Strong association between glucocerebrosidase mutations and Parkinson's disease in Sweden

Caroline Ran, Lovisa Brodin, Lars Forsgren, Marie Westerlund, Mehrfarin Ramezani, Sandra Gellhaar, Fengqing Xiang, Camilla Fardell, Hans Nissbrandt, Peter Söderkvist, Andreas Puschmann, Emil Ygland, Lars Olson, Thomas Willows, Anders Johansson, Olof Sydow, Karin Wirdefeldt, Dagmar Galter, Per Svenningsson, Andrea Carmine Belin

A R T I C L E   I N F O
Article history:
Received 8 February 2016
Accepted 26 April 2016
Available online 3 May 2016

Keywords:
Genetics
Lysosome
α-Synuclein
Gaucher's disease
GBA

A B S T R A C T
Several genetic studies have demonstrated an association between mutations in glucocerebrosidase (GBA), originally implicated in Gaucher’s disease, and an increased risk of Parkinson’s disease (PD). We have investigated the possible involvement of genetic GBA variations in PD in the Swedish population. Three GBA variants, E326K, N370S, and L444P were screened in the largest Swedish Parkinson cohort reported to date: 1625 cases and 2025 control individuals. We found a significant association with high effect size of the rare variant L444P with PD (odds ratio 8.17; 95% confidence interval: 2.51–26.23; p-value = 0.0020) and a significant association of the common variant E326K (odds ratio 1.60; 95% confidence interval: 1.16–2.22; p-value = 0.026). The rare variant N370S showed a trend for association. Most L444P carriers (68%) were found to reside in northern Sweden, which is consistent with a higher prevalence of Gaucher’s disease in this part of the country. Our findings support the role of GBA mutations as risk factors for PD and point to lysosomal dysfunction as a mechanism contributing to PD etiology.

© 2016 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction
The human glucocerebrosidase gene (GBA) is located on chromosome 1q21 and encodes a lysosomal protein, which cleaves the beta-glycosidic bond of glucosylceramide, an intermediate formed during glycolipid metabolism (Brady et al., 1965). Mutations in GBA, originally implicated in Gaucher’s disease (GD), have been identified both in familial and sporadic Parkinson’s disease (PD; Balicki et al., 2012). Genetic alterations of glucocerebrosidase (GCase) activity and decreased GCase activity, and protein levels have repeatedly been reported in brain tissue from PD patients with GBA mutations (Gegg et al., 2012; Lwin et al., 2004; Mazzulli et al., 2011). Interestingly, PD patients without GBA mutations were recently reported to have lower GCase activity as compared with healthy controls, suggesting GBA is involved in the pathologic mechanisms of PD even in the absence of known GBA gene body dementia with Alzheimer-like neuropathologic changes, but do not associate with Alzheimer’s disease (Clark et al., 2009; Tsuang et al., 2012). Genetic alterations of GBA, α-synuclein (SNCA), and leucine-rich repeat kinase 2 (LRRK2) together constitute the most common known genetic risk-factors for sporadic PD today (Ran and Belin, 2014).

Genetic variations in GBA are known to alter glucocerebrosidase (GCase) activity and decreased GCase activity, and protein levels have repeatedly been reported in brain tissue from PD patients with GBA mutations (Gegg et al., 2012; Lwin et al., 2004; Mazzulli et al., 2011). Interestingly, PD patients without GBA mutations were recently reported to have lower GCase activity as compared with healthy controls, suggesting GBA is involved in the pathologic mechanisms of PD even in the absence of known GBA gene
insight into the role of (Alcalay et al., 2012; Mata et al., 2015; Seto-Salvia et al., 2012). To gain symptoms, in particular cognitive impairment and dementia DOPA (Clark et al., 2007). However, patients with clinically similar to patients with sporadic PD and respond well to L-
characterized by relatively early disease onset, but are otherwise according to genetic background (Gan-Or et al., 2015; Sidransky et al., 2009). In particular, GBA mutations are more common in subjects with Ashkenazi Jewish ancestry (Aharon-Peretz et al., 2004; Gan-Or et al., 2008; Sidransky et al., 2009).

Not only rare mutations but also single nucleotide polymorphisms (SNPs) such as E262K have been suggested to affect the risk of developing PD (Clark et al., 2007; Liwin et al., 2004). E262K has been reported to associate with PD in European populations, although there are conflicting results (Lesage et al., 2011; Sidransky et al., 2009). The association with E262K is particularly strong in patients with early disease onset and suggested to result in an aggravated phenotype with cognitive decline (Duran et al., 2013; Mata et al., 2015). GCase activity measured in post-mortem brains of PD patients heterozygous for E262K ranged between 90% and 100% of the activity in wild-type carriers (Liwin et al., 2004), but in combination with other GBA mutations, for example L444P, the E262K mutation leads to further deterioration of enzymatic activity (Chabas et al., 2005; Montfort et al., 2004).

