Dementia with Lewy bodies (DLB) is the second most common form of degenerative dementia. Siblings of affected individuals are at greater risk of developing DLB, but little is known about the underlying genetic basis of the disease. We set out to determine whether mutations in known highly penetrant neurodegenerative disease genes are found in patients with DLB. Whole-exome sequencing was performed on 91 neuropathologically confirmed cases of DLB, supplemented by independent APOE genotyping. Genetic variants were classified using established criteria, and additional neuropathological examination was performed for putative mutation carriers. Likely pathogenic variants previously described as causing monogenic forms of neurodegenerative disease were found in 4.4% of patients with DLB. The APOE ε4 allele increased the risk of disease (P = 0.0001), conferred a shorter disease duration (P = 0.043) and earlier age of death (P = 0.0015). In conclusion, although known pathogenic mutations in neurodegenerative disease genes are uncommon in DLB, known genetic risk factors are present in > 60% of cases. APOE ε4 not only modifies disease risk, but also modulates the rate of disease progression. The reduced penetrance of reported pathogenic alleles explains the lack of a family history in most patients, and the presence of variants previously described as causing frontotemporal dementia suggests a mechanistic overlap between DLB and other neurodegenerative diseases.

INTRODUCTION
Dementia with Lewy bodies (DLB) is the second most common form of dementia. It affects 5% of the population over 75 years of age, and has a greater impact on healthcare provision than Alzheimer’s disease (AD). The neuropathological hallmark of DLB is widespread α-synuclein-positive neuronal inclusions (Lewy bodies and Lewy neurites) and in addition this is often associated with amyloid deposition. Siblings of affected individuals have a 2.3-fold increased risk of developing the disorder, but little is known about the genetic aetiology of the disease. Although genetic variants in APOE, GBA, SNCA and SCARB2 (ref. 7) have been associated with an increased risk of DLB, only a few families have been described with more than two first-degree relatives, and no single highly penetrant gene defects have been shown to cause familial forms of the disorder. Using exome sequencing in 91 autopsy-confirmed cases, here we determined whether confirmed or putative pathogenic mutations in genes in known neurodegenerative disease genes are found in patients with DLB.

MATERIALS AND METHODS
Subjects and sample preparation
We studied 91 post-mortem cases conforming to both the clinical and post-mortem diagnostic criteria for DLB. Two patients were first-degree relatives (mother and daughter) and two patients were siblings (brothers). The remaining 87 patients had no recorded family history of neurodegenerative disease. Age of onset, disease duration, age of death, neuropathological subtype of Lewy body disease according to McKee/Neurological criteria and Braak neurofibrillary tangle stage were recorded (Figure 1). In addition, we assessed Lewy body Braak stages, Aβ phases and stages of cerebral amyloid angiopathy. Of note, none of the cases showed intracytoplasmic TAR DNA-binding protein 43 (TDP-43) inclusions indicative for frontotemporal lobar degeneration associated with TDP-43 pathology, nor were there neuropathological features consistent with other types of frontotemporal lobar degeneration (see additional Supplementary Methods).

RESULTS
The mean exome sequencing base coverage depth was 84-fold (s.d. = 13) in the 91 DLB cases and 76-fold (s.d. = 12) in the 93 controls. There was no difference in the proportion of the exome target covered at > 30-fold depth between DLB cases and controls (DLB 84%, s.d. = 5; controls 84%, s.d. = 3, P = 0.588).
Known mendelian disease genes

A total 18 rare heterozygous mutations in 25 patients were observed in genes previously shown to cause autosomal dominant forms of neurodegeneration (Tables 1, 2 and Supplementary Table S1). Three of these variants have been described in patients with AD, PD or frontotemporal lobar degeneration and amyotrophic lateral sclerosis (Patient A: PSEN2 p.D439A,16,17 B: CHMP2B p.I29V,18 and C: SQSTM1 p.A33V,19,20). In two additional cases (Patient E: EIF4G1 p.M1134V and F: SQSTM1 p.P27L), variants in known disease genes affecting highly conserved residues and predicted to be pathogenic by in silico software algorithms, were deemed of uncertain significance. Two patients also had variants of uncertain significance in GIGYF2, which is also implicated in PD (H: GIGYF2 p.S66T; G: GIGYF2 p.S1029C, Table 2). In genes causing autosomal recessive PD, AD or frontotemporal dementia and amyotrophic lateral sclerosis, only one rare compound heterozygous mutation in PARK2 was seen (Patient D, p.R275W/p.G430D).

Only patient A had a relevant family history (father affected—deceased and no tissue/DNA available). A clinical description of these cases is shown in the Supplementary Information. All showed typical DLB pathology with cortical LB being present and moderate AD pathology (Table 2).

The mean age at the presentation for the four cases with previously described pathogenic mutations (Patients A–D) was 78.25 years (s.d. = 8.05). Motor symptoms developed in three cases (Patient A, B and D) at a mean of 1.33 years (s.d. = 0.58) after the onset of cognitive symptoms. When patients E and F were included, the mean age of onset was 78.6 (s.d. = 6.68), with motor symptoms developing in four patients (A, B, D and E), and a mean disease duration of 2.3 (s.d. = 1.16) years.

