The Movers and The Shakers: You Can't Dance Without Dopamine

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Parkinson's Disease (PD) is a progressive neurodegenerative illness characterized by rigidity, bradykinesia and resting tremor. Pathologically, PD involves the death of dopaminergic neurons in the substantia nigra of the brain. Two forms of PD exist: familial and sporadic. The causes of sporadic PD are still not fully understood, although α-synuclein, a neuronal protein, is found misfolded in neurons. Among familial forms, autosomal dominant PD (ADPD) is a result of mutations in one of two genes: α-synuclein, which accumulates in substantia nigra dopaminergic neurons, and UCH-L1, whose etiology remains unclear. Autosomal recessive PD (ARPD) involves mutation in parkin, a ubiquitin ligase involved in protein degradation. Until recently, the molecular mechanisms by which α-synuclein, UCH-L1, and parkin were involved in the pathogenesis of PD remained a mystery. Lately, scientists have made significant progress using *in vitro* models, and transgenic fly and mouse models to clarify α-synuclein dependent pathology and symptoms. Scientists have also discovered that mutant parkin leads to α-synuclein aggregation by preventing proper α-synuclein ubiquitination and degradation. Given this new information, and the inadequacies of current drug therapies, scientists are exploring gene therapy, stem cells, and protein therapies as alternative treatments for PD. In Parkinson's disease, the discovery of a future cure remains hopeful.

Say Hello to Parkinson's Disease

Parkinson's disease (PD) is a progressive neurodegenerative disorder that causes gradual, progressive loss of the ability to regulate muscle movements. It is characterized by a loss of neurotransmitter dopamine, which relays movement messages from the substantia nigra to parts of the brain (1). While there is currently no cure for PD, treatments for the disease do exist.

Traditionally, a drug called levadopa was the only treatment for PD and was mildly successful in lessening the symptoms of the disease. However, levadopa may wear off between doses, lessening its effectiveness over time. Other medications used to treat PD are longer-lasting dopamine agonists, which mimic dopamine and can be used with levadopa. Also, dopamine extenders are used to increase dopamine's action by disabling the enzymes that inhibits the neurotransmitter. Unfortunately, drugs cannot control all the symptoms. Surgeries, such as, deep brain stimulation and pallidotomy, have been performed to treat some of the symptoms of PD, but with marginal lasting success, due to the risks of brain cell death.

Symptoms: Shaking and Moving

Dr. James Parkinson first characterized the disease in 1817 by tremor at rest, slowness of movement, rigidity, and minimal facial expressions as symptoms of the disease (3). Symptom development is often slow, with slowness of movement or *bradykinesia* often develops first, making fluid movement difficult. In some cases, tremor is present; noticeable in the hands, limbs, head and neck. Stiffness and rigidity are also present in the early stages of PD (1).

Later stages of the disease bring posture and balance problems, including a shuffling walk. Furthermore, people with Parkinson's disease develop speech problems characterized by loss of voice volume, speed and clarity. Mood changes, such as anxiety and depression are also common. Gradually, memory loss and signs of dementia may develop in about a quarter of those affected (1).

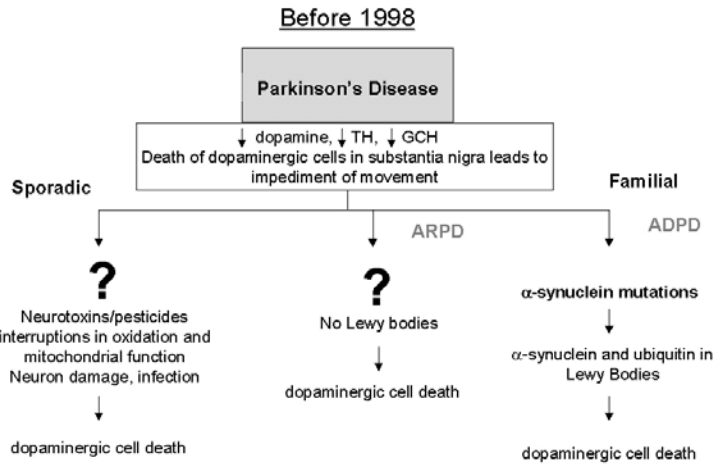

Figure 1: **A Model of the Past.** Prior to 1998, there were many unknowns in Parkinson's disease. Only one gene had been isolated in correlation with PD; the gene for the protein α-synuclein was indicated in familial ADPD. All that was known was that something was causing the death of dopaminergic neurons in the substantia nigra.