GBA mutations have not frequently reached significance in genome-wide association studies (GWAS), probably because of the low minor allele frequencies of PD associated GBA mutations and the methodological difficulties in avoiding contaminating signals from the pseudogene GBAP1 (glucosidase, beta, acid pseudogene 1). Other factors that might influence the outcome of a GWAS are which genetic markers are included and how these are linked to disease associated variants. Nevertheless, 2 GWAS studies published in 2011 and 2012 reported association between PD and N370S, and one of them further supports the E262K association (Do et al., 2011; Pankratz et al., 2012). These data have also been confirmed in large meta-analyses (Lill et al., 2012; Nalls et al., 2014).

Parkinson patients carrying heterozygous GBA mutations are characterized by relatively early disease onset, but are otherwise clinically similar to patients with sporadic PD and respond well to L-DOPA (Clark et al., 2007). However, patients with GBA mutations have been reported to be more likely to suffer from nonmotor symptoms, in particular cognitive impairment and dementia (Alcalay et al., 2012; Mata et al., 2015; Seto-Salvia et al., 2012). To gain insight into the role of GBA in the etiology of PD in the relatively homogenous Swedish population, we investigated the presence of 3 most reported nonsynonymous GBA variants in PD, N370S, L444P, and E262K, among Swedish Parkinson patients and controls. In the light of the particularly elevated prevalence of GBA mutations in individuals with Ashkenazi Jewish descent, the Swedish population, which has not previously been screened for GBA mutations, is especially interesting to study as there is a known occurrence of GD type III (Norrbottian type) in northern Sweden (Dahl et al., 1990).

### 2. Materials and methods

#### 2.1. Human DNA and tissue

A total of 1625 Swedish PD patients were recruited from the neurology clinics at Karolinska University Hospital, Stockholm, Sahlgrenska University Hospital, Gothenburg, Skåne University Hospital, Lund, Umeå University Hospital, Umeå and Linköping University Hospital, Linköping. Patient material was obtained after informed consent and approval of the local ethics committees in Stockholm, Gothenburg, Lund, Umeå, and Linköping, respectively (http://www.epn.se). Information on cognitive decline was only available for patients from Lund and was reported for 33 of the 122 patients (27.9%) which were also included in the genetic screening. The PD patients were diagnosed according to the United Kingdom Parkinson’s Disease Society Brain Bank criteria for idiopathic PD, including patients who declared having one or more first, second, or third degree relatives with PD to get a large material (Gibb and Lees, 1988). The 2025 Swedish control subjects were recruited from the corresponding catchment areas as the patient material and consisted of spouses of PD patients, individuals visiting the neurology clinic, blood donors, and subjects recruited from an ongoing longitudinal study, SNAC-K (The Swedish National Study on Aging and Care in Kungsholmen, http://www.snac-k.se/), see Table 1 for further site specific demographic information. Screenings of known PD mutations in LRRK2 and SNCA have been performed previously on a subset of the joint case-control material (Carmine Belin et al., 2006; Puschmann, 2011; Westerlund et al., 2008). All subjects were unrelated and a majority of Caucasian origin (~95%). DNA was extracted from whole blood according to standard protocols.

#### 2.2. Genotyping

##### 2.2.1. Pyrosequencing

The 3 genetic GBA variants were genotyped by pyrosequencing (Ronaghi et al., 1998), except for a fraction of samples which were genotyped for N370S with TaqMan (see paragraph 2.2.2 on TaqMan SNP genotyping for details). Primer sequences are available on request (Thermo Scientific, MA, USA). Primers were designed using free online software (Primer 3 v4.0.0 and mFold v3.6; Koressaar and
One of the primers in each primer-pair was biotinylated at the 5'-end to allow subsequent immobilization to streptavdin-coated beads. All primers were designed to bind specifically to GBA by targeting parts of the sequence that varied between GBA and the pseudogene GBAP1. A polymerase chain reaction (PCR) reaction was run for 45 cycles at 95 °C for 20 seconds, annealing for 20 seconds at different temperatures: 57 °C for N370S (rs76763715), and 60 °C for L444P (rs421016) and E326K (rs2230288). After PCR, the biotinylated PCR product was immobilized to streptavidin-coated beads (GE Healthcare, Buckinghamshire, United Kingdom), mixed for 10 minutes at room temperature and captured onto filter probes (PyroMark Vacuum Prep Tool, Qiagen, Venlo, the Netherlands). The filter probes were flushed with 70% ethanol, 1M NaOH denaturation solution, and washing buffer and the single-stranded template was annealed to the sequencing primer at 80 °C for 370S, L444P, and 90 °C for E326 K for 2–3 minutes. All solutions used in sample preparation were made according to manufacturer’s instructions (Qiagen). Samples were analyzed on an automated pyrosequencer (PSQ 96 System with SNP Software and PyroMark Gold Reagent Kits; Qiagen).

