Chapter from the book *Gene Duplication*
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1. Introduction

Parkinson disease (PD) is caused by several pathogenic mutations in genes such as alpha-synuclein (SNCA; MIM#163890), Leucine-rich repeat kinase 2 (LRRK2; MIM#609007), PARKIN (parkin; MIM#602544), PTEN-induced kinase 1 (PINK1; MIM#608309), and DJ-1 (MIM#602533) (Farrer, 2006). The alpha-synuclein protein is also a major component of Lewy bodies (LB), the pathologic substrate that is observed in PD patients at autopsy (Spillantini et al., 1997). LB are generally localized to the mid-brain in patients with PD, however a widespread distribution of LB, including cortical regions, is seen in dementia with Lewy bodies (DLB) (Braak et al., 2003, McKeith et al., 2005). The observation of SNCA multiplications co-segregating with PD and dementia in families led to the hypothesis that over-expression of the alpha-synuclein protein is an important mechanism of disease. Herein, we place the gene dosage effect of SNCA in PD in perspective and describe the recent molecular insights underlying them.

2. SNCA triplication and duplication in hereditary PD

PD is the second most frequent neurodegenerative disorder following Alzheimer disease in the elderly. The main symptoms of PD are tremor, bradykinesia, and gait disturbance. PD genetics is categorized into two groups; one is sporadic PD and the other is familial PD. Familial PD has two forms; autosomal dominant heredity (ADPD) and autosomal recessive heredity (ARPD). ADPD has been observed to be caused by mutations in SNCA and LRRK2. ARPD is caused by homozygous or compound heterozygous mutations in PARKIN, PINK1, and DJ-1(Farrer, 2006). This review will focus on SNCA which is located on chromosome 4q21-22 and encodes the 140 amino acid alpha-synuclein protein. SNCA has three point mutations; c.88G>C (Ala30Pro), c.188G>A (Glu46Lys) and c.209G>A (Ala53Thr) (Kruger et al., 1998, Zarranz et al., 2004), but they are very rare. SNCA duplications and triplications have also been identified as a genetic cause of ADPD. Duplication has two SNCA copies on one allele (50% dose increase) and triplication has three, 100 percent dose increase (Figure 1). Rarely, compound heterozygote forms (two duplication alleles) are seen as SNCA triplication events (Ikeuchi et al., 2008). These multiplications generate higher SNCA expression of mRNA and protein, the so called gene...
dosage effect. Increasing the levels of protein appears to influence the clinical manifestations of PD patients. A subtle increase in alpha-synuclein expression may increase the risk of developing typical sporadic PD, whereas higher expression may cause severe forms of Parkinsonism similar to DLB. Pathologically, the burden of LB correlates with a PD or DLB clinical diagnosis, and it is still unclear whether PD and DLB are a continuum within the disease spectrum.

Fig. 1. A model for gene dosage of deletion, duplication and triplication

Singleton et al. first reported SNCA gene triplication within a large family with PD and dementia family (the Iowa kindred) (Singleton et al., 2003). The family members had been followed both clinically and pathologically by Mayo Clinic doctors for 100 years (Muenter et al., 1998, Gwinn-Hardy et al., 2000). The pattern of inheritance is autosomal dominant. The patients show a severe clinical course, early age at onset, complicating with dementia, or Parkinsonism. The pathological features are similar to DLB; (1) widespread LB pathology in the brain, (2) neuronal loss in the CA2/3, and (3) neuronal loss in the substantia nigra (Muenter et al., 1998). SNCA triplication was confirmed with quantitative PCR and FISH methodology. The size of the triplication region was over 2.0Mb. The expression values of messenger RNA and protein in peripheral blood and brain were twice the amount that is present in control subjects as predicted (Miller et al., 2004). Apart from the Iowan family, a
Swedish-American family was also reported as a SNCA triplication family and the patients had DLB-type prognosis and pathological findings. Their expression level of messenger RNA and protein of alpha-synuclein was also double the dosage of normal subjects in frontal cortex (Farrer et al., 2004).

These findings provided two novel insights regarding the underlying mechanisms of PD; (1) over-expression of alpha-synuclein may cause a more severe form of Parkinsonism such as DLB and (2) the gene dosage of alpha-synuclein may directly correlate with the clinical features of PD. Furthermore, these findings give us hints into the development of potential therapeutic avenues of treatment for PD by decreasing the expression of alpha-synuclein. The suppression of alpha-synuclein by siRNA approaches has proven successful in decreasing the levels of LB pathology in animal models (Lewis et al., 2008, McCormack et al., 2010).

