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Genome-wide association study of Parkinson's disease in East Asians

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Abstract

Genome-wide association studies (GWAS) on Parkinson's disease (PD) have mostly been done in Europeans and Japanese. No study has been done in Han Chinese, which make up nearly a fifth of the world population. We conducted the first Han Chinese GWAS analyzing a total of 22,729 subjects (5,125 PD cases and 17,604 controls) from Singapore, Hong Kong, Malaysia, Korea, mainland China and Taiwan. We performed imputation, merging and logistic regression analyses of 2,402,394 SNPs passing quality control filters, adjusted for the first three principal components. 88 SNPs with association $P < 10^{-4}$ were validated in 9 additional sample collections and the results were combined using fixed-effects inverse-variance meta-analysis. We observed strong associations reaching genome-wide significance at *SNCA*, *LRRK2* and *MCCCI*, confirming their important roles in both European and Asian PD. We also identified significant ($P < 0.05$) associations at 5 loci (*DLG2*, *SIPA1L2*, *STK39*, *VPS13C* and *RIT2*), and observed the same direction of associations at 9 other loci including *BST1* and *PARK16*. Allelic heterogeneity was observed at *LRRK2* while European risk SNPs at 6 other loci including *MAPT* and *GBA-SYT11* were non-polymorphic or very rare in our cohort. Overall, we replicate associations at *SNCA*, *LRRK2*, *MCCCI* and 14 other European PD loci but did not identify Asian-specific loci with large effects ($OR > 1.45$) on PD risk. Our results also demonstrate some differences in the genetic contribution to PD between Europeans and Asians. Further pan-ethnic meta-analysis with European GWAS cohorts may unravel new PD loci.

Introduction

Parkinson's disease (PD; OMIM:168600) is among the most common age-related neurodegenerative diseases worldwide. It occurs at similar incidence in Europeans and Asians, and this is expected to increase over the coming decades as the size of the aged population grows. Large-scale meta-analyses of genome-wide association studies (GWAS) on PD in the European population analyzing up to 13,708 cases and 95,282 controls have led to the identification of 32 loci significantly associated with disease risk (1). One of the first PD GWAS was conducted in Japan (2), which led to the identification of loci such as *PARK16* and *BST1* that were subsequently replicated in Europeans (1-9), and also demonstrated the absence of the *MAPT* risk variant in Asians. The Han Chinese population is the largest worldwide, with 1.37 billion people in mainland China alone. This ethnic group makes up about 19% of the world population and a significant fraction of PD patients worldwide, thus it will be important to elucidate the genetic risk factors underlying morbidity in this population. To date, no study has been done in Han Chinese samples. Here, we conducted the first Han Chinese GWAS in 779 Singapore Chinese cases and 13,227 controls with validation in samples from Hong Kong, Malaysia, Korea, mainland China and Taiwan, analyzing a total of 22,729 subjects (5,125 cases and 17,604 controls) from across East Asia.

Results

Discovery-stage GWAS

At the discovery stage, we analyzed a total of 2,402,394 SNPs in 779 cases and 13,227 controls after quality control filtering (Supplementary Figure 1). Genome-wide inflation of association evidence was low ($\lambda_{GC}=1.017$) in the discovery analysis, suggesting minimal bias resulting from population stratification (Supplementary Figure 2). We observed strong association at the *SNCA* locus crossing the threshold for genome-wide significance (Supplementary Figure 1). There was no secondary signal observed within the

SNCA locus, although we might not have had sufficient power in the current study to identify independent associations at variants with smaller effects or lower minor allele frequencies.