2.2.2. TaqMan SNP genotyping

Two hundred forty-nine samples from Karolinska University Hospital, 165 samples from Skåne University Hospital, Lund, and 854 samples from Umeå University were genotyped for N370S using TaqMan SNP genotyping (ABI 3730 DNA Analyzer instrument; Applied Biosystems, Carlsbad, CA, USA). We used a custom-designed assay (forward primer: 5’ GTG ACC CTT ACC TAC ACT CTC T, reverse primer: 5’ GGT TCA GGG CAA GGT TCC), the reverse primer being specific for the GBA gene, thus excluding the pseudogene GBAP1. Allelic discrimination was run with 2'5' fluorescence-labeled allele-specific probes: C_2286559_30 and C_2268560_20, genotyping master mix (TaqMan, Applied Biosystems), and 20 ng of genomic DNA. We used the default PCR fast cycling conditions, 10 minutes at 95 °C, 15 seconds at 92 °C, and 1 minute at 60 °C for 40 cycles. Analysis was performed with software supplied with the instrument (QuanStudio 6 and 7, Flex Real-Time PCR System Software v1.0). Mutation carriers detected with TaqMan were resequenced by pyrosequencing to confirm results.

2.3. Statistical analysis

We achieved a call rate of 95.3% for E326K, 95.8% for N370S, and 97.4% for L444P. Genotypic and allelic associations were evaluated separately for southern and northern Sweden because of uneven geographical distribution of mutation carriers. Analysis was performed under a dominant model, using appropriate software (Prism 5.03; GraphPad Softwares Inc, La Jolla, CA, USA) with chi-square ($\chi^2$) and Fisher’s exact test, significance level 5%, 2-sided $p$-values. Results were compared between southern and northern Sweden by means of a meta-analysis based on the odds ratio (ORs) obtained from the dominant genotype analysis. Meta-analysis included Cochran’s Q statistics and calculation of the heterogeneity index $I^2$ as a measure of consistency between studies and was performed in PLINK v1.07 (Purcell et al., 2007). The meta-analysis was run under a random-effects model. Bonferroni correction for multiple testing was used in both the association analysis and the meta-analysis. We used a $\chi^2$ Hardy-Weinberg equilibrium (HWE) test to calculate HWE for each variation in the control population (Rodriguez et al., 2009). To compare demographic characteristics between groups, unpaired Student’s $t$ test was used; 2-tailed $p$-values (Prism 5.03).

### 3. Results

#### 3.1. Association analysis

Our study included 3 genetic variations in GBA, 2 rare mutations: N370S and L444P, and the more common SNP, E326K. Parkinson patients and healthy control individuals from 5 different geographic locations in Sweden were analyzed. We found that the 2 rare variants occurred more often in PD patients than in healthy individuals (Table 2). Furthermore, E326K was overrepresented in PD patients, with a minor allele frequency of 3.05%, versus 1.75% in controls. None of the identified GBA mutation carriers have been reported to carry SNCA and/or LRRK2 mutations to date (Carmine Belin et al., 2006; Puschmann, 2011; Westerlund et al., 2008). When comparing site-specific genotype data, we discovered that L444P was more common in the cohort collected in Umeå in northern Sweden where 4.11% of the PD patients were heterozygous for L444P as compared with 0.79% of the patients in Stockholm, 2.47% in Gothenburg, and 1.72% in Lund. In total, 26 of the identified L444P carriers (68%) were from the northernmost Swedish provinces (Norrbotten and Västerbotten). Considering the skewed geographical distribution of L444P carriers in favor of Norrbotten and Västerbotten, and the elevated incidence of GD type III in the north provinces, we decided to analyze genotype data from southern Sweden (Gothenburg, Linköping, Stockholm, and Lund) and northern Sweden (Umeå) separately (Dahl et al., 1990). In performing a separate analysis for these 2 groups, we further avoid any bias that might be introduced in our analysis by the genetic population stratification that occurs in the Swedish population between northern and southern counties (Humphreys et al., 2011). The E326K variation was in HWE in controls from southern Sweden but not in controls from northern Sweden. Because of the lack of homozygous mutation carriers, we could not perform HWE analysis for N370S and L444P.