Epidemiology

PD affects approximately one million people in the United States, with 50,000 new casing being diagnosed this year (2). The disorder can affect both sexes, but it seems to be more prevalent in men. Typically, onset of the disease does not set in until after the age of 60 (2). In Genetic forms of PD, symptoms may appear before the age of 40 (2).

Pathology of PD

Researchers still do not know the underlying causes of Parkinson's disease. It is evident, however, that dopaminergic cells within the substantia nigra are dying at an accelerated rate. With the loss of these cells, dopamine levels decrease and muscular function becomes greatly impared (1). The causes of dopaminergic cell death are still unknown. Scientists have observed however, that about 240,000 cells die in the nigral region before symptoms of PD become noticeable. Normally, humans lose about 2400 nigral cells a year, but in PD this cell loss is intensified. (2). Motor functions controlled by the basal ganglia suggest how these symptoms emerge. (3)

The basal ganglia is a large, functionally diverse set of nuclear structures deep within the cerebral hemispheres of the brain. Its major component's are the caudate, putamen, and globus pallidus, including the substantia nigra and subthalamic nucleus. Normally, in the basal ganglia, the active inhibitory neurons of caudate and putamen (or corpus striatum) project to tonically active inhibitory neurons in the globus pallidus and the substantia nigra pars reticula, which project to the thalamus complex which generates the transiently excitatory input to the upper motor neurons. Therefore, the basal ganglia contributes to the initiation of the performance of voluntary movement. If, however, the nigral (dopaminergic) cells are damaged, the input of the substantia nigra decrease, stressing the inhibition of corpus striatum, which will make the inhibition of globus pallidus more tonic. Consequently, the ability of the thalamus complex to excite the motor cortex will weaken, causing the symptoms of PD. (3).

Studies suggest that gene mutations, either genetic or environmental in nature cause the onset of PD (4). We will largely discuss how genetics factors are involved in pathology of the PD, specifically looking at the genes that code for the proteins α-synuclein and parkin.

What Is It About α-Synuclein?

The neuronal protein α-synuclein has been a major focus of PD research. Structurally, α-synuclein has very little conformational shape past secondary structure (5). Although it is regarded as a presynaptic protein with a potential role in neuronal plasticity, little is understood of its actual normal function (5). What scientists do know is that α-synuclein aggregates in the brains of PD patients, and that in some cases, α-synuclein-rich inclusions known as Lewy bodies form in the nuclei of dopaminergic neurons. Because PD involves the selective death of such neurons, scientists speculate that α-synuclein is

directly involved in the pathogenesis of PD. In the past, the mechanisms of how α-synuclein was related to PD, and why the protein abnormally aggregated remained a mystery. All of this changed with the discovery of a genetic link in PD.

ADPD – The Breakthrough

In 1997 scientists made a landmark discovery, showing that not only was PD heritable in an autosomally dominant form, but that missence mutations in the gene encoding α-synuclein produced two distinct mutant forms of the protein, A30P and A53T (6). These families showed abnormally high levels of aggregate α-synuclein in the brain in addition to the presence of Lewy bodies. With this knowledge, scientists were able to conclude that the accumulation of mutant α-synuclein was directly related to the onset of PD symptoms (6). From the basis of this research, scientists have begun to characterize these two mutant forms of α-synuclein, A30P and A53T.

Recently, studies utilizing immunohistochemistry suggest that α-synuclein is concentrated in the cytoplasmic matrix surrounding synaptic vesicles in presynaptic terminals, supporting its definition as a presynaptic protein (7). Specifically, it has also been shown that α-synuclein binds small, acidic phospholipid synaptic vesicles within the synaptic terminal (7). Given α-synuclein's lack of structural conformation, binding these synaptic vesicles might provide the additional stabilization needed for proper protein function (7). Indeed, in researching the pathology of A30P and A53T, it was discovered that A30P and A53T mutant α-synuclein do not bind to synaptic vesicles, and instead aggregate within the neuronal nuclei (8). This indicates that normal (wild-type) α-synuclein is tightly associated with vesicle membranes, while mutant forms directly involve membrane dissociation, misfolding, and aggregation (9).

