Beta-Glucocerebrosidase Gene Mutations P.Asn409Ser and P.Leu483Pro in Polish Patients with Parkinson’s Disease

Abstract

Background: The aim of his study was to evaluate the frequency of two most frequent GBA gene mutations in heterozygote state in patients with early and later onset Parkinson’s disease in Polish populations.

Methods and findings: Patients with Parkinson’s disease; 115 non-demented with early onset disease (<45 year-old) and 155 patients with onset over 45 year-old were recruited to the study. The p.Asn409Ser and p.Leu483Pro screening was performed with the PCR-RFLP and direct sequencing methods. In the group of 270 PD patients, 11 heterozygotes for mutations in the GBA gene were identified (carrier frequency 4.07%). Among patients with early onset disease carrier frequency was 4.34% compared to 3.87% in the group of later onset.

Conclusion: The study yielded that carrier frequency of GBA mutation in polish population was comparable to other European populations p.Asn409Ser mutation dominates in patients with early onset PD disease.

Keywords: GBA Gene; Parkinson’s Disease; Mutation; Dementia

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Introduction

Parkinson’s disease (PD) is one of the most common neurodegenerative diseases with an incidence of 100-200 cases per 100,000 population. It belongs to the group of synucleinopathies and is characterized by slowness of movement, tremors, muscle rigidity, bradykinesia, and postural instability. Mutations in the SNCA, LRRK2, UCHL-1, PARK2, PINK1, and DJ-1 genes [MIM_163890, MIM_609007, MIM_191342, MIM_602544, MIM_608309, MIM_606324] are thought to be the pathogenic causes of familial PD. However, the pathomechanism of these sporadic forms of PD are not fully understood, although several explanatory hypotheses have been proposed.

It has been suggested that mutations in the GBA gene (MIM_606463) coding for lysosomal beta-glucocerebrosidase (GBA; EC 3.2.1.45) are a probable risk factor for PD and Levy body dementia (LBD) [1-4], but only one study has also linked it to other synucleinopathies [5]. The reported incidence of GBA gene mutations in PD patients has varied according to geographical area, population studied, methods of DNA testing (sequencing versus evaluating only the most common mutations), and control groups [6-9].

Beta-glucocerebrosidase is a lysosomal hydrolase located in the lysosomal membrane and involved in the degradation of a sphingolipid glucocerebroside (glucosylceramide, GicCer). It is known that mutations in both alleles of the GBA gene lead to the Gaucher’s disease (GD), an autosomal recessive metabolic disorder characterized by accumulation of undegraded GicCer and secondary macrophage activation. The most frequent mutations causing GD are p.Asn409Ser and p.Leu483Pro (historical names N370S and L444P) [10].

Recent reports, however, suggest that mutations in the GBA gene present in a heterozygous state (i.e. in one allele of the gene) are associated with familial parkinsonism and an earlier age at the onset of PD [11-14]. Our study was conducted to evaluate the
presence of the two most common GBA gene mutations in Polish patients with early onset PD.

**Material and Methods**

Two groups of PD patients were included in the study; a group of 115 non-demented PD patients with early onset of the disease (<45 years of age) and a group of 155 patients with PD onset above 45 years of age. Patients were recruited based on the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria. The presence of dementia was investigated in all PD patients according to the MMSE protocol.

To identify mutations in the GBA gene, genomic DNA was extracted from white blood cells using standard techniques. Molecular analysis was performed using restriction fragment length polymorphism analysis of polymerase chain reaction-amplified fragments (PCR-RFLP) and direct sequencing methods in conditions excluding amplification of the GBA pseudogene (pGBA). Screening for p.Asn409Ser and p.Leu483Pro mutations was performed in all patients as described in the literature (PCR-PFLP with NciI and XhoI, respectively) [15,16]. Additionally, in the group of later onset PD patients, sequencing of exons 2, 8, and 9 was performed (details on reaction conditions and starter sequences available on request), and the obtained sequences were compared to the rates reported for other general European populations and PD patients [7,8].

**Results**

In the group of 115 PD patients with early onset of the disease, we identified 5 heterozygotes for the examined mutations in the GBA gene, including 4 individuals with and p.Leu483Pro and one person with p.Asn409Ser (carrier frequency in this group was 4.34%). In the later onset PD group, 6 of the 155 patients were heterozygotes for the p.Asn409Ser mutation (carrier frequency 3.87%). Overall, 11 heterozygotes for mutations in the GBA gene were identified from the 270 PD patients in this study (carrier frequency 4.07%).

In the later onset PD group, the mean number of MMSE p.Asn409Ser mutation carriers was 26.2 (SD 3.7), similar to that obtained from patients without GBA gene mutation (X=27.4, SD 3.0, P=0.28).

**Discussion and Conclusions**

The findings of this study demonstrate that the type of mutation in the GBA gene is strongly related to the age of PD onset and that most probably these mutations promote alpha-synuclein accumulation. The approximate carrier incidence of 4% found in our group of PD patients, independent age at onset of the disease, is about 10-fold higher as compared to the results obtained for a general European population, and similar to that reported earlier in PD patients (Table 1) [8].

