

Global investigation and meta-analysis of the *C9orf72* (G₄C₂)_n repeat in Parkinson disease

OPEN

Jessie Theuns, PhD*
Aline Verstraeten, MSc*
Kristel Slegers, PhD
Eline Wauters, MSc
Ilse Gijssels, PhD
Stefanie Smolders, MSc
David Crosiers, PhD
Ellen Corsmit, BSc
Ellen Elinck, MSc
Manu Sharma, PhD
Rejko Krüger, MD
Suzanne Lesage, PhD
Alexis Brice, MD
Sun Ju Chung, PhD
Mi-Jung Kim, MD
Young Jin Kim, MD
Owen A. Ross, PhD
Zbigniew K. Wszolek, MD
Ekaterina Rogaeva, PhD
Zhengru Xi, PhD
Anthony E. Lang, MD
Christine Klein, PhD
Anne Weissbach, MD
George D. Mellick, PhD
Peter A. Silburn, PhD
Georgios M.

Hadjigeorgiou, MD
Efthimios Dardiotis, MD
Nobutaka Hattori, PhD
Kotaro Ogaki, PhD
Eng-King Tan, MD
Yi Zhao, PhD
Jan Aasly, PhD
Enza Maria Valente, PhD
Simona Petrucci, MD
Grazia Annesi, PhD
Aldo Quattrone, MD
Carlo Ferrarese, MD
Laura Brighina, MD
Angela Deutschländer, MD

Author list continued on next page

ABSTRACT

Objectives: The objective of this study is to clarify the role of (G₄C₂)_n expansions in the etiology of Parkinson disease (PD) in the worldwide multicenter Genetic Epidemiology of Parkinson's Disease (GEO-PD) cohort.

Methods: *C9orf72* (G₄C₂)_n repeats were assessed in a GEO-PD cohort of 7,494 patients diagnosed with PD and 5,886 neurologically healthy control individuals ascertained in Europe, Asia, North America, and Australia.

Results: A pathogenic (G₄C₂)_{n>60} expansion was detected in only 4 patients with PD (4/7,232; 0.055%), all with a positive family history of neurodegenerative dementia, amyotrophic lateral sclerosis, or atypical parkinsonism, while no carriers were detected with typical sporadic or familial PD. Meta-analysis revealed a small increase in risk of PD with an increasing number of (G₄C₂)_n repeats; however, we could not detect a robust association between the *C9orf72* (G₄C₂)_n repeat and PD, and the population attributable risk was low.

Conclusions: Together, these findings indicate that expansions in *C9orf72* do not have a major role in the pathogenesis of PD. Testing for *C9orf72* repeat expansions should only be considered in patients with PD who have overt symptoms of frontotemporal lobar degeneration/amyotrophic lateral sclerosis or apparent family history of neurodegenerative dementia or motor neuron disease. *Neurology*® 2014;83:1906-1913

GLOSSARY

ALS = amyotrophic lateral sclerosis; **FTLD** = frontotemporal lobar degeneration; **GEO-PD** = Genetic Epidemiology of Parkinson's Disease; **PD** = Parkinson disease; **RP** = repeat-primed; **STR** = short tandem repeat.

A substantial number of patients with frontotemporal lobar degeneration (FTLD)/amyotrophic lateral sclerosis (ALS) (14%–35%) carrying *C9orf72* (G₄C₂)_{>60} expansions^{1–3} present with atypical parkinsonism in early disease stages and increased incidence of parkinsonism with or without features of the FTLD/ALS complex in their relatives.^{4–9} Ten research groups have reported on *C9orf72* repeat expansions in Parkinson disease (PD) or atypical parkinsonism patients^{10–19} but none of these investigated the *C9orf72* repeat in large-scale cohorts, and European and Australian populations were underrepresented in the published data. Apart from the pathogenicity of (G₄C₂)_{>60} expansions, we provided in vitro evidence that the (G₄C₂) repeat size negatively correlated with the transcriptional activity of the *C9orf72* promoter.²⁰ Hence, it is conceivable that an increasing number of *C9orf72* repeats may affect transcription gradually and increase susceptibility to disease.²⁰ Three studies indicated a role of *C9orf72* repeats in PD susceptibility but associations were found using different dichotomizations of repeat length, muddling biological interpretation. In one study, a marginal increased risk of PD was observed for carriers of (G₄C₂)₁₀ repeats.¹² In the second, a significant increased frequency of (G₄C₂)_{>20} repeats was observed in patients clinically diagnosed with PD.¹⁵ In the third study, the authors

