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Investigating the genetic architecture of dementia with Lewy bodies: a two-stage genome-wide association study

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Summary

Background—Dementia with Lewy bodies is the second most common form of dementia in elderly people but has been overshadowed in the research field, partly because of similarities between dementia with Lewy bodies, Parkinson's disease, and Alzheimer's disease. So far, to our knowledge, no large-scale genetic study of dementia with Lewy bodies has been done. To better understand the genetic basis of dementia with Lewy bodies, we have done a genome-wide association study with the aim of identifying genetic risk factors for this disorder.

Methods—In this two-stage genome-wide association study, we collected samples from white participants of European ancestry who had been diagnosed with dementia with Lewy bodies according to established clinical or pathological criteria. In the discovery stage (with the case cohort recruited from 22 centres in ten countries and the controls derived from two publicly available database of Genotypes and Phenotypes studies [phs000404.v1.p1 and phs000982.v1.p1] in the USA), we performed genotyping and exploited the recently established Haplotype Reference Consortium panel as the basis for imputation. Pathological samples were ascertained following autopsy in each individual brain bank, whereas clinical samples were collected after participant examination. There was no specific timeframe for collection of samples. We did association analyses in all participants with dementia with Lewy bodies, and also only in participants with pathological diagnosis. In the replication stage, we performed genotyping of

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Declaration of interests

We declare no competing interests.

See Online for appendix

For the **GTEx website** see https://www.gtexportal.org/

For the **Harvard Brain Bank** website see www.brainbank.mclean.org

For the **PDGene database** see http://www.pdgene.org/

For **The Netherlands Brain Bank** see: www.brainbank.nl

For the **National Institutes of Health database of Genotypes and Phenotypes** see: http://www.ncbi.nlm.nih.gov/gap

For the full list of **contributing authors** see http://gnomad.broadinstitute.org/about

Contributors

JB, RG, JH, DJS, and AS designed the study. JB, AS, DJS, and OAR obtained funding for the study. JB, RG, OAR, CK-R, LD, SWS, and DGH did the data acquisition. JB, RG, OAR, and CK-R analysed and interpreted the data. CES, LP, SWS, OA, JC, LC, LSH, KMa, ALee, ALem, ALl, EL, ER, PStG-H, EL, HZ, IB, AB, KB, KMo, CT, SA-S, TL, JH, YC, VVD, JQT, GES, TGB, SL, DG, EM, IS, PP, PJT, LM, MO, TR, BFB, RCP, TJF, VE-P, NG-R, NJC, JCM, DJS, SP-B, DM, DWD, and GMH collected and characterised samples. JB, RG, OAR, CK-R, and TO wrote the first draft of the manuscript. All other co-authors participated in preparation of the manuscript by reading and commenting on drafts before submission.

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significant and suggestive results from the discovery stage. Lastly, we did a meta-analysis of both stages under a fixed-effects model and used logistic regression to test for association in each stage.

Findings—This study included 1743 patients with dementia with Lewy bodies (1324 with pathological diagnosis) and 4454 controls (1216 patients with dementia with Lewy bodies *vs* 3791 controls in the discovery stage; 527 *vs* 663 in the replication stage). Results confirm previously reported associations: *APOE* (rs429358; odds ratio [OR] 2·40, 95% CI 2·14–2·70; $p=1{\cdot}05 \times 10^{-48}$), *SNCA* (rs7681440; OR 0·73, 0·66–0·81; $p=6{\cdot}39 \times 10^{-10}$), and *GBA* (rs35749011; OR 2·55, 1·88–3·46; $p=1{\cdot}78 \times 10^{-9}$). They also provide some evidence for a novel candidate locus, namely *CNTN1* (rs7314908; OR 1·51, 1·27–1·79; $p=2{\cdot}32 \times 10^{-6}$); further replication will be important. Additionally, we estimate the heritable component of dementia with Lewy bodies to be about 36%.

Interpretation—Despite the small sample size for a genome-wide association study, and acknowledging the potential biases from ascertaining samples from multiple locations, we present the most comprehensive and well powered genetic study in dementia with Lewy bodies so far. These data show that common genetic variability has a role in the disease.

Introduction

Dementia with Lewy bodies is the second most common form of dementia after Alzheimer's disease.[1] Despite this fact, very little attention has been devoted to understanding the pathogenesis of this disorder, particularly when compared with the other common neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.

So far, the only fully penetrant genetic variants that have been identified and replicated as a specific cause of dementia with Lewy bodies are *SNCA* point mutations and gene dosage. Three major factors might have contributed to this low number of causative mutations. First, dementia with Lewy bodies, often a disease of old age, is not commonly seen in multiplex kindreds, meaning that successful linkage studies have been rare.[2] Second, the accurate clinical diagnosis of dementia with Lewy bodies is complex, with a high rate of misdiagnosis.[3] Third, even the largest cohorts of dementia with Lewy body samples have been generally small, in many instances including as few as 100 patients.[4,5] However, the fact that dementia with Lewy bodies has a strong genetic component is currently indisputable. The ε4 allele of *APOE* is recognised to be a strong risk factor,[6,7] as are heterozygous mutations and common polymorphisms in the glucocerebrosidase gene (*GBA*).[8] Both of these results have stemmed from candidate gene association studies; *APOE* was known to be strongly associated with Alzheimer's disease and *GBA* was known to be a strong risk factor for Parkinson's disease and Lewy body disorders. In addition to these genetic associations with susceptibility, in 2016, our group provided evidence that dementia with Lewy bodies has a heritable component.[9]

No overlap in common genetic risk has been shown to exist between Parkinson's disease and Alzheimer's disease,[10] a fact that is not entirely surprising in view of the differences in phenotype. However, it is reasonable to hypothesise that the overlaps and differences in clinical and pathological presentation between dementia with Lewy bodies and both Parkinson's disease and Alzheimer's disease stem, at least in part, from aspects in their

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underlying genetic architecture and, consequently, disease pathobiology. Specific genes and loci associated with disease and the strength of association are factors that can be expected to modulate these phenotypic overlaps and differences. However, despite these encouraging findings, large-scale, unbiased genetic studies of dementia with Lewy bodies have not yet been done, which is probably due to the difficulty in identifying large, homogeneous cohorts of people with the disease.