We selected 62 SNPs with $P < 10^{-4}$ representing 46 loci for further validation. Taking into consideration that some true signals may be masked by inclusion of other disease cases amongst our controls or by differential genotyping quality of SNPs across the different arrays, we included another 11 SNPs representing 9 loci with $P < 10^{-4}$ if we analyze only healthy controls (8,996 controls, excluding other disease cases), and another 23 SNPs representing 21 loci crossing the same threshold if we analyze only samples genotyped on similar arrays (OmniZhonghua and OmniExpress; 3,496 controls, 489,262 overlapping genotyped SNPs). For loci tagged only by imputed SNPs, we selected only those with more than 1 imputed SNPs crossing the $P < 10^{-4}$ threshold for validation. In total, 96 SNPs representing 76 independent loci were taken forward for validation in eight independent sample collections across East Asia (first validation) (Supplementary Table 2). These made up a total of 3,769 cases and 3,762 controls that were genotyped on the Sequenom or Taqman platforms. Of the 76 loci, only 4 (*MCCCI*, *LRRK2*, *SNCA* and *DLG2*) have been previously reported (Figure 1).

Validation of top-associated SNPs in 9 independent sample collections

90 SNPs (73 loci) passed design and genotyping successfully in at least 6 out of 8 validation cohorts (Supplementary Table 3). We tested each SNP for association in each collection using logistic regression analysis, and combined the results using a fixed-effects meta-analysis across six to nine sample collections. From this combined meta-analysis across the discovery and validation samples, only SNPs in three of the previously reported loci (*MCCCI*, *LRRK2*, *SNCA*) reached genome-wide significance (Figure 2). We brought four other SNPs (including *DLG2*) with $5 \times 10^{-8} < P < 10^{-4}$ in the meta-analysis for further validation in another independent collection of 577 cases and 615 controls from Taiwan

(second validation). We observed consistent association at *DLG2* rs7479949, another previously reported locus which was close to genome-wide significant ($P=5.90 \times 10^{-8}$; Figure 3). None of the other three loci were validated (with $P < 0.05$) nor reached genome-wide significance in the combined analysis of 5,125 cases and 17,604 controls (See Supplementary Table 4 for the other three loci).

Analysis of PD risk loci

We then investigated 32 SNPs that were previously reported to have reached genome-wide significance in the largest European PD GWAS(1) in our Han Chinese GWAS discovery dataset (Supplementary Table 5). Besides rs12637471 in *MCCC1* ($r_2 > 0.8$ with rs2270968), 13 other reported SNPs showed consistent directions of effect with that reported in European studies (Supplementary Table 5), out of which 4 (in *RIT2*, *VPS13C*, *STK39* and *SIP1AL2*) were statistically significant ($P < 0.05$) (Supplementary Figure 3a), although none of the SNPs within these loci crossed our threshold ($P < 10^{-4}$) in selecting SNPs for validation (Supplementary Figure 3b). This included *BST1* and *PARK16*, loci that were first reported in Japanese (2) and subsequently validated in Europeans (1). Another 6 reported SNPs were non-polymorphic in our samples and in East Asians samples from the 1000 genomes project (Supplementary Table 5) and none of the other SNPs across these loci (including *MAPT* and *GBA*; Supplementary Figure 3c) showed significant association in our samples, suggesting that they may be European-specific loci. There was no evidence of consistent association at 5 other SNPs, although larger numbers of samples will be needed to assess the small effects that were previously reported at these loci (Supplementary Figure 3d). There were also another 7 previously reported SNPs that could not be confidently imputed (with $> 98\%$ of samples with imputation genotype quality above 0.9, info score > 0.8). Within the loci tagged by these SNPs, we observed evidence of association at neighbouring SNPs for the previously reported *SNCA* SNP rs356182 ($r_2 > 0.5$ with reported SNP in 1000 genomes Asians) (10,11)

and the reported *DLG2* SNP rs3793947 ($r^2 > 0.8$ with reported SNP in 1000 genomes Asians)(10,11) (Figure 1), but there was little evidence of association at neighbouring SNPs within the other 5 loci (Supplementary Figure 3e).

Pathway analysis

To identify pathways and gene sets with a high proportion of genes significantly associated with PD, we ran a gene set enrichment analysis (GSEA) using the i-GSEA4GWAS (improved GSEA analysis for GWAS) web server (12). Among the top-ranked significant gene sets ($P < 0.001$), we identified potentially interesting pathways related to neurodegeneration (Alzheimer's disease), long-term potentiation, apoptosis and some immune-related pathways (Supplementary Table 6). Most of the contributing association signals are individually weak ($P \sim 10^{-2}$ to 10^{-3}), and future studies will confirm if these are true association signals.