As with α-synuclein, the gene coding for ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) also appears mutated in some cases of ADPD. UCH-L1 is involved in the routine tagging of proteins, like α-synuclein, that require degradation (10). Failure to properly degrade these proteins leads to their subsequent accumulation, as we see with A30P and A53T. Based on this analysis, scientists believe that in ADPD, mutations in either α-synuclein and/or UCH-L1 are preventing the proper tagging and disposal of the aggregating proteins (10). Based on this knowledge, many scientists hypothesize that Lewy bodies, instead of being pathological, may be neuro-protective, enclosing aggregate fibrillous proteins preventing them from harming the cell (10). Additional research is needed to prove this link.

Of Flies and Mice

To better understand the pathology of α-synuclein, scientists have turned to fly and mouse models as a way of manipulating the protein and observing its function. Initially, scientists were interested in discovering if normal, wild-type (w-t) human parkin was also prone to excessive aggregation. In order to test this, scientists expressed w-t human α-synuclein in transgenic mouse models, discovering that the mice showed excessive aggregation of w-t α-synuclein in neuronal inclusions resembling Lewy bodies in the substancia nigra (11). This suggested that w-t human α-synuclein was also prone to aggregation. The transgenic mice also showed characteristic deficits in motor performance as seen in human PD (11). The scientists also noted a loss of TH-positive nerve terminals within transgenic mouse brains, indicating that α-synuclein accumulation may lead to the injury of nerve terminals and synapses (11). This was the first discovery *in vivo* that α-synuclein may be directly linked to cellular damage.

In another study, scientists removed the α-synuclein gene from a group of mice, thus effectively preventing the expression of the gene, and the production of α-synuclein (12). These "knockout" mice, varying in age, showed no problems in dopamine release, uptake, or neuronal development over time (12). It was shown, however that the knockout mice did suffer from numerous neurochemical, electrophysical, and behavioral deficits, leading to the conclusion that α-synuclein is essential to activity-dependent negative regulation of dopamine neurotransmission (13). Scientists now understand that α-synuclein is directly involved in the regulation of dopamine.

The transgenic fly model also provides scientists with a look into the pathology of PD. *Drosphila* flies, expressing mutant and wild-type forms of α-synuclein showed a loss of dopaminergic neurons in the brain, not to mention decreased locomotor function over time (14). After 30 days, transgenic flies lost the ability to fly and climb, indicating a loss of dopamine transmission (14). Indeed, in 60 day old flies, nearly 90% of all dopaminergic

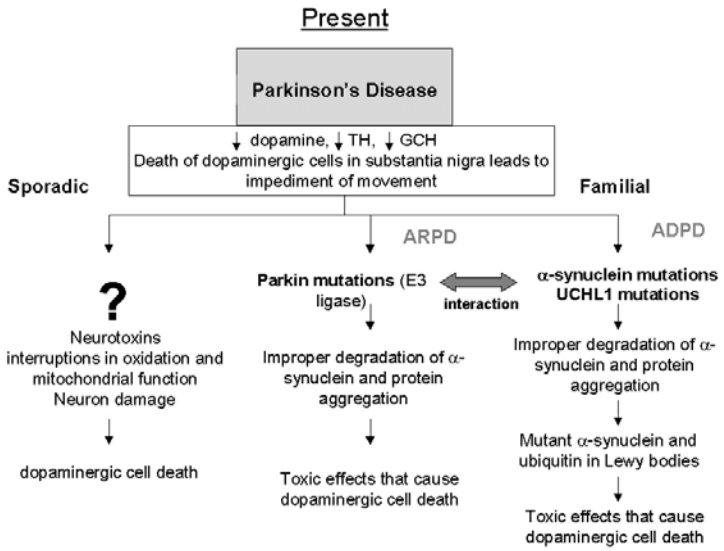

Figure 2: **And Now We Know…** Advances over the past five years have pinpointed more genes involved in familial PD. The proteins linked with these genes, parkin, α-synuclein, and ubiquitin, are connected by a cellular ubiquitination pathway responsible for α -synuclein degradation. However, the causes for sporadic PD remain a mystery.

neurons had died (14). These models provide researchers with a look at the pathology of PD in vivo, with the ability to manipulate the components genetically.