To address the need for more powerful and comprehensive genetic studies of dementia with Lewy bodies, we performed the first large-scale genome-wide association study in this disease.

Methods

Study design and participants

In this two-stage genome-wide association study, we examined data from white participants of European ancestry who had been diagnosed with dementia with Lewy bodies according to either clinical or pathological consensus criteria.[11] Most participants were diagnosed using pathological criteria and were included only when the likelihood of a diagnosis of dementia with Lewy bodies was “intermediate” or “high”.[11] Samples were collected at 22 different centres across ten countries in Europe, North America, and Australia. Pathological samples were ascertained following autopsy in each individual brain bank, whereas clinical samples were collected after participant examination. There was no specific timeframe for collection of samples. White control participants in the discovery stage are part of the “general research use” controls from the two studies publicly available at the database of Genotypes and Phenotypes (The Genetic Architecture of Smoking and Smoking Cessation [phs000404.v1.p1] and Genetic Analysis of Psoriasis and Psoriatic Arthritis [phs000982.v1.p1]). For the replication stage, white controls were from the Mayo Clinic Florida control database. Investigators at every site obtained written informed consent from patients and control individuals and approval from a local ethics committee.

Discovery stage: genotyping, quality control, imputation, and statistical analysis

Participants with dementia with Lewy bodies were genotyped in either the Illumina Omni2.5M array or the Illumina OmniExpress genotyping array (Illumina, San Diego, CA, USA). Controls were genotyped in either the Illumina Omni2.5M array or the Illumina Omni1M array (Illumina, San Diego, CA, USA). Autosomal variants with GenTrain scores of more than 0·7 were included in the quality control stage. We removed single nucleotide polymorphisms (SNPs) with a call rate of less than 95%, a Hardy-Weinberg equilibrium p value in controls of less than 1×10^{-7}, or a minor allele frequency of less than 0·01. Samples were removed if they had substantial non-European admixture, were duplicates or first-degree or second-degree relatives of other samples, had a genotype call rate of less than 98%, or had substantial cryptic relatedness scores (PI_HAT >0·1).

We determined population outliers by principal components analysis, using SNPs passing the aforementioned quality-control filters. We used PLINK (version 1.9)[12] to do linkage disequilibrium-based pruning. Genotypes for remaining SNPs were combined with

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1000Genomes phase 3 genotypes for samples from the YRI, CEU, JPT, and CHB reference populations, and subjected to principal components analysis. Individuals lying farther than a quarter of the distance between CEU and JPT/CHB/YRI when plotted on the axes of the first two principal components were deemed to have substantial non-European admixture and were excluded (appendix p 8).

Because samples were genotyped in a variety of arrays, we selected only variants that intersected between all arrays to be included in the imputation stage. We performed imputation using the most recent reference panels provided by the Haplotype Reference Consortium (version 1.1, 2016). We used Eagle (version 2.3) to prephase haplotypes on the basis of genotype data.[13,14] We did the imputation using the Michigan Imputation Server.[15] Following imputation, we kept variants passing a standard imputation quality threshold ($R^2 \geq 0{\cdot}3$) for further analysis.

We used logistic regression, implemented in PLINK1.9,[12] to test for association of hard-call variants with the binary case–control phenotype using sex as a covariate. We examined variants under an additive model (ie, effect of each minor allele) and estimated odds ratios (ORs) and 95% CIs. To control for population stratification, we used coordinates from the top six principal component dimensions as additional covariates in the logistic regression models. We used Q–Q plots and the genomic inflation factor (λ) to test for residual effects of population stratification not fully controlled for by the inclusion of the principal components analysis and cohort covariates in the regression model. Additionally, we have done a subanalysis in the discovery stage, including only participants with pathologically diagnosed dementia with Lewy bodies.

Moreover, to take into account the uncertainty of imputation, we have done the same association in PLINK1.9 using dosage data.

We did gene-wise burden tests using all variants with an effect in protein sequence and a maximum minor allele frequency of 5%, using SKAT-O[16,17] as implemented in EPACTS.[18] We used the top six principal components and sex as covariates in the burden test.

Replication stage: genotyping and power analysis

Replication was attempted for top variants showing a p value in the discovery stage of less than 5×10^{-6}. We tested a total of 32 signals for replication using a Sequenom MassARRAY iPLEX SNP panel (Sequenom, San Diego, CA, USA; appendix p 4). We did power calculations for replication sample size selection using the R package RPower. We estimated a mean statistical power of 81% for the 32 signals on the basis of sample size, variant frequency, and effect size in the discovery stage, and used a replication p value threshold of 0·05. We tested associations in the replication stage using logistic regression models adjusted for age (age at onset for the patients with clinically diagnosed dementia with Lewy bodies, age at death for the patients with a high pathological likelihood of dementia with Lewy bodies, and age at recruitment to study for controls) and sex.

We did a combined meta-analysis of stage 1 and 2 with GWAMA[19] under a fixed-effects model, using estimates of the allelic OR and 95% CIs.

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Estimation of phenotypic variance

To estimate the phenotypic variance explained by the genotyped SNPs in this cohort, we used genetic restricted maximum likelihood analysis as implemented in the Genome-wide Complex Trait Analysis tool.[20,21] We used the first ten principal components as covariates and a disease prevalence of 0·1%.[22] We also estimated the partitioned heritability by chromosome, for which a separate genetic relationship matrix was generated for each chromosome. Each matrix was then run in a separate restricted maximum likelihood analysis. We applied linear regression to determine the relation between heritability and chromosome length.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

This study included a total of 1743 patients with dementia with Lewy bodies and 4454 controls. The majority of patients with dementia with Lewy bodies were neuropathologically assessed (n=1324), providing a greater level of diagnostic detail. 987 participants with dementia with Lewy bodies were genotyped with the Illumina Omni2.5M array and 700 with the Illumina OmniExpress genotyping array. 1523 controls were genotyped with the Illumina Omni2.5M array and 2847 with the Illumina Omni1M array. Application of quality control filters to the dataset at the discovery stage yielded high-quality genotypes at 448 155 SNPs for 1216 participants with dementia with Lewy bodies and 3791 controls (table 1). A total of 52 participants with dementia with Lewy bodies were excluded for cryptic relatedness, 20 for genetic ancestry, and the remaining 399 for low call rates or poor genotyping. After imputation and quality control, genotypes for 8 397 716 variants were available for downstream analyses. After linkage disequilibrium-based pruning with PLINK (version 1.9)[12] to quasi-independence (variance inflation factor=2), 130 715 SNPs remained in the dataset. The Q–Q plot and genomic inflation factor (λ=1·01) indicated good control of population stratification (appendix p 9).

