LRRK2: where do we stand and where to go?

Bachelorthesis
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Wiebe Dam, s2370026
Supervisors: A. Kortholt & Y.F. Ho

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Parkinson's disease is the second most prevalent neurodegenerative disease and the most common genetic cause of late-onset Parkinson's is a mutated gene called LRRK2. Named after a domain it possesses, LRRK2 consists of multiple protein interaction domains like kinase, WD40, Roc and Cor domains and LRR repeats. It is also involved in a legion of processes and its interactome is huge. Where its suspected role in Parkinson's seems to be well substantiated, a lot is still unknown about LRRK2. Combining research, this thesis discusses several aspects of LRRK2 with the aims to clarify almost 15 years of research and to add a possible outlook for the future. LRRK2's different domains, mutations and mechanisms of activation are discussed and combined with possible therapeutic strategies that can be enforced when LRRK2 in Parkinson's disease is targeted. Where research has mainly had its focus on the inhibition of LRRK2 kinase, other therapeutic strategies like the disruption of dimerization, interference on the protein-protein interaction platform and disruption of localisation seem to remain underexposed. While research has put out some negative outcome, the development of current drugs and the possibilities that have yet to be researched look promising.

Introduction

Parkinson's disease (PD) is a common progressive neurodegenerative disease of the central nervous system that affects various parts of the brain, resulting in cognitive decline and deficits in motor movement and motor control. Most cases of PD are still labeled idiopathic with a mostly unknown etiology. PD comes in more than one variant and is characterized by either one or both of these two hallmarks: the loss of dopamine producing neurons in the substantia nigra pars compacta and the presence of Lewy bodies in the olfactory bulb, vagus nerve and lower brainstem regions like the medulla oblongata and the pontine tegmentum (Alexander, 2004; Alves et al., 2008). Lewy bodies are intracellular, abnormal protein aggregates in the brain that are first formed through the pathological accumulation of α-synuclein, a protein that is abundantly present in the human brain, and will come to cause neurodegeneration in the areas in which they are present (Beyer, Domingo-Sàbat, & Ariza, 2009). The loss of dopamine-releasing neurons or presence of Lewy bodies in the aforementioned affected areas constitutes the various symptoms of PD. For example, research showed that lesions of the substanta nigra pars compacta result in deficits in fine motor movement, explaining PD's akinesia (Pioli et al., 2008). But while disruption of regular movement is one of the first markers for the disease, different symptoms often occur at a later stage. Besides the 'regular' motor symptoms, other symptoms like sleep disturbances, neuropsychiatric problems, dementia, and sensory problems are also commonly found in PD patients (Sveinbjornsdottir, 2016).

Treatment for PD has yet to evolve. Momentarily, most patients are treated with levodopa (L-dopa, a dopamine precursor) for their motor problems. Treatments with L-dopa are found to be purely symptomatic and insufficient for long-term symptoms (Hely, Morris, Reid, & Trafficante, 2005). All other symptoms like depression, fatigue, or psychosis are also symptomatically treated, through palliative care. These non-motor complications are commonly categorized among neuropsychiatric and autonomic symptoms. The problem with treating PD patients might lie in the fact that the cause of PD is still mostly unknown. While the etiology of PD remains a mystery, a few risk factors have been clarified to some extent. These risk factors can be categorized into gender, non-genetic (for example environmental) and genetic factors. For example, men experience higher levels of motor impairment and require higher doses of levodopa during treatment than women, and pesticide exposure is significantly associated with PD (Baldereschi et al., 2003; Lubomski, Louise Rushworth, Lee, Bertram, & Williams, 2014). When looking into environmental factors, it is also worth mentioning that smoking is found to be negatively related to PD, meaning that heavy smokers tend to have a smaller chance of developing the disease (Fratiglioni & Wang, 2000).

