The Role of Genome-Wide Association Studies in Understanding Sporadic Parkinson's Disease Susceptibility

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Summary

Parkinson's disease is one of the most common neurodegenerative diseases, occurring mainly in individuals older than 60 years. The disease causes severe motor disabilities of which tremor is the most frequent. Although many mutations have been shown to cause early-onset Parkinson, the sporadic late-onset form remains largely idiopathic (no known cause). Recent Genome-wide association studies have revealed a large pool of single nucleotide variations that increase the risk of developing this disease. In this review the genetic basis of Parkinson's disease will be discussed. The most prominent risk loci, identified in multiple association studies, will be linked to known gene mutations and affected pathways. Showing that early- and late-onset PD share a common genetic and molecular basis. The limitations of association studies will also be addressed, showing that we still have a long way to go in fully understanding the genetics behind this disease.

Abbreviations

PD	Parkinson's Disease	LRRK2	Leucine Rich Repeat Kinase 2
SN	Substantia Nigra	BST1	Bone Marrow Stromal Cell Antigen 1
LB	Lewy Body	HLA	Human Leukocyte Antigen
SNP	Single Nucleotide Polymorphism	PINK1	PTEN-induced Putative Kinase 1
GWAS	Genome-Wide Association Study	GBA	Glucocerebrosidase
SNCA	α-synuclein		

Introduction

As we grow older, we get more susceptible to aging diseases such as Parkinson, Alzheimer and dementia. These diseases are characterized by neurodegeneration in the brain and have, for most cases, an unknown cause.

Parkinson's disease (PD) was first described in 1817 by James Parkinson in his "*An assay of the Shaking Palsy"* and is now the second most common neurodegenerative disease after Alzheimer. Approximately 1% of the population older than 60 years suffers from PD, increasing to 4-5% in the 85-year-old population [1]. The mean risk of developing PD at any time is 1.5%, with a mean age-of-onset of 60 years and an average duration, from diagnosis until death, of 15 years [2].

Disease Phenotype

Parkinson's disease is accompanied by several distinct clinical features, which mainly affect motor functions. The disease generally shows a slow progression and might not be noticed for several years (the usual lag between diagnosis and first symptoms is 2-3 years [2]), the end stage symptoms are however clearly defined. Patients generally suffer from rest tremor (tremor in limbs at rest), bradykinesia (slowness in execution of movements), rigidity and postural instability [3], which are collectively termed Parkinsonism [2, 4]. Besides motor dysfunctions, PD can be accompanied by non-motor symptoms. These are however hardly used for diagnosis, even though they generally occur before the onset of motor symptoms [5, 6]. The most common non-motor symptoms are depression, dysautonimia (dysfunction of the autonomous nervous system), sensory loss (mainly smell), sleep disturbance and mild cognitive impairment [5, 7, 8].

Pathologically, PD is characterized by the loss of *dopaminergic neurons* in the *substantia nigra* (SN) and the subsequent decrease of stratial *dopamine* levels in the basal ganglia, which is generally accepted as the cause of the motor dysfunction [2, 9].

The neuronal loss is accompanied by Lewy body (LB) formation in the remaining neurons [10]. Which are enclosures of protein aggregates, mainly composed of the α-synuclein protein [11]. Although the precise events that trigger the neuronal loss are still unclear [12], the aggregates have been shown to correlate with activated *microglia cells*, which have repeatedly been found in PD brains [13-18]. It is therefore argued that neuronal degeneration might result from inflammatory processes against these protein aggregates [19, 20]. It is however still a lively debated whether or not these α-synuclein containing LBs are neurotoxic or neuroprotective [11]. α-synuclein has also been implicated in synaptic transmission processes [21, 22], aggregation of the protein could therefore lead to impairment of neuronal function and subsequent degeneration.

Lewy bodies can be subdivided in two classes; classical and cortical. Classical LBs are located in the brainstem, are spherical, 8-30μm in size with a hyaline core and peripheral pale-staining halo [11]. While cortical Lewy bodies are located in the small-to-medium-sized pyramidal neurons of layers V and VI of the cortex and lack the inner core and halo [2].

Lewy bodies are found in many different brain regions in PD patients and it has been proposed that widespread distribution of LBs in the brain corresponds to a variety of motor and non-motor symptoms [5, 11]. Although the role of LBs in neuronal degeneration is still debated, it has been shown that, in a PD patient's brain, a constant proportion of the SN neurons (3-4%) contain Lewy bodies, independent of the disease duration. This is consistent with the finding that LBs are constantly forming and disappearing in the SN of a PD affected brain [23].

In typical PD cases, neuronal loss and Lewy body formation, starts in the olfactory bulb, which is in line with the reported loss of smell in many PD patients [24]. From there the disease spreads in well-defined stages [25]. First toward the midbrain and brainstem and finally to cortical areas [9, 25], although it has to be noted that a substantial portion of the PD patients do not follow this progression pattern [26, 27]. Motor dysfunctions become evident only at third stage of the disease, with the substantial loss of dopaminergic neurons in the SN [9]. The pathological lesions and characteristics of PD are therefore developing long before the first motor impairments become clinically visible [25]. With the larger spread of the Lewy body pathology and greater loss of neurons in brain regions, PD patients develop other symptoms besides motor dysfunctions, like dementia and hallucinations.

Progression and treatment

Progression of PD is believed to occur via the cell-to-cell spread of α-synuclein aggregates [28], which have been shown to spread via endo- and exocytotic pathways [29, 30]. α-synuclein aggregation is a self-progressing process, as miss-folded α-synuclein acts as a template for other proteins to deposit upon [10, 25]. Considering the cell-to-cell spread and the self-progression of α-synuclein aggregation, Parkinson's disease has to initiate only once in a single neuron. Making the disease a more stochastic event based on genetic susceptibility and environmental triggers than a truly heritable disease [10].

A lot of research has therefore been performed on identifying the events that trigger PD onset and the genetics that underlie its susceptibility. However up to today, the disease itself remains incurable. Treatments with either drugs or surgery only improve the quality of life and functional capacity of patients but are unable to stop the disease progression.

One of the most frequently used drugs in PD treatment is *L-DOPA* the precursor of dopamine, though a large pool of other drugs have been developed and tested [Reviewed by 31]. L-DOPA is used to re-establish the decreased dopamine level caused by loss of dopaminergic neurons in the SN.

Most patients receive a daily dose of 300-600mg for the first 5 years after diagnoses and within two weeks show an improvement of symptoms over the course of three months [32]. It is however still debated whether or

not L-DOPA is toxic and increases PD progression [33-35], though a good response to the drug is generally used as confirmation for the PD diagnosis.

Surgical treatments are applied as well, but usually only if the medication fails to improve the symptoms or induces too many side effects. Two recently developed treatments are deep brain stimulation [Reviewed by 36, 37, 38] and transplantation of neuronal stem cells in the affected brain region [39]. These treatments are performed in a relative small number of patients. Neuronal transplantation is for example only performed in the USA and in Sweden, as the long-term effects are still unclear [40, 41]. While deep brain stimulation is reserved for the most severe patients, as the exact mechanisms of how the treatment improves PD symptoms remains unknown [42].

Genetics of Parkinson's disease

Parkinson's disease is generally regarded as an aging disorder, occurring sporadically at older age (> 60y) and more frequent in men than in woman [43]. However, 4% of patients develop an early-onset form before the age of 50 [2], while ~20% of the PD patients have a first-degree relative with the disease [44]. These numbers indicate that PD is not merely a sporadic disease, caused by environmental factors, but contains a substantial genetic factor. Although clear forms of mendelian inheritance of the disease are rare [45], several genes have now been identified that, when mutated, contribute to the early-onset of the disease [See also: 10, 46-48].

The genetic contributors to late-onset PD have long remained elusive and the disease has therefore long been viewed as an environmental disease with no genetic contribution. Recent studies have however identified several genetic loci, that increase the risk of developing sporadic PD and the disease is now generally believed to result from a combination of genetic susceptibility and environmental factors [43, 49, 50].

The majority of genetic contributions to sporadic PD reside in *single nucleotide polymorphisms* (SNPs), which are spread across the human genome. The nature of genetic contribution in PD is therefore based on two opposing hypotheses. 1) Common variants with a high genetic frequency but low

Dopamine is the main neurotransmitter of dopaminergic neurons and is thought to modulate motor response processes by setting a "threshold" for movement initiation. High levels of dopamine lead to high motor activity and impulsive behavior while low dopamine levels causes stiffness and reduced movement (as found in Parkinson's disease patients).

Dopaminergic neurons are small pool of neurons, mainly located in midbrain regions. They are the main source of dopamine for the brain. Though few in numbers, they play important roles in brain functions such as voluntary movement and behavioral processes, including mood, reward and stress.

The **Substantia Nigra** is located in the midbrain region and involved in movement control, reward and addiction processes. The region is termed "Nigra" or black, as it appears to be darker then other midbrain regions. A characteristic of Parkinson's disease is the substantial loss of dopaminergic neurons in this region.

Microglia cells are resident immune cells in the brain and spinal cord and therefore the main immune defense of the central nervous system. They make up 10-15% of the total glia-cell population and are constantly searching the brain for damaged neurons and infectious agents. It is proposed that the neuronal loss in Parkinson's disease patients is due to the action of microglia cells against the α-synuclein aggregates.

L-DOPA is the precursor of dopamine and used in drug treatments to increase the quality-of-life for Parkinson's disease patients. The drug is used to increase the level op dopamine in patient's brain and improve motor control. As L-DOPA can pass the blood-brain barrier while dopamine cannot, the drug is usually co-administrated with dopa-decarboxylase inhibitors to prevent conversion and side effects outside the brain

penetrance are the major contributors to the disease. Suggesting that PD is caused by a combination of frequently occurring genetic variations, which individually have little effect [51, 52]. 2) Disease onset depends on several rare variations with high penetrance. Suggesting that a small pool of rarely occurring variants cause PD, each having a great effect on the disease susceptibility [50, 51, 53].

The last decade, several *genome-wide association studies* (GWAS; Table 1) have been performed on various populations to identify risk loci for sporadic PD. The first GWAS was performed among American patients and linked 13 SNPs to the onset of sporadic PD [54]. Subsequent replication studies have however failed to replicate these results and the role of these loci in disease onset is therefore highly debated [55-57].

In recent years, with the advance of better sequencing and analysis techniques, more GWAS on sporadic PD have been performed. This has resulted in a large number of risk loci and SNPs associated with the disease. Despite the difference in GWAS size, included SNPs and tested population, many risk loci have been independently identified in multiple studies (Table 3), confirming their role as risk loci for the development of sporadic PD.

