Genome-wide mapping of IBD segments in an Ashkenazi PD cohort identifies associated haplotypes

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The recent series of large genome-wide association studies in European and Japanese cohorts established that Parkinson disease (PD) has a substantial genetic component. To further investigate the genetic landscape of PD, we performed a genome-wide scan in the largest to date Ashkenazi Jewish cohort of 1130 Parkinson patients and 2611 pooled controls. Motivated by the reduced disease allele heterogeneity and a high degree of identical-by-descent (IBD) haplotype sharing in this founder population, we conducted a haplotype association study based on mapping of shared IBD segments. We observed significant haplotype association signals at three previously implicated Parkinson loci: LRRK2 (OR = 12.05, \(P = 1.23 \times 10^{-56}\)), MAPT (OR = 0.62, \(P = 1.78 \times 10^{-11}\)) and GBA (multiple distinct haplotypes, OR > 8.28, \(P = 1.13 \times 10^{-11}\) and OR = 2.50, \(P = 1.22 \times 10^{-9}\)). In addition, we...
identified a novel association signal on chr2q14.3 coming from a rare haplotype (OR = 22.58, \( P = 1.21 \times 10^{-10} \)) and replicated it in a secondary cohort of 306 Ashkenazi PD cases and 2583 controls. Our results highlight the power of our haplotype association method, particularly useful in studies of founder populations, and reaffirm the benefits of studying complex diseases in Ashkenazi Jewish cohorts.

INTRODUCTION

Parkinson disease (PD; OMIM: #168600) is a common neurodegenerative disorder, affecting \( \sim 0.5\% - 1\% \) of the population in the 65–69 age group and 1–3% of individuals 80 years of age and older (1). Causes of PD remain largely unknown, and the majority of patients do not report a family history of PD. Recent estimates of heritability of PD based on genome-wide complex trait analysis are between 0.16 and 0.49 (median 0.23), depending on the cohort (2). Causal mutations have been identified in \( > 10 \) genes, and the recent string of large genome-wide association studies (GWASs) in European and Japanese cohorts found \( > 30 \) genes and susceptibility loci associated with this disease (3–7).

We here investigate the genetic component of PD in Ashkenazi Jews, a well-characterized, homogeneous founder population particularly useful in genetic studies of diseases (8,9) and an important patient population in its own right. Improved control for population stratification (10,11) and reduced allelic heterogeneity facilitate identification of disease-associated variants in Ashkenazi Jews. About a third of Ashkenazi PD cases carry a mutation in either the leucine-rich repeat kinase 2 (LRRK2) or \( \beta \)-glucocerebrosidase (GBA) genes (12). The p.G2019S mutation in the LRRK2 gene occurs in 9.9–14.8% of Ashkenazi cases and 1.3–2.4% controls, compared with 2.7–3.1% of non-Jewish European cases and 0.4% of respective controls (13–15). Mutations in the GBA gene occur in 13.7–17.9% of Ashkenazi cases and 4.2–6.3% controls, compared with 5 and \( < 1\% \) of non-Jewish European cases and controls (12,16–18). The most frequent of these mutations is p.N370S (10.9% Ashkenazi cases, 5.9% controls) also present are c.84dupG (84GG, 1.9% cases, 0.2% controls) and p.R496H (1.67% cases, not observed in controls) (12,18,19). Another advantageous genetic feature of this founder population is the high frequency of identical-by-decent (IBD) segments sharing among Ashkenazi cases and 1.3–2.4% controls, compared with 6–7% in Europeans (3). Our findings reaffirm the benefits of using the Ashkenazi Jewish founder population in studies of complex diseases and highlight the power of our haplotype association method, which is particularly useful in genetic studies of founder populations.

RESULTS

We analyzed genome-wide association of shared IBD segments identified by the use of GERMLINE (23) and clustered using DASH (21). We concurrently analyzed association of imputed SNPs using EMMAX (24), a mixed model well suited to studies of populations with high degree of cryptic relatedness (25), as is the case with Ashkenazi Jews. Association results summarized as Manhattan and QQ plots are shown in Figure 1, Supplementary Material, Figures S1 and S2, and the top associated haplotypes and SNPs are listed in Tables 1 and 2, respectively.