The third category of risk factors is the genetic component of the disease. It has been known for some time that PD has a familial background (Nussbaum & Polymeropoulos, 1997), and since the first research on the genetic basis of PD a lot of genes that might play a role in its pathogenesis have been discovered. In the early 00's it has already been shown that mutations on a certain gene, the parkin-gene on chromosome 6, cause autosomal-recessive, early-onset PD (Bonifati et al., 2001). More recent research points us towards new genetic mutations that could play a major role in the progression and emergence of the disease. For example, a meta-analysis of GWASs (Genome Wide Association Studies) has recently identified 17 more risk-loci for PD

(Chang et al., 2017). A few of the promising genes that have been researched in the last years are SNCA, LRP10 and LRRK2. Scientists belief that the cure for PD probably lies in research focusing on the genetic cause of the disease, thus, since the discovery of some potentially important genes, scientists have had their focus on these genes. LRRK2, being discovered in 2004, has been researched thoroughly since then. This research is justified through the impact that LRRK2 mutations have on several ethnical groups worldwide: G2019S-LRRK2, the most known prevalent mutation, is accountable for 4% of all familial cases and 1% of sporadic cases worldwide (Healy et al., 2008; Mata et al., 2016). On top of that, within the population of North African Arabs and the Ashkenazi Jews the mutation is present in 40% of all PD cases, and that is even a low estimate for it excludes atypical cases and cases with a strong familial history (Lesage et al., 2006; Ozelius, Senthil, & Saunders-Pullman, 2006). Ever since, a lot of progress has been made. We now have substantial knowledge on various aspects of the gene: its mechanism of activation has been clarified to a great extent, a lot of its regulators and interactors have been identified and LRRK2's (interaction-) domains have been identified as well. This information can be put to use when designing drugs to specifically target LRRK2. This thesis aims to clarify and summarize the progress that research has made on LRRK2, its therapeutic targets and the drugs that target them. So, regarding LRRK2 research: where do we stand? And where to go?

Domains, mechanism of activation and mutations

LRRK2 belongs to the Roco (Ras of complex) family of proteins, is encoded by the PARK8 gene and is a relatively large protein with several important domains, under which LRR, Roc, Cor, WD40, ankyrin repeats and a kinase domain (Paisán-Ruíz et al., 2004). Ankyrin-like repeats, together with leucine-rich repeats and the WD40 domain all make the suspected role of LRRK2 a scaffold protein, meaning that it is involved in key regulating pathways of other proteins: this is because all mentioned domains are likely to be protein-protein interaction (PPI) motifs. The LRR domain, which stands for Leucine-Rich Repeats, consists of 20-29 residue sequence motifs and is found in a number of different proteins with diverse functions. It is commonly associated with the innate immune response through PAMPS (Pathogen-Associated Molecular Patterns) and is believed to play a big role in PPIs (Kobe & Kajava, 2001; Ng & Xavier, 2011). WD40 domains are abundantly present in eukaryotes and are thought to be among the most interacting eukaryotic domains. These interactions are mostly protein-DNA and PPIs. WD40 domains exhibit a 'ß-propeller' structure that often consists of seven blades, which are made out of four antiparallel ß-strands. This structure is commonly placed in the Gß subunit of heterotrimeric G-proteins, which regulate various cell functions through transmembrane signaling (Xu & Min, 2011). Deletion of the WD40 domain leads to impaired LRRK2 dimer formation (Rosenbusch & Kortholt, 2016). LRRK2's ability to form a dimer will be discussed later in this paragraph. The Roc, Cor and kinase domains play a large role in the activity of LRRK2: most of the pathogenic LRRK2 mutations find their place in this so-called 'catalytic core' of LRRK2. The kinase and Roc domain both are enzymatic domains, which both regulate their own activity as well as the activity of other proteins. These two domains are responsible for the intrinsic enzymatic activity of LRRK2: the GTPase and the kinase activity combined. Roc becomes active through its guanine nucleotide-binding domain, functioning as a GTP-binding protein that stimulates kinase activity when bound to GTP. The kinase domain regulates the Roc-domain through autophosphorylation, thus regulating the activity of the protein (Alexander, 2004; Gilsbach & Kortholt, 2014). However, how this process fully works still has to be figured out. Protein kinases are vital for biological processes like energy metabolism, orderly cell cycle progress and transcription. Protein kinases modulate various protein functions through the catalysis of the transfer of the γ-phosphate of ATP to the serine/threonine/tyrosine in protein substrates. Since LRRK2 and Roco-proteins are serine/threonine kinases, they make use of the kinase mechanism. The Cor domain functions as a regulator for the GTPase activity of Roc by dimerization in a nucleotide-dependent manner (Gotthardt et al., 2008; Rudenko, Chia, & Cookson, 2012). GTP hydrolysis might be nucleotide-dependent as well, but this has not been fully proven yet. Further research by Deng et al. (2008) suggested that loss of stabilizing forces in the Roc dimer is probably also related to decreased GTPase activity.

