LRRK2 and Parkinson's disease.

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ABSTRACT: The identification of the leucine-rich repeat kinase 2 (LRRK2) gene was a breakthrough in the area of Parkinson's disease (PD) genetics. Mutations throughout the whole gene have been associated with both familial and sporadic PD, in unprecedented percentages, with G2019S mutation being today the most common genetic cause of the disease. This review will describe current knowledge on G2019S mutation, the multidomain structure of LRRK2 protein, its function and implication in PD pathogenesis.

Key Words: LRRK2 gene, G2019S mutation, LRRK2 kinase.

INTRODUCTION

Parkinson's disease (PD) is one of the most common neurodegenerative diseases. Approximately 1-2% of the population over the age of 65 years suffers from PD, while this percentage arises to 3-5% at ages above 85 years[1]. PD is characterized by motor symptomatology including tremor at rest, rigidity, bradykinesia, postural instability and freezing episodes. However, the clinical spectrum of PD is more extensive covering a wide range of non motor symptoms, including cognitive and behavioral symptoms, sleep disorders, autonomic symptoms, sensory symptoms and fatigue[1]. The pathological hallmarks of the disease are the loss of the dopaminergic neurons in the substantia nigra, causing dopamine depletion in the striatum, and the presence of Lewy bodies in the remaining neurons.

PD occurs primarily in a sporadic form, although family studies in patients with PD have highlighted specific genes/loci associated with the disease, following an autosomal dominant or an autosomal recessive mode of inheritance. So far, PD has been associated with 15 genetic loci, however only five genes are definitely considered to be associated with the Mendelian inheritance of the disease[2]. One of the major genes associated with autosomal dominant PD is the Leucine-Rich Repeat Kinase 2 (LRRK2, also referred to as PARK8) gene the discovery of which was a milestone in the genetic history of PD. This review describes the main data on the LRRK2 gene and PD, with particular emphasis on the G2019S mutation, which is the most common LRRK2 mutation identified today. It also discusses the structural features of the LRRK2 protein, its function, the effects of LRRK2 mutations and the possible substrates of LRRK2 and the pathways in which it may participate.

LRRK2 GENE: G2019S MUTATION

The LRRK2 gene is located on chromosome 12q12, spans 144 Kb and contains 51 exons. In 2004 the first mutations in the LRRK2 gene were described, being now the most common genetic cause of both the hereditary and the sporadic form of the disease[2]. Although a large number of genetic changes is found throughout the whole gene, only in five LRRK2 mutations (R1441C, R1441G, Y1699C, G2019S and I2020T) the pathogenesis is fully established[3]. Of particular interest is the LRRK2 6055G→A transition, resulting in a glycine-to-serine substitution at amino acid position 2019 (G2019S), which is currently the most common LRRK2 mutation and is associated with both the hereditary and the sporadic form of the disease.

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Its incidence varies according to ethnicity, reaching very high levels in certain populations. In Europe, the G2019S mutation occurs at a rate of 2-5% in patients with inherited PD and of 1-2% in patients with sporadic PD[2]. Nevertheless, in some Asian countries[4,5], in South Africa[6] and in some European countries like Poland, Germany and Greece[7-9], the incidence of the G2019S mutation is very rare to zero. Instead, the G2019S mutation is found in particularly high percentages in populations such as North Africa (37% and 41% in patients with hereditary and sporadic PD, respectively) and the Jewish population (Ashkenazi Jews) (29% and 13% in patients with hereditary and sporadic PD, respectively)[10,11].

The G2019S mutation is likely to come from at least three different haplotypes[2]. Most patients with PD having this mutation live in different countries of Europe and North Africa, or belong to the Ashkenazi Jews population and seem to share a common founder haplotype (haplotype 1) suggested to have been created in the Middle East about 2000 years ago[2]. A second haplotype is also found in some European patients, and a third-one is found in patients from Japan[2].

Of particular interest is the penetrance of the G2019S mutation, which has been found to increase from 17% at age 50 years to 85% at age 70 years[12]. Nevertheless, in subsequent studies these percentages vary in regard to the sample size of the study and the methodology used[2]. According to a major study involving 21 centres in North America and Europe, the penetrance of the G2019S mutation is age-dependent, with rates of 28% at age 59 years, 51% at age 69 years and 74% at age 79 years, and is independent of sex[13]. However, ethnicity may influence the penetrance of the G2019S mutation which has been calculated at 45% in Arabs[14], while in Eskenazi Jews or Italians it was 35%[11,15]. This diversity of results may be explained by factors such as the fact that families with many members suffering from PD are overrepresented, resulting in an overestimation of the G2019S penetrance[16]. Additional genetic or environmental factors may also influence the penetrance of the G2019S mutation[16].