Statistical analysis showed that L444P was significantly associated with PD in southern Sweden (Table 2). L444P had an OR of

### Table 2: Genotype frequencies and test statistics of E326K, N370S, and L444P

| SNP   | Genotype | Southern Sweden | Northern Sweden | $\chi^2$ (df) | OR (C)   | $p$-value | $\chi^2$ (df) | OR (C)   | $p$-value |
|-------|----------|-----------------|-----------------|--------------|----------|-----------|--------------|----------|-----------|
|       | CTRL, n (%) | PD, n (%)      | CTRL, n (%)    | PD, n (%)    |           |           |              |           |           |
| E326K | CC       | 1731 (96.65)    | 858 (94.83)    | 4.93 (1)     | 1.57 (1.07–2.32) | 0.081 | 141 (95.92) | 552 (92.93) | NA        | 1.79 (0.75–4.29) | 0.78 |
|       | CT/TT    | 59 (3.35)       | 48 (5.17)      | 0.0          |          |           |              |           |           |
| N370S | TT       | 1768 (99.89)    | 971 (99.28)    | 5.29 (1)     | 6.73 (1.32–30.75) | 0.065 | 152 (100)  | 595 (99.50) | NA        | 1.79 (0.09–34.92) | 1   |
|       | TC/CC    | 2 (0.11)        | 7 (0.72)       | 0            | 3 (0.50) |           |              |           |           |
| L444P | AA       | 1812 (99.89)    | 971 (99.58)    | 10.27 (1)    | 9.33 (2.04–42.69) | 0.0039 | 151 (99.34) | 583 (95.89) | NA        | 6.49 (0.87–48.28) | 0.13 |
|       | AC/GG    | 2 (0.11)        | 10 (1.02)      | 1            | 25 (4.11) |           |              |           |           |

Southern Sweden comprises the geographic areas of Stockholm, Gothenburg, Linköping, and Lund; Northern Sweden comprises samples collected at Umeå University Hospital. Key: CI, 95% confidence interval; CTRL, control individuals; df, degrees of freedom; NA, not applicable; OR, odds ratio; $p$-value, $p$-value after Bonferroni correction for multiple testing; PD, Parkinson’s disease; SNP, single nucleotide polymorphism.
9.33, with a 95% confidence interval (95% CI) of 2.04—42.69, and a p-value after Bonferroni correction for multiple testing (pC) of 0.0039. Genotype analysis of N370S and E326K showed a trend for association with PD in southern Sweden (Table 2). N370S had an OR of 6.73; 95% CI: 1.32—30.75, and a crude p-value of 0.022, but the association did not remain significant after correction for multiple testing, pC = 0.065. The corresponding values for E326K were OR = 1.57, 95% CI: 1.07—2.32, p-value = 0.027, which also lost significance after Bonferroni correction. Allele analysis did not add substantial information (data not shown) with the exception of confirming the association between the minor allele (C) of N370S and an increased risk of developing PD (OR = 7.27; 95% CI, 1.54—34.26; pC = 0.028).

In the northern Swedish case-control material, all 3 GBA variants were overrepresented, and in particular L444P, which was present in 4.11% of the PD patients, as compared with 1.02% of the PD patients in southern Sweden. Statistical testing in this patient group was inconclusive, probably because of the low number of controls (Table 2). However, L444P showed a trend for association with an OR of 6.49 and a p-value of 0.042, but the CI overlaps 0 (95% CI, 0.87—48.28), and the p-value did not hold for Bonferroni correction (pC = 0.13).

The distribution of mutation carriers was equal between sexes; 24 female carriers and 26 male carriers. Five healthy individuals were discovered to carry N370S or L444P mutations. Three of these individuals (2 heterozygous for N370S and 1 for L444P) were anonymous blood donors, for whom future development of PD cannot be excluded. The remaining 2 were healthy individuals heterozygous for L444P, with no signs of PD when followed up to ages 66 and 75 years.

The importance of these 3 variants in PD etiology in the overall Swedish population was evaluated using a meta-analysis. Cochran’s Q statistics were nonsignificant and heterogeneity was estimated nonexistent, indicating that the results from these 2 studies are consistent and can be compared (Table 3). Data were analyzed under a random-effects model because we expected the effect of these mutations to vary between southern and northern Sweden. Pooled estimates confirmed the association between L444P and E326K and increased risk of PD, with a small effect size for E326K (OR 1.60; 95% CI, 1.16—2.22; pC = 0.026) and a large effect size for L444P (OR 8.17; 95% CI, 2.51—26.63, pC = 0.0020; Table 3). We also found a trend for association between N370S and PD with a p-value of 0.023, which did not remain significant after correction for multiple testing (OR 5.04; 95% CI, 1.10—23.04; pC = 0.068).

3.2. Clinical characteristics of mutation carriers

We furthermore investigated the phenotype of patients carrying L444P and N370S mutations (Table 4). In concert with previous reports on PD patients with GBA mutations, we observed that many patients had a relatively early onset (50% were aged ≤55 years), in particular individuals carrying L444P mutations, who had an average age of onset of 55.7 ± 10.9 years, p = 0.0052 as compared with 709 of the noncarrier patients (for whom information on age of onset was available). N370S patients had an average age of onset of 63.6 ± 12.2 years, which is not lower than that of the noncarrier group (p = 0.36). Apart from the age of onset, mutation carriers did not differ significantly from other PD patients, 24.4% had a known familial history of PD and there were no other features indicating a more severe or a more rapidly progressing phenotype based on the clinical information available. Information on cognitive decline was available for a subset of affected carriers (n = 34) which allowed us to estimate that around 20% of the mutation carriers suffer from cognitive decline.