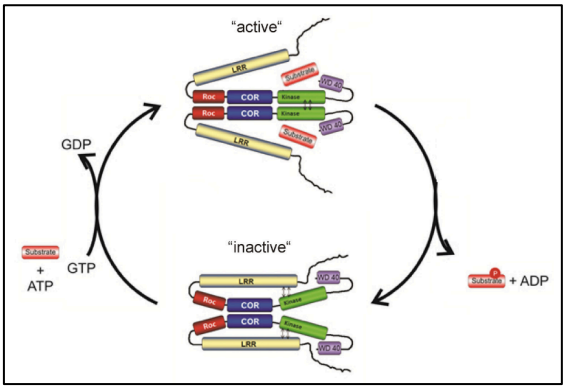

fig 1. – LRRK2's putative mechanism of activation. Note that the switch between the active state and inactive state is paired with GTP- and GDP-bound and monomeric and dimeric states (Gilsbach & Kortholt, 2014)

Ho et al. (2014) reviewed these activation mechanisms using a model in *Dictyostelium discoideum*, an amoeba that feeds on bacteria. The review suggests that a combination of the two activation mechanisms of LRRK2 makes it switch between two states: the low-activity monomeric state and the high-activity dimeric state. In figure 1 is seen that the Roc and Cor domains become active through the binding of GTP, and switch between the inactive GDP-bound state and the active GTP-bound state. After phosphorylation of the kinase domain, the structural conformation of the kinase switches to the 'active' state, which means that the N- and C-terminus move more closely towards one another. This change in structure also has its effect on other parts of the protein, because it allows the activation loops of the kinase protomers to be autophosphorylated and thus activated (Gilsbach and Kortholt, 2014). Localisation also seems to play a part in the mechanism. Early research by Berger, Smith & Lavoie (2010) showed that the kinase activity of LRRK2 is greater when LRRK2 is membrane-bound, compared to cytosolic LRRK2. Nichols et al. (2010) have shown that disruption of the bond between LRRK2 and 14-3-3 proteins resulted in altered LRRK2 cytoplasmic localisation, and recent work by Purlyte et al. (2018) stated that Rab29 is a master regulator of LRRK2, with control over its activation (through elevated phosphorylation on ser1292) and localisation. According to research, Rab29 mediates LRRK2 transport to the trans-Golgi network and controls its portion that is membrane-bound (De Wit, Baekelandt, & Lobbestael, 2018; Purlyte et al., 2017). Also worth noting is the fact that G2019S-LRRK2 enhanced phosphorylation at ser72 of Rab29, which resulted in an alteration in the morphology of the trans-Golgi apparatus (Fujimoto et al., 2018; Liu et al., 2018). Waschbüsch et al. (2014) stated that Rab32 co-localises with LRRK2 to the late endosome and transport vesicles, and that overexpression of active Rab32 decreases mitochondrial and thus active LRRK2. With this information we can conclude that Rab29 and Rab32 play a big role in LRRK2 activation and (co-)localisation.

