Genetics of familial Parkinson’s disease

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ABSTRACT: Parkinson’s disease (PD) is a complex, heterogenous neurodegenerative disorder. In the last century, the genetic contribution to the risk of PD was controversial. Recently, the identification of mutations in α-synuclein (SNCA), parkin (PARK2), ubiquitin C-terminal hydrolase L1 (UCHL1), PTEN-induced kinase 1 (PINK1), DJ-1, nuclear receptor-related 1 (Nurr1) and leucine-rich repeat kinase 2 (LRRK2) genes has confirmed the role of genetics in familial forms of PD. These genetic findings have greatly advanced our knowledge on the pathophysiological background of familial PD. In addition to these genes, several other loci have been implicated in PD, although the responsible genes have not been identified, yet. Possible interactions between various already known or new candidate genes and the influence of environmental agents on the expression of PD-linked genes are in the scrutiny of recent genetic studies.

Key Words: Familial Parkinson’s disease, α-synuclein, Parkinson’s disease genetics.

INTRODUCTION

Parkinson’s disease (PD) is a common neurodegenerative disorder affecting approximately 1% of the population over 60 years of age¹. PD is associated with progressive loss of the dopaminergic neurons of the substantia nigra (SN). The presence of Lewy bodies in some remaining nerve cells is necessary for a definite diagnosis. Clinically PD is characterized by resting tremor, bradykinesia, rigidity, postural instability and responsiveness to L-dopa therapy².

The molecular and cellular mechanisms underlying the specific loss of dopaminergic neurons is still unknown. Epidemiological studies have failed to resolve any single determinant as the cause of PD and suggest a complex etiology, with both environmental and genetic factors influencing the pathogenesis of the disease. Neurotoxins, pesticides, rural environment are some environmental factors that seem to contribute to the pathogenesis of PD. These toxins selectively destroy dopaminergic neurons in SN. On the other hand, the role of genetics in PD is outstanding as well. Although most of the PD cases are sporadic, a small proportion of them show Mendelian inheritance. In fact, 90-95% of PD patients have sporadic disease attributed to interactions between environmental conditions and the genetic constitution of each individual. However, approximately 5-10% of PD cases have a positive familial background and demonstrate an autosomal dominant or autosomal recessive inheritance. Recent progress in molecular genetic studies of families with PD has led to the identification of several genes and loci that are linked to certain inherited forms of PD. These include: α-synuclein (PARK1)³, parkin (PARK2)⁴, PARK3 locus⁵, UCH-L1 (PARK5)⁶, PINK1 (PARK6)⁷, DJ-1 (PARK7)⁸, LRRK2 (PARK8)⁹, PARK10 locus¹⁰, PARK11 locus¹¹, NR4A2¹² and NR4A2¹³ (Table 1). This review focuses on the genes and genetic loci that have been associated with familial cases of PD. The progress that has been made during the last years looks like a genetic bloom in the area of PD’s pathogenesis.

α-Synuclein (PARK1)

In 1997, α-synuclein was the first gene implicated in the genetic pathogenesis of Parkinson’s disease. A missense mutation A53T in exon 4 of the gene was identified in a large Italian-American family (Contursi
family) with autosomal dominant PD with a mean age of disease onset at around 55 years. The same mutation was also detected in a number of Greek families with PD and in two families of Greek ancestry living one in America (Family H) and the other in Australia. A second mutation (A30P) was later found in a small German family with PD and recently another mutation E46K was identified in a Spanish family. Pathological examination documented dopaminergic neuron degeneration with Lewy bodies in the SN of patients with these mutations. However, Lewy body pathology was more widespread than in sporadic cases. Duplications and triplications of a-synuclein gene have also been associated with familial cases of PD. In fact, a direct relationship between a-synuclein gene dosage, expression and the age of disease onset, progression and phenotypic severity has been observed. The genomic triplication of a-synuclein has been associated with an earlier disease onset and an evidently increased disease progression, later on characterised by weight loss, dementia and a limited lifespan. In contrast, patients with genomic duplication of a-synuclein had a milder clinical phenotype. Taking the above into consideration, increased expression of a-synuclein seems to reduce the age of PD onset and increase the disease severity.