4. Discussion

Here, we describe a genetic study on 2 rare GBA mutations and 1 common GBA SNP in the largest Swedish PD case-control study reported to date. We found known pathogenic GBA mutations predominantly in PD patients. In a meta-analysis, comprising patients from the entire country, L444P strongly associated with PD, whereas N370S showed a trend for association. The allele association found between the minor allele of N370S and increased risk for PD in southern Sweden further supports the importance of N370S also in our study population. Both mutations were associated with high ORs, and somewhat broad 95% CIs clearly separated from 0. We also found an association between E326K and PD, in agreement with previous observations. The allele frequency for E326K in PD described by others corresponds to our observation in the Swedish population (Lesage et al., 2011). Because of the suggested role of E326K as a modifier (Chabas et al., 2005; Montfort et al., 2004), it would be interesting to investigate the possible combined effect of carrying this SNP plus one of L444P or N370S, but allele frequencies of these 3 mutations were too low to allow for haplotype analysis. Only 1 of the subjects in this study carried more than 1 mutated GBA allele. This individual was heterozygous for E326K and L444P and, with an age of onset 55 years, is not exceptional, considering GBA mutation carriers overall have relatively early onset of symptoms (Gan-Or et al., 2008; Nichols et al., 2009).

The common variant E326K presented a lower OR (OR E326K = 1.60) than the rare mutations (OR N370S = 5.04 and OR L444P = 8.17), which was also expected considering the detrimental effect of N370S and L444P on enzyme activity. The effect of L444P was stronger than that of N370S, which is in agreement with the classification of the L444P mutations as severe, whereas N370S is classified as mild (Gan-Or et al., 2015; Mao et al., 2013).

The 2 mutations analyzed, L444P and N370S, were observed more often in PD patients than in control subjects. N370S was found in 10 patients (0.63%), 1 of which was homozygous, and 2 were controls (0.10%), whereas L444P was found in 35 patients (2.20%) and 3 control individuals (0.15%). Although individuals carrying homozygous GBA mutations usually suffer from GD, the homoyz- gous N370S mutation carrier had a clear idiopathic PD diagnosis with no signs of dementia, when followed up at the age of 75. Homozygous GBA mutation carriers with typical PD phenotypes have also been reported by others (Lesage et al., 2011). The overall occurrence of these 2 GBA variations was 2.77% in the Swedish PD population, which is comparable with what has been reported globally (around 3% in PD cohorts without Ashkenazi Jewish ancestry), although this number is very different depending on ethnicity (Sidransky et al., 2009). In Norway, the frequencies of L444P and N370S are also somewhat lower than 3%, indicating that these variations are not very frequent in the Scandinavian populations (Sidransky et al., 2009; Toft et al., 2006). We observed a clear stratification in the Swedish population, with a higher mutation load in the northern parts of the country. In total, 29 of the 50 identified GBA mutation carriers (controls and patients) were from northern Sweden. Of these, 26 individuals were heterozygous for

### Table 3

| SNP     | pC-value | Pooled OR | 95% CI    | Q     | I²  |
|---------|----------|-----------|-----------|-------|-----|
| E326K   | 0.026    | 1.60      | 1.16—2.22 | 0.99  | 0.00|
| N370S   | 0.068    | 5.04      | 1.10—23.04| 0.41  | 0.00|
| L444P   | 0.0020   | 8.17      | 2.51—26.63| 0.77  | 0.00|

Key: 95% CI, 95% confidence interval; I², I² heterogeneity index; OR, random-effects meta-analysis odds ratio; pC-value, random-effects meta-analysis p-value with Bonferroni correction for multiple testing; Q, p-value for Cochran’s Q statistic; SNP, single nucleotide polymorphism.
L444P, which corresponds to 4.11% of the PD patients in northern Sweden, as compared with 1.02% in southern Sweden. Only 3 individuals from northern Sweden were heterozygous for N370S, which was comparable with the percentage of heterozygous individuals from northern Sweden, but only constitute a rare characteristic of a large number of control subjects (Linder et al., 2010). Our study did not include all pathogenic mutations, known or yet to be discovered, are more predominant in the Swedish PD population. For example, the membrane-bound forms of GBA gene affect PD pathogenesis, but there is an interesting connection to another PD candidate gene, SNCA, which strengthens the hypothesis of involvement of the endosomal and/or lysosome-autophagy pathways in PD. For example, the membrane-bound forms of GBA, glucocerebrosidase, is further limited by the uneven distribution of controls between northern Sweden (Norrbotten and Västerbotten), which has been linked to the increased prevalence of Norrbottian type GD in northern individuals in the rest of the population. These data are consistent with the increased prevalence of Norrbottian type GD in northern Sweden (Norrbotten and Västerbotten), which has been linked to homozgyosity of the L444P mutation (Dahl et al., 1990).