Test-Tube Protein

In vitro studies of α-synuclein have given scientists a look into the causes of protein aggregation. In one study, wild-type, A30P, and A53T forms of α-synuclein were compared over time and different concentrations in vitro to determine any differences in protein aggregation. Both wild-type and A30P α-synuclein aggregated relatively slowly, while A53T aggregated at an exceptional rate (15). Scientists attribute this difference to slight conformational differences between A53T and w-t / A30P that enable A53T to form protein fibrils faster. α-synuclein aggregation was also found to be time dependent, with a steady rise in aggregate concentration over a 48 hour period (16). Time for threshold aggregation in vitro differed among the different types of α-synuclein, with w-t showing full aggregation at 280 hours, A30P at 180 hours, and A53T at 100 hours (17). This data seems to suggest that while A53T is a more pathogenic species of α-synuclein due to the speed at which the protein accumulates.

Another study attempted to discover the reason that α-synuclein aggregation was time dependent. By seeding in vitro assays of w-t, A30P, and A53T with aggregate α-synuclein fibrils, researchers have been able to induce the aggregation of α-synuclein (18, 20). This fibril "seed" speeds up the aggregation process, explaining the time dependent nature of fibril formation. Also of interest, scientists discovered that mutant A53T seeds succeeded in aggregating w-t α-synuclein, suggesting that the two are similar in conformation, and that both w-t and mutant forms of α-synuclein contribute to the pathology of PD (18, 20).

Anti-apoptosis α-synuclein?

Finally, one study has suggested a direct functional relationship between α-synuclein and anti-apoptotic function. It was found that wild-type α-synuclein exerts a negative control of caspase activity (anti-apoptosis) on neurons, and that mutant α-synuclein form A53T has directly lost this function (19). This is supported in the behavior of the mutant A53T, which interacts with normal neuronal membranes does not interact with those expressing an apoptotic phenotype (19). The implications for such findings are astounding, as the discovery of the true cause of neuronal death will fill the current gap in knowledge and bring scientists one step closer to a cure.

Parkin: ARPD Zone

Before 1998 it was unclear how and if a protein (now known as parkin) was linked to the autosomal recessive PD,(21). Scientists became

concerned when they observed a DNA sequence encoding a protein of 465 amino acids, which seemed fairly similar to unbiquitin at the amino terminus and a RING-finger motif at the carboxy terminus. Normally, the protein contains 12 exons. (22). However, scientists discovered that about five of the exons are missing in PD patients. Also, they observed that normally, the size of the transcript sequence of the protein is 4.5 kb, while shorter transcripts are observed in the PD brains. These abnormalities are attributed to a mutation in this gene. Therefore, the scientists identified parkin to be responsible for the pathogenesis of PD. Moreover, this gene is described to be liked to an early onset of the disease. (22).

In another scientific paper, scientists show how parkin interacts with other proteins in the brain, showing that parkin binds to the E2 ubiquitin-conjugating enzyme 8 (UbcH8; later discovered to actually bind to UbcH7) throught C-terminal ring-finger (23). Also, it is shown that parkin has a ubiquitin-protein ligase activity in the presence of UbcH8. Normally, it is detected that Parkin interacts with CDCrel-1 (a synaptic protein) in its ring-finger domain.(23)

Ubiquitination is characterized by activation of ubiquitin by E1, ubiquitin-activating protein, following by the transfer of ubiquitin to an E2 ubiquitin conjugating enzyme, which works with a ubiquitin-protein ligase, E3 to ubiquitinate substrate proteins. parkin seems to resemble this E3 protein in a way that it has an E2 binding site (ring-finger domain) and substrate binding site (N-terminus) (23). This suggests that parkin is involved in ubiquitination activity. Furthermore, a mutation (observed in ARPD) detected in the ring-finger domain, prevents UbcH8 from binding to parkin, disrupting the ubiquitin-protein ligase function, which interferes with the ubiquitination of CDCrel-1.