After the detection of SNCA locus triplication, SNCA duplications were also reported in two families from France and Italy (Chartier-Harlin et al., 2004, Ibanez et al., 2004). Chartier-Harlin et al detected one duplication family among nine ADPD. Ibanez et al detected two families with SNCA duplication among 119 ADPD by 250K Affymetrix microarray and semi-quantitative PCR method(Ibanez et al., 2004). The symptoms of the patients with SNCA duplication are milder than that of SNCA triplication, younger age at onset and have a good efficacy for levodopa therapy, similar to sporadic PD.

Following these findings, we started screening for SNCA multiplications within 216 ADPD and 271 sporadic PD patients originating from Japan (Nishioka et al., 2006, Nishioka et al., 2009). We found six ADPD families and one sporadic case with SNCA duplication. Haplotype analysis showed these seven patients are derived from four common founders. Interestingly, the clinical manifestations of these patients were quite diverse such as sporadic PD, DLB-type and also many elderly asymptomatic carriers. The estimated penetrance ratio is about 30-40%. One patient presented with a severe clinical course with no efficacy for levodopa therapy. He progressed to Hoehn and Yahr stage V in a few years and at autopsy demonstrated features similar to DLB (Obi et al., 2008).

In addition, we also detected five asymptomatic carriers over 65 years old. We therefore focused our work on the reasons why the asymptomatic carriers at later ages do not present any clinical features of parkinsonism. We started to assess Brain MRI, PET study with $[^{18}\text{F}]$-labeled 2-fluoro-2-deoxy-D-glucose (FDG) and $[^{11}\text{C}]$-labeled 2β-carbomethoxy-3β-(4-fluorophenyl)-tropane (CFT), polysomnography, and Sniffin’ sticks to explore the differences between the patients and the asymptomatic carriers with SNCA duplication. Against our expectation, the assessments for asymptomatic carriers did not show any abnormal results in our studies, with similar results as those obtained for normal individuals (Nishioka et al., 2009). It is an area of intense research why the asymptomatic carriers do not develop Parkinsonism at these late ages and its resolution will be a key in the puzzle regarding the late-onset of PD.

Apart from our cases, other teams have reported two families from Japan with SNCA duplication (Ikeuchi et al., 2008, Uchiyama et al., 2008). Interestingly, one family has both heterozygote and homozygote duplication (producing a pseudo-triplication) (Ikeuchi et al., 2008). The clinical features of the individual with the SNCA homozygote duplication showed severe Parkinsonism similar to that of a triplication carrier. These findings also confirm the gene dosage effect within the same family. Earlier studies had reported that the
Swedish family named the “Lister family complex” has both SNCA duplication and triplication patients within different branches of the pedigree suggesting a primary duplication event followed later by another resulting in the triplication (Fuchs et al., 2007). In this family also the patient with SNCA triplication presented with more severe symptoms than the patients with duplication. Recently one small pedigree with SNCA triplication was detected in Japan (Sekine et al., 2010).

The breakpoint of SNCA multiplication is different in each family. The largest multiplication about 4.9Mb is detected within a French family. The smallest one about 0.2 Mb is in a Japanese family (Nishioka et al., 2009). The size and gene make-up of each multiplication region does not seem to influence the clinical presentation of the carrier. The single common determining factor that appears between all patients with SNCA multiplication is the presence of the entire SNCA gene. To conclude, it is clear that SNCA multiplication alone is sufficient to result in the parkinsonian phenotype.

2.1 Sporadic PD and SNCA duplication

In 2007, Ahn et al reported two sporadic patients with SNCA duplication from a screen of 906 PD patients (Ahn et al., 2008). The age at onset was 65 and 50 years old for the two patients. Their clinical course was similar to typical sporadic PD without severe progression or cognitive decline. The estimated penetrance ratio was 33.3% among the Korean patients. Our studies have also detected one sporadic patient with SNCA duplication and this means that the low penetrance SNCA duplications may give the appearance of sporadic disease.

2.2 The frequency of SNCA multiplications in PD

The prevalence of SNCA multiplications is relatively low (table 1). Bruggemann et al reported one sporadic case among 403 PD cases from Germany (1/403=0.25%) (Brueggemann et al., 2008). Troiano et al reported one sporadic cases among 101 young onset PD cases from French (1/101=1%) (Troiano et al., 2008). Nuytemans et al reported one duplication patient with dementia among 219 sporadic PD cases from Belgium (Nuytemans et al., 2009). Sironi et al reported one duplication patient with dementia among 144 PD cases from Italy (1/144=0.7%) (Sironi et al., 2009). Furthermore some reports did not detect SNCA duplication; 0/50 and 0/290 (Hope et al., 2004, Xiromerisiou et al., 2007). To conclude, SNCA multiplication is not a common cause of sporadic or hereditary PD.