Association

LRRK2

The strongest SNP association signal was detected at the LRRK2 locus [rs1442190, OR = 3.72 (2.98, 4.64), \( P = 1.53 \times 10^{-27} \), Fig. 2A]. The best associated DASH segment refines this signal with OR = 12.05 (8.35, 17.41) and \( P = 1.23 \times 10^{-56} \). We found 170 copies of this segment in PD cases and 35 in controls, which accounted for all known p.G2019S PD cases in our cohort (independently genotyped, see Supplementary Material, Table S2). The two pericentromeric association signals on chr12 reported in Tables 1 and 2 are due to long-range LD in this region (chr12:33–40 Mb) (26). There was no residual signal on chr12 after conditioning on the independently genotyped p.G2019S carrier status, indicating that in our cohort, p.G2019S is responsible for the association at the LRRK2 locus.

Chr17q21.31/MAPT

Strong association signal coming from both SNPs [rs17577094, OR = 0.64 (0.56, 0.72), \( P = 4.51 \times 10^{-10} \)] and DASH segments [OR = 0.62 (0.54, 0.72), \( P = 1.78 \times 10^{-11} \), shared by 423 cases and 1351 controls] was observed within the 900-kb inversion polymorphism on chr17q21.31 (27). Association to PD at this locus has been previously reported in a European cohort (rs393152; OR = 0.77; \( P = 1.95 \times 10^{-10} \)) (7) and replicated in a subset of our Ashkenazi samples [OR = 0.54 (0.49, 0.74), \( P = 7.16 \times 10^{-5} \)] (28). The inverted haplotype (H2) frequency in Europeans varies in a gradient away from the Mediterranean Sea (37.5% in Sardinians to 4.3% in Finns) and is 25.6% in Ashkenazi Jews (29). H1 and H2 haplotypes do not recombine and are well tagged by common SNPs, which causes a peculiar LD.
Figure 1. Manhattan plots showing the association of (A) imputed SNPs and (B) DASH segments. Lower panels show association results conditioned on the LRRK2 and GBA mutation carrier status, for (C) imputed SNPs and (D) DASH segments. Markers color-coded red surpass the Bonferroni-corrected genome-wide significance thresholds.
Table 1. DASH segment association results with \( P \)-values of \( < 10^{-7} \) based on either Fisher's exact (single stratum analysis) or exact conditional test (samples stratified by the \( LRRK2 \) and \( GBA \) carrier status)

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Start (kb)</th>
<th>End (kb)</th>
<th>Length (kb)</th>
<th>Gene/locus</th>
<th>PD (%)</th>
<th>Controls (%)</th>
<th>( P ) (95% CI)</th>
<th>Fisher's exact ( P )</th>
<th>Exact conditional ( P )</th>
<th>( P )value (replicated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>33,191,462 33,320,612</td>
<td>1,970</td>
<td>( LRRK2 )</td>
<td>100</td>
<td>91</td>
<td>0.04</td>
<td>0.36 (0.27, 0.46)</td>
<td>1.23 ( \times 10^{-9} )</td>
<td>( \times 10^{-11} )</td>
<td>( \times 10^{-15} )</td>
</tr>
<tr>
<td>7</td>
<td>124,400,279 124,977,213</td>
<td>5,694</td>
<td>( MAPT )</td>
<td>1,313</td>
<td>91</td>
<td>0.04</td>
<td>0.26 (0.17, 0.38)</td>
<td>1.06 ( \times 10^{-9} )</td>
<td>( \times 10^{-10} )</td>
<td>( \times 10^{-13} )</td>
</tr>
<tr>
<td>12</td>
<td>153,010,843 153,612,127</td>
<td>6,104</td>
<td>( GBA ) ( N370S )</td>
<td>1,313</td>
<td>91</td>
<td>0.04</td>
<td>0.26 (0.17, 0.38)</td>
<td>1.06 ( \times 10^{-9} )</td>
<td>( \times 10^{-10} )</td>
<td>( \times 10^{-13} )</td>
</tr>
<tr>
<td>16</td>
<td>28,510,492 28,822,934</td>
<td>3,313</td>
<td>( CNTNAP5 )</td>
<td>3,313</td>
<td>91</td>
<td>0.04</td>
<td>0.26 (0.17, 0.38)</td>
<td>1.06 ( \times 10^{-9} )</td>
<td>( \times 10^{-10} )</td>
<td>( \times 10^{-13} )</td>
</tr>
</tbody>
</table>