LRRK2 not only regulates itself, it also regulates other proteins. We know that it plays a role in a diverse set of cellular signaling complexes and cellular functions, among which mitochondrial function, transcription, molecular structural integrity, autophagy and part of the immune system (Price et al., 2018; Wallings, Manzoni, & Bandopadhyay, 2015). Following a study by Biskup et al. (2006), in which LRRK2 was likely found to be localised in vesicular and membranous structures within neurons in the mammalian brain, researchers investigated possible links to this conclusion. LRRK2 is involved in various processes, among which synaptic endosomal vesicle trafficking, and as said before, trans-Golgi sorting and autophagy (Cirnaru et al., 2014). We also know that LRRK2 is involved with Rab29 and Rab32, but there are plenty of other interactions between LRRK2 and Rab proteins. For example, an interaction with Rab5 has shown to coordinate neurite outgrowth, and inhibition of LRRK2 has been found to promote the perinuclear clustering of lysosomes through Rab7 (Esteves et al., 2015; Heo, Kim, & Seol, 2010; Waschbüsch et al., 2014). Furthermore, research on LRRK2's role in autophagy also yielded good results: a study showed that where wt-LRRK2 seems to improve autophagy, G2019S-LRRK2 inhibited autophagy, facilitating age-related dopaminergic neuronal loss in nematodes (Saha et al., 2015). More connections between LRRK2 and Rab-proteins are found in the autophagy-lysosomal pathway. Eguchi et al. (2018) found connections between LRRK2 and Rab 8 (LRRK2 stabilizes Rab8, which results in suppressed lysosomal enlargement), Rab10 (stabilized by LRRK2, results in increased secretion by lysosomes). Their tests concluded that LRRK2, Rab29, and phosphorylated Rab8/10 are involved in lysosomal homeostasis.

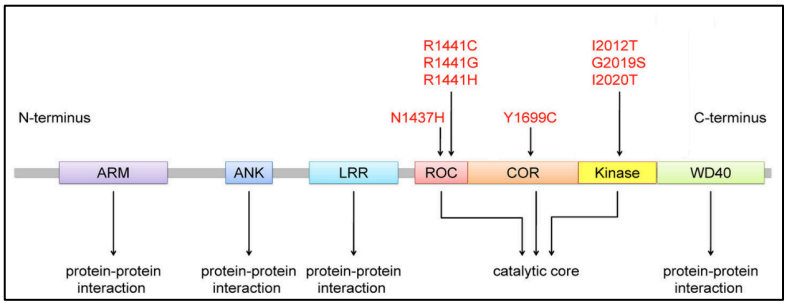

fig. 2 – Multidomain structure of LRRK2 displaying several potential pathogenic mutations and their locations, edited for clarity (Chen & Wu, 2018)

More than 40 mutations in LRRK2 have been identified so far, but only a handful of (missense) mutations are known to result in a pathogenic form of LRRK2 that causes PD. A small overview of the multi-domain structure of LRRK2 with known and corresponding mutations is shown in figure 2. These mutations all have a big impact on the way LRRK2 corresponds and behaves in its environment because they are located in the stretch of the gene that is, among other tasks, responsible for activation. The mutations that are located in the Roc and Cor domains (R1441C, R1441G, N1437H and Y1699C) result in decreased GTPase activity and increased kinase activity by either disrupting the GTP hydrolysis or local weakening of dimerization. Mutations in the kinase domain (G2019S, I2012T and I2020T) all result in increased kinase activity to differing extents (Daniëls et al., 2011; Lewis et al., 2007; X. Li et al., 2007; Puschmann et al., 2012). Chen & Wu (2018) have recently shown that the consequences of the aforementioned mutations, namely reduced GTP hydrolysis and increased kinase and GTP binding activity, will inherently lead to dysregulations and abnormal changes of mitochondrial dynamics, the autophagic-lysosomal pathway, intracellular trafficking and the ubiquitin-proteasome system. While most of the mutations that are discussed so far have been known for quite some time, new mutations are still being discovered and more information is gathered on these mutations as well. For example, new research indicates that G2019S knock-in mice models showed resilience (mostly in the brain) to kinase inhibition in comparison to wildtype LRRK2, and the R1441C, Y1699C and G2019S mutations have recently been found to dramatically increase LRRK2 phosphorylation of Rab7L1, thus promoting the active state of LRRK2 (Kelly et al., 2018; Liu et al., 2018).

Targeting LRRK2

Loosely based on Rudenko et al. (2012), there are a few possible therapeutic strategies that are based on targeting of LRRK2's enzymatic regions that can be enforced:

1. Inhibition of kinase activity
2. Disruption of LRRK2 dimerization
3. Interference of LRRK2 PPI platform
4. Disruption of LRRK2 localisation

In this section of the paper, current research on therapeutic strategies will be discussed. However, as the main focus of this research is on therapeutic inhibition on LRRK2 kinase activity, the last three strategies are grouped into one section.