SNCA

α-synuclein (SNCA) was the first gene associated with PD, discovered in a genetic heritability study among a Greek family with autosomal dominant PD [58]. SNCA encodes for the α-synuclein protein, which is present in high concentrations in neuronal tissues and found predominantly in presynaptic terminals. Altogether the protein makes up 1% of the total number of proteins in the brain [59].

The protein has been shown to play an important role in synaptic release, homeostasis of dopamine levels in synaptic vesicles and has been implied to be involved in the ubiquitin proteasome system [2, 22, 60]. Furthermore the protein promotes the formation of the SNARE complex, thereby regulating neurotransmitter release [21].

Knockout of the a-synuclein gene in mouse models showed that the protein is indeed essential for the regulation of neurotransmitter release from specific midbrain dopaminergic neurons. Loss of synuclein led to a change in dopamine handling by presynaptic terminals, making the brain vulnerable to PD [61]. Overexpression of a-synuclein in various animal models resulted in aggregation of the protein and subsequent cell death [62]. Transgenic mice that overexpress either wild type or mutant α-synuclein under various promoters show neuronal inclusions, mitochondrial abnormalities and neurodegeneration [63-65].

To date five SNCA mutations have been confirmed to cause autosomal dominant PD; Ala30Pro, Glu46Lys, His50Gln, Gly51Asp and Ala53Thr [3, 11]. Although these mutations all trigger the onset of PD, they are associated with different disease phenotypes. The mutation Glu46Lys is, for example, associated with the same clinical features as patients with Lewy body dementia, in contrast to patients with the Ala30Pro mutation, which do not develop severe dementia.

Gene mutations are not the only form of α-synuclein induced PD, 1-2% of the PD patients carries a duplication of the SNCA gene [3]. Triplications have also been reported but occur less frequently and are rarely found in sporadic PD cases [66, 67]. Individuals with a SNCA triplication generally develop an early onset form of PD with rapid progression and more extended neurodegeneration [68]. Overall, duplication and triplication results in similar pathologies as overexpression of the gene in mouse models.

Besides causing early-onset heritable PD, almost all GWAS studies identified SNCA as a major risk locus for sporadic PD. Most associated SNPs are located in the 3' region of the gene, suggesting that they influence expression, regulation, RNA stability or splicing of the gene [50].

Almost all SNPs were first identified in a Caucasian population, but have now been confirmed in many countries. The SNPs rs11931074, rs2736990, rs894278 and

Table 1 : Overview of all Genome-wide Association Studies performed in the last decade to identify risk loci for sporadic Parkinson's disease.

GWAS	Year	Population	Cases	Controls
Maraganore, et al [54]	2005	USA	775	775
Fung, et al [69]	2006	USA	273	271
Pankratz,et al [70]	2009	USA	857	867
Simon-Sanchez, et al [71]	2009	USA, Germany, UK	1713	3978
Satake W, et al [72]	2009	Japanese	2011	11381
Edwards, et al [73]	2010	USA	1752	1745
Hamza, et al [43]	2010	USA	2000	1986
Saad M, et al [74]	2010	French, UK, Australia	1039	1984
Simon-Sanchez, et al [12]	2011	Dutch	772	2024
Do CB, et al [75]	2011	USA	3429	29624

rs6532194 were confirmed in the Korean and Chinese population, though some displayed an opposite effect (protective instead of risk increasing) [76-79]. Further linkage study revealed that the SNP rs11931074 is highly correlated to either rs2736990 or rs6532194, but not with any other SNCA SNP [76]. Showing that risk associations can be the effect of several interlinked SNPs.

A meta-analysis of several GWAS studies identified another SNP (rs3562190) [80] and this association has been confirmed among the Chinese, Spanish, American and Norwegian population [81-85]. However, a study in Japan failed to replicate the result [86], only when taking smoking habits into account became the SNP significantly associated with PD. Two other SNPs, rs356220 and rs2736990, have now also been confirmed among the Japanese [86] and the latter also among patients from the UK [87]. Although GWAS studies are a powerful tool to identify risk loci, single small-scale linkage studies are still able to identify novel SNPs. So was the SNP rs7684318 not identified in the GWAS among the Japanese population, but in a small-scale linkage study [88]. The SNP is located in an intron of the SNCA gene and has been confirmed as risk loci among the Han Chinese population [89].

LRRK2

The Leucine Rich Repeat Kinase 2 (LRRK2) gene was linked to PD development in a Japanese family showing autosomal dominant parkinsonism [90]. Mutations in this gene, of which more then 20 have now been reported [91], are the most common cause of autosomal dominant PD [92, 93].

LRRK2 is a 51-exon protein with various functional domains [94, 95]. The protein is highly expressed in the brain, with high mRNA levels found in the stratium and hippocampus [96]. The exact biological functions of LRRK2 are unclear, as no substrates have been identified so far. The protein has however been shown to interact with parkin [97] and has been linked to various cellular processes. The protein is involved in signaling pathways as a kinase [98], has been shown to control synaptic morphogenesis in Drosophila [99] and is associated with cytoskeleton dynamics [100].

The most common LRRK2 mutation is Gly2019Ser in the MAP-KKK domain [101, 102], explaining up to 40% of the autosomal dominant cases [103]. Although the mutation has been shown to cause autosomal dominant PD, it should not be considered a strict Mendelian disease mutation, as environmental cues still play an important role in the onset of PD [45]. An individual with this mutation has a 28% risk of developing PD before the age of 60, increasing to a 74% risk at the age of 79 [104].

Like SNCA, LRRK2 is linked to both early- and late-onset PD, as recent GWAS have associated the gene region with sporadic onset of the disease [12, 43, 71, 72, 103]. These studies identified several SNPs close to the gene that either increased or decreased the risk of PD development. Most SNPs have now been confirmed in various American

populations [43, 71] and one SNP has been replicated among the Japanese [72]. Other small-scale linkage studies further confirmed and replicated SNPs in both the Korean (rs34778348) and Chinese (rs1994090, rs7304279, rs2046932) populations [76, 79].

Besides these SNPs, several mutations in LRRK2 are now also shown to be associated with sporadic PD. The Met1646Thr mutation, for example, increases the risk for PD with 50% in Caucasian population, while the Ala419Val mutation results in a 2.2-fold increased risk for developing PD among the Asians [105]. Furthermore the Asn551Lys-Arg1398His haplotype displayed a protective function among the Chinese population [106]. In line with the Asn551Lys-Arg1398His-Lys1423Lys (which is found in 5% of the population), that was shown to be protective among Caucasian and Asian populations [105]. These results indicate that besides SNPs located in non-coding regions, mutations in a gene coding region also influence the risk of developing PD sporadically and that mutations are not directly coupled to the deveopment of the disease.

BST1

Another identified risk locus for sporadic PD is the Bone Marrow Stromal Cell Antigen 1 (BST1) locus, located on 4q15. The locus has been associated with PD by GWAS studies in several populations, including Japanese, Caucasian Americans and Europeans [12, 43, 72, 74], where is showed a slightly higher effect in Asian populations then in Caucasians [72, 107].

The association of BST1 as risk factor for PD has been replicated in subsequent studies, where they showed that the SNP rs46989421 was associated with PD in the Europeans [74] and mainland Han Chinese [79]. Other studies have however failed to replicate this result in the Northern Han Chinese [78, 108] or Japanese population [109]. These results show that genetic variability and subsequent disease susceptibility are greatly dependent on the population's genetic background.

A large replication of GWAS meta-analysis later identified the SNP rs17724635, which was associated with a decreased risk for PD in the American, European and Asian populations [80, 107]. This association could however not be replicated in subsequent studies among the Japanese and Taiwanese populations, although the SNP reached a significant association when the consumption of well water was taken into account [109, 110]. The failure to replicate these results could be due to the smaller sample size of the replication studies, which lowers the statistical power to detect variations with a small size effect, and shows that environmental cues are of great influence in the development of PD. Overall the associations of the BST1 risk locus remains to be further investigated to determine its effect across different populations.

HLA-region

The Human Leukocyte Antigen (HLA) region is one of the most complex regions of the human genome, with a high density of closely linked genes. The genes in this region encode for the major histocompatibility proteins, which are antigen presenting proteins essential for a functional immune response against pathogens. The region is located on chromosome 6p21 and is associated with several autoimmune, infectious, malignant and neurologic disorders. HLA-DRA positive microglia cells have, for example, been shown to contribute to the neuroinflammation process in PD and Alzheimer brains [15, 111], linking the neurodegenerative diseases to the immune system.

The association of SNPs in the HLA region with PD development was identified in a study among the British population [112], where it was discovered that a SNP in the HLA-DRB gene was associated with an increased risk of developing PD. This association was later confirmed by a GWAS among the American, Dutch and French population, though these studies identified a SNP in the HLA-DRA region (rs3129882) [12, 43, 113].

A major problem with SNP association to a specific disease is the variation of genetic background in different populations. A clear

example is the rs3129882 SNP, which has different allele frequencies among different populations [114]. This variation could explain the recent conflicting studies, in which the loci could not be associated with PD among the Swedish, Norwegian, Spanish and Taiwanese population, while in Irish and Polish populations, the SNP displayed a protective function [84, 114-117].

Altogether, three SNPs (rs3129882, rs9268515 and rs2395163) have recently been replicated and confirmed to be associated with PD, although the real risk effect greatly depends on the population tested [118]. This suggests that the region is not a direct cause for the disease, but rather co-regulates disease onset.

MAPT

The MAPT gene encodes for the tau protein, which has been shown to aggregate together with α-synuclein in Lewy bodies [50]. Several GWAS studies have identified SNPs in both the American and European population [12, 71, 73-75], and these have been confirmed in an independent linkage study [84].

Surprisingly this risk locus is only associated with PD in the Caucasian population as no GWAS or other linkage studies in the Japanese population found any association [72]. This might be due to an ancient inversion of ~900kb in the MAPT region, that resulted in two distinct haplotypes in the European population [107, 119]. Haplotype2 is the most associated with PD, but is absent in the Asian population. Explaining the lack of association of the MAPT region in studies among Asians [72].

PARK16

The PARK16 risk locus was originally identified in the Japanese population and is located on chromosome 1q32 [72]. Several SNPs have been identified that increase the risk of developing PD. Later GWAS studies confirmed this locus in the USA, UK, Germany, Holland, Spain and China. [12, 71, 78, 84]. Furthermore the SNPs rs947211 and rs11240572 in this region have also been confirmed in the Korean population [76].

