RESEARCH ARTICLE

CME

Systematic Review and UK-Based Study of *PARK2* (parkin), *PINK1, PARK7* (DJ-1) and *LRRK2* in Early-Onset Parkinson's Disease

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ABSTRACT: Approximately 3.6% of patients with Parkinson's disease develop symptoms before age 45. Early-onset Parkinson's disease (EOPD) patients have a higher familial recurrence risk than late-onset patients, and 3 main recessive EOPD genes have been described. We aimed to establish the prevalence of mutations in these genes in a UK cohort and in previous studies. We screened 136 EOPD probands from a high-ascertainment regional and community-based prevalence study for pathogenic mutations in *PARK2* (parkin), *PINK1*, *PARK7* (DJ-1), and exon 41 of *LRRK2*. We also carried out a systematic review, calculating the proportion of cases with pathogenic mutations in previously reported studies. We identified 5 patients with pathogenic *PARK2*, 1 patient with *PINK1*, and 1 with *LRRK2* mutations. The rate of mutations overall was 5.1%. Mutations were more common in patients with age at onset (AAO) < 40 (9.5%), an affected first-degree relative (6.9%), an affected sibling (28.6%), or parental consanguinity (50%). In our study EOPD mutation carriers were more likely to present with rigidity and dystonia, and 6 of 7 mutation carriers had lower limb symptoms at onset. Our systematic review included information from >5800 unique cases. Overall, the weighted mean proportion of cases with *PARK2* (parkin), *PINK1*, and *PARK7* (DJ-1) mutations was 8.6%, 3.7%, and 0.4%, respectively. *PINK1* mutations were more common in Asian subjects. The overall frequency of mutations in known EOPD genes was lower than previously estimated. Our study shows an increased likelihood of mutations in patients with lower AAO, family history, or parental consanguinity. © 2012 *Movement* Disorder Society

Key Words: systematic review; PARK2; PINK1; PARK7; LRRK2; early-onset Parkinson's disease; parkin, DJ-1

Additional Supporting Information may be found in the online version of this article.

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Funding agencies: This work was supported by the Medical Research Council UK (G0700943), Parkinson's UK (Grant 8047), the Cambridge NIHR Biomedical Research Centre, the Midlands Neuroscience Teaching and Research Fund, the Sandwell and West Birmingham Hospitals NHS Trust, the Ipsen Fund, the Wellcome Trust, and a Patrick Berthoud Clinical Research Fellowship and Raymond and Beverly Sackler studentship.
Relevant conflicts of interest/financial disclosures: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received: 27 October 2011; **Revised:** 26 June 2012; **Accepted:** 12 July 2012
Published online 6 September 2012 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.25132

Although Parkinson's disease (PD) is generally considered a disorder of the elderly, it also affects a substantial number of younger individuals. The age-standardized prevalence rate for PD in the United Kingdom is around 140 per 100,000 population, and about 3.6% of PD patients develop symptoms before age 45, which is usually defined as early-onset PD (EOPD).[1,2] Previous studies have indicated an increased familial recurrence risk in PD,[3] and in EOPD in particular there is an increased risk in siblings compared with to parents, consistent with autosomal recessive disease.[4–6]

There are 3 autosomal recessive genes for EOPD: *PARK2* (NM_004562.2), coding for E3 ubiquitin-protein ligase parkin; *PINK1* (NM_032409.2, *PARK6*), which codes for a serine/threonine protein kinase (PTEN-induced putative kinase 1); and *PARK7* (NM_001123377.1), coding for DJ-1.[7–9]

Mutations in *PARK2* and *PINK1* are the most common causes of EOPD. However, reported mutation frequencies vary widely across studies, which may relate to differences in case ascertainment, ethnicity, and proportions of familial and/or consanguineous cases. Lücking and colleagues reported that up to 50% of familial and 18% of sporadic EOPD cases had pathogenic *PARK2* mutations,[10] whereas more recent studies have reported a pathogenic mutation frequency as low as 1.6%.[11] Frequency estimates for *PINK1* mutations tend to fall within a similarly broad range,[12,13] whereas *PARK7* mutations are generally very rare.[14,15]

Mutations in *LRRK2* (NM_198578.3), coding for leucine-rich repeat serine/threonine-protein kinase 2 (dardarin), are the most frequent cause of genetic PD, with the most common mutation (G2019S) accounting for 1% of sporadic and 4% of familial cases.[16] The penetrance of *LRRK2* mutations is age dependent—less than 20% at age 45 and more than 80% at age 80.[16]

We aimed to estimate the prevalence of *PARK2, PINK1*, *PARK7*, and *LRRK2* mutations in EOPD in a UK-based study. In addition, we aimed to provide an estimate of the proportion of EOPD patients with autosomal recessive mutations in a systematic review of previously published studies.