Inhibition of kinase activity

The first strategy, inhibition of kinase activity, is originally based on the assumption that kinase and GTPase activity contribute to neuronal death in LRRK2-linked PD (Smith et al., 2006). The inhibition of LRRK2 kinase activity was first brought up when it was shown that G2019S is a 'gain-of-function' mutation that resulted in enhanced kinase catalytic activity and increased cellular toxicity (West et al., 2005). Since the discovery of this mutation, LRRK2 research aimed for therapeutic strategies have focused on selective LRRK2 kinase inhibitors. Please note the word 'selective' in the last sentence; as research has pointed out, LRRK2 mainly reacts on non-specific kinase inhibitors. The downside to treatments with these compounds is that they also come with non-specific reactions.

It is apparent that extensive research has been conducted regarding LRRK2 kinase inhibitors in the past years. In a review by Kethiri & Bakthavatchalam (2014), it was pointed out that over the timespan of two years, over 20 LRRK2 inhibitors were applied for patents. But before we can see what happens when we inhibit LRRK2 kinase, we first need discuss its substrates. In a review by West & Cookson (2016), two bonafide 'substrates' are distinguished: autophosphorylation on ser1292 and the phosphorylation of Rab GTPases. These are discussed in *Domains, mechanism of activation and mutations* (West & Cookson, 2016). Being the only known site that is exclusively phosphorylated by LRRK2, ser1292 makes for an excellent therapeutic target. This is underlined in research by Sheng et al. (2012), in which ser1292 is first identified as an autophosphorylation site, and later on a relatively selective kinase inhibitor for ser1292, G1023, is tested with positive results, indicating that LRRK2 inhibition might yield good results for PD treatment. The second 'bonafide substrate', Rab proteins, is even more important. As mentioned before, Rab proteins are involved in numerous cellular processes and regulatory pathways. This is both a blessing and a disadvantage: as Rab proteins and their pathways can tell us a lot about LRRK2's true nature, inhibiting its kinase domain will inherently result in unpredictable responses due to the great number of interactions. Luckily, through the Rab Detection Initiative of the Michael J. Fox Foundation (MJFF), we might expect some light on the subject in the future.

As was said before, research by Dzamko et al. (2010) found that LRRK2 kinase inhibition leads to dephosphorylation on ser910 & ser935, disruption of 14-3-3 protein binding and relocalisation of LRRK2 to the cytosol. It is noteworthy that later research has pointed out that mutations on ser910 and ser935 did not alter phosphorylation in ser1292, so while these sites are important for the functioning of LRRK2, they are not solely a part of LRRK2's mechanism of activation.

