Genetic Testing in Parkinson Disease

Promises and Pitfalls

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Parkinson disease (PD) is the second most common neurodegenerative disease. The recent discoveries of a number of disease-causing genes (such as α-synuclein, parkin, UCHL1, PINK1, DJ-1, LRRK2) in PD have generated considerable interest and debate for both physicians and patients regarding diagnostic and presymptomatic genetic testing of PD in the clinic. Of particular significance are reports of a common G2019S mutation of the LRRK2 gene as a cause of familial and sporadic PD across different populations worldwide. This is the first time a common gene mutation has been reported in such an extensive manner across races. However, the feasibility of diagnostic and predictive testing of PD is still beset with many unanswered questions. We discuss the promises and limitations of genetic testing in PD and suggest that many more scientific studies are required before any meaningful guidelines and recommendations for genetic testing in PD can be formulated and broadly implemented.

Parkinson disease (PD) is a progressive neurodegenerative disease characterized by a loss of dopaminergic cells in the substantia nigra pars compacta and the presence of Lewy bodies. It is the second most common neurodegenerative disease and its incidence increases with age. With quickly aging populations in many countries, the medical and social consequences of PD will invariably have a significant impact globally. The recent discoveries of disease-causing genes in PD have generated considerable interest and debate for both physicians and patients regarding routine genetic testing of PD in the clinic. Genetic testing refers to the evaluation of an individual's genetic material (eg, DNA) to determine the predisposition to a specific disease or to confirm the diagnosis of genetic disease. Molecular genetics provide a powerful tool in the diagnosis of many neurological diseases. Genetic testing of mutations in disease-causing genes can help better define and classify many of the heterogeneous inherited neurodegenerative disorders. It not only allows early confirmation of diagnosis and appropriate institution of genetic counseling, but it may provide genotype-phenotype correlation, help select specific patients for clinical drug trials, and ultimately provide a better understanding of the pathogenesis and long-term clinical outcome of the disease. Here we discuss the implications and limitations of genetic testing in PD in the context of the recent genetic discoveries.

AGE-OLD DEBATE ON THE GENE-ENVIRONMENT ETIOLOGY

The relationship between genes and environment in the pathogenesis of PD has been debated for many decades. However, a major discovery in the early 1980s of MPTP (methyl-4-phenyl-1,2,3,6-tetrahydropyridine)–induced parkinsonism in intravenous drug users led to numerous epidemiologic studies evaluating potential environmental causative agents. Epidemiologic studies have identified protective associations (eg, smoking, caffeine) as well as adverse risk factors (eg, pesticide exposure) for PD. Biochemical models of selective nigrostriatal damage (such as neurotoxicity induced by
MPTP or rotenone) highlight the relative importance of environmental agents in PD pathogenesis. In this light, variability of genes involved in the development and maintenance of dopaminergic neurons, xenobiotism, oxidative stress, and cell death are potential candidates in studies of gene-environmental interaction. 

### POPULATION GENETIC STUDIES IN SPORADIC PD

Besides the intensive linkage study of familial PD, population studies of genetic variants of candidate genes by association analysis have also been attempted, but the results are far from conclusive. Because of various methodological inadequacies, findings from genetic association studies generally have little clinical relevance.

### DISCOVERY OF DISEASE-CAUSING GENES IN FAMILIAL PD

In the late 1990s, 2 major discoveries revolutionized the field of genetics in PD. In 1997, Polymeropoulos et al. discovered a missense mutation A53T in the α-synuclein gene in a family with an autosomal-dominant mode of inheritance for parkinsonism. This mutation was subsequently found in other families; all were of Greek and Southern Italian ancestry for parkinsonism. This mutation was subsequently covered in a family with an autosomal-dominant mode of inheritance. In 1998, Kitada et al. identified homozygous deletions of exon 4 or exons 7 of the parkin gene in 4 Japanese families with autosomal-recessive parkinsonism. More recently, mutations in the ubiquitin carboxy-terminal hydrolase L1 (UCHL-1) (PARK3), PTEN-induced putative kinase 1 (PINK1) (PARK6), DJ-1 (PARK7), leucine rich repeat kinase 2 (LRRK2) (PARK8), NURR1, synphilin, and serine protease 25 (PRSS25) genes have also been described in PD (see text). Refer to the Online Mendelian Inheritance in Man Web site for updates on new PD genes (http://www.ncbi.nlm.nih.gov/entrez/query).