Five regions were associated with dementia with Lewy bodies risk at genome-wide significance ($p<5 \times 10^{-8}$) in the discovery stage (figure 1; table 2). These regions included the previously described Alzheimer's disease and Parkinson's disease loci *APOE* (rs429358; OR 2·40, 95% CI 2·14–2·70; $p=1{\cdot}05 \times 10^{-48}$), *SNCA* (rs7681440; OR 0·73, 0·66–0·81; $p=6{\cdot}39 \times 10^{-10}$), and *GBA* (rs35749011; OR 2·55, 1·88–3·46; $p=1{\cdot}78 \times 10^{-9}$). Additionally, loci overlapping *BCL7C/STX1B* (rs897984; OR 0·74, 0·67–0·82; $p=3{\cdot}30 \times 10^{-9}$) and *GABRB3* (rs1426210; OR 1·34, 1·21–1·48; $p=2{\cdot}62 \times 10^{-8}$) were also genome-wide significant. A subanalysis including only participants with pathologically diagnosed dementia with Lewy bodies revealed that all but *GABRB3* maintained their genome-wide significance in that smaller dataset (table 2; appendix p 11). Furthermore, when undertaking the same associations in PLINK1.9 to take into account the uncertainty of imputation, results were identical to the best-guess calls (appendix p 8).

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A total of 527 participants with dementia with Lewy bodies and 663 controls from the Mayo Clinic were included in the replication stage (table 3). The replication stage of the genome-wide association study design provided independent replication ($p<0{\cdot}05$) for three of the loci (*APOE*, *SNCA*, and *GBA*), all of which were also genome-wide significant in the combined analysis of both stages (table 2; appendix p 4).

In the discovery stage, suggestive evidence of an association ($p<5 \times 10^{-6}$) with dementia with Lewy bodies was also seen for two loci: *SOX17* and *CNTN1*. The association at *SOX17* did not replicate (appendix p 4). For *CNTN1*, the association with dementia with Lewy bodies (rs7314908; 1·51, 95% CI 1·27–1·79; $p=2{\cdot}32 \times 10^{-6}$) improved slightly when performing the subanalysis on the participants with pathologically confirmed dementia with Lewy bodies (rs7314908; OR 1·58, 1·32–1·88; $p=4{\cdot}32 \times 10^{-7}$), and this candidate locus showed evidence of replication with very similar effect size to that in the discovery stage (rs79329964; OR 1·54, 1·32–1·79; p=0·03; rs79329964 was used in replication as a proxy for rs7314908).

A systematic assessment of genetic loci previously associated with Alzheimer's disease or Parkinson's disease showed no evidence of other genome-wide significant associations in this dementia with Lewy bodies cohort (appendix p 5). These loci include the *TREM2* locus, where the p.Arg47His variant has been shown to have a strong effect in Alzheimer's disease.[24] In our cohort this variant did not show genome-wide significant levels of association (OR 3·46, 95% CI 1·54–7·77; p=0·002), despite the over-representation in people with dementia with Lewy bodies compared with controls. Similarly, *MAPT*, which is strongly associated with Parkinson's disease and has been previously linked to dementia with Lewy bodies,[25] shows no strong evidence of association in this study (rs17649553; OR 0·86, 0·76–0·96; p=0·0126).

To examine whether the association with *SNCA* is independent of that seen in Parkinson's disease, we conditioned our analysis on the top Parkinson's disease variant (rs356182), which showed only a negligible effect on the DLB association (conditioned OR 0·70, 95% CI 0·63–0·78; $p=2{\cdot}89 \times 10^{-10}$; figure 2). To gain insight into potential regulatory effects of this distinct *SNCA* signal, we used expression quantitative trait loci (eQTL) data from the Genotype-Tissue Expression (GTEx) Project Consortium and the Harvard Brain Bank Resource Center to determine whether rs7681440 and rs7681154 (a variant shown to have an independent association for Parkinson's disease that is in strong linkage disequilibrium [R^2=0·91] with the rs7681440 *SNCA* variant) affect gene expression as eQTLs. In the GTEx data, the most associated SNP in dementia with Lewy bodies is a strong eQTL in the cerebellum for *RP11-67M1.1*, a known antisense gene located at the 5′ end of *SNCA*, with the alternative allele showing a reduction in expression of *RP11-67M1.1* (figure 3). Additionally, rs7681154 was associated with *SNCA* expression in the cerebellum using the Harvard Brain Bank Resource Center results ($p=2{\cdot}87 \times 10^{-11}$; figure 3), with the alternative allele associated with increased *SNCA* expression.

We assessed linkage disequilibrium across the *LRRK2* locus region and that analysis revealed that rs79329964 is in equilibrium with both p.Gly2019Ser (R^2=0·000043) and with the Parkinson's disease hit at this locus, rs76904798 (R^2=0·003), suggesting rs79329964 to

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be an independent association from the Parkinson's disease risk. Although samples were not screened for p.Gly2019Ser directly, the variant was well imputed (R^2=0·94). The exclusion of all samples that carried the p.Gly2019Ser variant showed no significant effect on the association at the *CNTN1* locus. Notably, the p.Gly2019Ser variant showed a higher minor allele frequency in participants with dementia with Lewy bodies (0·0021) than in controls (0·0003).