In yet another study, scientists study parkin substrates as possible disease mechanisms. As mentioned earlier, Parkin increases the degradation of its substrates in the healthy brains. In this study, toxic accumulation of the protein α-synuclein was observed in the ARPD brains, where Parkin is mutated. This means that normally, α-synuclein must bind to parkin, which will tag it for degradation to avoid the harmful accumulation of α-synuclein. If there is a mutation in the ubiquitin carboxyl-terminal (UCHL-1), parkin will not polyubiquitinate α-synuclein, leading to harmful accumulation. (24).

Furthermore, Parkin and Ubiquitin, contribute to the formation of α-synuclein-rich Lewy bodies. Evidence of polyubiquitinated α-synuclein in Lewy bodies, suggests the parkin contribution to Lewy body formation, because parkin functions as a ubiquitin-ligase, resulting in poluubiquitination of α-synuclein. (24).

Searching for Alternatives to Drug Use

Doctors have long been searching for an alternative to drug therapy. Most current drugs work to replace the dopamine lost in the PD brain. These oral drugs introduce L-dopa, a precursor to dopamine, into the body and enable the production of dopamine. Although it is currently the most prevalent treatment, dopamine drug therapy creates side effects, such as dyskinesias, nausea, and irregular blood pressure, and goes through “on” and “off” periods of effectiveness. L-dopa treatments become less effective and side effects worsen with prolonged use. Improved versions of L-dopa drugs, such as pramipexole and ropinerole, have been developed in the last five years. However, drug therapies are still a temporary solution to PD and cause problems when used for long periods of time (25).

A good alternative to drug therapy has been found in gene therapy. Instead of introducing L-dopa into the body through drugs, researchers have sought to introduce genes that can aid the brain’s dopamine production. Many trials have involved the gene for tyrosine hydroxylase (TH). The TH enzyme is the limiting factor for L-dopa production in dopaminergic brain cells. Once the TH gene was isolated, the problem became targeting gene insertion into the dopaminergic brain cells involved in PD. Recently, the possibility of using a virus as a vector for the gene has come about. It is possible to insert genes into viral genetic material. If the virus can be targeted to the affected area of the body, then the viruses will invade cells there and introduce the gene into those cells for expression. A contemporary study has examined the possibility of using viral vectors more closely (26).

The study, published in 1999, worked with 6-OHDA Parkinson’s model rats that showed behavioral indications of >90% depletion of striatal dopamine. Successful introduction of the TH gene carried by a vector virus (AdPGK·tet·hTH1) led to the expression of TH enzyme in brain cells. The increased levels of TH caused more dopamine production, which led to behavioral improvements in the rats. It was found that the gene expression could be controlled through oral treatments with doxycycline (dox), which blocked TH gene expression. This study lent credibility to viruses

as transport vectors for genes, and pointed towards possible future treatments for many different types of diseases, especially PD. However, the behavioral improvements in the rats wore off rather quickly (after six weeks). To be effective, the process would have to be refined.

In 2002, another study was carried out using viruses as gene vectors (27). In this experiment, two virus vectors were used, one carrying the TH gene and one carrying the GTP-cyclohydrolase-1 (GCH1) gene (rAAV-CBA-TH and rAAV-CBA-GCH1). GCH1 is the limiting factor in production of tetrahydrobiopterin, a compound that has been shown to influence dopamine synthesis as a cofactor with TH. By introducing both genes into the brain, researchers hoped to obtain higher levels of dopamine synthesis. The study worked, and the 6-OHDA rats in this study showed marked behavioral improvements for up to twelve weeks, nearly double that of the 1999 study (26).

Solutions in Stem Cells?

Both drug and gene therapies have their drawbacks and limitations. Some doctors have been exploring the possibility of transplanting new dopaminergic cells into the brain. At first, fetal tissue transplants were examined. However, sources of fetal tissue are highly restricted as well as morally questionable. More doctors have now turned towards stem cells. When trails were first carried out, it was found that embryonic stem (ES) cells implanted into the striatum would often develop into teratoma-like tumors. Scientists continued to work on refining ES cell transplant procedures however, and one experiment worked by injecting low concentrations of ES cells into the brain with the idea that fewer cells would lower the chance of tumor formation (28).