Patients and Methods

UK Cohort Mutation Analysis

EOPD was defined on the basis of the Queen Square Brain Bank criteria for the definition of PD (not including the presence of family history as an exclusion criterion) and age at onset (AAO) < 45 years. Unrelated cases were ascertained from an intensive community-based prevalence study in the city of Cardiff, South Wales, United Kingdom, and from referrals from neurologists and PD specialists in Wales and from the whole of the United Kingdom.[2] There were 3 nested EOPD groups: Cardiff, Wales, and the United Kingdom. The Cardiff series was ascertained via a primary care PD survey, the Welsh group was ascertained through frequent contact with PD specialists in Wales, and the UK cases were primarily recruited from the British National Surveillance Unit and collaborating genetics centers. Pathogenic mutation frequency data will be most accurate in actively recruited sample series, as these series will be less susceptible to referral bias. Clinical data were collated on ethnicity, consanguinity, AAO, family history, and presenting symptoms where available. Cases were defined as familial if they had at least 1 affected first-degree relative.

The cohort was screened for mutations in *PARK2*, *PINK1*, *PARK7*, and *LRRK2* exon 41. All exons in *PARK2*, *PINK1*, and *PARK7* and in exon 41 of *LRRK2* including approximately 40 bp of flanking intronic sequence were sequenced (Supplementary Table S1a–d). Fragments were amplified and sequenced using a BigDye Terminator Cycle Sequencing kit (Perkin Elmer Applied Biosystems, Cheshire, UK) and analyzed on an ABI3100 sequencer (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Sequences were analyzed using Lasergene SeqMan Pro v8.02 software (DNAstar, Inc., Madison, WI). Sequence variants were compared with known mutations and variants documented in the MDPD and PDGene databases.[17,18]

Exon copy number for all exons of *PARK2* and *PINK1*, as well as exons 1, 3, 5, 6, and 7 of *PARK7* was determined using the multiplex ligation-dependent probe amplification method (MLPA) with the SALSA P051 Parkinson's probe mix kit (MRC-Holland, Amsterdam, the Netherlands) according to the manufacturer's instructions. Amplification products were run on an ABI 3100 Analyzer and peak heights assessed using Genotyper software (Applied Biosystems, Foster City, CA).

Statistical analysis comparing cases with and without mutations was performed using Fisher's exact test and the Student t test as appropriate (STATA).

The study was approved by the Research Ethics Committee for Wales (studies 04/9/025, 05/9/058, and 09/09/35).

Systematic Review

For the systematic review we included all studies published from 2000 to March 2011 that had investigated the occurrence of pathogenic *PARK2*, *PINK1*, and/or *PARK7* mutations in a given patient cohort using standard diagnostic criteria and provided information about family history, proband status, consanguinity, ethnicity, and type of mutations found. Studies not satisfying strict inclusion criteria (see supplementary data) were excluded from analysis. The

TABLE 1. Summary of pathogenic mutations in *PARK2, PINK1*, and *LRRK2* found in 136 EOPD cases

	Number of mutations found (prevalence, %)					
	PARK2		*PINK1*		*LRRK2*	
Cohort (n)	Homozygous	Compound heterozygous	Homozygous	Compound heterozygous	Heterozygous	Total %
Cardiff (14)	0	0	0	0	0	0
Wales (82)	0	3 (3.7%)	0	0	0	3.7
UK (136)[a]	0	5 (3.7%)	1 (0.7%)	0	1 (0.8%)	5.1

[a]The United Kingdom (UK) includes England and Wales.

proportion of EOPD cases (AAO < 50) with 2 pathogenic mutations in *PARK2*, *PINK1*, and/or *PARK7* was calculated for each study and collectively. In about half the studies, family history was defined as positive if probands had at least 1 affected first-degree relative (usually restricted to siblings), but in about 15% of studies, family history was extended to second- and third-degree relatives, and no definition was provided in about 30% of studies. For the purposes of this study, all cases with any positive family history were classified as familial. Consanguinity was defined by pedigree information or patient self-reports; articles were included in the familial and consanguineous subanalyses if the prevalence of familial and consanguinity was reported in both mutation-negative and -positive patients. In some studies, cases had been prescreened for mutations (usually in *PARK2*), and if published results were available, this was taken into account.