Gene-based burden analysis of all low frequency and rare variants (minor allele frequency <0·05) changing the aminoacid sequence, showed a single genome-wide significant result comprised of six variants at *GBA* (p.Asn409Ser, p.Thr408Met, p.Glu365Lys, p.Arg301His, p.Ile20Val, and p.Lys13Arg; $p=1{\cdot}29 \times 10^{-13}$). No other gene showed evidence of strong association with disease or overlapped single variant analysis results (table 4).

Using the first ten principal components as covariates and a disease prevalence of 0·1%, estimation of the phenotypic variance attributed to genetic variants showed a heritable component of dementia with Lewy bodies of 36% (SD 0·03). As expected for a common complex disease, we found a strong correlation between chromosome length and heritability ($p=6{\cdot}88 \times 10^{-5}$; figure 4).

The heritability for dementia with Lewy bodies at chromosome 19 is much higher than what would be expected considering the chromosome's size and probably reflects the role of *APOE*. Notably, chromosomes 5, 6, 7, and 13 all have higher heritability for dementia with Lewy bodies than expected, although none of them has variants with genome-wide significant results.

Discussion

This is the first comprehensive, unbiased study of common and intermediate frequency genetic variability in dementia with Lewy bodies. We identified five genome-wide significant associations in the discovery stage (*APOE*, *BCL7C*/*STX1B*, *SNCA*, *GBA*, and *GABRB3*), with the associations regarding *APOE*, *SNCA*, and *GBA* being confirmed in the replication stage and in the combined analysis of both stages.

The most significant association signal is seen at the *APOE* locus (*APOE* ε4), which has been previously shown to be highly associated with dementia with Lewy bodies.[6,7] As described, *APOE* ε4 is the major genetic risk locus for Alzheimer's disease and has been implicated in cognitive impairment within Parkinson's disease, although not with the risk of Parkinson's disease itself. The locus has also been reported to affect the levels of both β-amyloid and Lewy body pathology in brains of patients.[27] In a small Finnish dataset,[28] the ε4 allele association with dementia with Lewy bodies was largely driven by the subgroup with concomitant Alzheimer's disease pathology.

The second strongest association is seen at the *SNCA* locus. Results from our conditioned analysis confirmed the different association profile between dementia with Lewy bodies and Parkinson's disease that we had previously reported.[7] *SNCA* is the most significant common genetic risk factor for Parkinson's disease, with rs356182 having a meta-analysis p value of $1{\cdot}85 \times 10^{-82}$ (OR 1·34, 1·30–1·38) in PDGene. This variant is located 3' to the gene,[29]

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whereas in dementia with Lewy bodies, no association was found in that region (figure 2). The most associated dementia with Lewy bodies SNP for the *SNCA* locus (rs7681440) has a Parkinson's disease meta-analysis p value of more than 0·05 in PDGene. When doing a conditional analysis on the top Parkinson's disease SNP (rs356182), Nalls and colleagues[29] reported an independent association at the 5′ region of the gene (rs7681154), and this variant is in strong linkage disequilibrium (R^2=0·91) with the rs7681440 *SNCA* variant identified in our study. It is tempting to speculate that these differences might reflect pathobiological differences between the two diseases, perhaps mediated by differential regulation of gene expression. The results in the GTEx data, showing that the most associated SNP in dementia with Lewy bodies is a strong eQTL in the cerebellum for *RP11-67M1.1*, are compatible with a model in which rs7681440 genotypes affect the expression levels of *SNCA* indirectly through the action of *RP11-67M1.1*. More specifically, the alternative allele associates with a decreased expression of *RP11-67M1.1* and consequently reduced repression of *SNCA* transcription (increased *SNCA* expression), which is in accordance with an increased frequency of the alternative allele in participants with dementia with Lewy bodies when compared with controls. Additionally, the relationship between rs7681154 and *SNCA* expression is supported by the high expression of *SNCA* in the brain and the association of rs7681440 with increased *SNCA* expression in whole blood ($p=2{\cdot}13 \times 10^{-38}$).[30,31] However, further investigation of the identified significant eQTLs is needed because the effect was seen for only one brain region. This localised effect could plausibly result from low overall expression of *RP11-67M1.1* and higher RNA quality in the cerebellum than in other assayed brain regions in these datasets. Notably, both eQTLs' effects fit with a model of increased *SNCA* expression in participants with dementia with Lewy bodies compared with controls.

The most significant marker at the *GBA* locus (rs35682329) is located 85 781 base pairs downstream of the gene and is in high linkage disequilibrium (D′=0·9; R^2=0·8) with p.Glu365Lys (also reported in the scientific literature as E365K, E326K, and rs2230288), which has been suggested as a risk factor for dementia with Lewy bodies.[8] The top associated variant for Parkinson's disease at this locus is the rs71628662 (PDGene meta-analysis OR 0·52 [95% CI 0·46–0·58; $p=6{\cdot}86 \times 10^{-28}$]). This variant is also in high linkage disequilibrium with the top SNP identified here (D′=0·9 and R^2=0·8). In this study, we show similar effect sizes for *APOE* (OR 2·40) and *GBA* (OR 2·55) in dementia with Lewy bodies. Gene burden-based analysis showed *GBA* as the only genome-wide significant association with dementia with Lewy bodies risk. The inexistence of other associations should be interpreted with some caution. Because we were not ascertaining the complete spectrum of genetic variability, other genes could have had a significant burden of genetic variants that were simply not captured in our study design, despite our use of the most recent imputation panel.