Because our study did not include all pathogenic GBA variations discovered, we might be overlooking important components of the GBA contribution to PD pathology in Sweden. It is possible that other GBA mutations, known or yet to be discovered, are more predominant in the Swedish PD population. On the other hand, our results suggest that GBA mutations contribute to the high incidence of PD reported from northern Sweden, but only constitute a rare risk factor for PD elsewhere in Sweden (Linder et al., 2010). Our study is further limited by the uneven distribution of controls between southern and northern Sweden and by the unknown age and specific characteristics of a large number of control subjects (n = 855) who were blood donors and can, therefore, be expected to have a lower mean age at enrollment than the PD patients in this study.

In agreement with previous studies, we found that L444P mutation carriers have lower age of onset of PD symptoms than non-carriers (p = 0.0052). A few controls were found to carry GBA mutations (0.25%), 3 were anonymous blood donors and they might all be at risk for developing PD in the future. Finding carriers of pathogenic mutations among controls is not unexpected, and healthy control subjects with a copy of the N370S or L444P allele have been reported in other populations as well (Sidransky et al., 2009; Toft et al., 2006). Moreover, the penetrance of the L444P mutation has been estimated to be 30% at the age of 70 (Anheim et al., 2012). It is possible that the L444P carriers in our study (aged 66 and 75) will develop PD with increasing age, but because one of the hallmarks of GBA mutations is early disease onset, we expect these individuals to remain healthy. Healthy mutation carriers are interesting subjects for further genetic studies, as they might carry protective genetic variants, either generally neuroprotective or counteracting the effect of lower GCase activity.

It is not clear how mutations in the GBA gene affect PD pathogenesis, but there is an interesting connection to another PD candidate gene, SNCA, which strengthens the hypothesis of involvement of the endosomal and/or lysosome-autophagy pathways in PD. For example, the membrane-bound forms of...
α-synuclein and GCase have been demonstrated to interact under lysosomal conditions, leading to inhibition of GCase (Yap et al., 2011, 2013). The levels of α-synuclein protein are affected by mutations in GBA both in cell culture and in vivo in GBA transgenic mice (Cullen et al., 2011; Mazzuolli et al., 2011). Similarly, knockdown of GBA results in increased α-synuclein levels (Mazzuolli et al., 2011). It should be noted that there are also data reporting no effect on α-synuclein or lysosomal function of GCase inhibition (Dermontzaki et al., 2013). However, untreated GD patients have increased plasma levels of oligomeric α-synuclein, whereas such an increase was not observed in GD patients having received GCase replacement therapy (Pinhelina et al., 2014). Inaccurate GCase handling is hypothesized to result in accumulation of α-synuclein (Mazzuolli et al., 2011). The elevated α-synuclein levels observed as a result of low GCase activity would then lead to further reduction in GCase activity and impairment of the intracellular trafficking of GCase, which becomes retained in the endoplasmic reticulum (ER; Mazzuolli et al., 2011). Interestingly, α-synuclein accumulation can interfere with vesicle transport between the ER and the Golgi apparatus (Cooper et al., 2006). Furthermore, GBA mutations might impair the unfolded protein response of the ER (Kurzawa-Akanbi et al., 2012). An alternative explanation suggests that GBA deficiency impairs autophagy and, thereby, results in α-synuclein accumulation (Du et al., 2015). Altered expression of proteins involved in autophagy has been observed as a result of impaired GCase activity (Du et al., 2015: Rocha et al., 2015). Moreover, α-synuclein accumulation can be reversed by the use of drugs inducing autophagy in cell culture (Cullen et al., 2011; Du et al., 2015).

5. Conclusions

This genetic study has been conducted on the largest PD case-control material yet reported from Sweden. We found N370S and L444P predominantly in PD patients and they were significantly associated with PD in Sweden. Both mutations are expected to contribute to PD pathology. How heterozygous GBA mutations affect cellular mechanisms and contribute to PD pathogenesis is unknown, but accumulating evidence suggest the mechanism involves α-synuclein accumulation and impairment of the lysosome and autophagy pathways. L444P mutations were more common in northern Sweden, which is consistent with the higher incidence of GD type III in this part of Sweden. The common variant E326K was also found to associate with increased risk for PD in Sweden, supporting a role for common genetic variations in PD etiology. Further genetic screening and studies of GBA should bring new insights into the pathophysiology of PD, which in turn may result in earlier diagnosis and design of relevant therapeutic strategies.

Disclosure statement

The authors have no actual or potential conflicts of interest.