In contrast to the previous hypothesis which links the overexpression and aggregation of a-synuclein to PD, the theory of haploinsufficiency has been also put in forward. According to it, a single copy of the wild-type gene is incapable of providing sufficient protein production as to assure normal function, leading to a disease state. In lymphoblastoid cell lines from family members carrying the A53T and A30P mutations, the mutant alleles were less or not at all expressed in most of the affected mutation carriers. Particularly, in severely affected patients, the reduction in the expression of the mutant allele of a-synuclein gene was obvious. Moreover, in some asymptomatic heterozygotes, mutant a-synuclein could not be detected, suggesting that lack of the protein seems not to be an immediate cause of PD. However, one affected member of the examined families did express the mutant allele, which argues against haploinsufficiency. Consequently, further studies are needed in order to confirm or dissociate the hypothesis of haploinsufficiency with PD progression and severity.

The a-synuclein gene, member of the synuclein family, has been mapped to human chromosome 4q21.3-q22, contains 7 exons, five of which are protein-coding (exons 2 to 6). The encoded gene product, a-synuclein is a 140 amino acid soluble protein expressed primarily in neural tissue and especially in presynaptic terminals. It is consisted of i) a highly conserved amino-terminal domain that includes a variable number of a repeated motif (KTKEGV) ii) a hydrophobic domain that has been described as a component of senile plaques in Alzheimer’s disease and iii) a carboxy-terminal domain with chaperone properties. A-Synuclein is implicated in the pathogenesis of PD either with its overexpressed wild type or with a mutated form. In its native state, alpha-synuclein is a soluble and unfolded protein. Due to its central hydrophobic region a-synuclein has an inclination to aggregate. It initially forms an intermediate structure called an oligomer or protofibril and in succession insoluble polymers or fibrils. These insoluble fibrils are the major component of Lewy bodies. The regulation of the levels of monomeric and/or oligomeric a-synuclein in neurons appears to be critical, and this regulation might be altered in the common PD forms due to mutations in the a-synuclein gene, increased a-synuclein expression or decreased clearance or both.

Parkin (PARK2)

One year after the discovery of a-synuclein mutations, mutations in another gene were identified in a Japanese family with autosomal recessive juvenile parkinsonism (ARJP). The gene was named parkin to reflect its connection with the disease. The early-onset of the disease, which is before the 40 years of age, and the absence of Lewy bodies were two first distinct characteristics of great interest. Additional clinical features include dystonia and diurnal fluctuations. Disease progression is slow, but levodopa-induced dyskinesia often occurs. Parkin mutations have been identified in patients of different ethnicity and have been considered as the major mutant factor for the 50% of familial ARJP. Until now, more than 100 different mutations have been identified. PD causing parkin mutations include deletions especially in exons 2-5, insertions, duplications, triplications and point mutations, the majority of them being in exons 6-12. Furthermore, the severity of the parkin
mutations, depending on their homozygous or heterozygous state, has also been associated with the PD age of onset. Heterozygous parkin mutations have also been referred to show incomplete penetrance and heterogeneous phenotype. On the other hand, arguments against the role of single heterozygous parkin mutations suggest that, this heterozygous, likely pathogenic state is a misleading and masked condition that has only to do with the current inability to detect a second DNA change in the regulatory, coding or non-coding gene regions. Moreover, heterozygous parkin mutations have also been detected in healthy controls, suggesting that these variants could be polymorphisms rather than disease-causing mutations.