Although in our meta-analysis we saw a genome-wide significant association with dementia with Lewy bodies at the *BCL7C/STX1B* locus, this association was mostly driven by the discovery-stage data (replication-stage results were OR 0·98; p=0·83) and further replication is needed. That being acknowledged, an association at the *BCL7C/ STX1B* locus has been previously reported for Parkinson's disease.[29,32] The top Parkinson's disease-associated variants at this locus were rs14235 (synonymous; located at *BCKDK*) and rs4889603

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(intronic; located at *SETD1A*). The top SNP identified in dementia with Lewy bodies at this locus (rs897984) shows the same direction of association seen in Parkinson's disease (OR 0·93, 95% CI 0·90–0·96), a Parkinson's disease meta-analysis p value of $1{\cdot}34 \times 10^{-5}$ (data from PDgene), and strong linkage disequilibrium with both Parkinson's disease hits (R^2=0·28–0·32; correlation p values<0·0001). This is a gene-rich region of the genome (appendix p 9), making accurate nomination of the gene driving the association difficult. Mining data from the GTEx project showed that rs897984 is not an eQTL for any gene in the locus. Nonetheless, in both Parkinson's disease studies, the nominated gene at the locus was *STX1B*, probably due to its function as a synaptic receptor.[33] Additionally, *STX1B* has a distinctive pattern of expression across tissues, presenting the highest expression in the brain. In this tissue, when compared with the closest genes in the locus (*HSD3B7, BCL7C, ZNF668, MIR4519, CTF1, FBXL19, ORAI3, SETD1A, STX4*), *STX1B* also shows the highest levels of expression (appendix p 10). In 2014, mutations in *STX1B* were shown to cause fever-associated epileptic syndromes[34] and myoclonic astatic epilepsy.[35]

Although not quite genome-wide significant in the discovery stage, the association between *CNTN1* and dementia with Lewy bodies risk replicated with a very similar association OR as the discovery stage. Interestingly, the locus has been previously associated with Parkinson's disease in a genome-wide study of identical-by-descent segments in an Ashkenazi cohort,[36] and with cerebral amyloid deposition, assessed with PET imaging in *APOE* ε4 non-carriers.[37] This locus also did not reach genome-wide significance with clinicopathological Alzheimer's disease dementia (p=$5{\cdot}21 \times 10^{-6}$).[38] The contact in 1 protein, encoded by *CNTN1*, is a glycosylphosphatidylinositol-anchored neuronal membrane protein that functions as a cell-adhesion molecule with important roles in axonal function.[39,40] Mutations in *CNTN1* were found to cause a familial form of lethal congenital myopathy.[41] Contactin 1 drives Notch-signalling activation and modulates neuroinflammation events, possibly participating in the pathogenesis of multiple sclerosis and other inflammatory disorders.[42] A functional protein association network analysis of *CNTN1* using STRING shows contactin 1 is in the same network as *PSEN2* (appendix p 11), supporting its potential role in neurodegeneration. Further replication will be important in view of the absence of a genome-wide significant association in the discovery stage; however, this association seems promising. Notably, *LRRK2* is located less than 500 000 base pairs away from the most associated SNP at this locus, which could suggest that the association might be driven by variation at the *LRRK2* locus. Further validation of the involvement of *CNTN1* variation in modifying risk of dementia with Lewy bodies will be important.

In addition to performing a genome-wide association study with clinicopathological Alzheimer's disease dementia, Beecham and colleagues[38] also analysed commonly comorbid neuropathological features seen in elderly individuals with dementia, including Lewy body disease. In this latter analysis, only the *APOE* locus was found to achieve genome-wide significance. However, when testing known common Alzheimer's disease risk variants with coincident neuropathological features, Beecham and colleagues identified hits at *SORL1* and *MEF2C*, finding them to be nominally associated. In our cohort of participants with dementia with Lewy bodies, we found no genome-wide significant associations between these variants and disease. Similarly, we had previously reported an

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association at the *SCARB2* locus with dementia with Lewy bodies.[7] In the larger dataset of the present study, the association remained at the suggestive level and did not reach genome-wide significance (most significant SNP in the present study, rs13141895; $p=9{\cdot}58 \times 10^{-4}$). No other variant previously reported to be significantly associated with Alzheimer's disease or Parkinson's disease in recent genome-wide association study meta-analyses showed a genome-wide significant association with dementia with Lewy bodies. The most significant Alzheimer's disease or Parkinson's disease variants at the following loci showed nominal ($p<0{\cdot}05$) association levels: *MAPT*, *BIN1*, *GAK*, *HLA-DBQB1*, *CD2AP*, *INPP5D*, *ECHDC3*, and *SCIMP*. Additionally, variants previously suggested to be associated with Lewy-related pathology in a Finnish cohort,[28] did not show evidence of association in this study (appendix p 5). See appendix pp 12–70 for colocalisation plots of association between dementia with Lewy bodies and either Parkinson's disease or Alzheimer's disease.

This study has notable limitations. The control population is not perfectly matched to the case cohort because it was derived from publicly available data. To address this, we have used all available information (both clinical and genetic) to create a control cohort that is as similar as possible to the case cohort. Additionally, despite using the same diagnostic criteria for all included participants with dementia with Lewy bodies, diagnostic measurements were collected in a variety of locations, suggesting that diagnostic accuracy might have been variable, with contamination from participants with Parkinson's disease or Alzheimer's disease. Notably, we do not see an over-representation of genetic risk factors from those diseases in our results (eg, *MAPT*, *CLU*, or *CR1*), suggesting minimum inclusion. Similarly, population stratification could bias the results because samples were collected in various countries. In the present study, we have used standard methodology to correct for any such bias and, consequently, our results show no evidence of population stratification as evidenced by the Q–Q plot as defined by the acquired unbiased genotype data. Additionally, participants with dementia with Lewy bodies were genotyped at three locations and controls were all derived from publicly available datasets, using a mixture of genotyping arrays, which could provide a source of genotyping bias. However, our approach was to select variants that were at the intersection of all used arrays before imputation, which makes use, effectively, of the same genotyping probes for all samples. This approach has been shown to remove any bias from this type of result and any effects of using different array scanners are negligible for high-quality variants.[43]

This is the first large-scale genome-wide association study in dementia with Lewy bodies. We estimate the heritability of dementia with Lewy bodies to be approximately 36%, which is similar to what is known to occur in Parkinson's disease.[44] This finding shows that, despite not having multiple causative genes identified so far, genetics has a relevant role in the common forms of dementia with Lewy bodies. Additionally, we provide evidence suggesting that novel dementia with Lewy bodies loci are likely to be found at chromosomes 5, 6, 7, and 13 in view of the high heritability estimates at these chromosomes. A significant majority of our case cohort in the present study was comprised of participants with neuropathological diagnoses, which provide a greater level of information for diagnostic accuracy. These results provide us with the first glimpse into the molecular pathogenesis of dementia with Lewy bodies; they reveal that this disorder has a strong genetic component and suggest a unique genetic risk profile. From a molecular perspective, dementia with