Study proportions and confidence intervals were calculated using Wilson's technique.[19] Weighted mean proportions and confidence intervals for different patient subgroups were calculated using a negative binomial regression model in order to model between study heterogeneity (overdispersion) as in a random-effects model.[20] Risk ratios (including 95% confidence intervals and *P* values) comparing prespecified subgroups were also calculated using this model. When calculating pooled proportions for different subgroups, studies with fewer than 2 patients in a given subgroup were excluded from analysis. All statistical calculations were made using STATA.

Results

UK Cohort Mutation Analysis

We ascertained 136 unrelated EOPD cases (56% male), resident in the UK. Fourteen cases were identified from the Cardiff community-based study and 68 from the regional ascertainment in Wales (see Supplementary Table S2). The mean AAO was 37 years (median, 39 years; interquartile range, 35–42 years). Most cases were white (97.8%) and sporadic (78.5%). We identified 1 homozygous mutation, 5 compound heterozygous mutations, and 1 pathogenic heterozygous mutation in our cohort, corresponding to an overall mutation rate of 5.1% (95% confidence interval [95% CI], 1.4%–8.9%) for all 4 genes combined (Table 1). No cases were identified in the Cardiff cohort, and mutation frequencies in the Wales and UK cohorts were comparable (Table 1). Clinical descriptions of these 7 cases with pathogenic mutations are available in the supplementary material. One *PARK2* compound heterozygous mutation and 1 *PINK1* homozygous mutation were found in a total of 29 familial cases (6.9%; 95% CI, 1.9%–21.9%). In comparison, only 3.8% (95% CI, 1.5%–9.2%) of sporadic cases had pathogenic *PARK2* mutations, and none had pathogenic *PINK1* mutations (Tables 1 and 2). Pathogenic mutations were detected in 1 of 2 consanguineous patients. No mutations or gene dosage changes were found in *PARK7*, and no cases with digenic mutations were identified.

Sequence analysis and gene dosage assessment of *PARK2* revealed 5 compound heterozygous mutations in 4 sporadic cases and 1 familial case. In exon 5 of *PINK1*, we identified a homozygous point mutation in a consanguineous familial case of Filipino descent (L347P); see Tables 1 and 2. One white patient with sporadic decease from the tertiary-referral cohort was found to be heterozygous for the G2019S mutation in exon 41 of *LRRK2*.

In cases with AAO < 30 years (n = 14), the rate of mutations was 28.6%; in cases with AAO between 30 and 40 years (n = 61), the rate of mutations was 4.9%, and no mutations were found in cases with an AAO between 40 and 45 years (n = 61). Cases with pathogenic mutations had a lower mean AAO (25.5 vs 38.1 years; $P = .02$). About a third of cases (28.6%) with pathogenic mutations had an affected sibling, whereas the number of affected siblings for cases without pathogenic mutations was much lower (3.9%; $P = 4.0 \times 10^{-4}$). Furthermore, 14.3% of cases with pathogenic mutations were from a consanguineous family compared with 0.8% of cases without mutations ($P = .1$). Cases with pathogenic mutations were more likely to report rigidity or dystonia at

TABLE 2. Pathogenic mutations identified with descriptive clinical features

	A	B	C	D	E	F	G
Gene	*PARK2*	*PARK2*	*PARK2*	*PARK2*	*PARK2*	*PINK1*	*LRRK2*
Mutation 1	42P	c.154delA frameshift	Del. X2-3	c.438del40 frameshift	Dup X3	L347P	G2019S
Mutation 2	Dup. X3	R275W	Del. X2	Del. X2	Dup X7	L347P	Normal
Age	49	58	49	53	50	58	37
AAO	37	35	8	20	20	29	26
Ethnicity	Welsh	Welsh	Welsh	British	British	Filipino	British
Sex	Male	Male	Male	Female	Male	Female	Female
Family history	None	None	1 Sibling	None	None	2 Siblings and 2 nephews[a]	None
Onset symptom	Lower limb stiffness/rigidity	Symmetrical resting tremor	Lower limb stiffness/rigidity	Lower limb dystonia	Lower limb stiffness/rigidity	Lower limb tremor and pain	Lower limb stiffness/rigidity

[a]Parents unaffected. AAO, age at onset.
More detailed clinical descriptions can be found in the supplementary material.

presentation (71.4% vs 28.4%, $P = .03$) but had a similar incidence of tremor (28.6% vs 36.3%, $P = 0.5$). It is also notable that 6 of the 7 mutation-positive cases had onset in the lower limbs (Table 2).