LRRK2 PROTEIN (DARDARIN)

The LRRK2 gene encodes a protein named dardarin[17], a very large protein consisting of 2527 amino acids (286 KD) which belongs to the ROCO superfamily of proteins (18). LRRK2 protein is characterized by the presence of multiple functional domains: (i) the N-terminal ankyrin (ANK) domain (ii) the leucine-rich repeat (LRR) domain (iii) the Roc (Ras of complex protein) domain sharing sequence homology to the Ras-related GTPase superfamily (iv) the COR domain, C-terminal of the Roc domain (v) the kinase domain showing some homology to mitogen-activated protein kinase kinase kinase (MAPKKK) and (vi) the WD40 domain[18]. The simultaneous presence of a kinase region and a GTPase region highlights the potential enzymatic function of the LRRK2 protein, while the presence of the ANK, LRR and WD40 domains indicates an increased likelihood of interaction of the LRRK2 protein with other proteins (18). The LRRK2 protein is expressed in many regions of the central nervous system such as cortex, medulla, cerebellum, putamen and substantia nigra[19]. It is also expressed in other organs like heart, kidneys, lungs, liver and peripheral leukocytes[19]. Inside cells the LRRK2 protein is predominantly expressed in the cytoplasm and in a small percentage in the outer membrane of mitochondria[20]. The LRRK2 protein has also been identified in structures such as plasma membrane, Golgi apparatus, cytoskeleton proteins, synaptic vesicles and lipid rafts[20].

The physiological function of LRRK2 protein is not yet clear and current data are primarily derived from its complex structure and indirectly through the functional effects of LRRK2 mutations. The structure of the LRRK2 protein is of particular interest. As mentioned before LRRK2 belongs to the ROCO superfamily of proteins which are characterized by the simultaneous presence of ROC-COR regions, while some of them, though not all, harbour an additional kinase region[18]. As a kinase, LRRK2 functions as serine/threonine kinase and has the ability to autophosphorylate[21-23]. The effect of LRRK2 as a GTPase has also been documented[23-26]. According to the current data, the Roc domain appears to be sufficient for LRRK2 GTPase activity, independently of its kinase activity, as in experiments with kinase-dead mutants, GTP binding was maintained[24-26]. On the contrary, LRRK2 kinase activity appears to be depended on GTPase activity, as isolation of the MAPKKK region from the rest of the protein, resulted in the inactivation

of LRRK2 kinase activity[24-26]. Roc domain is likely to control the action of LRRK2 kinase activity through intramolecular regulation, as increased GTP binding in the Roc domain or decreased GTPase action has been found to increase LRRK2 kinase activity, whereas the hydrolysis of GTP to GDP by GTPase leads to the inactivation of LRRK2 kinase[23-26].

EFFECTS OF LRRK2 MUTATIONS

Due to the complex structure of the LRRK2 protein and the simultaneous presence of Roc and MAPKKK regions which demonstrate the dual enzymatic activity of the protein (both as a kinase and as a GTPase), major attention has been focused on the effect of LRRK2 mutations on its enzymatic properties. Specifically, multiple mutations have been identified in the LRRK2 kinase domain, including the most common G2019S mutation. The G2019S mutation is located in a hinge like region which connects the N-terminal and C-terminal lobe of the MAPKKK domain and lies between the Asp-Phe-Gly (DFG) and Ala-Pro-Glu (APE) amino acids, respectively. The configuration of this region may prevent access of LRRK2 substrates, possibly playing an important role in regulating the action of LRRK2 as a kinase[18]. Importantly, the G2019S mutation has been found to increase LRRK2 kinase activity[21,23,27,28], perhaps "unlocking" this region. It is of note that, the G2019S mutation has also an autophosphorylation site whithin this region[29]. However, the impact of LRRK2 autophosphorylation is currently under investigation. Similar results have been found for the I2020T mutation[23,30], however in another study, this mutation was associated with reduced LRRK2 kinase activity[31]. No change or increase in the kinase activity was found for the Y1699C[28,31], R1441C or R1441G mutations[21,23,28,31]. This difference probably stems from different structures, expression systems and methodologies of the various experiments. However, in most of the studies it appears that LRRK2 mutations, especially the G2019S mutation, increases the activity of LRRK2 compared to wtLRRK2 (wild type LRRK2).

Of particular interest is also the study of LRRK2 mutations in the Roc domain. It has been found that R1441C/G mutations reinforce the action of LRRK2 as a kinase, prolonging GTP binding, thus reducing the action of GTPase[25]. Nevertheless, in other studies the LRRK2 kinase activity was not affected[24,32] and the influence of LRRK2 mutations in GTP binding was low to negligible[23-26]. It is also of note, that the Roc region has been found to interact with itself and with other regions of the LRRK2 protein, forming a dimer[29,33], whereas mutations in this region have been found to affect this interaction[29,33]. It is also worth mentioning that, the wtLRRK2 dimer has recently been found to possess greater kinase activity compared to its monomer form and this more active form was enriched in the plasma membrane compared to the cytoplasm[34].