### GENETIC TESTING IN PD

Although it is exciting that the number of disease-causing genes in PD have expanded in recent years, the feasibility of routine genetic testing of PD in the clinic is still fraught with many unanswered questions. Ideally, before one can contemplate introducing such a service, a number of questions need to be addressed. Do we know the causative gene? Do we know the frequency of disease-causing mutations? Are we able to prioritize patients for genetic testing based on suggestive clinical features? What is the sensitivity and specificity of the genetic test? How reliable is the laboratory carrying out the test? What are the problems associated with ambiguous results? Should testing be available for both symptomatic and presymptomatic subjects? Would testing be better conducted in a research or clinical setting? Is there a multidisciplinary team available with the experience and expertise to handle pretesting- and posttesting-related problems? Is current scientific information sufficient enough for us to advise the subjects on prognosis and to properly counsel the patients? Will the knowledge of genetic abnormality change the patient’s and physician’s attitudes toward the illness, and will it change the management of the disease? Unfortunately, there are no immediate or easy answers to these questions.

### HOW COMMON ARE DISEASE-CAUSING MUTATIONS?

Currently identified gene mutations collectively account for less than 10% of all cases of PD. α-synuclein missense mutations are very rare and limited to select races and familial cases (Table). Similarly, duplication and trip-
Modification of the gene is uncommon and thus far only reported in a handful of families.20

Initial studies suggest that the frequency of parkin mutations in Europe is about 49% in families with early-onset parkinsonism with autosomal-recessive inheritance and about 18% of patients with sporadic PD with age at onset less than 45 years of age.21 Later studies revealed that the prevalence of parkin mutations in young-onset sporadic PD in most populations probably ranges from 5% to 15%. The wide range may reflect actual ethnic difference or methodological differences. In patients with older-onset PD, the prevalence is lower, around 1% to 2%, although data in this group of patients are still limited.17 These figures have to be interpreted with caution because of the technical complexity of screening techniques and the lack of clarity regarding the pathogenicity of some parkin mutations/variants. For example, while most documented cases are compound heterozygotes, the phenotype of a single mutation and the penetrance of compound heterozygous mutations have not yet been fully characterized.22 Extensive gene dosage studies were conducted in some studies while others merely reported direct sequence analysis findings, making it difficult to directly compare the data across some populations.

Mutations in the PINK1 gene have been shown to underlie PARK6-linked PD. An estimated 1% to 7% prevalence of PINK1 mutations has been demonstrated among early-onset PD or autosomal-recessive PD in white patients,23-25 similar to the 2% to 9% frequency in Asia.26-28 Based on current published data, PINK1 may be the second most common cause of autosomal-recessive PD and early-onset PD after parkin. DJ-1 (PARK7) mutations are associated with early-onset PD and autosomal-recessive PD. However, the estimated prevalence of 1% to 2% suggests that DJ-1 mutations are generally rare.30,31

The most recent discovery of LRRK2 (PARK8) as a causative gene in autosomal-dominant PD is another major development as the clinical phenotype (such as age at onset) resembles typical sporadic PD, even though the pathology of brains of patients with mutations in the LRRK2 gene is extremely variable.12 Furthermore, a common mutation, the G2019S mutation (which encodes the 6055G > A transition), has been identified and accounts for 5% to 6% of healthy controls, the mutation is located in an important functional domain of the encoded protein, and lastly specific mutation of the gene detected, the mutation unambiguously segregates with the disease in family studies, the mutation could not be identified in a large group of healthy controls, the mutation is located in an important functional domain of the encoded protein, and lastly in both in vitro and in vivo proof that the mutation is associated with an increased cell death or dysregulated cellular function. The large number of different gene mutations and the current lack of evidence regarding the pathogenicity of some of these mutations invariably creates problems when it comes to interpreting results of the genetic test. Some of these concerns have recently been highlighted by Klein through illustrative clinical cases in this journal.

PROBLEMS WITH INTERPRETATION OF RESULTS

From the clinical standpoint, it is important to establish whether a particular genetic variant is responsible for the disease such that genetic testing would be meaningful. The pathogenicity of a genetic variant could be supported by several lines of evidence. The causative gene is identified through linkage analysis studies and specific mutation of the gene detected, the mutation unequivocally segregates with the disease in family studies, the mutation could not be identified in a large group of healthy controls, the mutation is located in an important functional domain of the encoded protein, and lastly there is both in vitro and in vivo proof that the mutation is associated with an increased cell death or dysregulated cellular function. The large number of different gene mutations and the current lack of evidence regarding the pathogenicity of some of these mutations invariably creates problems when it comes to interpreting results of the genetic test. Some of these concerns have recently been highlighted by Klein through illustrative clinical cases in this journal.

HETEROZYGOUS MUTATIONS

Haploinsufficiency of the α-synuclein gene has been demonstrated with the ratio of expression of the wild type to mutant alleles correlating with the severity of the clinical phenotype, suggesting that haploinsufficiency of
α-synuclein mutations may contribute to disease progression in familial PD. On the other hand, triplicate and duplicate gene copies of the gene have been found to cosegregate in some familial PD.