Discussion

We have conducted the largest East Asian GWAS on PD to date, analysing 22,729 subjects (5,125 PD cases and 17,604 controls) from six different countries across East Asia, namely Singapore, Hong Kong, Malaysia, Korea, mainland China and Taiwan. It is also the first comprehensive GWAS to be done on Han Chinese subjects at the discovery stage. We demonstrated strong association signals in *MCCCI*, *LRRK2* and *SNCA*, confirming these as the most important PD loci in both Asians and Europeans, and observed consistent association in 14 other previously-reported loci. Like Satake et al (2) we did not observe any evidence for association across the *MAPT* locus (Supplementary Figure 3c) and the reported risk SNPs were non-polymorphic in our samples (Supplementary Table 5), even though this locus showed one of the strongest associations in the European population (1). We did not identify any Asian-specific loci with large effects, e.g., odds ratios > 1.3 with minor allele

frequency >30% or odds ratios >1.45 for minor allele frequency >10% (Supplementary Figure 4)(13).

We observed evidence for allelic heterogeneity at *LRRK2*. We did not observe association at the *LRRK2* SNP rs76904798, which is independent ($r^2 < 0.2$) of the SNPs identified by us (including rs1384236) and by Satake et al (rs1994090)(1). We have previously performed direct genotyping of the Asian-specific *LRRK2* coding variant G2385R in a subset of the Singapore Chinese (discovery; 749 cases, 3925 controls) and Korean (first validation; 1078 cases, 665 controls) samples of this study (14). Consistent with what we have shown for rs1994090 (14), our top SNP rs1384236 is in moderate LD with G2385R in Chinese ($r^2 = 0.32$) and Koreans ($r^2 = 0.59$). Interestingly, rs1994090 and rs1384236 are in near-perfect LD in Koreans ($r^2 = 0.99$) and are also in high LD in the Chinese samples ($r^2 = 0.79$). Further studies comparing Chinese, Koreans and Japanese will help to dissect these subtle LD differences within the *LRRK2* locus among East Asian populations.

Overall, our results demonstrate the presence of some genetic heterogeneity in PD risk between Europeans and East Asians. Larger studies focused on this population may help to reveal novel loci that are Asian-specific or Han Chinese-specific. Furthermore, future trans-ethnic meta-analysis with the European samples will help to identify new loci and to fine-map shared loci, and may eventually contribute to our understanding of population-specific differences in the genetic risk underlying PD.

Materials and Methods

GWAS genotyping

We genotyped 834 PD cases and 50 controls on the Illumina Human OmniZhonghua8 v1A BeadChip for a total of 900,015 SNPs. For additional controls, we included samples from 13 other studies (Supplementary Table 1) that were genotyped on different Illumina arrays (Human OmniZhonghua: 1,705, Human OmniExpress: 1,819, Human610-Quad:

6,113, Human 660W-Quad: 485, HumanHap 550-Quad: 1,029 and Human1M-Duo: 2,764). In total, we analyzed 14,799 Han Chinese samples from Singapore that passed genotyping quality control (call rates >95%), comprising 834 PD cases and 13,965 controls. Patients were diagnosed with PD using the UK Brain Bank Criteria. This study was approved by the ethics committees or institutional review boards of the respective institutions.

Imputation and statistical analysis of GWAS samples

The software IMPUTE version 2 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html)(15,16) was used for imputation of untyped SNPs in each dataset following pre-phasing using SHAPEIT (https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html)(17,18), and using the multi-ethnic 1000 genomes reference panel (March 2012) consisting of 36,648,992 autosomal SNP genotypes in 1,092 individuals from Africa, Asia, Europe, and the Americas (https://mathgen.stats.ox.ac.uk/impute/data_download_1000G_phase1_integrated.html)(11) which has been shown to out-perform imputations with reference panels of matched ancestry. The imputation was run separately for each of the 14 study collections, after removing samples with call rates <95%, SNPs with call rates < 95%, minor allele frequencies (MAF) <1% and Hardy-Weinberg equilibrium (HWE) $P < 10^{-3}$ in controls and/or $P < 10^{-6}$ in all samples as well as all non-autosomal SNPs (X, Y and mitochondrial chromosomes). The number of samples and SNPs used for imputation of each dataset is given in Supplementary Table 1.