*These authors contributed equally to this work.

Authors' affiliations are listed at the end of the article.

GEO-PD Consortium coinvestigators are listed on the *Neurology*® Web site at Neurology.org.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article. The Article Processing Charge was paid by research funding obtained by the principal investigator.

This is an open access article distributed under the terms of the Creative Commons Attribution-Noncommercial No Derivative 3.0 License, which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially.

Andreas Puschmann, PhD
 Christer Nilsson, PhD
 Gaëtan Garraux, PhD
 Mark S. LeDoux, PhD
 Ronald F. Pfeiffer, MD
 Magdalena Boczarzka-Jedynak, PhD
 Grzegorz Opala
 Demetrius M. Maraganore, MD
 Sebastiaan Engelborghs, PhD
 Peter Paul De Deyn, PhD
 Patrick Cras, PhD
 Marc Cruts, PhD
 Christine Van Broeckhoven, DSc
 On behalf of the GEO-PD Consortium

Correspondence to Prof. Dr. Van Broeckhoven: christine.vanbroeckhoven@molgen.vib-ua.be

Supplemental data at Neurology.org

reported association of $(G_4C_2)_{\geq 7}$ repeats with PD in the Chinese Han population.¹⁶ All of these studies, however, were executed in ethnically distinct and medium scaled cohorts. We set out to clarify the role of the *C9orf72* $(G_4C_2)_n$ repeat in PD etiology in the first global multicenter study cohort of more than 7,000 patients with PD of 12 nationalities and 4 continents. First, we assess the global prevalence of pathogenic $(G_4C_2)_{>60}$ expansions. Second, the size of the combined study populations enables a detailed investigation of the specific *C9orf72* repeat allele or size threshold associated with increased risk of PD.

METHODS **Standard protocol approvals, registrations, and patient consents.** Genetic studies applied in this research were approved by the ethics committees of the ZNA (Hospital Network Antwerp), the Antwerp University Hospital, and University of Antwerp. Clinical protocols were approved by the ethics committees of the ZNA, the Antwerp University Hospital, and local ethical review boards of the participating research centers. All human biological samples were collected, fulfilling ethical approvals, and used in accordance with the terms of subjects' written informed consent.

Participants. The Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium includes investigators from 60 sites from 30 countries and 6 continents (<http://www.GEO-PD.org/about/>). All sites were invited to participate in this study. A total of 18 sites representing 12 countries and 4 continents contributed either DNA or genotypic data, and clinical data of

in total 15,123 individuals (tables 1 and 2). After thorough quality control as described in the procedures section below, 13,669 samples were included in this study. We excluded all duplicate samples, sex mismatches, and samples that failed in the DNA fingerprint analysis because of low quantity or quality of DNA or because of contamination of the sample. Demographics and diagnostic criteria of each series included in this study and the sample size breakdown from each site are provided in table 2. Controls were collected at the local sites as demographically matched neurologically healthy individuals.