However, the significance of a single heterozygous mutation is still being debated for genes associated with recessive parkinsonism. It has been thought that parkin-linked PD is recessively inherited where a deleterious alteration is presumed on both alleles and heterozygous carriers are unaffected. However, in some studies, a significant number of parkin mutation carriers possessed only a single heterozygous mutation. Decreased mean fluorodopa uptake in the striatum has been demonstrated in asymptomatic carriers of a single parkin mutation with an apparently normal allele. It can be argued that patients who had a loss of 1 parkin allele may suffer from haploinsufficiency as a consequence of a reduced expression and enzymatic activity, resulting in a greater risk for disease. Similarly, a single heterozygous PINK1 mutation has been described in PINK1 mutation carriers. Some of these mutations occur in a highly conserved amino acid position in the encoded protein kinase domain and are absent in a control population, and thus it is possible that these mutations are “risk alleles.” In 18-fluorodopa positron emission tomography studies, a 20% to 30% reduction in the caudate and putamen in asymptomatic PARK6-linked carriers has been demonstrated.

The problem with interpreting a single heterozygous mutation is compounded by the fact that one cannot be absolutely sure whether another causative mutation is present but remains undetected. The observation that gene dosage studies were not conducted in a number of parkin screening studies further limits proper interpretation of the result. Some presumably pathogenic heterozygous parkin mutations (eg, R275W) have been detected in elderly asymptomatic individuals.

**VARIED EXPRESSION OF MUTATIONS**

Some gene mutations may exhibit varied expression, making genetic counseling difficult. For instance, the parkin 438-477 del mutant is associated with both recessive and dominant patterns of inheritance in 2 different families.

**COMMON MUTATIONS AND VARIED PENETRANCE**

The identification of a common LRRK2 (G2019S) mutation across populations in patients with both familial and sporadic PD raises hope that finally a genetic screen can be made available for diagnostic purpose. Although the prevalence of 5% to 6% for familial and 1% to 2% for apparently sporadic cases of PD have been quoted, the number can vary from 0% to 40% depending on specific ethnic populations. Some LRRK2 mutations may have high prevalence in select populations with the R1441C accounting for about 3% of sporadic PD among Spanish patients.

However, the varied penetrance could pose problems for genetic counseling. For example, a woman with a compound heterozygous parkin mutation (T240M and EX 5_6 del) was free of any neurological symptoms at age 52 years whereas all other affected members of the family had onset of symptoms before 38 years of age. Zimprich et al demonstrated that the penetrance of G2019S-associated disease was highly age dependent, increasing in a close-to-linear fashion from 17% at age 50 years to 85% at age 70 years. Interestingly, a neurologically healthy octogenarian with the LRRK2 G2019S mutation has been reported, suggesting that other environmental and epigenetic factors may be involved in some mutation carriers. As a further illustration, the R1441C mutation was first described in 2 autosomal-dominant PD families. The mean age of symptoms in 1 family was 65 years and affected individuals reported typical PD symptoms. Two of the 24 unaffected individuals were mutation carriers older than 60 years old. In another family, the mean age at onset was 56 years with similar clinical presentations. The high penetrance of the R1441C contrasted with the second report of R1441C mutation by Zabetian et al, who described a patient with sporadic PD with onset at age 61 years. The 9 siblings of the patient were older than 61 years and remained asymptomatic. More recently, the R1441C mutation has also been described in a patient of Chinese ethnicity with sporadic PD. Hence, the penetrance of R1441C can be highly variable and complicates genetic counseling. Mutational hot spots have also been described for parkin and PINK1, but their relative rarity limits clinical utility.

**PATHOGENIC/PUTATIVE MUTATION OR COMMON POLYMORPHIC VARIANT**

A number of mutations (eg, in LRRK2) have not been demonstrated to segregate with the disease, and in most cases, they have been reported in only 1 proband. The pathogenicity of such putative mutations should be interpreted with caution until more evidence becomes available. Further studies are needed to determine the cellular pathogenic mechanism of these LRRK2 mutations and the effects of gene-gene interactions on cell survival and death. Interestingly, a LRRK2 Gly2385Arg variant reported in a PD family from Taiwan was originally thought to be a pathogenic mutation. However, subsequently in a separate large case-control sample of Taiwanese Chinese individuals, this variant was shown to be a common polymorphism, which was significantly more frequent among patients with PD than controls.