The 2002 study worked with 6-OHDA Parkinson's model rats exhibiting behavior characteristic of >97% depletion of striatal dopamine. The rat brains were examined, and it was found that the ES stem cells had developed into midbrain-like dopaminergic cells. This was indicated by the presence of cells that were colabeled for dopaminergic key proteins, such as TH, DAT, and AADC. Dopaminergic neurons were labeled by the M6 mouse-specific antibody, proving that they were derived from implanted mouse ES cells. Before death, the rats were observed for rotational behavior, and as the weeks progressed, the rats had shown marked improvement. In this case, transplants of stem cells were more successful than past attempts. Over half of the rats that underwent the stem cell transplants survived and showed marked improvement in their condition (14 out of 25 rats). Only five developed deadly brain tumors associated with stem cell transplants, and six showed no improvement because stem cell transplants did not survive. Stem cell transplants have good possibilities for the treatment of PD patients. However, they too have their drawbacks. Twenty percent of the ES cell transplants in this study were lethal. Obviously, the procedure needs more refining before human use.

How About Some Prevention?

Drug and gene therapies are often used to treat decreased dopamine levels. Some scientists, however, have been developing techniques to treat the causes of dopaminergic cell death. In 2002, a group of undertook a study that attempted to treat the expression of mutant forms of α-synuclein, one of the causes for autosomal dominant familial parkinsonism (ADPD) (29). Using a *Drosophila* fly model of Parkinson's induced by the expression of mutant forms of α-synuclein (A30P and A53T α-synuclein), the scientists investigated using chaperone proteins to correct mutant α-synuclein forms. They found that compromising the chaperone function of protein Hsp70 increased α-synuclein-induced death of dopaminergic neurons, while increasing the activity of Hsp70 reduced the α-synuclein toxicity. Augmented Hsp70 chaperone action decreased the death rate of dopaminergic neurons. This was a very exciting finding, as Hsp70 augmentation therapies could provide valuable preventative treatments for those suffering from PD.

Parkinsonism As It Stands Today

Today, causes for the two familial forms of PD have been pinpointed and have been found to be connected. Parkin, the cause of ARPD, is an E3 ligase that is responsible for ubiquitinating α-synuclein, while α-synuclein and ubiquitin are two proteins that interact with it. Mutations in any of these proteins will interrupt the protein degradation process and cause aggregation of α-synuclein, which, in turn, leads to dopaminergic cell death.

Sporadic PD, conversely, has defied explanation, but has spawned many hypothetical causes. On the top of the list of suspects are environmental toxins, which can come from pesticides or chemicals we use in daily life (30,31). Also, brain damage is thought to be a cause for neurodegeneration, an example being the many cases of neurodegenerative diseases seen in boxers. Although these theories are

plausible, they are hard to put to the test. The causes behind sporadic PD are thought to be complex and multifaceted. Further research may find preventative drugs to slow the process of PD (32).

Currently, research is directed towards gene therapies and cell transplants. Most of the gene therapies developed thus far involve introducing TH and GCH genes into the brain, thereby increasing natural dopamine production. These therapies have proved mildly successful in treating rat models of Parkinson's. Cell transplants using of ES cells, although promising, have yet to become safe and effective. In many cases, ES cell transplants do not work, or they result in lethal brain tumors. The procedure is under refinement, but it will undoubtedly be many years before it is available as a treatment for PD patients.

An innovative field in the search for PD treatments involves proteins. Scientists have researched correcting the mutant proteins that cause the disease. Some researchers have had success correcting mutant α-synuclein in fruit flies using chaperone proteins (29). However, this field is relatively new and unexplored. Future research might be directed towards gene therapies for mutant parkin, ubiquitin, and α-synuclein in the brain, thus creating a healthy protein degradation process and halting the progression of PD. Also, preventative gene treatments for those with a family history of Parkinson's could be explored, but this is far down the line from current capabilities.

Drug treatments for Parkinson's patients are under refinement but remain only a temporary alleviation of a devastating progressive illness. Dopamine therapies, though constantly under improvement, still remain effective for limited periods of time, after which they produce debilitating side effects. Drugs are increasingly losing ground in the search for a cure for PD. However, drugs may be the only hope of those who develop sporadic forms of the disease.

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