After imputation and merging, we performed identity-by-descent analysis using overlapping genotyped SNPs in PLINK (19) and first-degree relative pairs were identified; the relative with a lower sample call rate was excluded. We then ran further stringent quality control filtering on the SNP level, setting SNPs with genotype probabilities less than 0.9 to missing and removing SNPs with call rate < 98%, and further excluding those with MAF

<1%, info score <0.8, HWE P in controls <10⁻³, HWE P in all samples <10⁻⁶ and heterogeneity in controls P <10⁻⁶. We also ran principal components analysis (20) on 42,125 independent genotyped SNPs ($r^2 < 0.2$, pruned using PLINK(19) --indep-pairwise 50 5 0.2) overlapping across all the different arrays after exclusion of SNPs in the five conserved long-range LD regions in Chinese as previously described (21). We then excluded outliers on the first five principal components. Eventually, 2,402,394 SNPs remained for analysis in 779 cases and 13,227 controls. We ran logistic regression analyses adjusting for the first three principal components (Supplementary Figure 1) using PLINK (19). **The full set of summary association statistics can be requested for download at <https://www.nni.com.sg/research/research-platforms/Genomics/Pages/Home.aspx>.**

Pathway analysis was conducted using the i-GSEA4GWAS web server (<http://gsea4gwas.psych.ac.cn/>)(12) with default parameters and our genome-wide SNP logistic regression P-values as input. Power calculations were performed using the Genetic Power Calculator (<http://pngu.mgh.harvard.edu/~purcell/gpc/>) (13) and regional association plots were generated using LocusZoom (<http://locuszoom.sph.umich.edu/locuszoom/>)(10).

Validation samples

Patients and ethnically-matched controls from nine independent centres across East Asia were recruited by the respective hospitals and study groups (Supplementary Table 2). Patients were diagnosed with PD using the UK Brain Bank Criteria. Blood samples were collected from each participant and DNA extraction was performed.

Validation genotyping

Genotyping analysis of the 96 SNPs selected for validation was performed using the MassArray system from Sequenom. Locus-specific PCR and detection primers were designed using the MassArray Assay Design 3.0 software (Sequenom). For the second validation of the four selected SNPs, pre-designed TaqMan assays were obtained from Applied

Biosystems/Life Technologies. TaqMan reactions were carried out in 5- μ l volumes containing 10–20 ng DNA according to the manufacturer’s protocols. Fluorescence data were obtained in the ABI PRISM 7900HT, and SDS 2.4 software (Applied Biosystems) was used to call genotypes.

Meta-analysis of GWAS and validation samples

After removing samples with a SNP call rate of <90% from further analysis, all SNPs passed quality control filters (call rates >95%, HWE $P > 0.001$). For all SNP calls, we visually inspected the clustering patterns of genotypes from TaqMan and Sequenom assays and confirmed that the genotypes were of good quality. In each of the validation samples, we performed logistic regression analyses and combined the results using a fixed-effects inverse variance meta-analysis in PLINK(19).

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Conflict of Interest Statement

The authors declare no conflicts of interest.

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Legends to Figures

Figure 1. Recombination plot (10) showing association P-values of SNPs at 4 known loci taken forward for validation, with colours representing the LD with respect to the validated SNP (purple diamond and labelled on the plot).

Figure 2. Forest plot showing odds ratios, allele frequencies and P-values in each sample collection at validated SNPs within known loci *SNCA*, *MCCC1* and *LRRK2*.

Figure 3. Forest plot showing odds ratios, allele frequencies and P-values in each sample collection at validated SNPs within known locus *DLG2*.

Abbreviations

PD: Parkinson's disease

GWAS: genome-wide association study

GSEA: gene set enrichment analysis

SNP: single nucleotide polymorphism

MAF: minor allele frequency

OR: odds ratio

CI: confidence interval