Procedures. Sample quality control. Concentration and purity were checked spectrophotometrically using the Trinean DropSense96 UV/VIS droplet reader (Trinean, Gentbrugge, Belgium) for all consortium genomic DNA samples. Sex and DNA fingerprint were determined for all samples using an in-house-developed multiplex PCR panel composed of 13 short tandem repeat (STR) markers distributed over multiple autosomal locations: D20S480, D22S1174, D3S1287, D3S1744, D3S1764, D7S672, D7S2426, D8S1746, D14S1005, D20S866, D10S1237, D20S912, and D6S965. This panel also includes a marker specific for the X chromosome (DXS1187) and one for the *SRY* gene on the Y chromosome, and enables fast and accurate sample identification and sex determination in a single assay. After selective amplification of 20 ng genomic DNA, amplification products were size separated on an ABI 3730 automatic sequencer (Applied Biosystems, Foster City, CA) using GeneScan-600 LIZ (Applied Biosystems) as internal size standard and genotypes were assigned using in-house-developed TraI genotyping software (<http://www.vibgeneticservicefacility.be>).

Genetic analyses. To screen the GEO-PD cohorts for the pathogenic $(G_4C_2)_{>60}$ *C9orf72* repeat expansion, we designed a 2-step procedure: an STR fragment length assay with flanking PCR primers optimized for alleles with high GC content (STR-PCR) followed by 2 repeat-primed PCR assays (forward and reverse RP-PCR) as described earlier.^{1,20}

Four participating research groups performed the genotyping in their local facilities according to previously published procedures.^{2,17} For consistent allele scoring of repeat lengths between GEO-PD groups and accurate interpretation of the repeat length, we designed a reference DNA set of 14 samples covering a range of normal repeat sizes that was genotyped by each of these facilities. Furthermore, for 2 of the cohorts, a random set of samples homozygous for the STR-PCR assay were included in the RP-PCR analysis at the Antwerp site for independent validation of the absence of a pathogenic repeat expansion.

Statistical analyses. To investigate the association between repeat units and PD susceptibility, 3 explorative approaches were followed, based on (1) allele counts of the distinct repeat sizes, to determine whether one or more specific repeat sizes were associated with PD, (2) the total number of repeat units (sum of both alleles) per individual, and (3) the size of the longest repeat per individual (maximum allele). Summary statistics were computed in a random-effects meta-analysis (DerSimonian-Laird)^{12,21} for each approach in the *rmeta* package implemented in the R environment version 2.15.3. Based on the results obtained in the above-mentioned analyses, we performed hypothesis-driven dichotomized genotypic meta-analyses. Details are provided in the e-Methods on the *Neurology*[®] Web site at Neurology.org.

To take into account the number of tests performed ($n = 22$), a Bonferroni-corrected 2-sided p value of ≤ 0.002 was considered statistically significant. Population attributable risk of $(G_4C_2)_{10}$, $(G_4C_2)_{\geq 10}$, and $(G_4C_2)_{\geq 17}$ was estimated using the *epiR* package in R. For the meta-analyses, only the cohorts including both the patients with PD and the controls that were

Table 1 Synopsis of this global GEO-PD study		
	Patients	Controls
$(G_4C_2)_{>60}$		
Total	4/7,232	1/5,478
Europe	4/4,252	0/3,172
US + CA	0/1,261	0/1,313
Asia	0/1,364	1/656
Australia	0/355	0/337
Risk associated with $(G_4C_2)_n$		
Total ^a	7,050	5,886
Europe	4,215	3,379
US + CA	1,118	1,313
Asia	1,190	660
Australia	527	534

Abbreviations: CA = Canada; GEO-PD = Genetic Epidemiology of Parkinson's Disease.

^aFor the meta-analyses, only the cohorts including both patients with Parkinson disease and geographically matched controls that were size-corrected using the reference panel were included.