Surprisingly, two other SNPs (rs812128 and rs16856139) were associated with an increased risk for PD in the Japanese population, but were linked to a decreased risk in the Caucasian and Chinese population. These results again implicates the effect of allele frequencies on association studies, as the *minor allele frequency* of these SNPs is ~20% in the Japanese population but only 3% in the white population.

GWAS specific risk loci

The previous section focused on those risk loci that have been identified in multiple GWAS and that were subsequently confirmed in other population using smaller-scale linkage studies. However, as most GWAS are performed on distinct populations, risk loci that have been identified in a single GWAS, may indicate a population-specific factor.

Four GWAS specific risk loci are; BRDG1, 12q24, GAK/DGKQ and SCARB2. BRDG1 has been identified in one of the first GWAS performed on PD patients in the USA and has never been replicated [69]. The gene encodes for the STAP1 protein, which has been implicated in inflammatory processes and is found in activated microglia [120]. The real association with PD however remains to be confirmed by other studies.

The same goes for the 12q24 loci, although 2 GWAS studies identified the SNP rs4964469 as a risk factor for PD [12, 74]. This result could not be replicated, among the Chinese [77] and none of the genes, located in this region, have so far been shown to be involved in PD processes.

Another risk loci identified in only a single GWAS, is the GAK/DGKQ region. The GAK protein is a cyclin G associated kinase and is differently expressed in the SN in PD patients compared to healthy controls [121]. Furthermore, its kinase activity has been correlated with α-synuclein expression levels in neuronal cells [122]. The DGKQ protein is highly expressed in the hippocampus and cerebellum, but has so far not been functionally linked to PD.

The region has been identified in the Caucasian population [70], but never replicated or confirmed by other GWAS

studies. Recent genetic studies among Taiwanese and Chinese showed a mild association of this region with PD. Two SNPs (GAK:rs1564282 and DGKQ:rs11248060) have been confirmed to increase PD risk in the Chinese population [123], but no SNP reached a statistical significant association among the Taiwanese [116]. Indicating that the region might indeed be associated with PD development but needs further confirmation.

The last associated region was identified among Caucasians patients in the USA and is located close to the SCARB2 gene [75]. The identified SNP is located within the intron of the FAM47E gene, which is located upstream of SCARB2. The SCARB2 gene encodes for the LIMP-2 protein, is highly expressed in the brain and has a key role in lysosomal pathway. This association has been replicated in the Greek population [124]. However, its role in the onset of PD is still heavily debated. As both its association in the Caucasian population as well as among the Greek are contradicted by other studies and could not be replicated among the Han Chinese population [125-128].

Meta-analysis refines risk loci

As shown in the previous section, sporadic Parkinson's disease is a complex disease caused by interplay of various genetic risk factors, that individually only harbor a small effect on the disease onset. One of the main problems of GWAS studies is therefore that, with a relative small sample sizes, they are underpowered to identify loci with a small effect size or rare occurring variants. To overcome this problem, recent studies have combined and re-evaluated the data from GWAS studies in so-called meta-analyses [80, 129-132]. As these studies combine the genotypic data of multiple GWAS studies, the sample size greatly increases, making it possible to better identify and associate rare occurring variants and small effect variants.

One of these meta-analyses compared 8 different GWAS studies conducted among the European population [132] and revealed that 27% of the total observed variance is associated with any form of PD. Moreover, 15% explains early-onset and 31% of the identified variation was associated with late-onset PD. This analysis confirms that a large proportion of the genetic susceptibility has not yet been accounted for by the recent GWAS. They subsequently showed that the demographic history of the sampled population has a major impact on the identification of risk loci and calculated heritability. The more heterogeneous the genetic background of a population, the more difficult it is to identify weak associations. As was shown by comparing the relative homogeneous Icelandic population to the more heterogeneous French and British populations [132].

Several other meta-analyses have now been performed and many risk loci have been confirmed. The BST1 locus for example, has been confirmed [80] and was shown to have a more severe effect in the Asian population compared to the Caucasians [107]. Nalls et al 2011 reconfirmed the LRRK2 risk loci and identified new SNPs in the SNCA (rs356219) and MAPT (rs2942168) loci [80]. Another meta-analysis by Pankratz et al 2012 further confirmed the SNCA and GAK loci, while new SNPs were associated in the HLA-DRA (rs23951630) and MAPT (rs199515) loci [130].

A major drawback of meta-analysis is that not all GWAS studies included the same SNPs and that, due to the allele frequency differences, only loci that harbor a risk in all tested populations could be identified. Nevertheless, recent meta-analyses not only confirmed known risk loci, but were also able to reveal novel factors.

One of these novel associations is the RIT2 locus [130], of which the expression levels were previously shown to be decreased in the SN of PD patients [133]. Moreover, the protein can indirectly bind to α-synuclein, suggesting a role in PD associated pathways. This locus has been identified by the SNP rs12456492, however this result could not be replicated among the Taiwanese population and thus requires further evaluation [134].

Another novel locus is the MCCC1/LAMP3 locus [80], which has been identified with the SNP rs11711441 and has later been confirmed in a regular GWAS study among

American Caucasians [75], although they identified the locus with a different SNP. More recent linkage studies also confirmed the association to PD in the Chinese populations [135, 136]. However, as the precise functions of these genes are unclear, their role in PD development remains to be determined.

The last novel risk locus is the MTHFR gene on 1p36, which encodes for a methylenetetrahydrofolate reductase and has been identified in a meta-analysis between European and Asian GWAS [137]. The associated SNP (rs1801133) is located in the coding region of the gene, resulting in a C-to-A missense substitution and the Ala222Val mutation. This mutation reduces the proteins enzyme activity and has recently been shown to modulate the age of onset of PD patients, confirming its role as risk factor [138].

Genetic heritability in Parkinson's disease

The previously discussed GWAS and meta-analysis studies have been preformed to link specific genetic variations to the development of sporadic PD. However SNPs are not the only genetic factors that influence the disease susceptibility. As shown for SNCA and LRRK2 gene mutations are also involved, although they generally contribute to heritable early-onset PD rather than sporadic forms of the disease. Besides the above-mentioned mutations, several other genes have now been shown to cause early-onset PD. Though their mutations have not been identified by GWAS studies, they are worth short mentioning as they further illustrate the genetic complexity of the disease.

Parkin and Pink1

Parkin (PARK2) was the first genetic locus linked to autosomal recessive PD and is located on chromosome 6. The protein is associated with mitochondrial degradation processes, as it labels defective mitochondria [139, 140]. Parkin might also be involved in the ubiquitin proteasome pathway as an E3 ubiquitin ligase because the C-terminus of the protein binds with ubiquitin E2 enzymes [2, 60]. Moreover, a-synuclein has been identified as parkin substrate for ubiquitination [141, 142].

The gene is typically mutated in early onset forms of PD and explains approximately 50% of the autosomal recessive PD cases. Mutations in the gene have not been directly linked with PD pathology, but heterozygous loss of the functional protein has been associated with Lewy bodies, neurofibrillary degeneration and brain stem restricted neuronal loss [2].

PINK1 (PTEN-induced putative kinase 1) is located on chromosome 1p36 and has been associated with autosomal recessive early-onset PD with a slow disease progression and

Single Nucleotide Polymorphisms are the most common type of genetic variation between individuals, representing a single nucleotide sequence variation that occurs frequently in a population.

Genome-wide association studies are used to link specific genetic variations to the risk of developing a certain disease. In general, these studies compare a genome-wide pool of SNPs between a disease and healthy control group, to identify alleles that are found more often in the disease group. The identified allelic variations are termed risk loci, as they are associated with an increased risk of obtaining the disease.

The **Minor allele frequency** is a measure for the occurrence of a SNP in a population. A SNP is a site in the genome, were two different nucleotides can occur at the same position. The least frequently occurring base is called the minor allele. SNP occurrence is measured by the percentage of individuals that carry the minor allele, which may differ between populations. For example, a SNP can have a 5% occurrence in the American population, while in Asia this SNP occurs in 20% of the population.

In GWAS studies, the **odd ratio** depicts how strong a certain SNP is associated with a disease. For example, an odd ratio of 2 means that individuals that carry that SNP have a 2-fold risk increase of obtained the disease. An odd ratio lower then 1 subsequently means that a certain SNP is more frequently found in the control group and is thus deemed protective.

L-DOPA responsiveness. The PINK1 protein is believed to protect cells from stress-induced mitochondrial defects as the protein contains a mitochondrial targeting domain and mainly localized these organelles. Most identified mutations so far are missense, although whole-genome deletions have also been reported [143, 144]. PD caused by PINK1 mutations is associated with Lewy bodies and neuronal loss pathology in the SN [145].

DJ-1 and GBA

Both DJ-1 (PARK7) and Glucocerebrosidase (GBA) have also been implicated in autosomal recessive forms of PD and have not been identified in GWAS studies. Patients with DJ-1 mutations develop early-onset PD, similar to mutated parkin, in combination with scoliosis (curvature in the spine), blephalospasm (uncontrolled blinking) and psychiatric symptoms. DJ-1 is present in synaptic terminals and mitochondria and has been shown to regulate mitochondrial oxidative stress [146, 147]. To date three missense mutations have been associated with PD in the Italian, Dutch and Uruguayan populations (Lys166Pro, Met26Ile, Glu64Asp) [148, 149].

The relation of GBA with PD is more elusive. Homozygous loss of the protein causes Gaucher's disease, while heterozygous loss increases the risk of developing PD [150]. It has been shown that Gaucher patients who survive into adulthood, develop PD with Lewy body pathology [150, 151]. Of the Jewish individuals with PD, 29% have a GBA mutation and have a 30% overall change of carrying a mutation in either GBA or LRRK2 [152]. On the other hand only 4% of the UK PD patients carry a GBA mutation, showing a high diversity in PD risk across distinct populations. A recent study showed that mutated GBA increased the risk of developing PD 5-fold, making it the strongest genetic risk factor so far for mendelian PD [94].

Pathways in Parkinson's disease

Many sporadic PD risk loci are also associated with heritable PD, suggesting that there is a strong genetic link between the two disease forms and that, most likely, common pathways are affected. To identify these pathways, a recent study combined GWAS and meta-analysis data with known biological functions and pathways of the identified genes [153]. They identified two novel SNPs (rs17651549 and rs10445337) located in the MAPT gene that alter the amino acid sequence of the protein. These SNPs are associated to neurogenesis, regulation of neurogenesis and positive regulation of axongenesis. MAPT is known to regulate microtubule stability and might be involved in maintaining neuronal polarity. The here-identified SNPs could impair the function of the MAPT protein, similar to mutations that cause shorter isoforms of MAPT. Which have been associated with neuronal degenerative diseases [154]. Showing that PD-associated SNPs can be linked to a biological function or specific pathway. The authors postulate that by focusing on pathways it is more likely to find SNPs with modest association as it takes the genetic interplay between loci in account [153].