Only the single most associated segment is reported per locus. In order to get a finite OR estimate, for the \( GBA \) \( 84GG \) segment, a count of 1 was added to all fields in the contingency table (marked with \( \ast \)).
Table 2. Imputed SNPs associated with PD, with EMMAX P-value of < 3.0 × 10^{-9} either unconditioned and conditioned on LRRK2 and GBA mutation carrier status

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chromosome</th>
<th>Position</th>
<th>Gene/locus</th>
<th>Alleles</th>
<th>MAF (%)</th>
<th>MAF (%) OR (95% CI)</th>
<th>P-value (unconditional)</th>
<th>P-value (conditional)</th>
<th>P-value (replicated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1442190</td>
<td>12</td>
<td>39,651-907</td>
<td>LRRK2</td>
<td>T/C</td>
<td>9.16</td>
<td>2.64 × 10^{-8}</td>
<td>3.72 (2.98, 4.64)</td>
<td>1.53 × 10^{-27}</td>
<td>0.184</td>
</tr>
<tr>
<td>rs10560609</td>
<td>12</td>
<td>32,691-394</td>
<td>LRRK2</td>
<td>A/G</td>
<td>7.88</td>
<td>2.60 × 10^{-8}</td>
<td>3.20 (2.54, 4.02)</td>
<td>1.36 × 10^{-20}</td>
<td>0.499</td>
</tr>
<tr>
<td>rs17576904</td>
<td>17</td>
<td>41,543-275</td>
<td>MAPT</td>
<td>C/T</td>
<td>18.50</td>
<td>26.25 × 10^{-9}</td>
<td>0.64 (0.56, 0.72)</td>
<td>8.33 × 10^{-10}</td>
<td>4.51 × 10^{-10}</td>
</tr>
<tr>
<td>rs16305000</td>
<td>1</td>
<td>153,121-679</td>
<td>GBA</td>
<td>A/G</td>
<td>7.70</td>
<td>4.54 × 10^{-8}</td>
<td>1.75 (1.43, 2.15)</td>
<td>2.12 × 10^{-8}</td>
<td>0.036</td>
</tr>
<tr>
<td>rs10737170</td>
<td>1</td>
<td>154,330-504</td>
<td>GBA</td>
<td>C/A</td>
<td>19.25</td>
<td>13.83 × 10^{-8}</td>
<td>1.49 (1.30, 1.69)</td>
<td>8.62 × 10^{-8}</td>
<td>0.725</td>
</tr>
<tr>
<td>rs1715232</td>
<td>16</td>
<td>28,840-969</td>
<td>CDCA7</td>
<td>G/A</td>
<td>20.27</td>
<td>26.45 × 10^{-7}</td>
<td>0.71 (0.63, 0.80)</td>
<td>1.38 × 10^{-5}</td>
<td>1.98 × 10^{-5}</td>
</tr>
<tr>
<td>rs823114</td>
<td>2</td>
<td>203,986-155</td>
<td>PARK16</td>
<td>G/A</td>
<td>34.82</td>
<td>41.54 × 10^{-9}</td>
<td>0.75 (0.68, 0.83)</td>
<td>2.76 × 10^{-6}</td>
<td>0.0090683</td>
</tr>
<tr>
<td>rs1813134*</td>
<td>4</td>
<td>151,450-821</td>
<td>MAPT</td>
<td>A/G</td>
<td>35.13</td>
<td>29.68 × 10^{-9}</td>
<td>1.28 (1.16, 1.43)</td>
<td>5.69 × 10^{-6}</td>
<td>4.33 × 10^{-7}</td>
</tr>
<tr>
<td>rs6739381*</td>
<td>2</td>
<td>174,249-536</td>
<td>2q31.1</td>
<td>T/C</td>
<td>11.02</td>
<td>8.16 × 10^{-9}</td>
<td>1.39 (1.18, 1.64)</td>
<td>3.58 × 10^{-9}</td>
<td>9.58 × 10^{-9}</td>
</tr>
</tbody>
</table>

Only the single strongest associated SNP at each peak has been reported. For the three SNPs that had stronger association in the conditional analysis (marked with *), ORs and 95% CI are based on Mantel–Haenszel pooled odds ratios; otherwise they were single stratum sample ORs.

seen in only two out of the five sequenced samples (Supplementary Material, Table S4). We did not identify any coding variants. According to the 1000 Genomes Project, MAF of rs77182849 is 5% across all ethnic groups and 11% in samples of European ancestry, making it unlikely to be a rare risk allele for PD.