Table 2 Characteristics of the GEO-PD cohorts included in the study

Site PI	Country	Ethnicity	Controls				Patients				% Males	% Familial	AAO ± SD	Diagnostic criteria for PD
			In	QC OK	STR OK	2-step OK	In	QC OK	STR OK	2-step OK				
C. Van Broeckhoven	Belgium	Caucasian	1,119	1,118	1,039	953	593	593	585	569	50	24	63.8 ± 13	Gelb ²⁶
G. Garraux	Belgium	Caucasian	0	0	0	0	139	126	125	116	59	NA	60.9 ± 10.5	Gelb ²⁶
C. Klein	Germany	Caucasian	706	697	679	581	433	410	396	396	55	33	52.0 ± 14.2	Hoehn and Yahr ²⁷
A. Deuschländer	Germany	Caucasian	87	81	79	79	87	81	79	79	57	22	61.5 ± 11.3	UKPDBB ²⁸
C. Ferrarese	Italy	Caucasian	92	89	86	86	87	84	68	68	58	20	65.5 ± 9.6	Gelb ²⁶
E.M. Valente	Italy	Caucasian	92	83	83	83	92	90	89	89	47	33	52.9 ± 5.0	UKPDBB ²⁸
G. Annesi	Italy	Caucasian	100	100	95	95	100	92	74	74	51	0	59.7 ± 9.0	Gelb ²⁶
G.M. Hadjigeorgiou	Greece	Caucasian	300	232	223	220	300	269	264	264	50	13	63.3 ± 10.4	Bower ²⁹
A. Puschmann	Sweden	Caucasian	43	43	43	43	119	115	111	111	56	49	61.7 ± 9.7	Ward and Gibb ³⁰
G.D. Mellick	Australia	Caucasian	920	571	534	337	920	535	527	355	46	27	62.0 ± 11.5	Gelb ²⁶
M.S. LeDoux	US	Caucasian	0	0	0	0	184	150	143	143	61	32	60.1 ± 11.8	Gelb ²⁶
S.J. Chung	Korea	Asian	650	568	562	558	1,200	1,113	1,088	1,088	46	9	59.0 ± 10.0	Gelb ²⁶
E.-K. Tan	Singapore	Asian	200	100	98	98	200	102	102	100	67	8	53.2 ± 7.0	UKPDBB ²⁸
N. Hattori	Japan	Asian	69	0	0	0	183	177	176	176	52	2	41.6 ± 11.9	Gelb ²⁶ /Hoehn and Yahr ²⁷
R. Krüger/M. Sharma	Germany	Caucasian	647	625	625	605	1,386	1,367	1,367	1,304	62	NA	49.9 ± 17.1	UKPDBB ²⁸
S. Lesage	France	Caucasian	442	442	427	427	1,193	1,185	1,182	1,182	54	39	48.6 ± 12.0	UKPDBB ²⁸
Z.K. Wszolek	US	Caucasian	712	712	712	712	676	676	676	676	53	NA	69.2 ± 11.1	UKPDBB ²⁸
E. Rogaeva	Canada	Mixed	601	601	601	601	451	442	442	442	50	35	52.4 ± 13.7	UKPDBB ²⁸

Abbreviations: AAO = age at onset; GEO-PD = Genetic Epidemiology of Parkinson's Disease; NA = not available; PD = Parkinson disease; PI = principal investigator; QC = quality control; STR = short tandem repeat; UKPDBB = UK Parkinson's Disease Brain Bank.

At the initial quality control step (QC), we excluded all duplicate samples, sex mismatches, and samples that failed in the DNA fingerprint analysis because of low quantity or quality of DNA or because of contamination of the sample. Additional samples did not pass the 2-step genetic analysis because of DNA shortage or limited concentration of the DNA sample.

size-corrected based on the reference panel were included in the study.

RESULTS **Definite pathogenic *C9orf72* repeat expansions in PD.** A total of 12,710 samples, including 7,232 patients with PD and 5,478 control individuals, were successfully genotyped with the 2-step $(G_4C_2)_n$ repeat genotyping assay. RP-PCR analysis revealed the typical sawtooth tail pattern indicative of a pathogenic repeat expansion $(G_4C_2)_{>60}$ in one German (MS_RK cohort) (1/1,304; 0.08%) and 3 French (SL cohort) (3/1,182; 0.25%) patients but none in the other GEO-PD patient cohorts (table 1). Based on these results, we calculated a prevalence of pathogenic *C9orf72* repeat expansions in this global consortium cohort of 0.06%.