Systematic Review

The initial literature search identified 1228 studies, of which 63 including our own met inclusion criteria, encompassing 5877 unique cases. The depth of clinical and demographic data provided varied between studies. The weighted mean AAO was 39.2 years, with a maximum AAO of 40–50 years, depending on the study. About one third of cases (27.2%) were familial, and 3.9% were consanguineous. Most patients studied (52.9%) were white, with Asian and Latin American patients making up 17.4% and 5.7% of cases, respectively; 24.0% of cases were either of undisclosed or of other ethnicities, for example, Arabic, black African, or Caribbean. Only about one third of the 63 studies (31.7%) included 100 or more cases.

Forty-three studies assessed the prevalence of *PARK2* (parkin) mutations in a total of 3952 patients, in whom the weighted pooled proportion of mutation-positive cases was 8.6% (95% CI, 6.0%–12.4%; Fig. 1, Table S3). Of the *PARK2* mutations reported in completely described studies, 44.1% (95% CI, 34.4%–56.4%) were homozygous, and 55.9% (95% CI, 44.9%–69.7%) were compound heterozygous (Table S8a). Familial cases were more likely than sporadic cases to have mutations in *PARK2*, with a proportion of 15.5% (95% CI, 10.3%–23.4%) versus 4.3% (95% CI, 2.7%–6.8.). The risk ratio for familial to sporadic cases was highly significant at 3.6 (95% CI, 2.0–6.7; $P < 1.0 \times 10^{-4}$). Only a small number of studies provided comprehensive information on consanguinity; however, in those inbred cases reported, the frequency of pathogenic mutations was much higher (31.4%; 95% CI, 18.5%–53.2%; Table S4) than in cases who were demonstrably outbred (7.7%; 95% CI, 5.0%–11.7%), and the risk ratio was highly significant at 4.3 (95% CI, 1.7–10.6; $P = 2.0 \times 10^{-3}$). Pathogenic *PARK2* mutation frequencies among white and Asian cases were similar at 7.7% (95% CI, 4.6%–13.0%) and 10.5% (95% CI, 4.9%–22.6%), respectively, and slightly but not significantly lower for Latin American cases (4.6%; 95% CI, 1.8%–11.6%); see Table S7.

Twenty-five studies with 2324 cases analyzed *PINK1*, and the weighted pooled proportion of cases carrying 2 pathogenic *PINK1* mutations was 3.7% (95% CI, 1.3%–10.4%; Fig. 2, Table S3) The majority of mutations (81.8%) were homozygous (95% CI, 56.1%–100.0%; Table S8b). The proportion of familial cases with mutations was 8.4% (95% CI, 2.2%–32.7%), and the risk ratio of familial compared with sporadic cases was 9.3 (95% CI, 1.7–49.4; $P = .01$). The proportion of consanguineous cases with mutations was 31.1% (95% CI, 12.7%–76.2%; Table S5). Compared with outbred cases, this was a highly significant increase, with a risk ratio of 35.3 (95% CI, 9.6–129.0; $P < 1 \times 10^{-4}$). Pathogenic *PINK1* mutations were much more common in Asian patients than in white patients: 13.5% (95% CI, 1.9%–94.9%) versus 0.6% (95% CI, 0.2%–1.6%), with a risk ratio of 20.3 (95% CI, 2.1%–197.7) and a *P* value of .01 (Table S7). The proportion of Latin American cases with *PINK1* mutations was similar to the proportion of white cases, namely, 0.9% (95% CI, 0.1%–6.0%; Table S7).

Thirteen hundred fifty-one cases in 15 different studies had been screened for *PARK7* (*DJ1*) mutations (Fig. 3, Table S3). *PARK7* mutations appear to be much rarer than *PARK2* or *PINK1* mutations, with an overall mutation frequency of 0.4% (95% CI, 0.2%–1.0%). The proportion of mutation-positive cases appears marginally higher in familial cases, at 0.8% (95% CI, 0.2%–1.3%), compared with 0.4% in sporadic cases (95% CI, 0.1%–1.0%); see Table S6. However, the risk ratio of 2.1 (0.4–11.6) was not significant ($P = .4$). Sample sizes were too small to