Since the first LRRK2 kinase inhibitors appeared in 2007, research has gone through tremendous efforts to put more compounds to the test. In this section, we will discuss a few of the more prominent small-molecule kinase inhibitors that have been published over the years. A short overview of all findings can be found at the end of this paragraph in table 1. Deng et al. (2011) found the first selective and potent inhibitor of LRRK2, LRRK2-IN1. Later research endorsed the findings of Deng and colleagues: LRRK2-IN1 was able to disrupt the phosphorylation of ser910 and ser935 (Yao et al., 2013). Sadly, Koshibu et al. (2015) found that LRRK2-IN1 inhibits critical and ubiquitous enzymes, and that it has shown poor brain penetration. Choi et al. (2012) developed a compound that derived form LRRK2-IN1, called HG-10-102-1, that was able to cross the blood-brain barrier and had better selectivity, but still bared suboptimal characteristics when it came to selectivity, penetration and pharmacokinetics. Another compound called GSK2578215A, identified by Reith et al. (2012), has also shown great potential. It was found to be able to inhibit ser910 and ser935 phosphorylation in both wt-LRRK2 and G2019S-LRRK2, but failed to inhibit LRRK2 in the brain due to poor drug availability. A selective compound studied by Hatcher et al. (2015), named JH-II-172, was also found to effectively inhibit ser910 and ser935 and also had better brain penetration. Further developing the promising GSK2578215A, (Estrada et al., 2012; Estrada et al., 2014) published two studies on several compounds, under which GNE07915, GNE0877 and GNE9605. However, as is stated in the articles, the selectivity of GNE-0877 and GNE-9605 were less than desirable. Qin et al. (2017) tested GSK2578215A together with GNE-7915 and GNE-0877 on effect on dopaminergic neurotransmission. The article clearly states that only GNE-7915 was capable of constituting positive effects at the proper dose, namely enhancing the release and recovery of dopaminergic vesicles. More good news on GNE-7915 was found by Howlett et al. (2017): the compound was able to reverse G2019S-LRRK2-induced damage to mitochondrial DNA. MLi-2 is a selective kinase inhibitor that is believed to have good brain and CNS penetration. First identified by Fell et al. (2015), MLi-2 was found to reduce phosphorylation of LRRK2 kinase activity, measured through the dephosphorylation of ser935, both peripheral and in the brain. Intake of MLi-2 did not pose any adverse effects on behavioral activity, weight or food intake. Later research looked into several similar promising compounds that came to light in the search for potent LRRK2 kinase inhibitors with the same chemical properties as MLi-2 possesses (Scott et al., 2017). Refinement and testing of several identified compounds led the researchers to a refined version of the original compound Fell and colleagues had found 2 years prior, but more usable information has yet to be published. In the review by Kethiri & Bakthavatchalam (2014), it was stated that while most compounds looked promising, most of them lacked either one or more of the following characteristics: proper penetration over the blood-brain barrier, kinase selectivity, CNS permeability or efficient inhibition of G2019S-mutated LRRK2. The problems found in these compounds are found among other LRRK2 kinase inhibitors as well (Choi et al., 2012; Reith et al., 2012). In vivo health liabilities have also been found in prominent research on the harmful effects of LRRK2 kinase inhibitors

by Fuji et al. (2015) as well. In their study, they made use of two small-molecule kinase inhibitors, GNE-7915 and GNE-0877**,** and found that use of both compounds resulted in pulmonary toxicity. Furthermore, combining the research by Fuji and his colleagues with MLi-2, an unpublished article by Baptista et al. (2018) sought to check the pharmacology, reversibility after drug withdrawal, and association with deficits in pulmonary function of GNE-7915, PFE-360 and MLi-2. The study found answers on all three questions. Firstly, Baptista and his colleagues identified a no-effect dose of PFE-360 and MLi-2. However, for MLi-2 goes that the no-effect dose might be close to the threshold, because one out of the twelve test subjects did show lung effects. Secondly, they confirmed the conclusions of previous studies, namely that LRRK2 kinase inhibition does impose negative effects on the lungs. However, a two-week withdrawal from treatment with GNE-7915 and MLi-2 resulted in a total recovery from these adverse effects. Furthermore, tests with these two compounds in doses that invoke said adverse effects seemed to have no impact on pulmonary function in the lungs of non-human primates. Worthy of mentioning is that studies funded by the MJFF are still keen on putting out quality research. A press release from August 1st, 2018 that was put out by 'Denali Therapeutics' claims to have found a compound, named DNL201, which inhibits LRRK2 in a supposedly safe manner. After a double-blinded, randomized, placebo-controlled study with 100 healthy subjects, the compound is the first to be currently entering the clinical phase. Later on, on October 25th, Forbes Magazine went on and called the progression on DNL201 a 'milestone moment'.

Table 1 – An overview of (recent) small-molecule LRRK2 kinase inhibitors, based on table 1 from Atashrazm & Dzamko (2016)

Reference	Compound name	IC_{50} (nM)		Brain permeability
		Wt	*G2019S*	
Deng et al. (2011)	LRRK2-IN1	13,0	6,0	No
Estrada et al. (2012), Hatcher et al. (2015)	GNE-7915	9	-	Yes
Choi et al. (2012)	HG-10-102-1	20,3	3,2	Yes
Reith et al. (2012)	GSK2578215A	10,9	8,9	Yes
Estrada et al. (2014)	GNE-9605	19	-	Yes
Estrada et al. (2014)	GNE-0877	3	-	Yes
Hatcher et al. (2015)	JH-II-172	6,6	2,2	Yes
Fell et al. (2015)	MLi-2	0,8	0,76	Yes
-	DNL201	*Clinical trials have started in October, 2018*		