The German patient was diagnosed with idiopathic PD at the age of 57 years. One year after onset, clinical examination revealed hypomimia, hypokinesia, resting tremor of the right arm, minor postural instability, mild bilateral rigidity, and slowed shuffling gait but also short-term memory disturbances, social withdrawal, and minor apathy. The patient had a positive family history of neurodegenerative dementia. All 3 French patients were diagnosed with PD without cognitive dysfunction at disease onset, and a detailed clinical description has been reported previously.¹⁷ Briefly, the first patient developed left hemiparkinsonism at age 29 years and symptoms worsened progressively while dopamine agonists were only partially effective. In the second patient, parkinsonism began at age 48 years and a cognitive decline was noted at age 56 years. The third patient developed parkinsonism at age 64 years and developed a mild cognitive deficit at age 69. Although these 3 patients were clinically diagnosed with PD, they all had family histories of atypical parkinsonism, degenerative dementias, or ALS. No expansions were detected in patients with sporadic PD or patients with a familial history of PD. Moreover, mutations in known PD genes had previously been excluded in these 4 pathogenic expansion carriers.

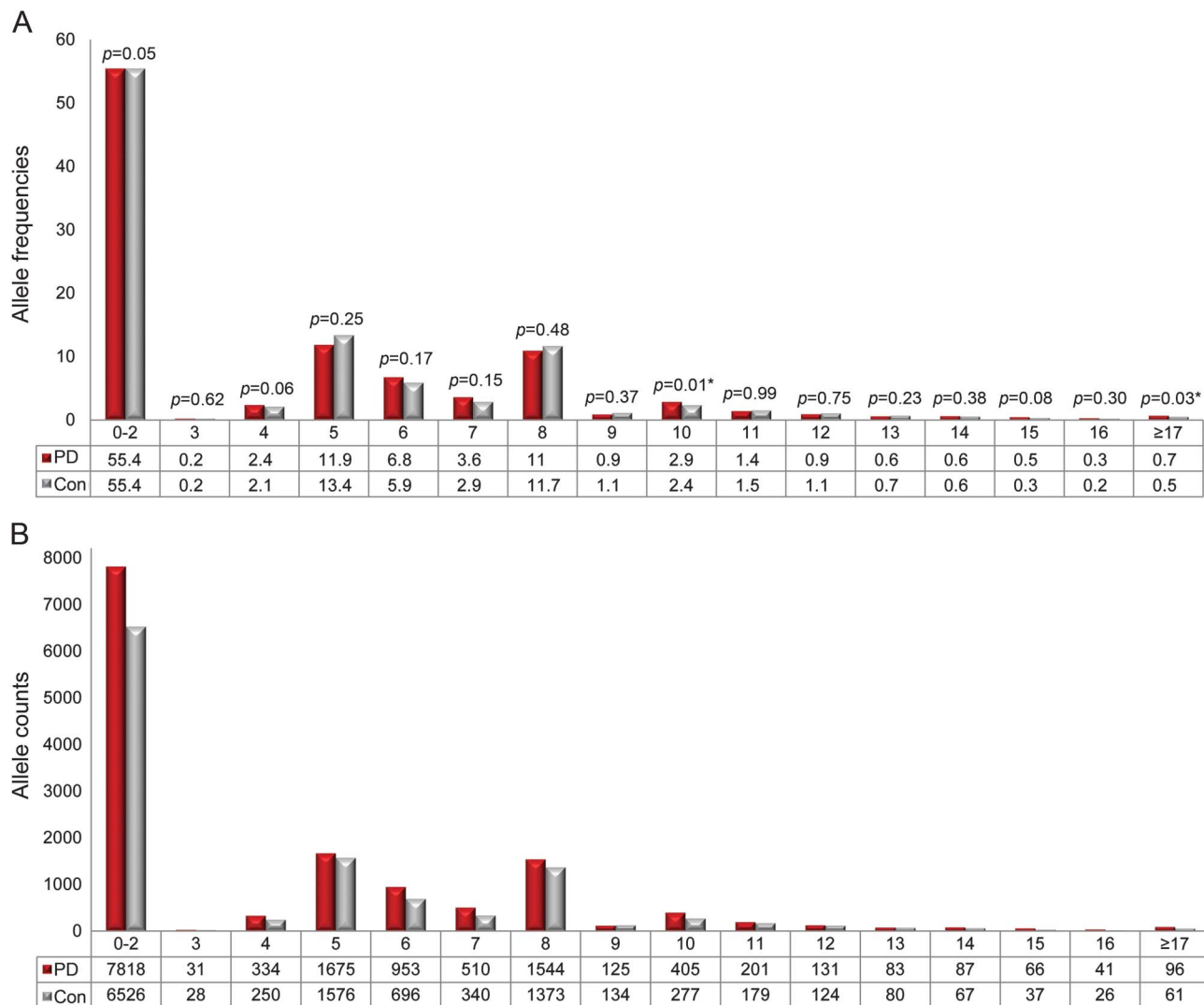
We identified one Asian control of Chinese origin with an age at inclusion of 52 years carrying a pathogenic $(G_4C_2)_{>60}$ expansion (table 1). Currently, there is no record of any symptoms related to PD, FTLN, or ALS in this individual. This brings the estimated prevalence of pathogenic repeat expansions in controls to 0.02% (1/5,478). Apart from the expansion mutations, the distribution of repeat lengths ranged from 0 to 32 in the Caucasian and from 7 to 14 in the Asian control persons.