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Lewy bodies does not simply sit between Parkinson's disease and Alzheimer's disease; instead, the combination of risk alleles is unique, with loci that are established risk factors for those diseases having no clear role in dementia with Lewy bodies (eg, *MCCC1*, *STK39*, *CLU*, *CR1*, or *PICALM*). Further increases in the size of dementia with Lewy body cohorts will probably reveal additional common genetic risk loci, and these will, in turn, improve understanding of this disease, its commonalities, and differences with other neurodegenerative conditions, ultimately allowing the identification of disease-specific targets for future therapeutic approaches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research in context

Evidence before this study

We searched PubMed using the keywords “dementia Lewy bodies” AND “genetics”, for manuscripts published in any language between database inception and June 21, 2017, and found no large-scale genome-wide studies of dementia with Lewy bodies. So far, most studies have focused on small cohorts and are frequently candidate gene association studies. However, in 2014, we showed that dementia with Lewy bodies has a genetic component, suggesting that a large unbiased genetic association study might provide novel loci that have a role in the disease.

Added value of this study

To our knowledge, this is the first large-scale genome-wide association study in dementia with Lewy bodies. The discovery stage included 1216 patients with dementia with Lewy bodies and 3791 controls, and the replication stage included 527 patients with the disease and 663 controls. The vast majority of people with the disease from both stages were neuropathologically diagnosed. Furthermore, despite the comparatively smaller size of the replication cohort, all samples were ascertained at the same centre, which reduces diagnostic heterogeneity.

Implications of all the available evidence

Our data show several genome-wide significant loci. Some of these loci had previously been implicated in Parkinson’s disease or Alzheimer’s disease, which could suggest that dementia with Lewy bodies is simply a combination of the genetic underpinnings underlying those diseases. However, our data suggest that dementia with Lewy bodies does not sit in the spectrum between Parkinson’s disease and Alzheimer’s disease, but instead, has a unique genetic profile. Additionally, we have also estimated the genetic heritability of dementia with Lewy bodies to be 36%, which is very close to what has been estimated for Parkinson’s disease, a disease now known to have a strong genetic component.

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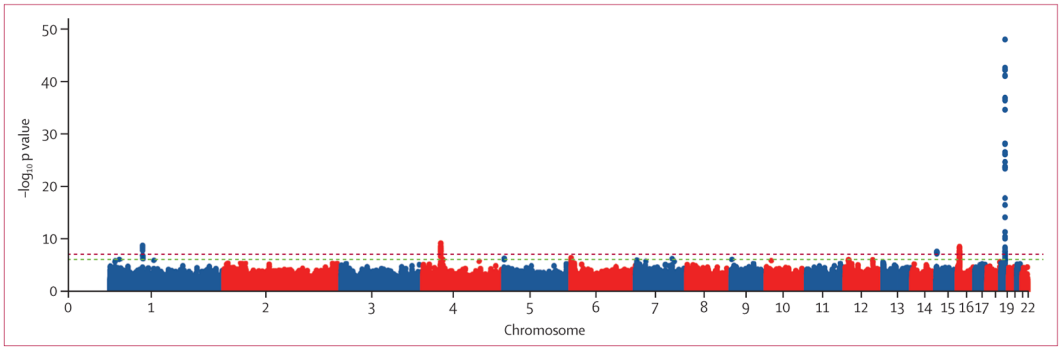

Figure 1. Manhattan plot showing genome-wide p values of association

The p values were obtained by logistic-regression analysis using the first six principal components and sex as covariates. The y axis shows $-\log_{10}$ p values of 8 397 716 single nucleotide polymorphisms, and the x axis shows their chromosomal positions. The horizontal red dotted line represents the threshold of $p=5 \times 10^{-8}$ for Bonferroni significance and the green dotted line represents the threshold of $p=5 \times 10^{-6}$ for selecting single nucleotide polymorphisms for replication.

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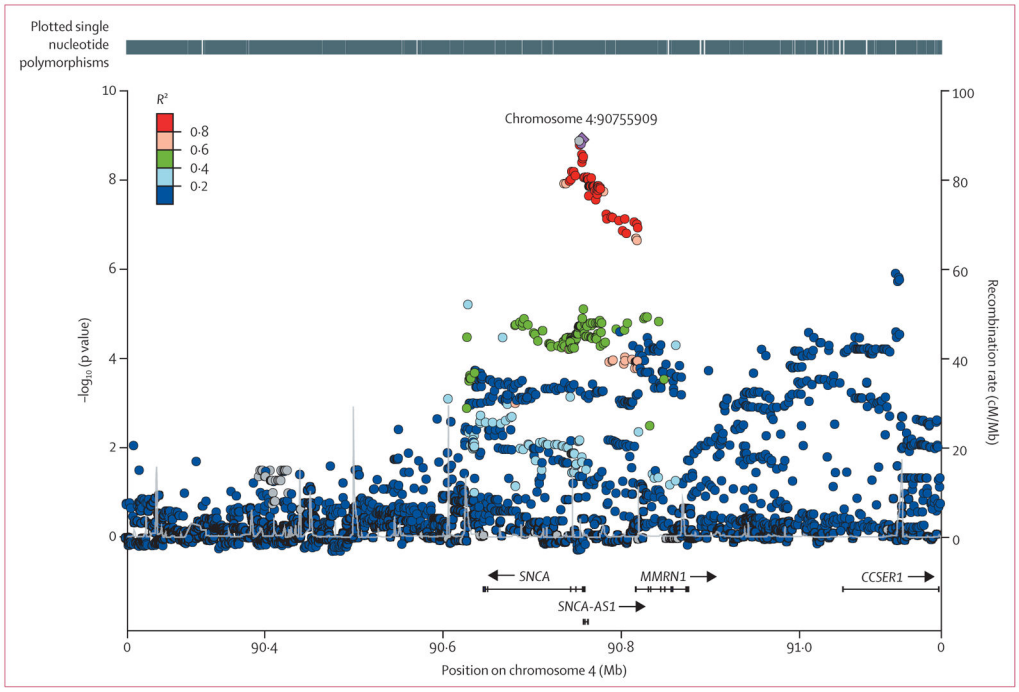

Figure 2. Regional association plot for the *SNCA* locus

Purple represents variant rs1372517, at chromosome 4, position 90755909, which is the most associated SNP at the locus also present in the 1000Genomes dataset. The variant rs1372517 is in complete linkage disequilibrium with rs7681440. Colours represent linkage disequilibrium derived from 1000Genomes between each variant and the most associated SNP. SNP=single nucleotide polymorphism. R^2 represents the level of pairwise linkage disequilibrium between the top variant and each other variant plotted, using data from the 1000 Genomes project.