Additional association signals

We observed an SNP hit on chr1q32.1, in the PARK16 locus [rs823114; OR = 0.75 (0.68, 0.83), P = 2.76 × 10^{-9}], with an effect consistent with the one seen in non-Jewish Europeans (rs823128, OR = 0.66 (7)), and recently in two cohorts of Ashkenazi PD patients and controls (28.32). The best DASH segment at this locus captures a rare haplotype that occurs in 11 cases and 2 controls [OR = 12.77 (2.83, 57.64), Supplementary Material, Fig. S4].

Among the three common loci that were nearing significance—chr2q31.1, chr4q31.3 and chr16p11.2 (Table 2)—that we attempted to replicate in the secondary cohort, we observed moderate replication signal at chr2q31.1 (P = 0.012), in the intergenic region between cell division cycle-associated seven gene CDC47 and Sp3 transcription factor gene SP3.

Two additional genome-wide significant rare DASH segments on chr3q31.1 [OR = 44.25 (5.92, 330.90), P = 1.79 × 10^{-5}] and chr1q12.2 [OR = 12.82 (4.41, 37.25), P = 3.13 × 10^{-8}] did not replicate in the secondary cohort (Supplementary Material, Tables S5 and S6).

Homozygosity mapping, typically informative in studies of founder populations, in our study did not imply any regions at the genome-wide significance level.

Clinical and demographics variables

Information about sex, age-at-onset (AAO, defined as the age of first symptoms) and whether the case was sporadic or familial (defined as having a first degree relative with PD) is summarized in Supplementary Material, Table S2. Columbia had a higher fraction of male PD cases (67.4% compared with 56.1 and 62.6%, \( \chi^2 P = 4.3 \times 10^{-5} \)), and Beth Israel had a higher fraction of familial cases (38.6% compared with 17.8 and 16.8%, \( \chi^2 P = 2.7 \times 10^{-11} \)). There was no significant difference in the AAO between the three centers (logrank \( P = 0.55 \), Supplementary Material, Fig. S6A). The differences in family history are likely because of differences in sampling strategy between the three centers: predilection towards early onset PD cases at Columbia, towards familial cases at Beth Israel and consecutive recruitment at Tel Aviv Sourasky.

The average p.G2019S carrier frequency was 14.9% (Supplementary Material, Table S2). Although some heterogeneity was observed among centers (19.8, 13.3 and 14.0% in Beth Israel, Columbia and Tel Aviv Sourasky cohorts, respectively), it was not statistically significant (Fisher’s exact \( P = 0.085 \)). Observed frequency of p.G2019S is similar to what was previously published (14.8%), although we should note that there is a sample overlap between this and the previous study. The frequency of GBA mutation carriers was 17.6% in the combined sample, with significant heterogeneity between centers (12.0, 16.7 and 20.2%, \( P = 0.021 \)), and not significantly different from the previously published frequency range of 13.7–17.9% (12,18), although again some samples overlapped between this and the earlier study.

LRRK2 and GBA mutation carriers have an earlier AAO than non-carriers (mean ± SD, 56.4 ± 11.7 and 54.0 ± 11.3%, respectively, compared with 60.6 ± 11.6%, logrank \( P = 1.2 \times 10^{-5} \), Supplementary Material, Fig. S6B). Familial cases are enriched in LRRK2 mutation carriers (29.1% of familial cases carry p.G2019S, \( P = 7.15 \times 10^{-6} \)), comparable to the previously published estimate of 26%, but are not enriched in GBA carriers. We did not observe evidence in support of a gender effect for either LRRK2 (\( P = 0.098 \)) or GBA (\( P = 0.70 \)), and there was no difference in AAO between males and females (\( P = 0.97 \)), nor between familial and sporadic cases (\( P = 0.27 \), Kaplan–Meier curves are shown in Supplementary Material, Fig. S6).