In our group of PD patients, the L444P mutation was more frequently observed in individuals with an early onset of the disease, while the p.Asn409Ser mutation was more common in later onset PD patients, which is in line with what has previously been reported [17-19]. In patients with GD, the presence of the p.Asn409Ser mutation protects from neurologial involvement, whereas homozygosity of the p.Leu483Pro mutation leads to the neuronopathic types 2 and 3 of GD. It seems that the presence of p.N370S in PD patients results in a later onset of the disease as compared to p.Leu483Pro carriers.

Alfa-synuclein is a protein encoded by the SNCA gene and expressed mainly in presynaptic terminals. Mutations in the SNCA gene lead to autosomal dominant familial PD. Alfa-synuclein binds to lipids present in plasma membrane, synaptic vesicles, and elsewhere. Under pathological conditions, alfa-synuclein aggregates to form various oligomeric structures and insoluble amyloid fibrils which characterize the synucleinopathies [20]. A number of different explanations for alfa-synuclein aggregation have been put forward including ones related to the role of proper lysosomal and autophagy functions, to reticulum-associated protein degradation (ERAD) system, and to influence of altered membrane lipid composition [21]. A prion-like hypothesis has been also been described, in which alfa-synuclein aggregates are transmitted through exocytosis and subsequent endocytosis between neighbouring cells (donors and recipients) and the misfolded protein then acts as a template to promote misfolding of alfa-synuclein in recipient cells [21,22]. Recently, Yap et al. have proposed that membrane-bound alfa-synuclein interacts with GBA, inhibiting its enzymatic function. Thus, mutations reducing GBA level/activity or interfering with alfa-synuclein may lead to reduced lysosomal degradation and result in alfa-synuclein aggregation as well as GlcCer accumulation, which together mediate an interaction with the protein-enzyme complex in vesicles leading to further loss of activity [23].

Although the effect of GBA gene mutations on PD development is undisputed, other pathological factors must also be considered in the pathomechanism of alfa-synuclein aggregation, since not
all GD patients and GD carriers suffer from PD or parkinsonism. The low proportion of heterozygous carriers of the tested GBA mutations who develop PD suggests that heterozygosity for these mutations is neither the only nor the predominating factor in the pathophysiology of parkinsonism (Table 2).

The nonsignificant MMSE difference between the two patient subgroups in our study resulted from a relatively small patient sample and a small difference in the mean age between these groups. The study yielded that carrier frequency of GBA mutation in polish population was comparable to other European populations and p.Asn409Ser mutation dominates in patients with early onset PD disease.

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**Competing Interests**

Jamrozik Z and Ługowska A contributed equally in preparing manuscript. No other competing interests exist.

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**Table 2** Frequency of carriers for GBA mutations in different groups of patients with PD (partly from Velayati et al.).

| Population studied     | Carrier frequency | Author and Year          |
|------------------------|-------------------|--------------------------|
|                        | PD patients       | Controls                 |                           |
| Mixed                  | 21                | -                        | Lwin et al., 2004         |
| Ashkenazi Jews         | 31.3              | 6.2                      | Aharon-Peretz et al., 2004|
| Ashkenazi Jews         | 10.7              | 4.3                      | Clark et al., 2005        |
| Caucasians             | 5.68              | 0.8                      | Sato et al., 2005         |
| Norwegian              | 2.3               | 1.7                      | Toft et al., 2006         |
| Mixed (no Jewish)      | 12                | 3.2                      | Eblan et al., 2006        |
| Chinese                | 2.4               | 0                        | Tan et al., 2007          |
| Chinese                | 4.3               | 1.1                      | Ziegler et al., 2007      |
| Taiwanese              | 3.1               | 1.2                      | Wu et al., 2007           |
| Mixed (64% Jewish)     | 13.7              | 4.5                      | Clark et al., 2007        |
| Italian                | 2.8               | 0.2                      | de Marco et al., 2008     |
| Brazilian              | 3                 | 0                        | Spitz et al., 2008        |
| Mixed                  | 2.9               | 0.4                      | Mata et al., 2008         |
| Ashkenazi Jews         | 17.9              | 4.2                      | Gan-Or et al., 2008       |
| Portuguese             | 6.1               | 0.7                      | Bras et al., 2009         |
| Greek                  | 6.4 (early onset11.5) | 3.0                      | Kalinderi et al., 2009    |
| Mixed (<10% Jewish)    | 12.6              | 5.3                      | Nichols et al., 2009      |
| British                | 4.18              | 1.17                     | Neumann et al., 2009      |
| Japanese               | 9.4               | 0.37                     | Mitsui et al., 2009       |
| Korean                 | 3.2               | 0                        | Choi et al., 2012         |
| Greek                  | 10.2              | 3.4                      | Moraitou et al., 2011     |
| Brazilian              | 3.4               | 0                        | Guimaraes et al., 2012    |
| French                 | 6.7               | 1.0                      | Lesage et al., 2011       |
| Spanish                | 9.8               | -                        | Seto-Salvia et al., 2011  |
| Ashkenazi Jewish Non-Jewish | 15.0             | 3.0                      | Sidransky et al., 2009    |
| Polish(early onset)    | 4.3               | -                        | This report               |
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