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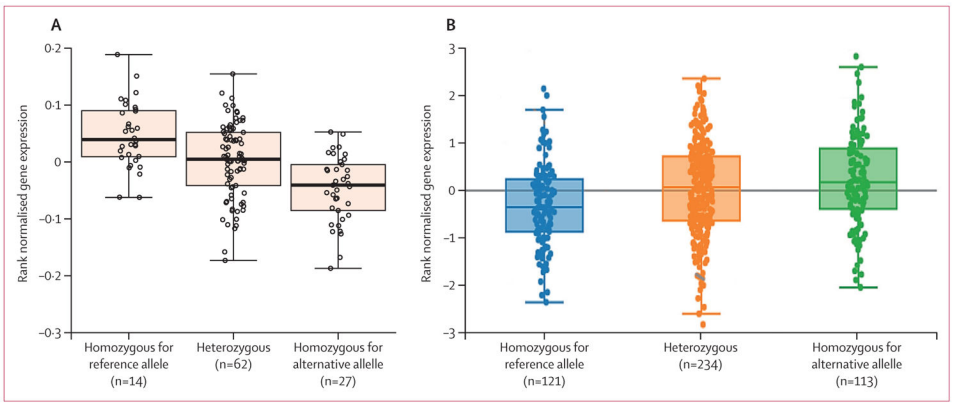

Figure 3. Associations between genotypes and gene expression in the cerebellum of post-mortem controls

(A) Association between rs7681440 genotypes and *RP11-67M1.1* expression in 103 disease-free post-mortem cerebellum samples ($p=2{\cdot}00 \times 10^{-7}$) from the Genotype-Tissue Expression (GTEx) Project Consortium. Carriers of the GG genotype (alternative allele) show the lowest levels of expression of the gene. Details on methods are on GTEx website. (B) Association between rs7681154 and *SNCA* expression ($p=2{\cdot}87 \times 10^{-11}$) in 468 disease-free cerebellum samples from postmortem individuals from the Harvard Brain Bank Resource Center.[26] Individuals with the alternative allele C had increased *SNCA* expression in the cerebellum, on average, compared with individuals with the reference allele G. Details on the subjects, experiments, and analytical methods of the expression quantitative trait loci study of the Harvard Brain Bank Resource Center samples are described by Zhang and colleagues[26] and on the Harvard Brain Bank website. Boxplots for both panels show medians, IQRs, and individual data points.

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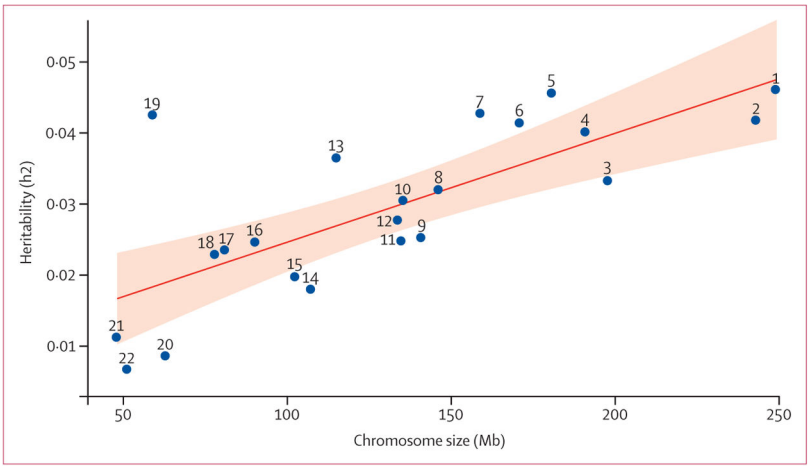

Figure 4. Dementia with Lewy bodies heritability by chromosome

Heritability (y axis) per chromosome is plotted against chromosome length (x axis). The red line represents heritability regressed on chromosome length and the shaded area represents the 95% CI of the regression model.

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Table 1

Characteristics of the discovery cohort

	N	Neuropathological diagnosis	Men:women ratio	Age at onset (years)	Passed quality controls	
					Total	Neuropathological
Patients with dementia with Lewy bodies						
Australia	79	79 (100%)	1·93	65·2 (10·3)	72 (91%)	72 (91%)
Canada	29	15 (52%)	2·22	67·9 (7·8)	6 (21%)	3 (10%)
Finland	34	34 (100%)	0·94	94·3* (3·5)	24 (71%)	24 (71%)
France	18	18 (100%)	3·5	64·8 (10·3)	16 (89%)	16 (89%)
Germany	58	0	2·41	67·8 (6·7)	0	0
The Netherlands	133	133 (100%)	1·71	78·6* (7·4)	132 (99%)	132 (99%)
Portugal	13	0	0·63	68·8 (8·2)	11 (85%)	0
Spain	133	16 (12%)	0·94	73·3 (7·0)	132 (99%)	15 (11%)
UK	404	308 (76%)	2·12	69·7 (10·1)	284 (70%)	245 (61%)
USA	786	705 (90%)	1·93	71·2 (9·9)	539 (69%)	467 (59%)
Total	1687	1308 (78%)	1·83	70·1 (9·5)	1216 (72%)	974 (58%)
Controls						
USA (controls from PSA)	2847	0	0·88	NA	2832 (99%)	0
USA (controls from SC)	1523	0	0·78	38 (5·7)	959 (63%)	··
Total	4370	0	0·83	NA	3791 (87%)	0

Data are n (%) or mean (SD), unless stated otherwise. NA=not applicable. PSA=Genetic Analysis of Psoriasis and Psoriatic Arthritis database. SC=Genetic Architecture of Smoking and Smoking Cessation database.