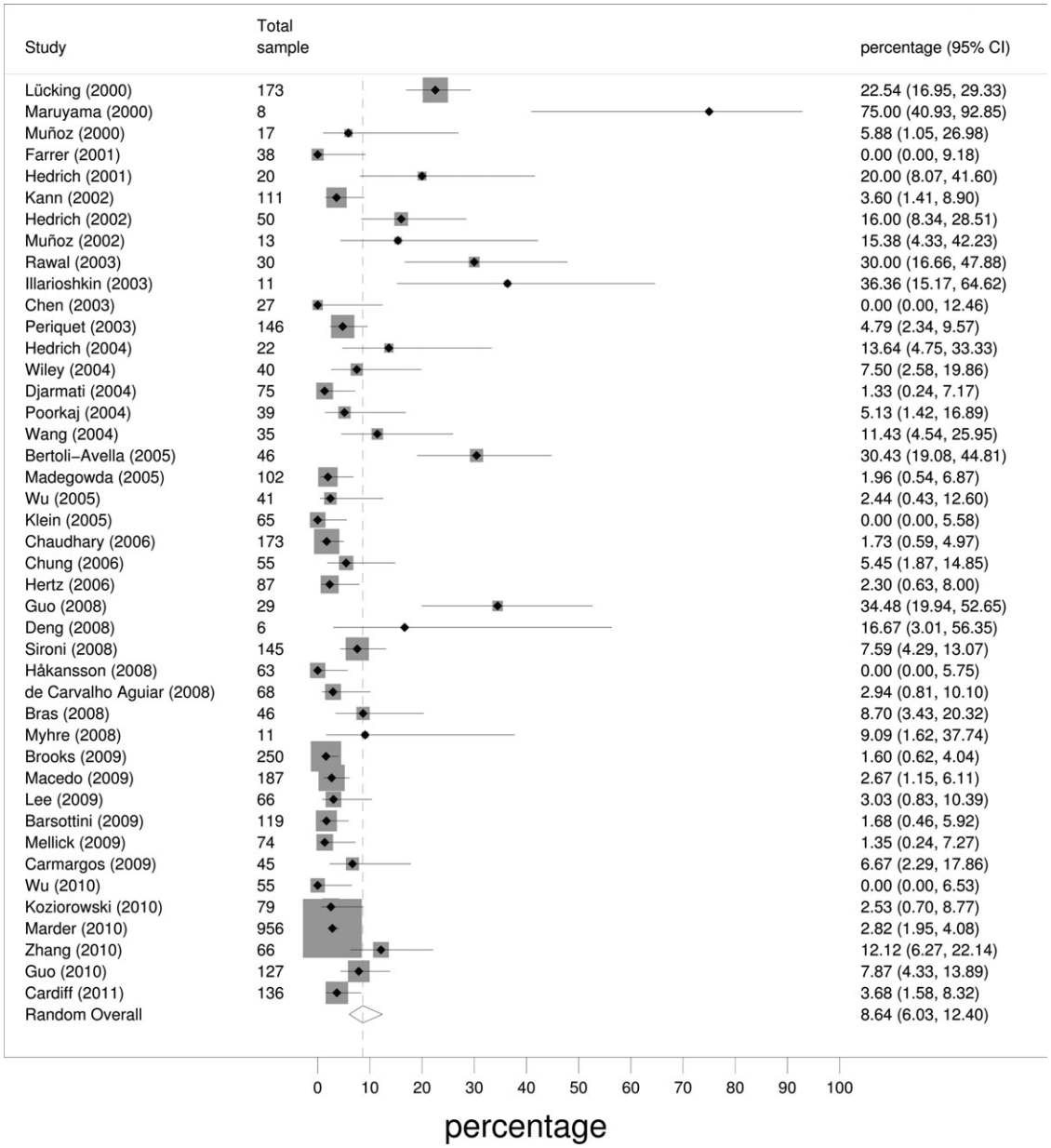

FIG. 1. Forest plot showing percentage of *PARK2* mutation-positive cases and 95% confidence intervals for each study included in the systematic review. The right-hand columns show per-study proportions of mutation-positive cases for *PARK2* (%) and 95% confidence intervals. The overall proportion, weighted in a random-effects model, is 8.6% (95% CI, 6.0%–12.4) and denoted by a gray diamond and dotted line. Gray areas are in proportion to the weighting of each study, and black bars show confidence intervals. The number of cases in each study is shown as well.

compare *PARK7* mutation frequencies across different ethnic groups.