To investigate genetic factors that may influence clinical and demographic variables, we performed case-only genome-wide associations to family history, gender and AAO using Fisher’s exact and logrank tests. We did not observe any site that increases gender-specific risk (Supplementary Material, Fig. S7). The only locus strongly associated with familial forms of PD was LRRK2 (Supplementary Material, Fig. S8). One locus on chr17q24.2 captured by DASH segments in three samples had a genome-wide significant effect on AAO (chr17:661 453–63 110 659; mean ± SD 92.7 ± 1.5, \( P < 1.0 \times 10^{-8} \), Supplementary Material, Figs S9C and S10). It contains two genes, proteasome 26S subunit (PSMD12) and cytoplasmic phosphatidylinositol transfer protein, cytoplasmic (PITPNC1).
Figure 2. Association of imputed SNPs and DASH segments at the four genome-wide significant loci either also associated in other studies, or replicated as part of this study: (A) chr12q12 (with the associated LRRK2 gene), (B) chr17q21 (MAPT), (C) chr1q21 (GBA) and (D) chr2q14 (CNTNAP5).
Replication of previously published loci

In order to evaluate the overlap between genetic risk factors contributing to PD in Ashkenazi Jews and other populations, we tested all hits with P-values of $<10^{-5}$ reported in previously published GWASs (3.5–7.33,34). For the first time in the Ashkenazim, we observed an association signal at the SNCA locus [rs56220; OR = 1.22 (1.11, 1.35), P = 5.6 $\times 10^{-5}$], with an odds ratio comparable to that in Europeans (1.23–1.30). At the significance level Bonferroni-corrected by the number of SNPs that we attempted to replicate ($\alpha = 0.05/66 = 0.00076$), we were able to capture association signals at the MCCCI1/LAMP3 [rs9290751; OR = 0.80 (0.71, 0.91), P = 3.55 $\times 10^{-4}$] and PARK16 loci [rs947211; OR = 0.77 (0.69, 0.87), P = 6.68 $\times 10^{-4}$]. At the nominal significance level, we were able to capture four additional signals (GAK/DGKQ, RIT2/SYT4, ITGA8 and CCDC62/HIP1R, Supplementary Material, Table S7).

In addition, association of IBD segments was able to capture signals at the GAK/DGKQ and BST1 loci (Supplementary Material, Table S8). At the BST1 locus—similar to what we observed at the GBA locus—there was more than one distinct group of haplotypes carrying the variants that increase the risk of PD: chr:4:1499745–15727723 [OR = 23.2 (3.0, 181.4), P = 4.98 $\times 10^{-5}$, 0.44% in PD, 0.019% in controls] and chr:4:1521529–15350349 [OR = 2.2 (1.3, 3.6), P = 0.0018, 1.4% in PD, 0.65% in controls]. However, when we corrected the nominal P-values by the number of DASH segments at the GAK/DGKQ and BST1 loci and the number of loci tested genome wide, these two hits were not significant. Nonetheless, carriers of the BST1 segments, and in particular the high-risk haplotype, are interesting candidates for sequencing follow-ups.

Using both association strategies, we were able to replicate known signals at three loci (SNCA, MCCCI1/LAMP3 and PARK16), and we have seen nominal significance at five additional loci (GAK/DGKQ, RIT2/SYT4, ITGA8, CCDC62/HIP1R and BST1). Together with the loci genome-wide significant in this study, we have in all been able to capture association signals at 11 out of 33 currently known PD loci. Top SNPs at the three genome-wide significant loci (LRRK2, GBA and MAPT) taken together explain 8.1% of phenotypic variance (Nagelkerke’s pseudo $R^2$ from logistic regression), and top SNPs at seven loci with replication P-value of $<0.05$ (PARK16, MCCCI1/LAMP3, GAK/DGKQ, SNCA, ITGA8, CCDC62/HIP1R and RIT2/SYT4) explain additional 2.0%, for a total of 10.1%. Genome-wide significant and replicated DASH segments (LRRK2, GBA, MAPT and CNTNAP5) explain 15.6% of the phenotypic variance and, together with the segments that have corrected P-values of $<0.05$, explain a total of 16.5% of phenotypic variance. As expected, this is significantly higher than variance explained in outbred European populations, estimated to be $\sim 6–7\%$ (3).