**WHO SHOULD UNDERGO GENETIC TESTING?**

Genetic testing can be used for diagnostic purpose or for presymptomatic testing. The diagnosis of PD is frequently made on clinical grounds, sometimes supported by traditional imaging (to exclude secondary causes) and by functional imaging studies (eg, fluorodopa positron emission tomography). In expert hands, the diagnostic accuracy is thus very high. Hence, it is contentious whether a genetic test for PD (beset with many limitations as discussed here) really contributes to the clinical care of patients. However, we are of the opinion that genetic testing (for parkin, PINK1, and DJ-1) may have a role in patients who have a family history of PD or those with very young age at onset. In such scenarios, the patients may request genetic confirmation to
assist them in future career planning and for other reasons. In addition, the recent discovery of the common LRRK2 G2019S mutation provides an opportunity for testing in families with autosomal-dominant pattern inheritance. Testing of this mutation in sporadic late-onset PD may be useful in populations with high carrier status.

Presymptomatic testing for PD in the general population is not warranted because current knowledge of some of the mutations is still limited. Such testing, when done with the appropriate genetic counseling, may be useful where a family member has already been diagnosed to carry a known mutation.

In a recent survey of 111 patients with PD in Germany, about half apparently had some knowledge about presymptomatic and prenatal testing, and about 70% would take a presymptomatic test if the test were available.60 Partnership and family were the most important reasons given for taking the test. These attitudes were largely independent of socio-demographic and disease variables. Whether responses to such hypothetical questions truly reflect actual uptake of genetic testing in the clinic remains to be clarified.

It is also worth noting that transcultural issues may confound sensitivity of testing. For instance, in some cultures, a positive family history may not be always volunteered, and for those who see it as a curse on the family, false information may be given. In this light, genetic testing should be conducted in a manner that is respectful of the religious and cultural perspectives and traditions of individuals. A de novo mutation may be associated with disease with apparent negative history. Other factors, such as early parental deaths, adoption, and nonpaternity, should also be considered when selecting subjects for testing.

**SHOULD TESTING BE DONE IN A RESEARCH OR CLINICAL SETTING?**

In clinical testing, an individual chooses to undergo genetic testing to know the outcome, whereas in a research setting, the individual may choose not to know the result of the test or the research protocol might specify that individual’s test result not be divulged. Because of potential problems in interpreting the results, some would argue that blood testing for PD genes be carried out preferentially under a research setting. If carried out under a clinical setting, the laboratory carrying out the test should preferably be accredited by the relevant authorities. A multidisciplinary team comprising a neurologist, a genetic counselor, a psychologist, nurse clinicians, and other support paramedical staff should be available before genetic testing can be undertaken. Interpretation of the tests should preferably be conducted by professionals who are appropriately qualified or have sufficient experience. Specifically, the limitations of testing should be clearly explained to the patients, including the potential for future denial of employment opportunities or medical, disability, or life insurance. A negative test may be uninformative, and not all abnormal results are diagnostic or useful. Based on current information, an abnormal test cannot accurately provide prognosis (such as rate of progression) in affected individuals nor exact age at onset of symptoms in asymptomatic subjects. Because DNA testing may have serious implications not only for the patient, but also for other family members, it should be performed only after very careful consideration of all potential factors. Because of the mostly adult-onset symptoms and the absence of preventive, neuroprotective, or disease-modifying strategies, skilled genetic counseling is essential for addressing the ethical, social, legal, and psychological issues associated with genetic testing. Psychological problems with predictive testing should be anticipated and properly managed. The lessons and experience with genetic testing in Huntington disease provide a useful framework and stepping stone for testing in PD.61-63 These include confirmatory testing, predictive testing, asymptomatic testing for children, confidentiality, insurability, finances, employment, disability, and marriage.64 However, the technical and scientific issues with testing in PD are more complicated than in Huntington disease, and hence a common set of guidelines is not feasible. Prenatal testing or testing in asymptomatic children should be strongly discouraged. Cost-effect analysis is needed because screening may involve genes like parkin where more than 100 different mutations have been described.

**CONCLUSIONS**

There is a remarkable convergence of clinical, biochemical, and genetic data that have unraveled a number of possible pathogenic mechanisms underlying PD. The discovery of new genes adds vital information to the complicated molecular puzzle and pathways involving gene and environmental interactions. Because genetic testing in PD is likely to be increasingly available to the public via various commercial channels, the onus is on the medical community to institute programs with appropriate safeguards to properly guide and educate our patients and family members. The advertising of direct genetic tests to the public without proper education should be discouraged. The clinical utility of genetic testing would require cautious evaluation of the potential risks and benefits of testing and the availability of interventional measures to address the risks in susceptible individuals. Large-scale collaborative studies are needed to investigate genotype-phenotype correlation, long-term susceptibility outcome, and clinical therapeutic potential of novel drugs in at-risk individuals. The field of genetics in PD will continue to evolve, but many more scientific studies are required before any guidelines and recommendations for genetic testing in PD can be formulated and applied.

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