Discussion

We present the results of a comprehensive screening study of all 3 EOPD genes and *LRRK2* G2019S in a large UK EOPD cohort, together with a systematic review of previous studies. Previously, high mutation frequencies have been reported for *PARK2*[10,21,22] and to some extent for *PINK1*.[12] Our results indicate a 5.1% rate of pathogenic mutations in *PARK2*, *PINK1*, and *DJ1* in UK EOPD patients. No cases with pathogenic mutations were identified in our community-based study. Although samples with high ascertainment are desirable for accurate genetic epidemiology, the rarity of EOPD mutations in the Cardiff cohort indicates that local community-based studies may be underpowered. The similar rates in Wales and the United Kingdom indicate that referral bias from specialist centers is not likely to have a significant effect. Our study confirms the previous reports of an increased likelihood of pathogenic mutations in patients with an earlier age of onset, with 30% of patients with age at onset < 30 years having pathogenic mutations in known genes.

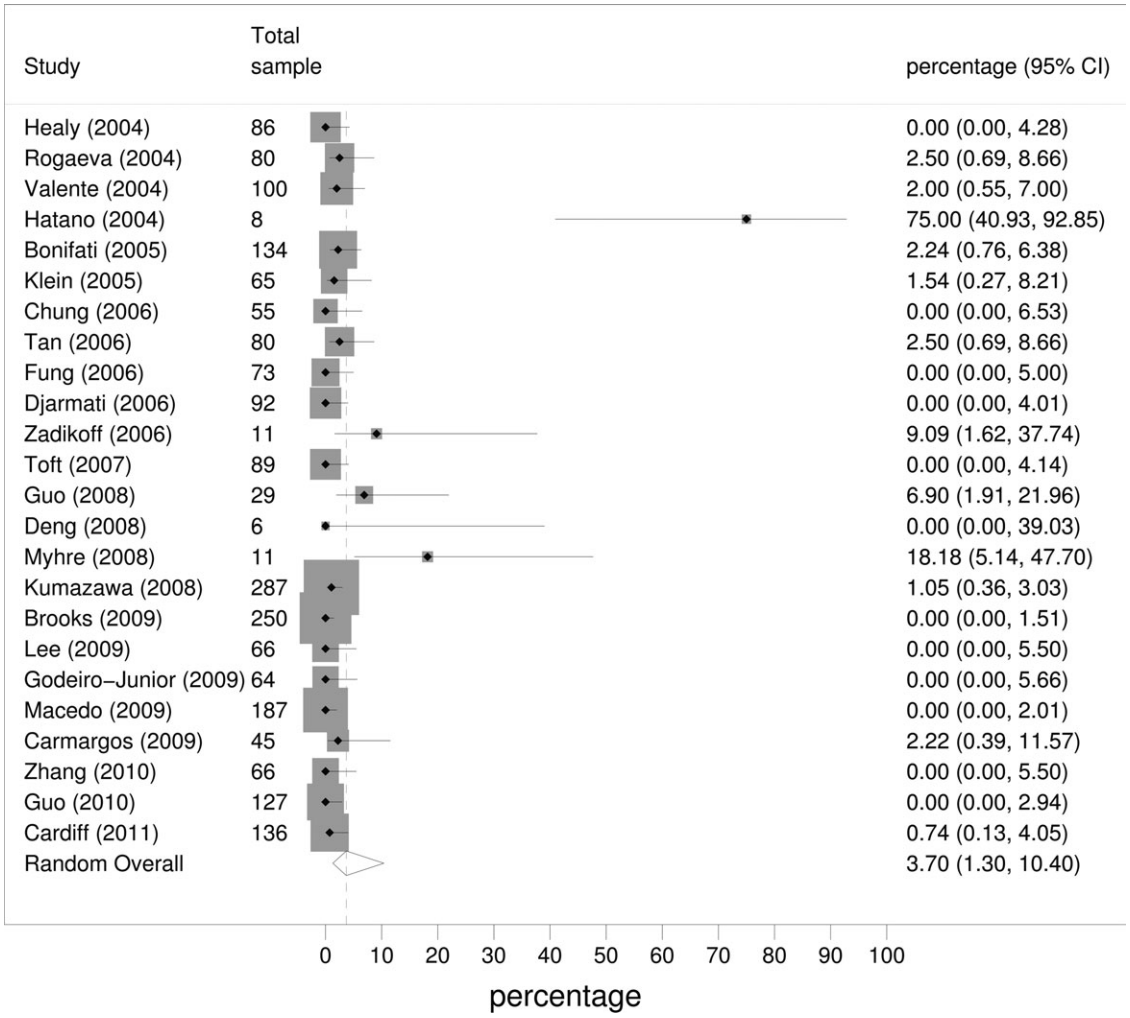

FIG. 2. Forest plot showing percentage of *PINK1* mutation-positive cases and 95% confidence intervals for each study included in the systematic review. The right-hand columns show per-study proportions of mutation-positive cases for *PINK1* (%) and 95% confidence intervals. The overall proportion, weighted in a random-effects model, is 3.7% (95% CI, 1.3%–10.4%) and is denoted by a gray diamond and dotted line. Gray areas are in proportion to the weighting of each study, and black bars show confidence intervals. The number of cases in each study is shown as well.