LRRK2 SUBSTRATES AND PATHWAYS

The recognition of substrates and proteins that interact with LRRK2 and of pathways that LRRK2 is implicated, is an important step in order to improve our understanding regarding the precise role of LRRK2 in PD pathogenesis. A number of substrates and pathways have already been associated with LRRK2. However, their exact relationship with PD is currently under investigation.

Some of the currently identified LRRK2-interacting proteins are proteins functioning in the cytoskeleton and trafficking. LRRK2 has been found to phosphorylate moesin, member of the ERM family of proteins (ezrin, radixin, moesin) involved in anchoring the actin cytoskeleton to the plasma membrane. The G2019S mutation was found to increase phosphorylation and binding of moesin to F-actin and the plasma membrane[31]. Interestingly, in a recent study[35], increased ERM phosphorylation, due to the G2019S mutation, was associated with inhibition of neuronal development. Another protein that LRRK2 has been found to interact, is the elongation factor 1A (EF1A) protein[36]. *In vitro*, EF1A was found to reduce the activity of LRRK2 as a kinase, however LRRK2 GTPase activity was not affected[36]. The expression of the EF1A protein is wide at a cellular level and it is estimated that 60% of that is associated with the cytoskeleton, interacting with both microtubules and actin[36]. In the study of Gillardon et al. the *in vitro* interaction of LRRK2-EF1A proteins was found to disrupt the stability of microtubules. Notably, LRRK2 has previously been documented to interact with microtubules[37]. Moreover, in a recent study the G2019S LRRK2 mutation was associated with dendrite degeneration through hyperphosphorylation of tau, a protein known to bind

to microtubules, by the GSK3-β kinase[38]. How these proteins participate in the "puzzle" of PD pathogenesis needs to be further investigated. LRRK2 has also been found to interact with the Rab5b protein. Rab5b is a small GTPase that regulates vesicle transport from the plasma membrane to endosomes[39]. When co-expressed with LRRK2, Rab5b was found to eliminate the negative effects of the intracellular overexpression of wtLRRK2, which led to decreased endocytosis. This observation supports a potential role of LRRK2 in normal synaptic function[40].

Recently, LRRK2 has been found to interact with proteins encoded by genes associated with familial PD. LRRK2 kinase has been found to phosphorylate alpha-synuclein in residue Ser129[41]. Phosphorylation of Ser129 was higher for the G2019S mutation compared to the wtLRRK2[41]. In another study in transgenic mice[42], overexpression of the LRRK2 protein resulted in rapid development of the neuropathology phenotype associated with the A53T alpha-synuclein mutation, reinforcing the possible action of the two proteins in a common functional pathway. LRRK2 has also been shown to directly interact with parkin. Parkin promoted the formation of LRRK2 aggregates and LRRK2 influence parkin to act as a ligase[27]. In a recent study, in overexpression of the G2019S mutant, parkin rescued dopaminergic neuron loss[43]. Further studies regarding these two proteins are expected to further clarify this interaction. Apart from parkin, in a recent study LRRK2 was found to interact with PINK1 and DJ-1, as well[44], however these data are preliminary and need to be further examined.

Another substrate of the LRRK2 kinase is the 4E-BP protein (eIF4E-binding proteins)[45]. This protein forms a protein complex with the eIF4E protein (eukaryotic translation initiation factor 4E)[45] inhibiting its activation and therefore protein synthesis. When phosphorylated by the LRRK2 kinase, 4E-BP is released from this complex, leading to protein overexpression, reduced resistance to oxidative stress and loss of dopaminergic neurons[45]. Nevertheless, according to a recent study, 4E-BP is not a direct substrate for the LRRK2 kinase[46].

In *in vitro* conditions, LRRK2 has also been found to interact with 14-3-3 proteins, small (28-33kDa) acidic polypeptides that form homo- and hetero-dimers. In the study of Nichols et al. the 14-3-3 protein was found to bind to the amino terminus of LRRK2 in residues Ser910 and Ser935[47] influencing the stability of LRRK2, perhaps *via* affecting dimer formation and LRRK2 kinase activity[47]. This connection is hampered by LRRK2 mutations leading to the formation of inclusions[47].

Members of the MAPKK family such as MKK 3, 4, 6 and 7 proteins have also been recognized as substrates for the LRRK2 kinase, whereas mutations in the LRRK2 gene have been found to increase the binding and phosphorylation of MKK proteins[48,49]. These observations suggest that LRRK2 indeed functions as a MAPKK.

