, while patients with this form of PD are virtually clinically indistinguishable from sporadic PD patients (besides their earlier age of onset), only a minority of these patients are pathologically the same as sporadic PD – only a small proportion of the individuals with this form of PD have LB pathology. It is therefore questionable as to whether they can be said to be the same disease.

Conclusion

In conclusion, genetics has an indispensable role in understanding the pathogenesis of PD. GWAS studies can identify common variation that is enriched in patients suffering with sporadic PD compared to controls, while genetic sequencing of mendelian families can identify causative mutations. Both of these techniques identify genes whose function can be studied to investigate potential pathogenic mechanisms. These functional studies also benefit from genetics, whereby genetic manipulation techniques can be used to mutate proteins and study the effects *in vitro*, or

knock-out/knock-in genes in living organisms to study the biological consequences of these mutations.