***C9orf72* repeat and PD susceptibility.** We investigated the role of $(G_4C_2)_n$ repeats in risk of PD. First, we assessed the distribution of the alleles in patients with PD vs controls in the GEO-PD cohort (figure 1). The

frequencies of the $(G_4C_2)_{10}$ allele and of $(G_4C_2)_{\geq 17}$ were nominally increased in PD vs the controls but the differences were not statistically significant after Bonferroni correction (figure 1, table 3, figure e-1, A and B). Genotypic frequencies for $(G_4C_2)_{10}$ (table 3, figure e-1, C) and $(G_4C_2)_{\geq 17}$ (table 3, figure e-1, D) were not significantly different between patients and controls after correction for multiple testing. The estimated attributable fractions in the population are very low (table 3). When considering the sum of the alleles and the size of the maximum allele as a quantitative variable, we observed a small but significant increase of disease risk with a rising number of repeat units (sum of alleles $p = 0.0012$, summary effect $[\beta] = 0.0128$ [0.00504–0.0205], figure e-2, A; maximum allele $p = 0.0010$, summary effect $[\beta] = 0.0181$ [0.00731–0.029], figure e-2, B). Together, these results suggested that the risk effect may not only be linked to the $(G_4C_2)_{10}$ repeat but may be increasing with length while the effect in the larger alleles is probably masked by the small number of carriers. Therefore, we decided to analyze the risk effect of *C9orf72* repeat expansions as a binary categorical value with a cutoff between 9 and 10. However, neither allelic nor genotypic meta-analysis of the GEO-PD cohorts revealed significant association with PD for $(G_4C_2)_{\geq 10}$ repeat alleles after Bonferroni correction (table 3, figure e-3, A and B). Furthermore, the estimated population attributable risk is low (table 3).