Parkin is one of the largest genes in the genome mapped to human chromosome 6q25.2-27. It is consisted of 12 exons, spanning over 1.53 Mb of genomic DNA and encoding a 465 amino acid, highly conserved protein. Parkin protein is expressed mainly in the nervous system and has a role of E3 ubiquitin ligase, in the degradation system of cellular proteins called proteasome. The structure of this protein is consisted of different motifs: an amino-terminal Ubl domain (Ubiquitin like domain), followed by two RING (Really-Interesting-New-Gene) finger domains separated by an IBR (In-Between-Ring) domain. The amino-terminal Ubl domain catalyses the conjugation of activated ubiquitin to target proteins prior to their destruction via the proteasome and is prone to most of the parkin mutations. Mutations in parkin result in a loss of its E3 ligase function, failure of ubiquitination of the target proteins and therefore a toxic build-up of proteins that are no longer effectively degraded by the parkin-dependent ubiquitin/proteasome pathway. The formation of these toxic aggregates to neurons of the substantia nigra seems to have a crucial role to the pathogenesis of PD. However, current data show that ARJP with parkin mutations can not be solely attributed to the catalytic impairment of the E3 ligase activity of parkin. Even more, experimental evidence support that parkin is also implicated in the regulation of mitochondrial function. Oxidative stress and damage is increased in experimental models with null parkin mutations.

**PARK3**

Linkage studies in 6 families with autosomal dominantly inherited PD led to the identification of the PARK3 locus to a 10.6 cM region on chromosome 2p13. Affected patients in these families displayed typical signs of PD including an average age of disease onset at 59 years and the presence of Lewy bodies. All of the families linked to PARK3 are of German ancestry, and two of these families share a common haplotype mapped on chromosome 2, suggesting a possible founder effect. Nevertheless, the majority of the individuals carrying the risk haplotype were not affected, indicating a reduced penetrance of the disease-causing mutation. Many genes have been screened in the candidate region but no disease-causing mutation has been found yet. However, independent linkage and association studies in large cohort of patients with familial PD support the presence of a disease-modulating gene in the PARK3 region.

**UCHL1 (PARK5)**

A missense mutation (I93M) in the ubiquitin C-terminal hydroxylase L1 (UCHL1) gene was identified in a single German PD family (6). The clinical features were typical of idiopathic PD and the age of disease onset at around 50. UCHL1 represents 1 to 2% of total soluble brain protein and is found in Lewy bodies and other protein aggregations.

As UCHL1 protein plays an important role in the labelling of abnormal proteins in the ubiquitin–proteasome system, the mutation affects protein degradation. In fact, UCH-L1 seems to have two opposing enzymatic activities: an ubiquitin C-terminal hydrolase and a dimerization-dependent ubiquityl ligase, which may be important in PD pathogenesis.

The failure to detect neither this nor any other mutations in the UCHL1 gene in additional linkage studies can be attributed to a reduced penetrance inheritance pattern.

**PINK1 (PARK6)**

The PARK6 locus has been mapped to chromosome 1p35-p36 in a large Italian family with autosomal recessively inherited form of PD. Patients have an early-onset of PD, between 30-40 years and early occurrence of levodopa associated dyskinesia. Recently an homozygous missense (G309D) and an homozygous nonsense mutation (W437X) were detected in
Spanish and Italian kindreds with PD, respectively. Furthermore a number of additional mutations have been reported in the PINK1 gene in families of different ethnicity. Many studies emphasize to the role of heterozygous PINK1 mutations supporting that these variations act as a susceptibility factor for PD. However, the results from a recent study were not in favor of the haploinsufficiency hypothesis. The phenotypic variability associated with PINK1 mutations could be attributed to other genetic or environmental factors such as the theory of «double hit». Digenic inheritance of PD has been recently demonstrated for heterozygous mutations of PINK1 and DJ-1. Furthermore, parkin has a rescuing role in experimental models with PINK1 mutations, preventing the otherwise expected mitochondrial dysfunction.