**DISCUSSION**

We have identified genome-wide significant haplotypes at LRRK2, GBA, MAPT and chr2q14.3 loci, ranging in size from 40 to 800 kb. The first three of these were previously known and were also captured using imputed SNPs as part of our study; however, haplotype associations consistently refined the SNP association signals. In addition, we replicated SNPs at SNCA, MCCCI1/LAMP3 and PARK16 loci. An earlier GWAS conducted on partially overlapping set of samples (268 cases and 178 controls ascertained at Columbia University Medical Center) replicated association signals at the MAPT (P = 7.16 $\times 10^{-5}$), LRRK2 (P = 1.56 $\times 10^{-4}$), PARK16 (P = 6.12 $\times 10^{-4}$) and GBA loci (seven SNPs with nominally significant P-values); however, owing to the small sample size, it was underpowered to reach genome-wide significance at any locus (28). Interestingly, we were not able to replicate the exact previously reported SNPs at the LRRK2 and GBA loci, although LRRK2 and GBA were genome-wide significant in our study. This points to the differences in LD structure between the Ashkenazim and non-European Jews at these two loci.

A point worth making is that we did not observe a significant p.N370S DASH segment that overlaps the GBA gene, although two significant p.N370S DASH segments were found proximal and distal to the GBA gene. This may indicate a limitation of our method to resolve haplotypes in a complex genetic region, or a possibility that there might be additional mutations contributing to the risk of PD, linked with p.N370S, located within the flanking DASH segments. We observed a secondary distal linked SNP signal [rs10737170; OR = 1.49 (1.30, 1.69), P = 8.62 $\times 10^{-8}$] that corresponds to the distal DASH segment; however, after conditioning on the GBA mutation carrier status, there was no residual signal at the GBA locus. On the other hand, a recent study reported an association hit in Europeans [OR = 1.67 (1.50, 1.84), P = 5.7 $\times 10^{-9}$] related to the nearby SYT1 gene (5), close to GBA but apparently independent of the mutations in GBA in Europeans.

Assembling a sizeable cohort of samples from the Ashkenazi Jewish founder population came at the expense of genotyping platform heterogeneity, which had to be carefully addressed. To provide a uniform, dense set of markers, we created a custom Ashkenazi reference panel (22) and imputed the missing SNPs. Pooling samples and imputation of missing SNPs, as opposed to a more straightforward meta-analysis, allowed consistent quality control across all samples and enabled us to phase SNPs and call shared IBD segments. Some of the control samples used in this study were diagnosed with Crohn disease (CD); nonetheless, CD and PDs are clinically, pathologically and etiologically distinct, and for the variants reported in this study, we did not observe significant differences in allele frequencies between healthy controls and Crohn cases (Supplementary Material, Table S9 and S10). Although variants at LRRK2 and HLA loci have been associated with both PD and CD, separate analysis of PD and CD in our cohort showed no overlap in terms of risk loci, which together with the increase in power coming from having a larger number of control samples (Supplementary Material, Fig. S5) justifies the use of CD cases as non-Parkinson controls. In addition, we concurrently performed a GWAS of CD in Ashkenazi Jews (22), and this provided an opportunity to verify the association signals. In contrast to CD, which has population prevalence two to four times higher in the Ashkenazim, limited surveys of PD prevalence remain inconclusive. One study found no significant difference between Ashkenazi Jews and non-Jewish Europeans (35). A more
recent study indicated that PD prevalence in Israel is elevated compared with Europe and the USA (36), although it did not specifically focus on the Ashkenazim.