Acknowledgements

The authors thank Laura Fratiglioni for providing control samples from the SNAC-K project. Research nurse Christin Karremo compiled Lund patient data. For samples from Lund, Biobank services were performed at Biobank, Labmedicin Skåne, University and Regional Laboratories Region Skåne, Sweden.

This work was supported by Swedish Brain Power, the Swedish Research Council (K2013-99X-22248-01-3), the Swedish Parkinson Foundation (613/13, 712/14), the Swedish Brain Foundation (PO2013-0213), Åke Wibergs Stiftelse (756194137), Karolinska institutets Funds (2013fbi37223), the Karolinska DPA, governmental funding for clinical research within the Swedish National Health Services (ALF), the Neurology Department Karolinska University Hospital 100-year Fund, ERC Advanced Investigator Grant (#322744), the Swedish Parkinson Academy, the Swedish Board of National Health and Welfare for contributions to National Treatment Guidelines for Parkinson Disease, Odd Fellow Luleå; Umeå University (Insamlingstiftefalen), Bundy Academy, Sweden, Lions Research Foundation Skåne, Elsa Schmitz Stiftelse, Skåne University Hospital Foundations and Donations program, NEURO förbundet, Sweden. None of these funds have been actively involved in the conduct of the research included in this report or the preparation and submission of this article.

References

Aharon-Peretz, J., Rosenbaum, H., Gershoni-Baruch, R., 2004. Mutations in the glucocerebroside gene and Parkinson’s disease in Ashkenazi Jews. N. Engl. J. Med. 351, 1972–1977.
Alcalay, R.N., Caccappolo, E., Mejia-Santana, H., Tang, M., Rosado, L., Orbe, R.M., Ruiz, D., Ross, B., Verbitsky, M., Kisselev, S., Lewis, E., Comella, C., Colcher, A., Jennings, D., Nance, M., Bressman, S., Scott, W.K., Tanner, C., Mickel, S., Andrews, H., Waters, C., Fahn, S., Cote, L., Frucht, S., Ford, B., Rezek, M., Novak, R., Friedmann, J.H., Pfeiffer, R., Marsh, L., Hiner, B., Siderowf, A., Payami, H., Molho, E., Factor, S., Ottman, R., Clark, L.N., Marder, K., 2012. Cognitive performance of GBA mutation carriers with early-onset PD: the COR-PD study. Neurology 78, 1433–1440.
Alcalay, R.N., Levy, O.A., Waters, C.C., Fahn, S., Ford, B., Kuo, S.H., Mazzoni, P., Pascuilo, M.W., Nichols, W.C., Gan-Or, Z., Rouleau, G.A., Chung, W.K., Wolf, P., Oliva, P., Keutzer, J., Marder, K., Zhang, X., 2015. Glucocerebrosidase activity in Parkinson’s disease with and without GBA mutations. Brain 138, 2648–2658.
Anheim, M., Elbaz, A., Lesage, S., Durr, A., Condroyer, C., Viallet, F., Pollak, P., Bonaiti, B., Bonafetti-Pellie, C., Brice, A., 2012. Penetration of Parkinson disease in glucocerebrosidase gene mutation carriers. Neurology 78, 417–420.
Balicki, D., Beutler, E., 1995. Gaucher disease. Medicine (Baltimore) 74, 305–323.
Brady, R.O., Kanfer, J., Shapiro, D., 1965. The metabolism of glucocerebrosides. I. Purification and properties of a glucocerebroside-cleaving enzyme from spleen tissue. J. Biol. Chem. 240, 39–43.
Carmel, R., Belin, A., Westerlund, M., Sydow, O., Lundström, K., Håkansson, A., Nissbrandt, H., Olson, L., Galter, D., 2006. Leucine-rich repeat kinase 2 (LRRK2) mutations in a Swedish Parkinson cohort and a healthy nonagenarian. Mov. Disord. 21, 1731–1734.
Chabas, A., Gort, L., Díaz-Font, A., Montfort, M., Santamaria, R., Cidras, M., Grinberg, D., Villegelú, L., 2005. Perinatal lethal phenotype with generalized ichthyosis in a type 2 Gaucher disease patient with the [L444P:E326K][P182] genotype: effect of the E326K change in neonatal and classic forms of the disease. Blood Cells Mol. Dis. 35, 253–256.
Chiasseri, D., Paciotti, S., Eusebi, P., Persichetti, E., Tasegian, J., Kurzawa-Akanbi, M., Chinnery, P.F., Morris, C.M., Calabresi, P., Parnetti, L., Beccari, L., 2013. Selective loss of glucocerebrosidase activity in sporadic Parkinson’s disease and dementia with Lewy bodies. Mol. Neurodegener. 10, 15.
Clark, L.N., Kartsaklis, L.A., Wolf, G.R., Dorado, B., Ross, B.M., Kisselev, S., Verbitsky, M., Mejia-Santana, H., Caccappolo, E., Mejia-Santana, H., Tang, M., Rosado, L., Bressman, S., Scott, W.K., 2015. Al-pha-synuclein blocks ER-Golgi trafficking in human neuronal cells. PLoS One 8, e60674.
Cressman, J.A., Tian, L., Zhou, J., Cady, K., Wilkins, B., 2015. 
Cullen, V., Sardi, S.P., Ng, J., Xu, Y.H., Tomlinson, J.J., Kolodziej, P., Kahn, I., 2011. Genetic screening and studies of GBA should bring new insights into the pathophysiology of PD, which in turn may result in earlier diagnosis and design of relevant therapeutic strategies.