The association of the LRRK2 protein with chaperones, have also been reported. More specifically, LRRK2, through the MAPKKK region, has been found to interact with Hsp90. Inhibition of Hsp90 disrupted the Hsp90-LRRK2 complex, resulting in LRRK2 degradation *via* the proteosome, thus protecting cells from the neurotoxic effect of the LRRK2 kinase[50]. Furthermore, CHIP has been found to bind and ubiquinate LRRK2, promoting its breakdown by the proteasome and Hsp90 complex[51]. Overexpression of CHIP appeared to be protective towards the toxicity caused by LRRK2 mutations[51].

Focusing on LRRK2 pathways, one possible mechanism is the MAPK/ERK pathway. Through this pathway, wtLRRK2 has been documented to exert a protective role against oxidative stress, an ability which was reduced in LRRK2 mutations[52]. The activation of proteins involved in this pathway such as Src, Hsp27, and JNK was found decreased in PD patients compared to the healthy study group, an observation that might indicate that changes in this pathway are common in PD patients[52]. The exact role of LRRK2 in the MAPK pathway is not yet clear. A recent study found that LRRK2 kinase phosphorylates a variety of MAPK kinases[48], whereas the MAPK pathway has currently been associated with increased transcription and expression of alpha-synuclein[53].

The process of apoptosis has also been associated with the LRRK2 protein. Recently, Ho et al. found that mutations associated with PD increased the association of LRRK2 with FADD (Fas-associated protein with death domain), leading to activation of caspase

8 and apoptosis, resulting in neurodegeneration[54]. In contrast, inhibition of FADD or inactivation caspase 8 was found to prevent neurodegeneration[54]. It is also interesting that in a previous study, LRRK2 mutations were associated with neurotoxicity due to mitochondrial dependent apoptosis, thus apoptosis through the release of cytochrome c and the activation of caspase 3[55]. In this study, a key role in the neurotoxicity of LRRK2 mutations was attributed to Apaf1 (Apoptotic peptidase activating factor 1), as ablation of Apaf1, led to the inhibition of the activation of caspase 3 and neurotoxicity[55].

Of particular interest is the recent implication of the LRRK2 protein in the autophagy pathway[56]. More specifically, the R1441G mutation was associated with impaired autophagy and accumulation of vesicles with incompletely digested products. In contrast, inactivation of LRRK2 resulted in increased activity of autophagy[56]. A previous study had also indicated the potential role of LRRK2 in autophagy, as the G2019S mutation was associated with neurite shortening and an increase in the presence of autophagy vesicles[57]. Autophagy seems to be involved in many neurological diseases[58], thus it is very important to investigate the precise role of LRRK2 in this pathway.

An additional pathway recently associated with LRRK2 is Wnt, which plays an important role in the normal neuronal development and function. LRRK2 was found to interact with proteins of the Dishevelled family (DVL1-3), which are key regulators of Wnt pathways[59], generally involved in the regulation of small GTPases.

CONCLUSION

Since the discovery of the LRRK2 gene and its relationship with autosomal dominant and sporadic PD, extensive research has been focused on LRRK2, in an attempt to elucidate its implication in the pathogenesis of the disease. However, this research effort is still in its infancy; unravelling the relationship of LRRK2 with PD is a huge ongoing scientific challenge in the area of PD genetics.

Abbreviations

LRRK2: Leucine-rich repeat kinase 2
PD: Parkinson's disease
wtLRRK2: wild type LRRK2
EF1A: Elongation factor 1A
4E-BP: eIF4E-binding proteins

LRRK2 και νόσος του Πάρκινσον.

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ΠΕΡΙΛΗΨΗ: Η ανακάλυψη του γονιδίου της πρωτεϊνικής κινάσης 2 πλούσιας σε επαναλήψεις λευκίνης (LRRK2) αποτέλεσε σταθμό στον τομέα της γενετικής της νόσου του Parkinson. Μεταλλάξεις σε ολόκληρο το γονίδιο έχουν συσχετισθεί τόσο με την κληρονομική όσο και με τη σποραδική μορφή της νόσου Parkinson, σε ασυνήθιστα υψηλά ποσοστά, με την μετάλλαξη G2019S να αποτελεί σήμερα τη πιο συχνή γενετική αλλαγή που έχει συσχετισθεί με τη νόσο. Στην παρούσα ανασκόπηση θα γίνει εκτενής αναφορά στη συγκεκριμένη μετάλλαξη, θα περιγραφεί επίσης η πολύπλοκη δομή και λειτουργία της πρωτεΐνης LRRK2, καθώς και τα έως τώρα δεδομένα σχετικά με τη συμμετοχή της στην παθογένεση της νόσου του Parkinson.

Λέξεις Κλειδιά: Γονίδιο LRRK2, Μετάλλαξη G2019S, Κινάση LRRK2.

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