PINK1 gene encodes a 581 amino acid protein with two predicted domains: a mitochondrial targeting motif and a protein kinase domain that shows a high degree of homology to the serine/threonine kinases of the calcium/calmodulin family. It has been suggested that the mutant form of the PINK1 protein could damage neurons by stress-induced apoptosis and mitochondrial dysfunction. Actually, the in vivo presence of PINK1 protein in mitochondria was recently demonstrated. Since mitochondrial impairment and oxidative stress have been associated with the aetiopathogenesis of PD, further analysis of the coding and non-coding regions of PINK1 gene is in the first line of genetic research.

**DJ-1 (PARK7)**

Close to the PARK6 locus, an additional locus was identified in one Dutch family. The DJ-1 gene that has been recognised in this locus is another reported gene causal for ARJP. Except from the initially found large homozygous chromosomal deletion in the Dutch kindred, L166P point mutation was subsequently detected in an Italian family. However, mutations in DJ-1 are until now responsible for a low number of ARJP cases (1%-2%).

The DJ-1 gene contains eight exons the first two of which are non-coding and alternatively spliced. DJ-1 is expressed in different cell types with high levels of the transcript found in astrocytes of human brain tissue. DJ-1 is not an essential component of Lewy bodies and Lewy neurites. DJ-1 has been associated with several different biological processes, but its implication with PD has given emphasis to its role as a sensor for oxidative stress. DJ-1 is believed to be protective against oxidative and mitochondrial damage. Further studies are needed to understand the exact functions and interactions of this gene.

Taking into consideration the mentioned PD genes, so far, three of them have been identified as responsible for autosomal recessive early-onset parkinsonism (Parkin, DJ-1 and PINK1 genes). Mutations in the parkin gene (PARK2) are a relatively frequent cause of parkinsonism with a prevalence of ~50% in families with autosomal recessive early-onset parkinsonism. The phenotype of patients with parkin mutations is mild and is recognized mostly in cases with onset before the age of 45. DJ-1 mutations (PARK7) are much less common than parkin mutations (frequency <1%), the phenotype is similar though. At last, several studies have described point mutations or deletions in the PINK1 gene, which are less frequent than parkin but more frequent than DJ-1 mutations.

**LRRK2 (PARK8)**

Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) have recently been identified in families with autosomal dominant late-onset PD and have shed new light on finding possible causes of PD. The locus, PARK8 on chromosome 12q12, was originally identified by linkage analysis in a Japanese family. The gene underlying this linkage was recently determined to be LRRK2. The LRRK2 4321C>G (R1441G) mutation was originally identified in Spanish families originating from the Basque region. Today, more than 20 LRRK2 mutations have been linked to autosomal-dominant parkinsonism, accounting for ~7% of familial PD and for a significant fraction of sporadic PD cases. The available data suggest that the prevalence of the LRRK2 mutation varies markedly across populations. The most prevalent LRRK2 amino acid substitution, G2019S, is responsible for ~40% of familial and sporadic PD in Arab samples from North Africa (68, 69), ~30% of familial PD in Ashkenazi Jewish populations, up to 6% of familial cases in Europe and up to 3% of apparently sporadic PD in Europe and North Amer-
The effect for the LRRK2 G2019S mutation can be dated back to as far as the 13th century with a common founder. Even more, the penetrance of LRRK2 G2019S mutation appears to be age-dependent, as it increases from 17% at 50 years of age to 85% at 70 years of age. The R1441G mutation has been associated with an apparently high incidence among Spanish and Basque PD patients, with prevalence up to 8%. The age of onset is variable ranging from the fourth to the ninth decade and the clinical presentation is compatible with typical sporadic PD. Moreover, the pathological findings in patients with LRRK2 mutations are diverse comprising Lewy body pathology and tauopathy with neurofibrillary tangles. This diversity in pathology strongly suggests that LRRK2 is involved in multiple cellular processes and may be a central component of multiple signaling pathways that are crucial for proper functioning of neurons.