Table 1. Genes causing monogenic forms of PD, AD, FTLD-ALS, which were analysed for rare protein altering mutations in patients

| Inheritance      | Disease | PD       | AD       | FTLD-ALS |
|------------------|---------|----------|----------|----------|
| Autosomal dominant | SNCA    | APP      | C9orf72  |          |
|                   | LRRK2   | PSEN-1   | SOD1     |          |
|                   | UCHL1   | PSEN2    | MAPT     |          |
|                   | GIGYF2  |          | PGRN     |          |
|                   | Omi/HTRA2 |        | TARDBP  |          |
|                   | EIF4G1  |          | OPTN     |          |
|                   |         |          | ANG      |          |
|                   |         |          | CHMP2B   |          |
|                   |         |          | SQSTM1   |          |
|                   |         |          | FUS      |          |
|                   |         |          | VCP      |          |
|                   |         |          | OPTN     |          |
| Autosomal recessive | PARK2  | PINK1    |          |          |
|                   | ATP13A2 | PLA2G6   |          |          |
|                   | FBX07   | DJ-1     |          |          |

Abbreviation: AD, Alzheimer’s disease; ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; PD, Parkinson’s disease.

Table 2. Clinical and pathological characteristics of the 91 dementia with Lewy body (DLB) cases. Top left: frequency of each pathological category (BS, brain stem; L, limbic; N, neocortical; UC, unclassified). Top right: BRAAK neurofibrillary tangle stage of patients (UC, unclassified). Bottom: table of the clinical and pathological data for all the 91 cases of DLB. Data are mean (s.d.). Motor features were defined by documented evidence of a Parkinsonian movement disorder by an assessing clinician.

Figure 1. Clinical and pathological characteristics of the 91 dementia with Lewy body (DLB) cases. Top left: frequency of each pathological category. Top right: BRAAK neurofibrillary tangle stage of patients (UC, unclassified). Translational Psychiatry (2016), 1
Table 2. The frequency of potentially pathogenic variants in DLB cases and controls

| Patient | Pathogenicity | Gene       | Chromosome | Position | R/V     | Predicted protein change | Previously reported phenotype | MAF ESP 6500 | MAF 1000G | ExAC MAF | SIFT   | PolyPhen2 | MutationTaster | CADD score (scaled) | NFT Braak stage | Braak PD stage | Aβ phase | TDP-43 | CAA | ACMG criteria |
|---------|---------------|------------|------------|----------|---------|--------------------------|----------------------------|----------------|------------|-----------|--------|-----------|----------------|----------------|----------------|-------------|---------|--------|----|--------------|
| A       | P             | CHMP2B     | 3          | 87289899 | A/G     | p.I29V                   | FTLD                       | 0.00015        | —          | 0.0001237 | T      | N         | D              | 14.1                       | 4              | 5/6         | 4          | +ve CA1 | 2   | (1) Same amino acid as previously reported (PS1) (2) Well established functional studies show a deleterious effect (PS3) |
| B       | P             | PARK2      | 6          | 162206852| G/A     | p.R275W                  | PD                         | 0.001999       | 0.0005     | 0.00206   | D      | D         | D              | 33                        | 4              | 6           | 3          | NT      | —   | |
| C       | LP            | PARK2      | 1          | 267083249| A/C     | p.G430D                 | AD                         | 0.000231       | —          | 0.0001076 | D      | D         | D              | 34                        | 4              | 6           | 4          | NT      | 2   | (1) Same amino acid as previously reported variant (PS1) (2) Well established functional studies show a deleterious effect (PS3) |
| D       | LP            | SQSTM1     | 5          | 179250906| C/T     | p.A33V                   | FTLD                       | 0.000769       | 0.0018     | 0.001523 | T      | N         | N              | 11.9                       | 3/4            | 6           | 3          | +ve CA1 | 2   | |
| E       | US            | EIF4G1     | 3          | 184046450| A/G     | p.M1134V                | U                          | 0.000115       | —          | 0.000224 | D      | D         | D              | 26.3                       | 3              | 5/6         | 2          | +ve CA1 | 2   | |
| F       | US            | SQSTM1     | 5          | 179250888| C/T     | p.P27L                   | U                          | 0.00008        | —          | 0.0000339| T      | D         | N              | 12.8                       | 1              | 6           | 4          | NT      | 2   | (1) Computational evidence supports a deleterious effect (1) Computational evidence supports a deleterious effect |
| G       | US            | GIGYF2     | 2          | 233709083| C/G     | p.S1029C                | U                          | 0.00123        | —          | 0.0007833| D      | D         | D              | 23.2                       | 5              | 6           | 4          | −ve     | 2   | |
| H       | US            | GIGYF2     | 2          | 233655546| G/C     | p.S66T                  | U                          | 0.00008        | —          | 0.0001813| T      | D         | D              | 23.7                       | 4              | 5           | 3          | NT      | 2   | |