Other therapeutic strategies

As we have already established, LRRK2's mechanism of activation, while not known in its entirety, relies on an interaction between dimerization and kinase activity. Thus, LRRK2 dimerization also follows as a natural target: if dimerization is inhibited, LRRK2 cannot become activated. This is underlined in research by Deyaert et al. (2017): their model, based on *Chlorobium tepidum*, showed that a certain mutation, namely the L487A mutation, lead to decreased GTPase activity by stabilizing the Roco protein in either the monomeric or the dimeric state. Much earlier research also showed the significance of the dimerization process: it was found that a kinase-inactivating LRRK2 mutation (D1994A) resulted in a reduction in ability to stabilize dimer-sized structures. Further research on similar mutations found that G2019S, I2020T and I1122V all showed increased dimer-sized proportions in comparison to wildtype LRRK2 (Sen, Webber & West, 2009). When further looking into these mutations, all research focuses on the impact of the mutations on kinase activity instead of LRRK2 dimerization. Besides that, it seems that membrane localisation of LRRK2 is vital for the forming of its dimers (Berger et al., 2010). When looking into the current research regarding LRRK2's dimerization process and its potential for becoming a prominent therapeutic strategy, it becomes apparent that more research in this field needs to be conducted.

Even though much about LRRK2 is unknown, most researchers agree on the fact that LRRK2 could very well be a scaffold protein that serves as a platform on which several different proteins can assemble to perform specific functions. This is mostly due to the fact that LRRK2 possesses a number of domains that are well known to act as protein-interacting platforms, such as WD40, ankyrin and armadillo repeats. Interference on the PPI platform could thus be an important therapeutic target. Recent research by Tomkins et al. (2018), aimed towards determining PPIs with human Roco as a working example, investigated the Roco family (LRRK1, LRRK2, DAPK1 and MASL1/MFHAS1) for PPIs. The study found 113 interactors for LRRK2 within their own confidence threshold, compared to 38, 14 and 4 for the other members of the human Roco family. This tells us a lot about the impact the gene has on its environment and could possibly lead to new insights in different therapeutic strategies. Important targets in LRRK2's PPI platform may be 14-3-3 proteins. As was said before in *Inhibition of kinase activity*, inhibition of LRRK2 kinase leads to the dephosphorylation of LRRK2 at ser910 and ser935 and the disruption of 14-3-3 binding. This binding of 14-3-3 proteins is on itself already an important fact: these regulatory proteins have the ability to bind a diverse array of signalling proteins and are implicated in the regulation of several neurological disorders, among which PD (Foote & Zhou, 2012; Stevers et al., 2017). As was referred to before, Nichols and colleagues (2010) found that 14-3-3 binding influenced cytoplasmic localisation of LRRK2. Further research by Muda et al. (2014) showed that 14-3-3 binding is regulated by cAMP-dependent protein kinase (PKA) and that ser1444 in the LRRK2 Roc-domain is a target of PKA. Binding of 14-3-3 proteins to the ser1444 resulted in a decrease of LRRK2 kinase activity, hinting that the binding of 14-3-3 proteins will result in increased recruitment of LRRK2 to the cytosol and thus decreased LRRK2 activity. Well enough, same study also found that substitution of ser1441 to alanine all result in increased kinase activity. Interestingly, the same goes for R1441C/G/H mutations. More research on the effects of 14-3-3 binding was done in a later point of time. Lavalley et al. (2016) investigated the question whether 14-3-3θ (a human subtype of the 14-3-3