**Table 1. Genes implicated in familial Parkinson’s disease.**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Map position</th>
<th>Protein/gene</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK1</td>
<td>4q21-q23</td>
<td>a-synuclein</td>
<td>Autosomal Dominant</td>
</tr>
<tr>
<td>PARK2</td>
<td>6q25.2–27</td>
<td>Parkin</td>
<td>Autosomal Recessive</td>
</tr>
<tr>
<td>PARK3</td>
<td>2p13</td>
<td>Unknown</td>
<td>Autosomal Dominant</td>
</tr>
<tr>
<td>PARK5</td>
<td>4p14</td>
<td>UCHL1</td>
<td>Autosomal Dominant</td>
</tr>
<tr>
<td>PARK6</td>
<td>1p35–p36</td>
<td>PINK1</td>
<td>Autosomal Recessive</td>
</tr>
<tr>
<td>PARK7</td>
<td>1p36</td>
<td>DJ-1</td>
<td>Autosomal Recessive</td>
</tr>
<tr>
<td>PARK8</td>
<td>12p11.2-q13.1</td>
<td>LRRK2</td>
<td>Autosomal Dominant</td>
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<tr>
<td>PARK10</td>
<td>1p32</td>
<td>Unknown</td>
<td>Autosomal Dominant (possibly)</td>
</tr>
<tr>
<td>PARK11</td>
<td>2q36–37</td>
<td>Unknown</td>
<td>Autosomal Dominant (possibly)</td>
</tr>
<tr>
<td>Pending</td>
<td>2q22-q23</td>
<td>Nurr1</td>
<td>Autosomal Dominant</td>
</tr>
</tbody>
</table>

LRRK2 gene has 51 exons and encodes a protein of 285 kDa called dardarin. Dardarin belongs to a group within the Ras/GTPase superfamily, termed Roco. It has five predicted functional domains: a leucine-rich repeat domain (LRR), a Roc (Ras GTPase) domain, a C-terminal of Roc (COR) domain, a kinase domain (MAPKKK) and a WD-40 domain. A remarkable number of mutations have been identified throughout the whole gene notifying its direct linkage with the genetic pathogenesis of PD.

**PARK10**

Another locus likely to be a genetic susceptibility factor was identified on chromosome 1p32 (PARK10), after the gene scanning of 51 families with more than one affected individual. Patients presented onset of disease at around 50-60 years of age. However, no disease-causing gene has been found, yet.
**PARK11**
Autosomal dominant PD has also been connected with PARK11 locus, mapped on chromosome 2q36-q37. This significant linkage was observed after the genetic analysis of 150 families fulfilling the diagnostic criteria of PD\(^{77}\).

**NR4A2/Nurr1**
Nuclear receptor-related 1 gene (Nurr1 or NR4A2) encodes a transcription factor, member of the nuclear receptor superfamily, highly expressed in the central nervous system and with a critical role in the development of midbrain dopaminergic neurons. The functions of this gene have an obvious association with neurological procedures. Nurr1 not only enhances the expression of dopamine transporter\(^{78}\) but is a transcriptional activator of tyrosine hydroxylase, an enzyme with central role in the biosynthesis pathway of dopamine, as well\(^{79}\). Genetic analysis of this gene located on chromosome 2q22-23, revealed the presence of two heterozygous mutations (-291T del and -245 T to G) in 10 out of 107 individuals with familial late-onset PD\(^{13}\). A remarkable decrease in Nurr1 mRNA levels was observed in both of these exon 1 mutations. The affected PD patients are Caucasians of European descent. Additional studies have provided further evidence for the possible association of Nurr1 gene with PD’s disease\(^{80}\). Nevertheless, other studies question the disease-modulating role of the Nurr1 gene\(^{81}\).