Our results highlight the usefulness of our haplotype association method. Until whole-genome sequencing in large cohorts becomes widespread, associated DASH segments can be used to guide targeted region resequencing efforts or to prioritize samples for whole-genome sequencing. Although the use of DASH is not limited to founder populations, pervasive IBD sharing in these populations facilitates haplotype mapping, as demonstrated here. DASH was able to dissect the signal at the GBA locus into distinct groups of haplotypes that carry distinct mutations and provide initial evidence that multiple causal variants might also be present at the BST1 locus. We were also able to identify and replicate a novel risk haplotype on chr2q14.3 occurring at ~1% frequency in PD cases. This haplotype contains a single gene expressed in the brain, CNTNAP5, previously not implicated in PD. As rare alleles tend to be population-specific, it remains to be seen whether the risk variant that arose on the backbone of this haplotype, or possibly different variants in the CNTNAP5 gene, are present in other populations. Taken together, our results reaffirm the complex genetic background of PD and emphasize the need for studying both common and rare variants in PD, with the goal of understanding the molecular processes these variants affect.

MATERIALS AND METHODS

The study was approved by the Institutional Review Boards at all participating institutions, including Beth Israel Medical Center, Columbia University, Tel Aviv Sourasky Medical Center, Mount Sinai School of Medicine, Albert Einstein College of Medicine, The Hebrew University of Jerusalem, Yale University, Children’s Hospital of Philadelphia and North Shore University Hospital-Long Island Jewish Medical Center. All patients provided written informed consent (in English or Hebrew) for the collection of samples and subsequent analysis.

Primary cohort

A total of 1248 Ashkenazi PD cases have been ascertained at Tel Aviv Sourasky Medical Center (Tel Aviv, Israel), Center for Movement Disorders at Columbia University and Department of Neurology at Beth Israel Medical Center (New York, USA). Subjects at Tel Aviv Sourasky Medical Center (n = 597) were consecutively recruited individuals of Ashkenazi Jewish origin; details of the cohort recruitment, diagnostic criteria and interview procedures are as described in (12,14). The cohort of cases recruited at the Center for Movement Disorders at Columbia University was created by combining participants from two studies of PD: genetic Epidemiology of PD (n = 168, enriched for cases with AAO < 50 y/o) and the Ashkenazi Jewish PD study (n = 184, selected based on self-reported ancestry). Details of the ascertainment procedures, evaluation criteria and structured interviews are provided in (28,37). Ashkenazi PD cases recruited from the Department of Neurology at Beth Israel (n = 299) have been evaluated as previously described in (15). PD cases that carried mutations in the GBA gene did not have symptoms of Gaucher Disease. To account for differences in procedures employed to verify subjects’ Ashkenazi Jewish ancestry at different centers, as part of this study ancestry of all subjects was uniformly verified using principle component analysis. Our recent results justify the use of a single population model for Ashkenazi Jews from Israel and the USA (38) and show that after accounting for European admixture, there is no evidence for significant population stratification (39).

The set of 2865 Ashkenazi controls contain both neurologically healthy individuals (n = 422) from Tel Aviv Sourasky, Columbia and Beth Israel Medical Centers and pooled controls who have not undergone neurological examination (n = 2443) from The Hebrew University of Jerusalem, Albert Einstein College of Medicine, Mt. Sinai School of Medicine, Yale University and Children’s Hospital of Philadelphia. Pooled controls consist of apparently healthy individuals (controls in their respective studies, n = 1474) as well as individuals diagnosed with CD (n = 969). A subgroup of the apparently healthy pooled controls (n = 646) was recruited for a study of healthy aging (40). Based on PD population prevalence of ~1% (41), ~24 pooled control samples can be expected to have or will have developed PD. Details of the sample cohorts are summarized in Supplementary Material, Table S1.

Samples were genotyped on Illumina Human 300 k, 500 k, 660 k and Omni1M and Affymetrix GeneChip Human Mapping 500K and Genome-Wide Human SNP 6.0 arrays (Supplementary Material, Table S1). Details about quality control, ancestry filtering, etc., are provided in Supplementary Material, Methods.

Replication cohort

A selected set of SNPs was genotyped in the replicate cohort of 306 Ashkenazi PD cases from Tel Aviv Sourasky Medical Center using ABI TaqMan assays (Applied Biosystems, Foster City, CA, USA). Frequencies of LRR2/GBA mutations in the replicate PD cohort were comparable to the primary sample. Replication controls consisted of 2583 Ashkenazi schizophrenia cases and controls from the North-Shore-Long Island Jewish Health System typed on Illumina HumanOmni1-Quad BeadChip, imputed using the same procedure and passing the same quality control and ancestry filtering as the discovery cohort.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG online.

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