*Represents age at death, which was available for these cohorts; these values were not used for calculation of the complete mean age at onset.

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Table 2

Top signals of association at each locus that passed genome-wide threshold for significance and their replication and meta-analysis p values

	General characteristics					Discovery					Replication					Meta-analysis	
	Chromosome	Position	Variant	R^2	Eur_AF*	MA	MAF_A	MAF_U	OR (95% CI)	p value	Power†	MAF_A	MAF_U	OR (95% CI)	p value	OR (95% CI)	p value
Global cohort																	
APOE	19	45 411 941	rs429358	0·949	0·149	C	0·283	0·140	2·40 (2·14–2·7)	$1·05 \times 10^{-48}$	1	0·282	0·148	2·74 (2·15–3·49)	$4·00 \times 10^{-16}$	2·46 (2·22–2·74)	$3·31 \times 10^{-64}$
SNCA	4	90 756 550	rs7681440‡	0·996	0·52	C	0·411	0·483	0·73 (0·66–0·81)	$6·39 \times 10^{-10}$	0·95	0·38	0·47	0·68 (0·56–0·82)	$6·00 \times 10^{-5}$	0·73 (0·67–0·79)	$9·22 \times 10^{-13}$
GBA	1	155 135 036	rs35749011	0·957	0·014	G	0·033	0·014	2·55 (1·88–3·46)	$1·78 \times 10^{-9}$	0·83	0·044	0·022	1·81 (1·05–3·11)	0·033	2·27 (1·75–2·95)	$6·57 \times 10^{-10}$
BCL7C/STX1B	16	30 886 643	rs897984‡	0·984	0·609	T	0·334	0·405	0·74 (0·67–0·82)	$3·30 \times 10^{-9}$	0·96	0·368	0·388	0·98 (0·81–1·19)	0·83	0·77 (0·71–0·85)	$1·19 \times 10^{-8}$
GABRB3	15	26 840 998	rs1426210	0·982	0·315	G	0·348	0·293	1·34 (1·21–1·48)	$2·62 \times 10^{-8}$	0·9	0·281	0·307	0·84 (0·68–1·04)	0·1	1·22 (1·11–1·33)	$2·05 \times 10^{-5}$
Neuropathologically diagnosed cases																	
APOE	19	45 411 941	rs429358	0·949	0·149	C	0·292	0·140	2·52 (2·23–2·85)	$2·77 \times 10^{-49}$	··	··	··	··	··	··	··
SNCA	4	90 756 550	rs7681440‡	0·996	0·52	C	0·409	0·483	0·73 (0·66–0·81)	$2·82 \times 10^{-9}$	··	··	··	··	··	··	··
GBA	1	155 135 036	rs35749011	0·957	0·014	G	0·037	0·014	2·87 (2·10–3·90)	$2·67 \times 10^{-11}$	··	··	··	··	··	··	··
BCL7C/STX1B	16	30 886 643	rs897984‡	0·984	0·609	T	0·332	0·405	0·73 (0·65–0·81)	$4·32 \times 10^{-9}$	··	··	··	··	··	··	··
GABRB3	15	26 840 998	rs1426210	0·982	0·315	G	0·350	0·293	1·34 (1·20–1·45)	$1·21 \times 10^{-7}$	··	··	··	··	··	··	··

R^2=imputation R^2 of each specific variant from Haplotype Reference Consortium. OR=odds ratio. MA=minor allele. MAF_A=minor allele frequency in cases. MAF_U=minor allele frequency in controls.

*Eur_AF is the alternative allele frequency derived from the European population of the Genome Aggregation Database (gnomAD).[23]

†Power refers to the calculated statistical power to replicate the discovery signal, taking into account the replication sample size, effect, and frequency in discovery and an association threshold of p<0·05.

‡Represents variants for which the gnomAD allele frequency corresponds to the alternative allele and not the effect allele.

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Table 3

Characteristics of the replication cohort

	Neuropathological diagnosis	Men:women ratio	Age at onset (years)*
USA: patients with dementia with Lewy bodies	350/527 (66%)	2·01	76·3 (8·2)
USA: controls	0/663	0·75	67·8 (10·0)

Data are n (%) or mean (SD), unless stated otherwise.

*Denotes age at examination for controls; for patients with dementia with Lewy bodies, the age reflects age at onset for those diagnosed clinically and age at death for those pathologically diagnosed.

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Table 4

Top gene burden results

	Chromosome number	Begin*	End*	Number of samples with non-missing genotypes	Fraction with rare allele†	Number of all variants defining the gene group	Number of variants passing the frequency and call-rate thresholds	Number of singletons among variants passing the frequency and call-rate thresholds	p value
GBA	1	155 204 797	155 210 498	5 016	0·05622	8	6	1	$1·29 \times 10^{-13}$
CBX6	22	39 262 224	39 267 761	5 016	0·010965	6	3	0	$1·66 \times 10^{-5}$
ST14	11	130 058 428	130 079 477	5 016	0·076754	20	11	2	$4·29 \times 10^{-5}$
NPS	10	129 347 767	129 350 889	5 016	0·076555	5	3	1	$6·74 \times 10^{-5}$
RBM47	4	40 428 010	40 434 855	5 016	0·0099681	3	2	1	0·0001
TPH1	11	18 047 141	18 057 637	5 016	0·0091707	8	5	0	0·0002
HLA-C	6	31 237 124	31 239 829	5 016	0·1262	32	10	1	0·0003
FOSB	19	45 971 941	45 976 122	5 016	0·00079745	4	2	1	0·0004
DPH2	1	44 435 905	44 438 171	5 016	0·004386	9	6	2	0·0004
RAMP1	2	238 785 923	238 820 379	5 016	0·00079745	2	2	1	0·0005

*Begin and end refer to the locations defining each gene or marker group tested.

†Fraction of individuals carrying rare variants below the allele frequency threshold (0·05).