Disclosure statement

The authors have no actual or potential conflicts of interest.

Acknowledgements

The authors thank Laura Fratiglioni for providing control samples from the SNAC-K project. Research nurse Christin Karremo compiled Lund patient data. For samples from Lund, Biobank services were performed at Biobank, Labmedicin Skåne, University and Regional Laboratories Region Skåne, Sweden.

This work was supported by Swedish Brain Power, the Swedish Research Council (K2013-99X-22248-01-3), the Swedish Parkinson Foundation (613/13, 712/14), the Swedish Brain Foundation (PO2013-0213), Åke Wibergs Stiftelse (756194137), Karolinska institutets Funds (2013fbi37223), the Karolinska DPA, governmental funding for clinical research within the Swedish National
Murphy, K.E., Gysbers, A.M., Abbott, S.K., Tayebi, N., Kim, W.S., Sidransky, E., Nalls, M.A., Pankratz, N., Beecham, G.W., DeStefano, A.L., Dawson, T.M., Doheny, K.F., Factor, S.A., Hamza, T.H., Hung, A.Y., Hyman, B.T., Ivison, J., Krainc, D., Lauterberg, B.E., Clark, L.N., Marder, K., Martin, E.R., Mayeux, R., Ross, O.A., Scherzer, C.R., Simon, D.K., Tanner, C., Vance, J.M., Wszolek, Z.K., Zabetian, C.P., Myers, R.H., Payami, H., Scott, W.K., Foroud, T., 2012. Meta-analysis of Parkinson’s disease: identification of a novel locus, RTN. Neurol. 71, 370–384.

Pechina, S.N., Nuzhnyi, E.P., Emelyanov, A.K., Boukina, T.M., Usenko, T.S., Nikolaev, M.A., Salogub, G.N., Yakimovski, A.F., Zakhareva, O.Y., 2014. Increased plasma oligomeric alpha-synuclein in patients with lysosomal storage diseases. Neurosci. Lett. 583, 188–193.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, M., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575.

Puschmann, A., 2011. Heredity in Parkinson’s disease. From rare mutations to common genetic risk factors, first ed. Media Trixy, Lund University, Lund, Sweden.

Ran, C., Belin, A.C., 2014. The genetics of Parkinson’s disease: review of current and emerging candidates. J. Parkinsonism Restless Legs Syndr. 4, 63–75.

Roba, D., Aboud, N., Tenen, S., Mercier, P., Robert, A., Kräutler, A., Gavrilov, S., Etienne, A., de Reuver, P.R., Pearson, M.J., Gao, Y., 2015. Alpha-synuclein in the substantia nigra of idiopathic Parkinson’s disease. Brain 138, 3098–3108.

Rosas, D.H., Gutzwiller, M., Riederer, P., Frangione, B., Forno, L., 2002. Parkinson’s disease: a metabolic disease of alpha-synuclein. J. Neural Transm. 109, 1–27.

Ryoo, S.J., Yu, M., Averbuch-Pouchot, E., Fulbright, R., Perry, T., Irizarry, C., Rojas, J., St Louis, J., 2015. Glucocerebrosidase expression and plasma oligomeric alpha-synuclein in patients with lysosomal storage disease. Neurosci. Lett. 602, 89–94.

Sahay, S., Nanda, A., Panchal, S., Das, S., Misra, K., Chaudhuri, K.R., 2014. Mutations in SLC25A5 are associated with idiopathic Parkinson’s disease. Ann. Neurol. 75, 220–227.

Sahay, S., Nanda, A., Panchal, S., Das, S., Misra, K., Chaudhuri, K.R., 2013. Mutations in SLC25A5 are associated with idiopathic Parkinson’s disease. Ann. Neurol. 75, 220–227.

Sahay, S., Nanda, A., Panchal, S., Das, S., Misra, K., Chaudhuri, K.R., 2014. Mutations in SLC25A5 are associated with idiopathic Parkinson’s disease. Ann. Neurol. 75, 220–227.

Sahay, S., Nanda, A., Panchal, S., Das, S., Misra, K., Chaudhuri, K.R., 2013. Mutations in SLC25A5 are associated with idiopathic Parkinson’s disease. Ann. Neurol. 75, 220–227.