DISCUSSION Molecular reclassification of complex brain diseases based on genetic etiology is of utmost importance to improve differential diagnosis and to rationalize drug development. Assessment of the contribution of novel disease genes to clinically and pathologically overlapping diseases is instrumental in this reclassification. In this global study, we assessed the prevalence of $(G_4C_2)_n$ repeat alleles and expansions in an extended PD cohort ascertained within the GEO-PD Consortium and excluded a major role for pathogenic $(G_4C_2)_{>60}$ repeat expansions in the causation of PD. The low frequency of these expansions (0.06%) in the GEO-PD cohort is in agreement with earlier findings in distinct patient groups^{10–15,18,19} and falls in the range of frequencies observed in controls by us (0.02%) and others (0–0.6%).^{1–4,22} Furthermore, 75% of the pathogenic expansion carriers in this global study showed a decline in cognitive functions within 1 to 8 years after onset. In the absence of autopsy diagnoses, we therefore cannot exclude that some if not all of these expansion carriers are primarily FTLN/ALS patients with pronounced early parkinsonian symptoms or comorbidity of PD and FTLN/ALS. This hypothesis is supported by the identification of only one pathogenic mutation carrier

Figure 1 Overall distribution of *C9orf72* repeat alleles in the GEO-PD cohorts



Only cohorts including both patients with PD and controls that were size-corrected based on the reference panel were included in the study. When the highest count for a specific allele was 5 or less across cohorts, the allele was clumped with the next allele for each cohort. (A) Allele frequencies. The p values for individual alleles were calculated using a Dersimonian-Laird random-effect meta-analysis. (B) Allele counts. *Nominally significant p values. Con = controls; GEO-PD = Genetic Epidemiology of Parkinson's Disease; PD = Parkinson disease.

in 826 (0.1%) autopsy-confirmed PD cases.^{23,24} Of note, this carrier presented, in addition to Lewy body pathology, with frontotemporal degeneration and *C9orf72*-ALS/FTLD pathology with numerous p62-positive inclusions. Furthermore, although substantia nigra involvement is common in *C9orf72*-positive ALS, it can be clearly distinguished from PD-related mechanisms by the presence of p62-positive inclusion and absence of Lewy body pathology.²⁴

Altogether, it is not advisable to include *C9orf72* (G_4C_2)_n repeat expansion testing in a medical genetic diagnostic setting for typical PD patients. Exceptions can be made for patients with PD who have cognitive and/or behavioral deficits early in the disease process or in patients with a personal or familial history of FTLD/ALS.

Given differences in the existing literature on *C9orf72* (G_4C_2)_n repeat length as risk factor for PD,^{12,15,16} we used the size of this global cohort to estimate a PD-related threshold of *C9orf72* repeats. Calculation of the risk for each of the observed *C9orf72* (G_4C_2)_n alleles in the GEO-PD cohorts suggested a role for the 10-units repeat and for the pooled alleles of 17 units or more in PD susceptibility. Genotypic meta-analysis supported a possible link between (G_4C_2)₁₀ and increased risk of PD but the association did not reach significance after correction for multiple testing. In addition, the number of carriers of these intermediate alleles is small and one should be cautious with the interpretation of these results. Furthermore, it is difficult to envisage the biological relevance of risk associated with a single

Table 3 Overview of DerSimonian-Laird meta-analyses

	Allelic			Genotypic		
	OR (95% CI)	p	AF (95% CI)	OR (95% CI)	p	AF (95% CI)
(G ₄ C ₂) ₁₀ ^a	1.3 (1.06-1.60)	0.01	0.53% (0.13-0.93)	1.31 (1.06-1.62)	0.012	1.05% (0.26-1.84)
(G ₄ C ₂) _{≥17} ^a	1.44 (1.03-2.01)	0.03	0.16% (-0.03 to 0.35)	1.47 (1.05-2.05)	0.025	0.35% (-0.03 to 0.72)
(G ₄ C ₂) _{≥10} ^b	1.16 (1.04-1.3)	0.009	0.69% (0-1.39)	1.18 (1.03-1.35)	0.02	1.47% (0.07-2.85)

Abbreviations: AF = attributable fraction; CI = confidence interval; OR = odds ratio.

For the meta-analyses, only the cohorts including both patients with Parkinson disease and geographically matched controls that were size-corrected using the reference panel were included. The *p* values were calculated using DerSimonian-Laird meta-analysis; *p* = 0.002 is considered statistically significant (22 tests).

^a Allele count approach.