Abbreviations: ACMG, American College of Medical Genetics; AD, Alzheimer’s disease; CA1, CA1 division of the hippocampus; CAA, cerebral amyloid angiopathy; DLB, dementia with Lewy body; FTLD, frontotemporal lobar degeneration; MAF, minor allele frequency; PD, Parkinson’s disease; R, reference allele; U, unknown or not described; V, variant allele. The number of patients covered at 430-fold sequence depth, and the number of case and control patients carrying each mutation is shown. Functional predictions were performed by SIFT, PolyPhen2 and MutationTaster. Variants were classified as: (1) pathogenic, if the same alleles had previously been described in patients with neurodegenerative disease; (2) likely pathogenic, if the alleles were in previously known neurodegenerative disease genes and in silico predictions supported a pathogenic role; and (3) possibly pathogenic, if in silico predictions supported a pathogenic role, and the gene had previously been associated with a Mendelian neurodegenerative disease. See Supplementary Material for citations. Neuropathology scores according to existing accepted diagnostic criteria as outlined in Supplementary Methods are shown.
DISCUSSION

Exome sequencing of 91 cases of pathologically confirmed DLB identified four patients harbouring previously described pathogenic mutations neurodegenerative disease genes based on current diagnostic criteria (PSEN2, CHMP2B, SQSTM1, PARK2); possible pathogenic mutations in two (EIF4G1 and SQSTM1); and two further cases with mutations in GIGYF2, which has previously been associated with autosomal dominant PD. The central question is: are these variants causing DLB, or are they co-incidental findings? The role of GIGYF2 in PD remains contentious, and the P.D439A variant in PSEN2 may have incomplete penetrance, and is thus found in control databases along with the CHMP2B and SQSTM1 variants. Providing definitive proof of pathogenicity is therefore challenging, and there are arguments in both directions.

On one hand, the variants detected in PSEN2, CHMP2B, SQSTM1 and PARK2 are exceptionally rare in the general population. Given the clinical, pathological and mechanistic overlap between DLB and the neurodegenerative disorders where these disease genes were first described, it is plausible that they are contributing to the neuropathology. For example, in families with familial AD due to PSEN2 mutations, up to 64% of cases have extensive Lewy body deposition at autopsy. The CHMP2B protein has been identified as having a role in alpha-synuclein accumulation in mice. This may explain why none of the four patients harbouring established pathogenic mutations reported a relevant family history.

On the other hand, the clinical and pathological phenotype of these five cases was wholly typical of DLB: how can this be reconciled with known pathogenic compound heterozygous mutations in PARK2, which typically presents with dystonia in early adult life? These findings highlight the challenges of using exome or whole-genome sequencing in a clinical context: is rare pathogenic mutation in a known disease gene more likely to be causing a variant phenotype, or is the phenotype so unusual that the variants must be a co-incidental finding? This will be difficult to resolve in individual cases, but the ongoing reporting of rare putative disease alleles, linked to rich phenotypic data, is an essential step in generating global data sets, which will ultimately provide definitive evidence of pathogenicity.

Although the size of our study cohort limited the potential to discover new disease genes and risk loci, and did not include exclusion of repeat expansions such as C9orf72, we saw enrichment of GBA alleles and APOE ε4 alleles in DLB. In total, 48 patients (55.2%) possessed an APOE ε4 allele, with 5 (5.7%) having a variant in GBA, together with four (4.4%) having likely pathogenic alleles (potentially with incomplete penetrance). Therefore, 62.6% of patients harbour a risk factor or potentially pathogenic allele. This could explain why DLB is a relatively common disorder in the population, with an increased risk of

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Table 3. APOE genotype of all cases (excluded confirmed pathogenic variants) and controls

| Study size | 4/4 | 3/3 | 2/2 | 4/3 | 3/2 | 2/2 | ε4 carrier |
|-----------|-----|-----|-----|-----|-----|-----|-----------|
| Controls  | 93  | 1   | 54  | 2   | 24  | 12  | 0         |
| DLB patients | 87  | 3   | 33  | 0   | 45  | 6   | 0         |
| P-value   | 0.35| 0.0076 | 0.50 | 0.0004 | 0.22 | 1.0 | 0.0001    |

Abbreviation: DLB, dementia with Lewy body. Comparison between groups (patients n = 87, controls n = 91) performed by Fisher’s exact test. APOE ε4 carrier determined by the presence of at least one APOE ε4 allele.

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Figure 2. Kaplan–Meier survival curves for DLB patients by APOE allele. Kaplan–Meier survival curves for DLB patients by APOE allele carrying at least one APOE ε4 allele (n = 43, blue line), compared with non-APOE ε4 carriers (n = 39, green line). Despite there being no significant difference in the age of onset of the DLB (see Results), APOE ε4 carriers (a) lived for a shorter period of time following diagnosis (P = 0.036, log rank, Mantel–Cox test), and thus (b) died at a younger age (P = 0.005, log rank, Mantel–Cox test) that non-APOE ε4 carriers. DLB, dementia with Lewy body.
CONFLICT OF INTEREST

The authors declare no conflict of interest.

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