Mitochondrial pathway

The link between PD and defective mitochondria was made after the discovery of mitochondrial complex I deficiency in the SN of PD patients [155] and confirmed with the discovery that mitochondrial toxins can induce PD in animal models [156].

Mitochondrial defects are mainly found in patients with a mendelian form of PD and have been linked to mutations in either parkin or PINK1 [10], as both genes have known roles in the clearance of defective mitochondria [157, 158]. Several studies have further confirmed their roles and showed that PINK1 binds and accumulates on the outer membrane of defective mitochondria. There the protein recruits parkin, which ensures mitochondrial degradation via autophagy [159-161].

Furthermore loss of PINK1 results in a similar phenotype as that of mutated parkin, namely the accumulation of defective mitochondria. Overexpression of parkin rescues this phenotype, showing that parkin acts downstream of PINK1 [157, 158, 162]. Recently it was shown that USP30 opposes the function of parkin and PINK1 by inhibiting

mitochondrial degradation. Inhibition of USP30 led to a better survival of flies with induced PD, further confirming that mitochondrial clearance defects are indeed a major contributor to PD development [163].

Lysosomal pathway

Accumulation of α-synuclein aggregates is a predominant characteristic for both early and late onset PD brains, likely due to defects in the lysosomal pathway. Defective lysosomes have been linked to PD via the GBA protein, which has been associated with an increased risk for sporadic PD in the American Caucasian population and linked to autosomal recessive early-onset PD [2, 75]. Mutated GBA leads to a reduced lysosomal function, causing accumulation of α-synuclein aggregates [3].

Another gene linked to lysosomal processes is LRRK2 [62]. The gene is mutated in many mendelian PD cases and associated with sporadic PD by various GWAS and meta-analysis studies, showing that the gene has a major impact on the disease onset. Though the exact molecular role in the brain remains unclear, the gene has been linked to lysosomal processes and the Golgi apparatus via vesicle transport processes [164, 165]. LRRK2 has been shown to interact with the Rab7L1 protein, which is located on the PARK16 locus. Rab7L1 deficiency results in similar neurodegeneration as LRRK2 mutations and LRRK2 mutations can be rescued by overexpression of Rab7L1. Rab7L1 itself has been shown to play a role in endo- and exocytotic pathways [72]. Therefore, LRRK2 likely collaborates with Rab7L1 in vesicle trafficking pathways and defects in these systems could cause accumulation of α-synuclein, increasing the risk for PD [100, 164].

LRRK2 has also been proposed to directly influence α-synuclein aggregation, as the Gly2019Ser mutation led to an increase in phosphorylation of α-synuclein at position Ser129. It still needs to be determined if LRRK2 directly interacts with α-synuclein [62, 166]. Indirect genetic interactions have however been shown, as mice with the SNCA Ala53Thr mutation display a more rapid neuronal loss with overexpression of LRRK2

Table 2: Overview of the here discussed genes that are either mutated in Parkinson's disease cases or carry SNPs that influence the risk of developing the disease.

Gene	Processes	Role in Parkinson's disease
SNCA	Signaling, membrane trafficking	Regulates dopamine levels in synaptic terminals and neurotransmitter release
LRRK	Signaling, vesicle trafficking	Controls synaptic morphology, regulates vesicle trafficking to lysosomes
BST1	Immune response	Immune-response; Facilitates b-cell growth
HLA-DRA/B	Immune response	Subunits of antigen presenting proteins
MAPT	Neuronal polarity	Promotes microtubule assembly and stability
BRDG1	Immune response	Regulates microglia morphology and migration
GAK	Vesicle coating	Clathrin-mediated endocytosis
DGKQ	Signal transduction	Unknown
SCARB2	Lysosomal pathway	Targets GBA to lysosomes
RIT2	GTPase	Binds α-synuclein and tua proteins
MCCC1	amino acid catabolism	Unknown
LAMP3	Tumor cell migration	Unknown
MTHFR	Methyl cycle	Unknown
Parkin	Mitochondrial clearance	Labels and ubiquitinates defective mitochondria
PINK1	Mitochondrial clearance	Targets Parkin to defective mitochondria
DJ-1	Mitochondrial stability	Protects neurons against oxidative stress
GBA	Glycolipid metabolism	Unknown
GRIN2A	Glutamate receptor	Regulator of neurotransmitter release, regulates behavior and movement

Gly2019Ser compared to overexpression of wild-type LRRK2. Furthermore, mice with only the LRRK2 Gly2019Ser mutation did not develop this phenotype [62, 166]. This indicates that LRRK2 and SNCA genetically interact and that mutated LRRK2 might contribute to the disease progression by accelerating α-synuclein aggregation.

Overall, mutations in LRRK2 most probably influence α-synuclein accumulation by accelerating aggregation and inhibiting the degradation via lysosomes [167]. However, these interactions need to be further examined as other studies have failed to recreate the observed phenotype in mice [168, 169].

Inflammatory pathways

The lack of control over motor functions for PD patients is caused by increased neuron degradation as the disease progresses. This degradation is likely aggravated by inflammatory process against the accumulation of α-synuclein aggregates. An *in vitro* study showed that extracellular aggregates activate microglia in a neuron-glia cell culture [13]. Suggesting that upon neuronal degeneration, α-synuclein aggregates are released in into the SN, activating microglia and further enhancing the disease progression.

Inflammatory response in PD can be linked to the BST1 gene, which has been associated with sporadic PD in both Asian and Caucasian population [12, 43, 71, 72, 74]. Although the precise effect on microglia cells is yet unknown, the gene has a 33% similarity with CD38, which is a known protein found on immune cells. The gene has been shown to facilitate B-cell growth and might activate microglia cells in PD patients [12, 73]. The role of inflammatory response in respect to neurodegeneration has recently been reviewed, further supporting the notion that chronic inflammation is an important factor in PD development [170].

Parkinson and other diseases

Neurodegeneration is not only observed in PD, but is also a major contributor to other age related diseases like Alzheimer, suggesting that similar genes or pathways might underlie these diseases. Several studies therefore recently compared the identified PD risk loci with those of other diseases.

Maybe the most striking result of these studies is that hardly any correlation between the risk loci for PD and the risk to develop another disease has been identified. Implying that neurodegenerative diseases have a distinct set of risk loci. For example, Vitamin D processing deficiency has been linked to Alzheimer, but evaluation of the specific risk loci revealed no correlation with PD [116], although two SNP were weakly associated in the Taiwanese and Korean population in other studies [171, 172].

Lewy body pathology in the SN is another clear hallmark of PD development, which can also be found in the brains of patients with Gaucher's disease. Besides, PD has been functionally linked to Gaucher's disease as loss of GBA causes accumulation of α-synuclein aggregates [173]. None of the identified risk loci for PD are however also a significant risk factor for this disease and vice versa [76, 174]. Other suggested diseases are melanomas [175], Crohn's Disease [176], breast cancer [177, 178] and rheumatoid arthritis [179, 180]. Although different linkage studies suggested interplay between the diseases, the underlying molecular mechanisms remain unclear and the associations are weak if at al present [20, 181].

Environmental factors

Despite the recent GWAS studies and meta-analysis performed to identify genetic risk factors for PD, ~40% of the cases remain unexplained [80, 131] and many susceptibility loci remain to be found [50]. This is partly due to the large environmental factors that play a role in the onset of the disease.

Studies to the triggers of PD have revealed environmental factors that increase the risk of developing PD, while others tend to have a protective function. Factors that mildly increase the risk of developing PD are, among others, rural living, middle-age obesity, lack of exercise and long-term exposure to toxins and heavy metals [182-184]. Frequent head

injury has also been suggested to increase the risk for PD, although this result is still debated [185, 186].

Opposite to increased risk, some environmental factors have been implicated to be protective for PD. These factors include smoking and the consumption of coffee and alcohol. Smoking has been associated with a decreased risk of PD in several studies and it has been shown that never smokers are twice as likely to develop PD [76, 86, 110]. Similarly, drinking no or little amounts of coffee has been shown to increase PD risk by approximately 25% [187, 188], while mild alcohol consumption has been weakly associated with PD protection [189, 190].

GWAS limitations

GWAS analyses are powerful tools to identify genetic contributions to a specific disease but have a few clear limitations. First of all, the term genome-wide is rather misleading, as an average GWAS study only includes SNPs with a minor allele frequency of 5% or higher, resulting in an average of ~500.000 SNPs. Therefore approximately 20% of the common SNPs are not or only partially tagged via linkage disequilibrium, while rare variants are generally not tagged [51]. Furthermore, most risk loci have only minor effect sizes, making it necessary to use large sample sizes to identify them with a statistical significance.

GWAS studies are based on the simultaneous analysis of thousands of SNPs, the general accepted rule is therefore that a SNP has to obtain a p-value of less then $5*10^{-6}$ to assure a very low prior probability that any given locus is associated with the disease [191]. This results in the problem that small studies have little power to detect significant associations [192]. When considering the hypothesis that the genetic burden is the result of many independent genetic factors of small effect size, a substantial portion of genetic factors might not reach this significance threshold. The general idea is that to obtain *odd ratios* of ~1.1, association studies should be in the 60.000 sample scale [51].

Overall, GWAS studies are relative expensive, harbor a low cost-benefit ratio and require large-scale collaborations with only a minor yield in terms of identified risk loci. Despite the recent meta-analyses, association studies have been unable to explain the heritability and have difficulty to link associated loci to a functional basis of the disease [51, 153]. For example, the exact mechanisms by which the SNCA and MAPT loci influence PD onset remain unclear [73, 193].

The low yield and minor significance of associations could be due to population differences, although most GWAS studies have been performed in white Caucasian populations [194]. Variance in the genetic background of a population are a primary source of false-positives and reduce the change to identify risk loci with a small effect size, due to differences in allele frequencies between different genetic background. A good example is the PARK10 locus, which has been identified in the relative genetic homogenous Icelandic population [195], but which could not be replicated in the American population [196]. Similar results have been shown for the HAS2 gene which was shown to have a minor effect on PD but which did not reach genome-wide significance in GWAS studies covering American and Chinese populations [43, 197].