2.3 SNCA multiplications in multiple system atrophy

Multiple system atrophy (MSA) is characterized by specific clinical features such as Parkinsonism, autonomic dysfunction, poor response to levodopa therapy, and cerebellar ataxia (Wenning et al., 2004). Glial cytoplasmic inclusions (GCIs) are the pathologic hallmark of the disease. As alpha-synuclein is a major protein component of GCIs, MSA is categorized within the group of neurodegenerative disorders classified as the alpha-synucleinopathies. Interestingly common variation at the SNCA locus has been associated with MSA risk (Scholz et al., 2009, Ross et al., 2010). Two studies did not identify any SNCA multiplications in a combined total of 258 MSA patients (Lincoln et al., 2007, Ahn et al., 2008). Although the number of assessed samples may be small, these findings suggest that SNCA dosage is not a common cause of MSA. It is speculated that PD or DLB may be cause by lysosomal dysfunction, however, MSA may be caused by the oligodendrocytic changes in myelin basic protein (Wenning et al., 2008).
<table>
<thead>
<tr>
<th>SNCA duplication in AD cases</th>
<th>Country</th>
<th>The number of pedigrees</th>
<th>The number of screening samples</th>
<th>Frequency (%)</th>
<th>Average age at onset</th>
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<tr>
<td>Nishioka et al. 2006 and 2009</td>
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<td>Uchiyama et al. 2008</td>
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<tr>
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<td>Sweden (Lister complex)</td>
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<th>Frequency (%)</th>
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<td>Belgium</td>
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<td>France</td>
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<th>The number of screening samples</th>
<th>Frequency (%)</th>
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<td>Fuchs et al. 2007</td>
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<td>Singleton et al. 2003</td>
<td>Iowa</td>
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Table 1. The clinical manifestations and prevalence of SNCA duplication and triplication
2.4 The Synuclein family in Parkinson disease

The SNCA gene is located on chromosome 4q21-22 and is associated with susceptibility to PD and DLB. Alpha-synuclein has two paralogous genes, beta- (SNCB; MIM#602569) and gamma-synuclein (SNCG; MIM#602998) with which it shares a highly conserved N-terminal domain. SNCB is located on chromosome 5q35 and SNCG is located on chromosome 10q23 associated with breast and ovarian cancer (Ji et al., 1997, Goedert, 2001). All three synuclein genes are highly expressed in brain; thalamus, substantia nigra, caudate nucleus, and amygdala (Lavedan, 1998, Lavedan et al., 1998). A phylogenic tree indicates that alpha- and beta-synucleins are related more closely to each other than to gamma-synuclein (Lavedan, 1998). Interestingly, two putative pathogenic mutations in SNCB are reported to cause DLB, however no significant co-segregation with disease could be shown and no other studies have identified these variants (Ohtake et al., 2004). A murine model with over-expressed gamma-synuclein is reported as a PD model with motor deficits (Ninkina et al., 2009). Our recent studies on common variation in the synuclein family of genes also suggested association for variants in both SNCA and SNCG with diffuse LB disease (Nishioka et al., 2010). Given these findings, it is postulated that there is a connection between not only SNCA, but also SNCB and SNCG and susceptibility to PD, however multiplications of the SNCB and SNCG loci have not yet been observed.

3. Conclusion and future work

Research focused on copy number variation has made remarkable progress in recent years. Genome-wide studies for copy number variants (CNV) indicate 1447 copy number variable regions (CNVRs) (Redon et al., 2006). Presumably, many of these CNV polymorphisms result in differential expression levels of proteins and dictate the phenotypic presentation at the individual level. Interestingly in Alzheimer disease multiplications of the APP gene have also been identified in families with autosomal dominantly inherited forms of the disease (Cabrejo et al., 2006, Rovelet-Lecrux et al., 2006). Robust and comprehensive studies are now warranted for CNV across the genome and may not only help develop new treatments for PD but perhaps several other neurodegenerative diseases.

4. Reference


SNCA Gene Multiplication: A Model Mechanism of Parkinson Disease


Xiromerisiou G, Hadjigeorgiou GM, Gourbali V, Johnson J, Papakonstantinou I, Papadimitriou A, Singleton AB (2007) Screening for SNCA and LRRK2 mutations...
The book Gene Duplication consists of 21 chapters divided in 3 parts: General Aspects, A Look at Some Gene Families and Examining Bundles of Genes. The importance of the study of Gene Duplication stems from the realization that the dynamic process of duplication is the "sine qua non" underlying the evolution of all living matter. Genes may be altered before or after the duplication process thereby undergoing neofunctionalization, thus creating in time new organisms which populate the Earth.

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