protein family) could regulate the neurite shortening and elevated kinase activity that is induced by G2019S-mutated LRRK2. The results showed that 14-3-3θ was able to reduce LRRK2 kinase activity, provided that it was directly bound to LRRK2. Making use of a 14-3-3 inhibitor, the study also confirmed that 14-3-3 proteins could reduce G2019S-mutated LRRK2 induced toxicity by reversing neurite shortening. Whether 14-3-3 proteins will form a legitimate therapeutic target in the future will depend on a few things, for example whether we will be able to selectively upregulate 14-3-3 binding on ser1441 or find a way around the downregulation of 14-3-3 binding in R1441C/G/H mutations. Still, when looking into localisation as a therapeutic target, it might be better to look at Rab29 and Rab32. As was constituted in *domains, mechanism of activation and mutations*, both Rab GTPases seem to function as recruiters of LRRK2 to the membrane. This might be an easier target, since inhibiting these compounds might mean that (pathogenic) LRRK2 is unable to form a dimer and/or become active. Another target that can be enforced when looking into LRRK2 localisation are GTP inhibitors. Recent research has pointed out that most known pathogenic mutations of LRRK2 cause it to relocalise to filamentous structures associated with microtubule-mediated vesicular transport processes in a GTP-dependent manner (Ramírez et al., 2017). GTP inhibitors are found to successfully interfere in this process, and thus make excellent therapeutic targets. Following this, recently research has not been able put out a lot of proper, selective GTP inhibitors, aside from the compound FX2149 (Li et al., 2015). This makes for another promising therapeutic target.

What now?

While PD is the most common known neurodegenerative disease, there is still little knowledge about any other type of treatment than symptomatic treatment. This paper has focused on the most promising therapeutic target to date: the LRRK2 gene. While research on LRRK2 already dates back to 2004, knowledge on how LRRK2 becomes activated (or how it activates itself), how it exactly interacts with its environment and thus how it can be targeted in an efficient way is still fairly insufficient. Research on LRRK2 as a therapeutic target in PD has mainly focused itself on the inhibition of kinase activation. Throughout the years, several LRRK2 kinase inhibitors have seen the light of day, yet none except DNL201 have made it through research as of yet. Problems like poor brain penetration and bad selectivity that are found with small-molecule LRRK2 kinase inhibitors keep popping up and the fact that kinases are an inherently complicated target (for they usually are involved in several or more pathways) does not help either. So while studies with compounds like MLi-2 seem to have a lot of potential and more auspicious research (such as the study on DNL201) is still being conducted, time has to tell if our efforts are justified. Studies on negative effects like the ones from Fell and colleagues and Baptista and colleagues (2018) pressure us to face the fact that extensive in vivo research has yet to tell us if LRRK2 kinase inhibition is as promising as we deem it to be.

The study by Tomkins et al. (2018) has showed us that LRRK2 interacts with a whole lot of proteins and genes that could all either potentially function as a target or help us understand more about LRRK2. The significance of these results underlines the importance of PPI's and the understanding of them. Please note that when it comes to PPI's and LRRK2, a lot has been left out for overview's sake. Furthermore, this thesis has discussed research regarding the interaction between LRRK2 and 14-3-3 proteins. This relation is deemed significantly important: results indicated that a 14-3-3 subtype was able to reduce kinase activity and reduce further toxicity inflicted in by G2019S-LRRK2. 14-3-3 proteins have also been confirmed to aid in LRRK2 localisation to the cytosol, and thus play a big part in the activation process of the protein. The antagonists in this process are Rab29 and Rab32, which recruit LRRK2 to the membrane where it becomes active. Studies on the inhibition of Rab GTPases in the light of LRRK2 inhibition have yet to be published, but for now seem like a promising subject to look into. When it comes to the disruption of dimerization, a lot of work still has to be done. Literature research made it apparent that there still is a great lack of knowledge on LRRK2 dimerization as a therapeutic target. However, whereas the inhibition of Rab proteins is likely to involve a legion of side effects, drugs synthesized to target the LRRK2 dimerization process might be less harmful. For instance, drugs designed to specifically competitively bind the GTP pocket of LRRK2 might be implicated in less regulatory pathways than Rab proteins.

There are still many more unexplored aspects of the gene that require our attention besides kinase inhibition: dimerization, localisation, GTP binding and PPIs are important examples of underexposed topics that could very well come to play a big role in targeting LRRK2 in PD treatment. In the long run it might be plausible that when we have gained more knowledge about the way LRRK2 interacts with its environment and how it becomes activated, different strategies can be combined into joint therapies for more effectiveness. For now, the future for LRRK2 research does seem bright: we just need some time to work it out.

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