A recent study among the Ashkenazi Jews identified 6 new risk loci [198]. While another large-scale study replicated the association of 11 SNPs across 21 countries (of which 16 were Caucasian) [107] and was able to reproduce most of the risk loci, except for the variable HLA region. Together these studies stress the influence of the genetic background on GWAS. The more heterogeneous the genetic background of a population, the more difficult it is to significantly identify risk loci with only minor effects on the disease.

Future Perspective

For the coming years, replication of GWAS studies will be essential to allow investigation if a risk loci is also associated with PD in other populations and exclude spurious associations that could be the result of undetected

population structure or genotyping errors [199]. Further advances in statistical analysis of GWAS results and more technological advance to detect rare and structural variants (insertions, deletions, inversions and copy number variations) are essential to better understand how genetic variation can be linked to PD phenotypes [200, 201].

To perform more and larger GWAS and in different populations (e.g. Chinese and Africans) should not be the main theme for the coming years. Although they are still underrepresented in GWAS studies, most risk loci have now also been confirmed among these populations. New GWAS could reveal more risk loci but, as we still don't fully understand how a risk locus affects the disease onset or progression, it would be more beneficial to further investigate the role of the now known loci (Table 2). Strict sample selection and differentiation between PD types based on pathology could increase the power of GWAS to link individual loci to distinct pathologies.

However, the problem remains that PD has a major environmental component. Selection of random sporadic PD cases may therefore include a substantial proportion of cases with no or little genetic basis for the disease. While in familial cases many individuals may be unaffected by the disease because of the lack of essential environmental exposures but still carry the risk allele [57]. The combination of GWAS with environmental assessment is therefore essential to reveal the interplay between the different susceptibilities.

A good example of the interplay between genetic burden and environmental factors is the risk loci GRIN2A. Which is a subunit of the NMDA-glutamate-receptor, a known regulator of neurotransmission in the brain

Table 3: Risk loci with associated Single Nucleotide Polymorphisms that have been identified by multiple GWAS studies.

Risk Loci	Location	SNP	Population	Reference
BST1	4p15	rs11931532	Japanese, Dutch	[12, 72]
		rs45538475	Japanese, Dutch	[12, 72]
		rs4698412	Japanese, Dutch, USA, UK, French, Australian	[12, 43, 72, 74]
HLA-DRA	6p21	rs3129882	USA, Dutch	[12, 43]
LRRK2	12q12	rs11564162	Dutch, German, USA, UK	[12, 71]
		rs1491923	Dutch, German, USA, UK	[12, 71]
		rs2708453	Japanese, USA	[43, 72]
		rs2896905	Dutch, German, USA, UK	[12, 71]
MAPT	17q21	rs12185268	USA, French, UK, Australia	[74, 75]
		rs17563986	Dutch, German, UK, USA, French, Australian	[12, 71, 74]
		rs1981997	Dutch, German, UK, USA, French, Australian	[12, 71, 74]
		rs199533	USA, Dutch, German, UK	[12, 43, 71]
		rs2532269	Dutch, French, UK, Australian	[12, 74]
		rs2532274	Dutch, French, UK, Australian	[12, 74]
		rs2668692	Dutch, French, UK, Australian	[12, 74]
		rs393152	Dutch, German, UK, USA, French, Australian	[12, 71, 74]
		rs8070723	USA, French, UK, Australian, Dutch	[12, 73, 74]
PARK16	1q32	rs11240572	Japanese, Dutch, USA, German, UK	[12, 71, 72]
		rs708730	Japanese, Dutch	[12, 72]
		rs823128	Japanese, Dutch, USA, German, UK	[12, 71, 72]
		rs823156	Japanese, Dutch, USA, German, UK	[12, 71, 72]
		rs947211	Japanese, Dutch	[12, 72]
SNCA	4q21	rs11931074	French, UK, Australian, Japanese, Dutch, USA, German	[12, 71, 72, 74]
		rs2736990	USA, French, UK, Australian, Dutch, UK, German	[12, 71, 73, 74]
		rs356220	USA, French, UK, Australia	[43, 74, 75]
		rs3857059	Japanese, Dutch, French, UK, Australian, German, USA	[12, 71, 72, 74]

and plays a role in the control of behavior and movement [202]. Standard GWAS studies were unable to detect gene-environment interactions [51]. A recent GWAS study did however take environmental factors into account and identified a protective SNP in this locus, but only in combination with coffee consumption [202]. Nicely showing that PD is a combinatory disease and is influenced by both genetic heritability and environmental cues.

Despite their limitations, GWAS studies have helped to understand the genetics susceptibility behind Parkinson's disease, though a major component is still unidentified. To date, more then 800 genetic association studies have been performed to identify or confirm genetic variations to the onset of PD. However, GWAS and meta-analysis studies generally only highlight their most significant results and mostly neglect variants with only subtle associations or variants that were previously identified but could not be replicated. This bias results in an increasingly difficulty to follow, evaluate and interpret the genetic findings of these studies. A recent consortium therefore systematically collected all published association studies and performed a large meta-analysis to create a large association database that can now be freely accessed online [131]. This dataset contributes to the accessibility of information on genetic susceptibility for Parkinson's disease and can be used as starting point for further research.

An interesting research direction for the coming years will be to compare the identified risk loci with the recently published ENCODE data [203]. Of all associated SNPs with any trait or disease only a minor fraction is located in protein coding genes. 80% of the SNP is located in either intergenic regions or introns, suggesting that they might influence regulatory sequences like enhancers or insulators [10, 204]. An increasing number of publications show that these non-coding regions are essential for the tight regulation of gene expression and cell differentiation. Much can therefore be learned on how SNPs influence regulatory processes of the genome and how they affect gene expression and the disease onset or progression.

References

1. de Lau, L.M. and M.M. Breteler, *Epidemiology of Parkinson's disease.* Lancet Neurol, 2006. **5**(6): p. 525-35.
2. Lees, A.J., J. Hardy, and T. Revesz, *Parkinson's disease.* Lancet, 2009. **373**(9680): p. 2055-66.
3. Trinh, J. and M. Farrer, *Advances in the genetics of Parkinson disease.* Nat Rev Neurol, 2013. **9**(8): p. 445-54.
4. Shulman, J.M., et al., *Association of Parkinson disease risk loci with mild parkinsonian signs in older persons.* JAMA Neurol, 2014. **71**(4): p. 429-35.
5. Langston, J.W., *The Parkinson's complex: parkinsonism is just the tip of the iceberg.* Ann Neurol, 2006. **59**(4): p. 591-6.
6. Muzerengi, S., D. Contrafatto, and K.R. Chaudhuri, *Non-motor symptoms: identification and management.* Parkinsonism Relat Disord, 2007. **13 Suppl 3**: p. S450-6.
7. Hely, M.A., et al., *The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years.* Mov Disord, 2008. **23**(6): p. 837-44.
8. Litvan, I., et al., *Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines.* Mov Disord, 2012. **27**(3): p. 349-56.
9. Dexter, D.T. and P. Jenner, *Parkinson disease: from pathology to molecular disease mechanisms.* Free Radic Biol Med, 2013. **62**: p. 132-44.
10. Hardy, J., *Genetic analysis of pathways to Parkinson disease.* Neuron, 2010. **68**(2): p. 201-6.
11. Wakabayashi, K., et al., *The Lewy body in Parkinson's disease and related neurodegenerative disorders.* Mol Neurobiol, 2013. **47**(2): p. 495-508.
12. Simon-Sanchez, J., et al., *Genome-wide association study confirms extant PD risk loci among the Dutch.* Eur J Hum Genet, 2011. **19**(6): p. 655-61.
13. Zhang, W., et al., *Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease.* FASEB J, 2005. **19**(6): p. 533-42.
14. Chen, H., et al., *Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease.* Arch Neurol, 2003. **60**(8): p. 1059-64.
15. McGeer, P.L., et al., *Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration.* Ann Neurol, 2003. **54**(5): p. 599-604.
16. Esposito, E., et al., *Non-steroidal anti-inflammatory drugs in Parkinson's disease.* Exp Neurol, 2007. **205**(2): p. 295-312.
17. Barcia, C., et al., *Evidence of active microglia in substantia nigra pars compacta of parkinsonian monkeys 1 year after MPTP exposure.* Glia, 2004. **46**(4): p. 402-9.
18. Wang, X.J., et al., *Parkinson disease IgG and C5a-induced synergistic dopaminergic neurotoxicity: role of microglia.* Neurochem Int, 2007. **50**(1): p. 39-50.
19. Hirsch, E.C. and S. Hunot, *Neuroinflammation in Parkinson's disease: a target for neuroprotection?* Lancet Neurol, 2009. **8**(4): p. 382-97.
20. Rugbjerg, K., et al., *Autoimmune disease and risk for Parkinson disease: a population-based case-control study.* Neurology, 2009. **73**(18): p. 1462-8.
21. Burre, J., et al., *Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro.* Science, 2010. **329**(5999): p. 1663-7.
22. Cabin, D.E., et al., *Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha-synuclein.* J Neurosci, 2002. **22**(20): p. 8797-807.
23. Greffard, S., et al., *A stable proportion of Lewy body bearing neurons in the substantia nigra suggests a model in which the Lewy body causes neuronal death.* Neurobiol Aging, 2010. **31**(1): p. 99-103.
24. Doty, R.L., S.M. Bromley, and M.B. Stern, *Olfactory testing as an aid in the diagnosis of Parkinson's disease: development of optimal discrimination criteria.* Neurodegeneration, 1995. **4**(1): p. 93-7.
25. Braak, H., et al., *Staging of brain pathology related to sporadic Parkinson's disease.* Neurobiol Aging, 2003. **24**(2): p. 197-211.
26. Kalaitzakis, M.E., et al., *Controversies over the staging of alpha-synuclein pathology in Parkinson's disease.* Acta Neuropathol, 2008. **116**(1): p. 125-8; author reply 129-31.
27. Parkkinen, L., T. Pirttila, and I. Alafuzoff, *Applicability of current staging/categorization of alpha-synuclein pathology and their clinical relevance.* Acta Neuropathol, 2008. **115**(4): p. 399-407.
28. Desplats, P., et al., *Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein.* Proc Natl Acad Sci U S A, 2009. **106**(31): p. 13010-5.