Duplication of *PARK2* exon 3, deletion of exon 2, and the joint deletion of exons 2 and 3 were first reported in European families in 2000 and are relatively common.[10,13] Duplication of exon 7 occurs less frequently.[23,24] Two small deletions, each resulting in a codon frameshift, have also been found in our study and have been reported recurrently in Europe. We identified a single base-pair deletion in exon 2 of *PARK2* (c.255delA) leading to a premature stop codon insertion (N52fsX8)[22,25,26]—once in the heterozygous state and once in a compound heterozygous state in combination with R275W. The second small deletion involves 40 base pairs in exon 3 (c.438-477del40) and is thought to result in aberrant exon splicing.[27] We also discovered 2 compound heterozygous point mutations in *PARK2*, the R275W missense mutation, which has been widely reported and is thought to be the most prevalent *PARK2* mutation in Europe,[23,24] and R42P.[28] The *PINK1* point mutation L347P appears to be restricted and common in Filipinos.[12,29,30] Parkin, PINK1, and DJ-1 proteins have been shown to be involved in mitochondrial function,[31] and recent data suggest that they may have complementary functions.[23,32,33] Compound heterozygous mutations across several PARK genes have been described previously[34–36]; however, in our study no case was found to harbor digenic mutations.

We identified 1 sporadic case with the G2019S mutation in *LRRK2*. Although this mutation more commonly results in typical late-onset PD, it can also cause PD at a much younger age.[37] In this cohort of EOPD cases, it was found as frequently as *PINK1* mutations, confirming recent findings by Alcalay and colleagues.[38] Interestingly, this patient presented with typical clinical PD, including nonmotor features, and did not have the early dystonia seen in some carriers of pathogenic mutations in autosomal recessive disease genes.

To put our findings into context, we undertook a systematic review assessing the proportion of *PARK2*,