**CONCLUSIONS**
Over the last few years, there has been a major shift in investigating the genetic background of PD. A number of genes and loci have been identified leading to major advances in our understanding of the physiopathology of this movement disorder. The presence of at least 10 disease-related genes or loci linked to familial PD provides a clear evidence for the molecular basis of the disease. Three genes have been identified as responsible for autosomal recessive early-onset parkinsonism (Parkin, DJ-1 and PINK1 genes). In addition to the three well-confirmed genes linked to autosomal recessive PD, mutations in the a-synuclein, UCHL1, Nurr1 and recently the leucine-rich repeat kinase 2 (LRRK2) genes have been identified in families with autosomal dominant PD. SNCA encoding a-synuclein was the first gene to be linked to familial parkinsonism for which three missense and several multiplication mutations have now been described\(^{15-21}\). Recently, the identification of pathogenic mutations in a novel gene, the leucine-rich repeat kinase 2 (LRRK2) gene, in families with autosomal dominant PD from different populations came as a major breakthrough in the area of PD genetics. According to present data, the LRRK2 gene and the encoded dardarin protein seem to be key players in PD pathogenesis. The whole gene is under thorough investigation. One of its domains, where the G2019S LRRK2 mutation has been detected, is responsible for the kinase activity of LRRK2, which seems to have critical role in PD pathogenesis. However, the multidomain structure of LRRK2 and its multifunctional encoded protein can harbor many possible disease-causing mutation hot-spots that can either lead to different or common neuropathologic pathways. Further studies would provide additional data for the importance not only of LRRK2, but of other genes implicated in familial PD, as well.

In conclusion, the genes that have until now been associated with familial PD have highlighted several pathways involved in this disease, such as protein misfolding and aggregation, defects in the ubiquitin-proteasome system and aggregation, impaired oxidative stress, mitochondrial dysfunction and altered kinase signalling pathways\(^{82}\). However, the exact pathogenic molecular and cellular mechanisms remain elusive. The PD research is ongoing and the analysis of larger series of families with the use of genetic informations derived from the Human Genome Project will soon provide novel insight into the causes of PD.
Γενετική της οικογενευούσας μορφής της νόσου Πάρκινσον

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ΠΕΡΙΛΗΨΗ: Η νόσος του Πάρκινσον είναι ένα συχνό νευροεκφυλιστικό νόσημα. Τον τελευταίο αιώνα, η συμβολή της γενετικής όσον αφορά την πιθανότητα εμφάνισης νόσου του Πάρκινσον ήταν αμφιλεγόμενη. Πρόσφατα, η αναγνώριση μεταλλάξεων στα γονίδια α-συνουκλεΐνη (α-ΣΝΚΑ), παρκίνη (αΡΚ2), καρβοξυτελική υδροξυλάσης της υδροξυλάσης του δικινοειδούς δικαίωμα L1 (UCHL1), κινάση Pten 1 (PINK1), DJ-1, πυρηνικό υδροξυλάση της ομιλίας της α-συνουκλεΐνης (α-ΣΝΚΑ) και κινάση LRRK2 (LRRK2) έχει επιβεβαιωθεί. Αυτό σημαίνει ότι το παθοφυσιολογικό υπόστρωμα της νόσου του Πάρκινσον είναι γενετικά διαφορετικό. Παρατηρήθηκαν δεδομένα οικογενειακών μορφών της νόσου του Πάρκινσον, επιπρόσθετα των γονίδιων αυτών, πολλές άλλες γενετικές θέσεις έχουν αναγνωρισθεί. Πιθανές αλληλεπιδράσεις μεταξύ πολλών ήδη γνωστών νόσων υποψηφιών γονίδιων και επιδράσεις περιβαλλοντολογικών παραγόντων στην επαγγελματική ζωή με την νόσο Πάρκινσον υπόκεινται στον άλλοκλειστικό έλεγχο των σύγχρονων γενετικών μελετών.

Αξίες Κλειδί: Οικογενειακή νόσος Πάρκινσον, α-συνουκλεΐνη, Γενετική της νόσου Πάρκινσον.

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