29. Lee, H.J., S. Patel, and S.J. Lee, *Intravesicular localization and exocytosis of alpha-synuclein and its aggregates.* J Neurosci, 2005. **25**(25): p. 6016-24.
30. Hansen, C., et al., *alpha-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells.* J Clin Invest, 2011. **121**(2): p. 715-25.
31. Stayte, S. and B. Vissel, *Advances in non-dopaminergic treatments for Parkinson's disease.* Front Neurosci, 2014. **8**: p. 113.
32. Fahn, S., et al., *Levodopa and the progression of Parkinson's disease.* N Engl J Med, 2004. **351**(24): p. 2498-508.
33. Agid, Y., *Levodopa: is toxicity a myth?* Neurology, 1998. **50**(4): p. 858-63.
34. Olanow, C.W., et al., *Levodopa in the treatment of Parkinson's disease: current controversies.* Mov Disord, 2004. **19**(9): p. 997-1005.
35. Parkkinen, L., et al., *Does levodopa accelerate the pathologic process in Parkinson disease brain?* Neurology, 2011. **77**(15): p. 1420-6.
36. Li, Q., et al., *Cortical effects of deep brain stimulation: implications for pathogenesis and treatment of Parkinson disease.* JAMA Neurol, 2014. **71**(1): p. 100-3.
37. Munhoz, R.P., A. Cerasa, and M.S. Okun, *Surgical treatment of dyskinesia in Parkinson's disease.* Front Neurol, 2014. **5**: p. 65.
38. Potter-Nerger, M. and J. Volkmann, *Deep brain stimulation for gait and postural symptoms in Parkinson's disease.* Mov Disord, 2013. **28**(11): p. 1609-15.
39. Hallett, P.J., et al., *Long-term health of dopaminergic neuron transplants in Parkinson's disease patients.* Cell Rep, 2014. **7**(6): p. 1755-61.
40. Olanow, C.W., et al., *A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease.* Ann Neurol, 2003. **54**(3): p. 403-14.
41. Kurowska, Z., et al., *Signs of degeneration in 12-22-year old grafts of mesencephalic dopamine neurons in patients with Parkinson's disease.* J Parkinsons Dis, 2011. **1**(1): p. 83-92.
42. Tarazi, F.I., et al., *Emerging therapies for Parkinson's disease: From bench to bedside.* Pharmacol Ther, 2014.
43. Hamza, T.H., et al., *Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease.* Nat Genet, 2010. **42**(9): p. 781-5.
44. Sellbach, A.N., et al., *Parkinson's disease and family history.* Parkinsonism Relat Disord, 2006. **12**(7): p. 399-409.
45. Bonifati, V., *Genetics of Parkinson's disease--state of the art, 2013.* Parkinsonism Relat Disord, 2014. **20 Suppl 1**: p. S23-8.
46. Saiki, S., S. Sato, and N. Hattori, *Molecular pathogenesis of Parkinson's disease: update.* J Neurol Neurosurg Psychiatry, 2012. **83**(4): p. 430-6.
47. Klein, C. and K. Lohmann-Hedrich, *Impact of recent genetic findings in Parkinson's disease.* Curr Opin Neurol, 2007. **20**(4): p. 453-64.
48. Cookson, M.R. and O. Bandmann, *Parkinson's disease: insights from pathways.* Hum Mol Genet, 2010. **19**(R1): p. R21-7.
49. McCulloch, C.C., et al., *Exploring gene-environment interactions in Parkinson's disease.* Hum Genet, 2008. **123**(3): p. 257-65.
50. Gandhi, S. and N.W. Wood, *Genome-wide association studies: the key to unlocking neurodegeneration?* Nat Neurosci, 2010. **13**(7): p. 789-94.
51. Frazer, K.A., et al., *Human genetic variation and its contribution to complex traits.* Nat Rev Genet, 2009. **10**(4): p. 241-51.
52. Bodmer, W. and C. Bonilla, *Common and rare variants in multifactorial susceptibility to common diseases.* Nat Genet, 2008. **40**(6): p. 695-701.
53. Fearnhead, N.S., B. Winney, and W.F. Bodmer, *Rare variant hypothesis for multifactorial inheritance: susceptibility to colorectal adenomas as a model.* Cell Cycle, 2005. **4**(4): p. 521-5.
54. Maraganore, D.M., et al., *High-resolution whole-genome association study of Parkinson disease.* Am J Hum Genet, 2005. **77**(5): p. 685-93.
55. Elbaz, A., et al., *Lack of replication of thirteen single-nucleotide polymorphisms implicated in Parkinson's disease: a large-scale international study.* Lancet Neurol, 2006. **5**(11): p. 917-23.
56. Goris, A., et al., *No evidence for association with Parkinson disease for 13 single-nucleotide polymorphisms identified by whole-genome association screening.* Am J Hum Genet, 2006. **78**(6): p. 1088-90; author reply 1092-4.
57. Myers, R.H., *Considerations for genomewide association studies in Parkinson disease.* Am J Hum Genet, 2006. **78**(6): p. 1081-2.
58. Polymeropoulos, M.H., et al., *Mutation in the alpha-synuclein gene identified in families*

with Parkinson's disease. Science, 1997. **276**(5321): p. 2045-7.
59. Kim, H.J., *Alpha-Synuclein Expression in Patients with Parkinson's Disease: A Clinician's Perspective.* Exp Neurobiol, 2013. **22**(2): p. 77-83.
60. Olanow, C.W. and K.S. McNaught, *Ubiquitin-proteasome system and Parkinson's disease.* Mov Disord, 2006. **21**(11): p. 1806-23.
61. Anwar, S., et al., *Functional alterations to the nigrostriatal system in mice lacking all three members of the synuclein family.* J Neurosci, 2011. **31**(20): p. 7264-74.
62. Hyun, C.H., et al., *LRRK2 as a Potential Genetic Modifier of Synucleinopathies: Interlacing the Two Major Genetic Factors of Parkinson's Disease.* Exp Neurobiol, 2013. **22**(4): p. 249-57.
63. Kahle, P.J., et al., *Subcellular localization of wild-type and Parkinson's disease-associated mutant alpha -synuclein in human and transgenic mouse brain.* J Neurosci, 2000. **20**(17): p. 6365-73.
64. Masliah, E., et al., *Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders.* Science, 2000. **287**(5456): p. 1265-9.
65. Giasson, B.I., et al., *Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein.* Neuron, 2002. **34**(4): p. 521-33.
66. Theuns, J. and C. Van Broeckhoven, *alpha-Synuclein gene duplications in sporadic Parkinson disease.* Neurology, 2008. **70**(1): p. 7-9.
67. Singleton, A.B., et al., *alpha-Synuclein locus triplication causes Parkinson's disease.* Science, 2003. **302**(5646): p. 841.
68. Venda, L.L., et al., *alpha-Synuclein and dopamine at the crossroads of Parkinson's disease.* Trends Neurosci, 2010. **33**(12): p. 559-68.
69. Fung, H.C., et al., *Genome-wide genotyping in Parkinson's disease and neurologically normal controls: first stage analysis and public release of data.* Lancet Neurol, 2006. **5**(11): p. 911-6.
70. Pankratz, N., et al., *Genomewide association study for susceptibility genes contributing to familial Parkinson disease.* Hum Genet, 2009. **124**(6): p. 593-605.
71. Simon-Sanchez, J., et al., *Genome-wide association study reveals genetic risk underlying Parkinson's disease.* Nat Genet, 2009. **41**(12): p. 1308-12.
72. Satake, W., et al., *Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease.* Nat Genet, 2009. **41**(12): p. 1303-7.
73. Edwards, T.L., et al., *Genome-wide association study confirms SNPs in SNCA and the MAPT region as common risk factors for Parkinson disease.* Ann Hum Genet, 2010. **74**(2): p. 97-109.
74. Saad, M., et al., *Genome-wide association study confirms BST1 and suggests a locus on 12q24 as the risk loci for Parkinson's disease in the European population.* Hum Mol Genet, 2011. **20**(3): p. 615-27.
75. Do, C.B., et al., *Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease.* PLoS Genet, 2011. **7**(6): p. e1002141.
76. Chung, S.J., et al., *Alzheimer's disease and Parkinson's disease genome-wide association study top hits and risk of Parkinson's disease in Korean population.* Neurobiol Aging, 2013. **34**(11): p. 2695 e1-7.
77. Liu, J., et al., *Analysis of genome-wide association study-linked loci in Parkinson's disease of Mainland China.* Mov Disord, 2013. **28**(13): p. 1892-5.
78. Tan, E.K., et al., *Analysis of GWAS-linked loci in Parkinson disease reaffirms PARK16 as a susceptibility locus.* Neurology, 2010. **75**(6): p. 508-12.
79. Chang, X.L., et al., *Association of GWAS loci with PD in China.* Am J Med Genet B Neuropsychiatr Genet, 2011. **156B**(3): p. 334-9.
80. Nalls, M.A., et al., *Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies.* Lancet, 2011. **377**(9766): p. 641-9.
81. Li, N.N., et al., *SNCA rs356219 variant increases risk of sporadic Parkinson's disease in ethnic Chinese.* Am J Med Genet B Neuropsychiatr Genet, 2013. **162B**(5): p. 452-6.
82. Cardo, L.F., et al., *A search for SNCA 3' UTR variants identified SNP rs356165 as a determinant of disease risk and onset age in Parkinson's disease.* J Mol Neurosci, 2012. **47**(3): p. 425-30.
83. Myhre, R., et al., *Multiple alpha-synuclein gene polymorphisms are associated with Parkinson's disease in a Norwegian population.* Acta Neurol Scand, 2008. **118**(5): p. 320-7.