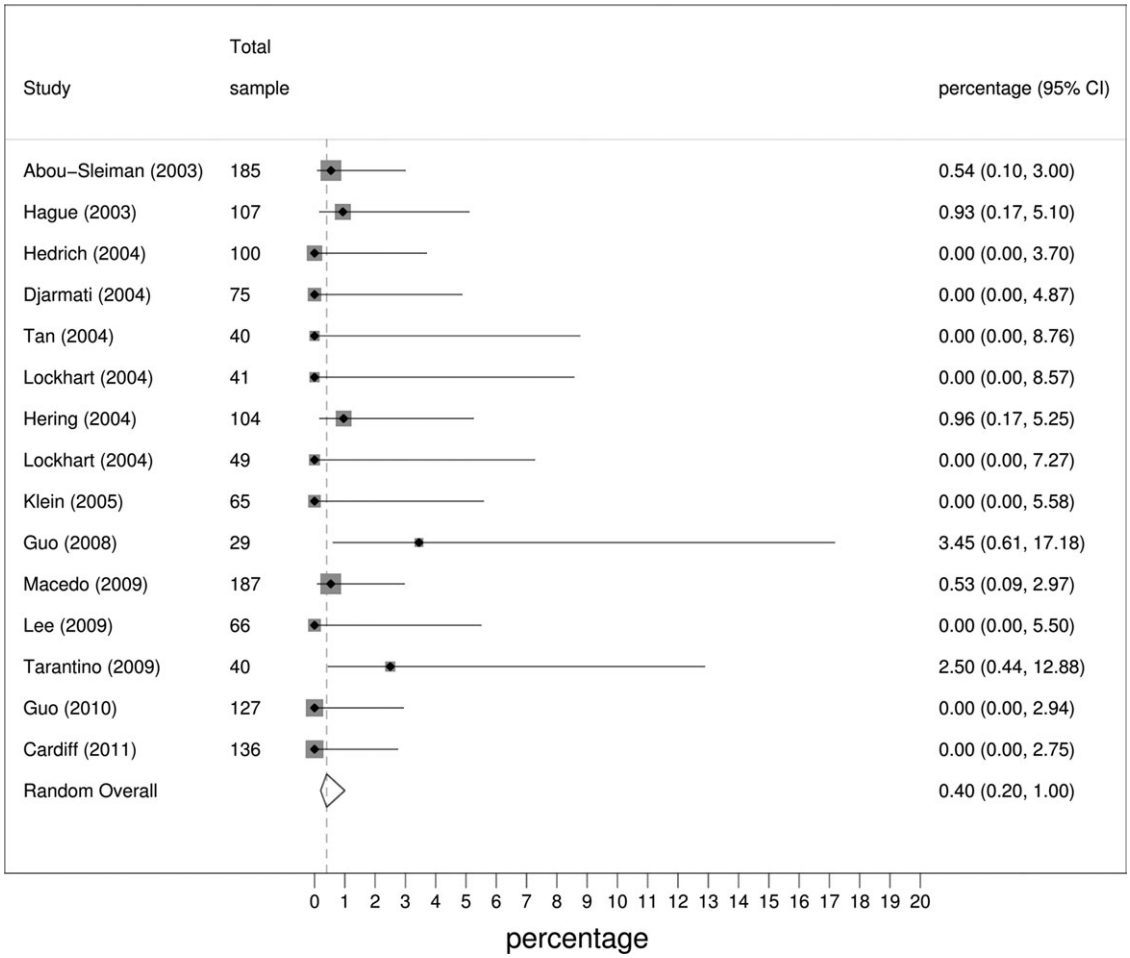

FIG. 3. Forest plot showing percentage of *PARK7* mutation-positive cases and 95% confidence intervals for each study included in the systematic review. The right-hand columns show per-study proportions of mutation-positive cases for *PARK7* (%) and 95% confidence intervals. The overall proportion, weighted in a random-effects model, is 0.4% (95% CI, 0.2%–1.0%) and is denoted by a gray diamond and dotted line. Gray areas are in proportion to the weighting of each study, and black bars show confidence intervals. The number of cases in each study is shown as well.

PINK1, and *PARK7* mutation-positive cases in more than 5800 patients across 63 articles from 2000 to 2011 including our own. *PARK2* was the most commonly mutated gene, with pathogenic mutations found in 8.6% of EOPD cases, followed by *PINK1* mutations, occurring in 3.7%, and *PARK7* mutations, in 0.4% of patients. *LRRK2* was not assessed as it is not (yet) considered a classical Mendelian EOPD gene and has not been investigated in EOPD cases to any great depth. The number of mutations reported varied widely across studies, especially for *PARK2*, with older and smaller studies generally reporting higher frequencies. Our systematic review has some limitations, which relate to variability between studies in case ascertainment, definitions of family history, maximum AAO, and the study of different ethnic groups. In addition, prescreening and exclusion of cases for mutations in the gene not under study would have led to an "enrichment" of mutations in the other 2 genes in any given cohort, and preferred selection of familial or consanguineous cases would have had a similar effect. There was an increased rate of pathogenic mutations in our UK study in familial and consanguineous patients, and the systematic review showed that these differences were highly significant across reported studies for *PARK2* and *PINK1*. For *PARK2*, consanguinity increased the likelihood of a mutation 3.6-fold, whereas for *PINK1*, it was 8-fold. Interestingly, *PINK1* mutations were also more likely to occur in the homozygous state (~81% for *PINK1* vs ~44% for *PARK2*) and to occur more commonly in Asian subjects.

We aimed to provide a realistic estimate of *PARK2*, *PINK1*, and *PARK7* mutation prevalence in EOPD—first with our own screening efforts on local, regional, and national levels and then on an international level by a systematic review of the literature. Although the proportion of cases with mutations was higher in familial EOPD patients than in sporadic patients, about 82% of familial EOPD patients did not have mutations in *PARK2*, *PINK1*, or *PARK7*. *LRRK2* mutations also appeared to be a relatively rare cause of EOPD. Recently, *ATP13A2, PLA2G6, FBXO7*, and *Spatacsin* have been described as additional autosomal

recessive EOPD genes[39]; however, the mutations in these genes are likely to be rare and largely restricted to patients with atypical clinical and neuroimaging features. Some of the familial clustering of EOPD may be related to environmental factors, although it is also likely that there are further Mendelian genes that remain to be identified in EOPD. ■

Acknowledgments: We thank Roger Harbord for his statistical advice on the meta-analysis. We also thank the Queen Square Brain Bank, UCL Institute of Neurology.

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