84. Mata, I.F., et al., *Replication of MAPT and SNCA, but not PARK16-18, as susceptibility genes for Parkinson's disease.* Mov Disord, 2011. **26**(5): p. 819-23.
85. Mata, I.F., et al., *SNCA variant associated with Parkinson disease and plasma alpha-synuclein level.* Arch Neurol, 2010. **67**(11): p. 1350-6.
86. Miyake, Y., et al., *SNCA polymorphisms, smoking, and sporadic Parkinson's disease in Japanese.* Parkinsonism Relat Disord, 2012. **18**(5): p. 557-61.
87. Ding, H., et al., *Association of SNCA with Parkinson: replication in the Harvard NeuroDiscovery Center Biomarker Study.* Mov Disord, 2011. **26**(12): p. 2283-6.
88. Mizuta, I., et al., *Multiple candidate gene analysis identifies alpha-synuclein as a susceptibility gene for sporadic Parkinson's disease.* Hum Mol Genet, 2006. **15**(7): p. 1151-8.
89. Yu, L., et al., *SNP rs7684318 of the alpha-synuclein gene is associated with Parkinson's disease in the Han Chinese population.* Brain Res, 2010. **1346**: p. 262-5.
90. Funayama, M., et al., *A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1.* Ann Neurol, 2002. **51**(3): p. 296-301.
91. Berwick, D.C. and K. Harvey, *LRRK2 signaling pathways: the key to unlocking neurodegeneration?* Trends Cell Biol, 2011. **21**(5): p. 257-65.
92. Di Fonzo, A., et al., *Comprehensive analysis of the LRRK2 gene in sixty families with Parkinson's disease.* Eur J Hum Genet, 2006. **14**(3): p. 322-31.
93. Singleton, A.B., M.J. Farrer, and V. Bonifati, *The genetics of Parkinson's disease: progress and therapeutic implications.* Mov Disord, 2013. **28**(1): p. 14-23.
94. Spatola, M. and C. Wider, *Genetics of Parkinson's disease: the yield.* Parkinsonism Relat Disord, 2014. **20 Suppl 1**: p. S35-8.
95. Schulte, C. and T. Gasser, *Genetic basis of Parkinson's disease: inheritance, penetrance, and expression.* Appl Clin Genet, 2011. **4**: p. 67-80.
96. Galter, D., et al., *LRRK2 expression linked to dopamine-innervated areas.* Ann Neurol, 2006. **59**(4): p. 714-9.
97. Smith, W.W., et al., *Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration.* Proc Natl Acad Sci U S A, 2005. **102**(51): p. 18676-81.
98. Dauer, W. and C.C. Ho, *The biology and pathology of the familial Parkinson's disease protein LRRK2.* Mov Disord, 2010. **25 Suppl 1**: p. S40-3.
99. Lee, S., et al., *LRRK2 kinase regulates synaptic morphology through distinct substrates at the presynaptic and postsynaptic compartments of the Drosophila neuromuscular junction.* J Neurosci, 2010. **30**(50): p. 16959-69.
100. MacLeod, D., et al., *The familial Parkinsonism gene LRRK2 regulates neurite process morphology.* Neuron, 2006. **52**(4): p. 587-93.
101. Bardien, S., et al., *Genetic characteristics of leucine-rich repeat kinase 2 (LRRK2) associated Parkinson's disease.* Parkinsonism Relat Disord, 2011. **17**(7): p. 501-8.
102. Haugarvoll, K. and Z.K. Wszolek, *Clinical features of LRRK2 parkinsonism.* Parkinsonism Relat Disord, 2009. **15 Suppl 3**: p. S205-8.
103. Tan, E.K., *Rare and common LRRK2 exonic variants in Parkinson's disease.* Lancet Neurol, 2011. **10**(10): p. 869-70.
104. Healy, D.G., et al., *Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study.* Lancet Neurol, 2008. **7**(7): p. 583-90.
105. Ross, O.A., et al., *Association of LRRK2 exonic variants with susceptibility to Parkinson's disease: a case-control study.* Lancet Neurol, 2011. **10**(10): p. 898-908.
106. Tan, E.K., et al., *Multiple LRRK2 variants modulate risk of Parkinson disease: a Chinese multicenter study.* Hum Mutat, 2010. **31**(5): p. 561-8.
107. Sharma, M., et al., *Large-scale replication and heterogeneity in Parkinson disease genetic loci.* Neurology, 2012. **79**(7): p. 659-67.
108. Zhu, L.H., et al., *Lack of association between three single nucleotide polymorphisms in the PARK9, PARK15, and BST1 genes and Parkinson's disease in the northern Han Chinese population.* Chin Med J (Engl), 2012. **125**(4): p. 588-92.
109. Miyake, Y., et al., *Lack of association between BST1 polymorphisms and sporadic Parkinson's disease in a Japanese population.* J Neurol Sci, 2012. **323**(1-2): p. 162-6.
110. Chen, M.L., et al., *BST1 rs11724635 interacts with environmental factors to increase the risk of Parkinson's disease in a Taiwanese population.* Parkinsonism Relat Disord, 2014. **20**(3): p. 280-3.

111. Lampe, J.B., et al., *HLA typing and Parkinson's disease.* Eur Neurol, 2003. **50**(2): p. 64-8.
112. Saiki, M., et al., *Association of the human leucocyte antigen region with susceptibility to Parkinson's disease.* J Neurol Neurosurg Psychiatry, 2010. **81**(8): p. 890-1.
113. Ahmed, I., et al., *Association between Parkinson's disease and the HLA-DRB1 locus.* Mov Disord, 2012. **27**(9): p. 1104-10.
114. Ran, C., et al., *The HLA-DRA variation rs3129882 is not associated with Parkinson's disease in Sweden.* Parkinsonism Relat Disord, 2013. **19**(7): p. 701-2.
115. Chiang, H.L., et al., *Genetic analysis of HLA-DRA region variation in Taiwanese Parkinson's disease.* Parkinsonism Relat Disord, 2012. **18**(4): p. 391-3.
116. Lin, C.H., et al., *Reaffirmation of GAK, but not HLA-DRA, as a Parkinson's disease susceptibility gene in a Taiwanese population.* Am J Med Genet B Neuropsychiatr Genet, 2013. **162B**(8): p. 841-6.
117. Puschmann, A., et al., *Human leukocyte antigen variation and Parkinson's disease.* Parkinsonism Relat Disord, 2011. **17**(5): p. 376-8.
118. Wissemann, W.T., et al., *Association of Parkinson disease with structural and regulatory variants in the HLA region.* Am J Hum Genet, 2013. **93**(5): p. 984-93.
119. Zody, M.C., et al., *Evolutionary toggling of the MAPT 17q21.31 inversion region.* Nat Genet, 2008. **40**(9): p. 1076-83.
120. Stoecker, K., et al., *Induction of STAP-1 promotes neurotoxic activation of microglia.* Biochem Biophys Res Commun, 2009. **379**(1): p. 121-6.
121. Grunblatt, E., et al., *Gene expression profiling of parkinsonian substantia nigra pars compacta; alterations in ubiquitin-proteasome, heat shock protein, iron and oxidative stress regulated proteins, cell adhesion/cellular matrix and vesicle trafficking genes.* J Neural Transm, 2004. **111**(12): p. 1543-73.
122. Dumitriu, A., et al., *Cyclin-G-associated kinase modifies alpha-synuclein expression levels and toxicity in Parkinson's disease: results from the GenePD Study.* Hum Mol Genet, 2011. **20**(8): p. 1478-87.
123. Chen, Y.P., et al., *GAK rs1564282 and DGKQ rs11248060 increase the risk for Parkinson's disease in a Chinese population.* J Clin Neurosci, 2013. **20**(6): p. 880-3.
124. Michelakakis, H., et al., *Evidence of an association between the scavenger receptor class B member 2 gene and Parkinson's disease.* Mov Disord, 2012. **27**(3): p. 400-5.
125. Chen, S., et al., *Association study of SCARB2 rs6812193 polymorphism with Parkinson's disease in Han Chinese.* Neurosci Lett, 2012. **516**(1): p. 21-3.
126. Li, K., et al., *Association study between two novel single nucleotide polymorphisms and sporadic Parkinson's disease in Chinese Han population.* Neurosci Lett, 2012. **517**(1): p. 56-9.
127. Kalinderi, K., et al., *Association study of rs6812193 polymorphism with Parkinson's disease in a Greek population.* Neurosci Lett, 2013. **541**: p. 190-2.
128. Maniwang, E., N. Tayebi, and E. Sidransky, *Is Parkinson disease associated with lysosomal integral membrane protein type-2?: challenges in interpreting association data.* Mol Genet Metab, 2013. **108**(4): p. 269-71.
129. Evangelou, E., D.M. Maraganore, and J.P. Ioannidis, *Meta-analysis in genome-wide association datasets: strategies and application in Parkinson disease.* PLoS One, 2007. **2**(2): p. e196.
130. Pankratz, N., et al., *Meta-analysis of Parkinson's disease: identification of a novel locus, RIT2.* Ann Neurol, 2012. **71**(3): p. 370-84.
131. Lill, C.M., et al., *Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: The PDGene database.* PLoS Genet, 2012. **8**(3): p. e1002548.
132. Keller, M.F., et al., *Using genome-wide complex trait analysis to quantify 'missing heritability' in Parkinson's disease.* Hum Mol Genet, 2012. **21**(22): p. 4996-5009.
133. Bossers, K., et al., *Analysis of gene expression in Parkinson's disease: possible involvement of neurotrophic support and axon guidance in dopaminergic cell death.* Brain Pathol, 2009. **19**(1): p. 91-107.
134. Lin, C.H., et al., *RIT2 variant is not associated with Parkinson's disease in a Taiwanese population.* Neurobiol Aging, 2013. **34**(9): p. 2236 e1-3.
135. Wang, Y.Q., et al., *Association analysis of STK39, MCCC1/LAMP3 and sporadic PD in the Chinese Han population.* Neurosci Lett, 2014. **566**: p. 206-9.
136. Li, N.N., et al., *MCCC1/LAMP3 reduces risk of sporadic Parkinson's disease in Han Chinese.* Acta Neurol Scand, 2013. **128**(2): p. 136-9.

137. Zhu, Z.G., et al., *Meta-analysis supports association of a functional SNP (rs1801133) in the MTHFR gene with Parkinson's disease.* Gene, 2013. **531**(1): p. 78-83.
138. Vallelunga, A., et al., *The MTHFR C677T polymorphism modifies age at onset in Parkinson's disease.* Neurol Sci, 2014. **35**(1): p. 73-7.
139. Narendra, D., et al., *Parkin is recruited selectively to impaired mitochondria and promotes their autophagy.* J Cell Biol, 2008. **183**(5): p. 795-803.
140. Park, J., G. Lee, and J. Chung, *The PINK1-Parkin pathway is involved in the regulation of mitochondrial remodeling process.* Biochem Biophys Res Commun, 2009. **378**(3): p. 518-23.
141. Chung, K.K., et al., *Parkin ubiquitinates the alpha-synuclein-interacting protein, synphilin-1: implications for Lewy-body formation in Parkinson disease.* Nat Med, 2001. **7**(10): p. 1144-50.
142. Shimura, H., et al., *Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease.* Science, 2001. **293**(5528): p. 263-9.
143. Marongiu, R., et al., *Whole gene deletion and splicing mutations expand the PINK1 genotypic spectrum.* Hum Mutat, 2007. **28**(1): p. 98.
144. Hatano, Y., et al., *Novel PINK1 mutations in early-onset parkinsonism.* Ann Neurol, 2004. **56**(3): p. 424-7.
145. Samaranch, L., et al., *PINK1-linked parkinsonism is associated with Lewy body pathology.* Brain, 2010. **133**(Pt 4): p. 1128-42.
146. Olzmann, J.A., et al., *Selective enrichment of DJ-1 protein in primate striatal neuronal processes: implications for Parkinson's disease.* J Comp Neurol, 2007. **500**(3): p. 585-99.
147. Usami, Y., et al., *DJ-1 associates with synaptic membranes.* Neurobiol Dis, 2011. **43**(3): p. 651-62.
148. Abou-Sleiman, P.M., et al., *The role of pathogenic DJ-1 mutations in Parkinson's disease.* Ann Neurol, 2003. **54**(3): p. 283-6.
149. Neumann, M., et al., *Pathological properties of the Parkinson's disease-associated protein DJ-1 in alpha-synucleinopathies and tauopathies: relevance for multiple system atrophy and Pick's disease.* Acta Neuropathol, 2004. **107**(6): p. 489-96.
150. Goker-Alpan, O., et al., *Parkinsonism among Gaucher disease carriers.* J Med Genet, 2004. **41**(12): p. 937-40.
151. Wong, K., et al., *Neuropathology provides clues to the pathophysiology of Gaucher disease.* Mol Genet Metab, 2004. **82**(3): p. 192-207.
152. Gan-Or, Z., et al., *Genotype-phenotype correlations between GBA mutations and Parkinson disease risk and onset.* Neurology, 2008. **70**(24): p. 2277-83.
153. Song, G.G. and Y.H. Lee, *Pathway analysis of genome-wide association studies for Parkinson's disease.* Mol Biol Rep, 2013. **40**(3): p. 2599-607.
154. Rademakers, R., M. Cruts, and C. van Broeckhoven, *The role of tau (MAPT) in frontotemporal dementia and related tauopathies.* Hum Mutat, 2004. **24**(4): p. 277-95.
155. Schapira, A.H., *Mitochondria in the aetiology and pathogenesis of Parkinson's disease.* Lancet Neurol, 2008. **7**(1): p. 97-109.
156. Cannon, J.R., et al., *A highly reproducible rotenone model of Parkinson's disease.* Neurobiol Dis, 2009. **34**(2): p. 279-90.
157. Clark, I.E., et al., *Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin.* Nature, 2006. **441**(7097): p. 1162-6.
158. Park, J., et al., *Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin.* Nature, 2006. **441**(7097): p. 1157-61.
159. Narendra, D., et al., *p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both.* Autophagy, 2010. **6**(8): p. 1090-106.
160. Matsuda, N., et al., *PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy.* J Cell Biol, 2010. **189**(2): p. 211-21.
161. Kawajiri, S., et al., *PINK1 is recruited to mitochondria with parkin and associates with LC3 in mitophagy.* FEBS Lett, 2010. **584**(6): p. 1073-9.
162. Yang, Y., et al., *Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink1 is rescued by Parkin.* Proc Natl Acad Sci U S A, 2006. **103**(28): p. 10793-8.
163. Bingol, B., et al., *The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy.* Nature, 2014. **509**(7505): p. 370-5.
164. MacLeod, D.A., et al., *RAB7L1 interacts with LRRK2 to modify intraneuronal protein*

sorting and Parkinson's disease risk. Neuron, 2013. **77**(3): p. 425-39.
165. Beilina, A., et al., *Unbiased screen for interactors of leucine-rich repeat kinase 2 supports a common pathway for sporadic and familial Parkinson disease.* Proc Natl Acad Sci U S A, 2014. **111**(7): p. 2626-31.
166. Lin, X., et al., *Leucine-rich repeat kinase 2 regulates the progression of neuropathology induced by Parkinson's-disease-related mutant alpha-synuclein.* Neuron, 2009. **64**(6): p. 807-27.
167. Orenstein, S.J., et al., *Interplay of LRRK2 with chaperone-mediated autophagy.* Nat Neurosci, 2013. **16**(4): p. 394-406.
168. Herzig, M.C., et al., *High LRRK2 levels fail to induce or exacerbate neuronal alpha-synucleinopathy in mouse brain.* PLoS One, 2012. **7**(5): p. e36581.
169. Daher, J.P., et al., *Neurodegenerative phenotypes in an A53T alpha-synuclein transgenic mouse model are independent of LRRK2.* Hum Mol Genet, 2012. **21**(11): p. 2420-31.
170. Russo, I., L. Bubacco, and E. Greggio, *LRRK2 and neuroinflammation: partners in crime in Parkinson's disease?* J Neuroinflammation, 2014. **11**: p. 52.
171. Kim, J.S., et al., *Association of vitamin D receptor gene polymorphism and Parkinson's disease in Koreans.* J Korean Med Sci, 2005. **20**(3): p. 495-8.
172. Han, X., et al., *Vitamin D receptor gene polymorphism and its association with Parkinson's disease in Chinese Han population.* Neurosci Lett, 2012. **525**(1): p. 29-33.
173. Mazzulli, J.R., et al., *Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies.* Cell, 2011. **146**(1): p. 37-52.
174. Moskvina, V., et al., *Analysis of genome-wide association studies of Alzheimer disease and of Parkinson disease to determine if these 2 diseases share a common genetic risk.* JAMA Neurol, 2013. **70**(10): p. 1268-76.
175. Dong, J., et al., *Susceptibility loci for pigmentation and melanoma in relation to Parkinson's disease.* Neurobiol Aging, 2014. **35**(6): p. 1512 e5-10.
176. Barrett, J.C., et al., *Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease.* Nat Genet, 2008. **40**(8): p. 955-62.
177. Inzelberg, R., et al., *The LRRK2 G2019S mutation is associated with Parkinson disease and concomitant non-skin cancers.* Neurology, 2012. **78**(11): p. 781-6.
178. Rugbjerg, K., et al., *Malignant melanoma, breast cancer and other cancers in patients with Parkinson's disease.* Int J Cancer, 2012. **131**(8): p. 1904-11.
179. Melikoglu, M.A., I. Sezer, and C. Kacar, *Rheumatoid-like hand deformities in Parkinson disease.* J Clin Rheumatol, 2007. **13**(4): p. 236-7.
180. Kogure, T., et al., *Rheumatoid arthritis accompanied by Parkinson disease.* J Clin Rheumatol, 2008. **14**(3): p. 192-3.
181. Kravitz, E., et al., *Parkinson's disease genes do not segregate with breast cancer genes' loci.* Cancer Epidemiol Biomarkers Prev, 2013. **22**(8): p. 1464-72.
182. Demartini, D.R., et al., *Alzheimer's and Parkinson's diseases: an environmental proteomic point of view.* J Proteomics, 2014. **104**: p. 24-36.
183. Forte, G., et al., *Trace and major elements in whole blood, serum, cerebrospinal fluid and urine of patients with Parkinson's disease.* J Neural Transm, 2004. **111**(8): p. 1031-40.
184. Chung, S.J., et al., *Genetic susceptibility loci, environmental exposures, and Parkinson's disease: a case-control study of gene-environment interactions.* Parkinsonism Relat Disord, 2013. **19**(6): p. 595-9.
185. Lehman, E.J., et al., *Neurodegenerative causes of death among retired National Football League players.* Neurology, 2012. **79**(19): p. 1970-4.
186. Savica, R., et al., *High school football and risk of neurodegeneration: a community-based study.* Mayo Clin Proc, 2012. **87**(4): p. 335-40.
187. Ascherio, A., et al., *Caffeine, postmenopausal estrogen, and risk of Parkinson's disease.* Neurology, 2003. **60**(5): p. 790-5.
188. Benedetti, M.D., et al., *Smoking, alcohol, and coffee consumption preceding Parkinson's disease: a case-control study.* Neurology, 2000. **55**(9): p. 1350-8.
189. Brighina, L., et al., *Alpha-synuclein, alcohol use disorders, and Parkinson disease: a case-control study.* Parkinsonism Relat Disord, 2009. **15**(6): p. 430-4.
190. Kiyohara, C., et al., *APOE and CYP2E1 polymorphisms, alcohol consumption, and Parkinson's disease in a Japanese population.* J Neural Transm, 2011. **118**(9): p. 1335-44.
191. Wang, W.Y., et al., *Genome-wide association studies: theoretical and practical concerns.* Nat Rev Genet, 2005. **6**(2): p. 109-18.

192. Kara, E., et al., *Assessment of Parkinson's disease risk loci in Greece.* Neurobiol Aging, 2014. **35**(2): p. 442 e9-442 e16.
193. Devine, M.J. and P.A. Lewis, *Emerging pathways in genetic Parkinson's disease: tangles, Lewy bodies and LRRK2.* FEBS J, 2008. **275**(23): p. 5748-57.
194. Sharma, M., R. Kruger, and T. Gasser, *From genome-wide association studies to next-generation sequencing: lessons from the past and planning for the future.* JAMA Neurol, 2014. **71**(1): p. 5-6.
195. Hicks, A.A., et al., *A susceptibility gene for late-onset idiopathic Parkinson's disease.* Ann Neurol, 2002. **52**(5): p. 549-55.
196. Wan, J.Y., et al., *Association mapping of the PARK10 region for Parkinson's disease susceptibility genes.* Parkinsonism Relat Disord, 2014. **20**(1): p. 93-8.
197. Yuan, Y., et al., *Marginal association between SNP rs2046571 of the HAS2 gene and Parkinson's disease in the Chinese female population.* Neurosci Lett, 2013. **552**: p. 58-61.
198. Liu, X., et al., *Genome-wide association study identifies candidate genes for Parkinson's disease in an Ashkenazi Jewish population.* BMC Med Genet, 2011. **12**: p. 104.
199. Pastor, P., *Genetic heterogeneity in Parkinson disease: the meaning of GWAS and replication studies.* Neurology, 2012. **79**(7): p. 619-20.
200. Wang, M., et al., *A robust and efficient statistical method for genetic association studies using case and control samples from multiple cohorts.* BMC Genomics, 2013. **14**: p. 88.
201. Laurie, C.C., et al., *Quality control and quality assurance in genotypic data for genome-wide association studies.* Genet Epidemiol, 2010. **34**(6): p. 591-602.
202. Hamza, T.H., et al., *Genome-wide gene-environment study identifies glutamate receptor gene GRIN2A as a Parkinson's disease modifier gene via interaction with coffee.* PLoS Genet, 2011. **7**(8): p. e1002237.
203. Bernstein, B.E., et al., *An integrated encyclopedia of DNA elements in the human genome.* Nature, 2012. **489**(7414): p. 57-74.
204. Hindorff, L.A., et al., *Potential etiologic and functional implications of genome-wide association loci for human diseases and traits.* Proc Natl Acad Sci U S A, 2009. **106**(23): p. 9362-7.