^b Dichotomized approach.

allele. Of note, we observed a small but significant increase in risk with an accumulative number of repeats supporting the idea of a threshold size rather than a single allele as the culprit of increased risk. We therefore decided to study the combined effect of (G₄C₂) alleles of 10 units and larger in the global GEO-PD cohort. Although meta-analyses implicated a potential role for these intermediate-sized repeats in PD risk, none of the associations survived Bonferroni correction suggesting that if *C9orf72* repeats of 10 units or larger have a role in PD susceptibility, the effect is small. This is supported by the fact that none of the published genome-wide association studies revealed the *C9orf72* locus as a risk factor for PD.²⁵ A limitation of this study is that we did not yet include all published association studies of *C9orf72* in PD; however, we chose to include only those studies that were corrected for allele scoring bias based on a reference panel.

Altogether, these data support the current hypothesis that pathogenic (G₄C₂)_n repeat expansions in *C9orf72* appear to be specific for the FTL/ALS spectrum with little or no contribution to the wider spectrum of movement disorders. It will be of interest to study the role of intermediate repeats ≥10 units in other neurodegenerative disorders, however, to obtain a more profound knowledge on their role in neurodegenerative diseases and a better understanding of the underlying mechanism.

AUTHOR AFFILIATIONS

From the Neurodegenerative Brain Diseases Group (J.T., A.V., K.S., E.W., I.G., S.S., D.C., E.C., E.E., M.C., C.V.B.), Department of Molecular Genetics, VIB, Antwerp; Institute Born-Bunge (J.T., A.V., K.S., E.W., I.G., S.S., D.C., E.C., E.E., S.E., P.P.D.D., P.C., M.C., C.V.B.), University of Antwerp; Department of Neurology (D.C., P.C.), Antwerp University Hospital, Edegem; Department of Neurology and Memory Clinic (S.E., P.P.D.D.), Hospital Network Antwerp, Middelheim and Hoge Beuken, Antwerp, Belgium; Department of Neurology and Alzheimer Research Center (P.P.D.D.), University of Groningen and University Medical Center Groningen, the Netherlands; Department of Neurodegenerative Diseases (M.S., R.K.), Hertie-Institute for Clinical Brain Research and DZNE-German Center for Neurodegenerative Diseases, Tuebingen; Institute for Clinical Epidemiology and Applied

Biometry (M.S.), University of Tuebingen, Germany; INSERM, UMR_S975 (S.L., A.B.), Université Pierre et Marie Curie-Paris, CNRS, UMR 7225, AP-HP, Pitié-Salpêtrière Hospital; CNRS (S.L., A.B.), UMR 7225, Paris; AP-HP (A.B.), Pitié-Salpêtrière Hospital, Department of Genetics and Cytogenetics, Paris, France; Department of Neurology (S.J.C., M.-J.K., Y.J.K.), Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; Departments of Neuroscience (O.A.R.) and Neurology (Z.K.W.), Mayo Clinic, Jacksonville, FL; Tanz Centre for Research in Neurodegenerative Diseases (E.R., Z.X.), Department of Medicine, University of Toronto; Toronto Western Hospital Research Institute (A.E.L.), University Health Network, Toronto, Canada; Institute of Neurogenetics (C.K., A.W.), University of Luebeck, Germany; Eskitis Institute for Drug Discovery (G.D.M.), Griffith University, Queensland; University of Queensland (P.A.S.), Centre for Clinical Research, Queensland, Australia; Department of Neurology (G.M.H., E.D.), Neuroscience Unit, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece; Department of Neurology (N.H., K.O.), Juntendo University School of Medicine, Tokyo, Japan; Department of Neurology (E.-K.T., Y.Z.), Singapore General Hospital, National Neuroscience Institute, Singapore; Duke NUS Graduate Medical School (E.-K.T., Y.Z.), Singapore; Department of Neurology (J.A.), St. Olav's Hospital, Trondheim; Department of Neuroscience (J.A.), Norwegian University of Science and Technology (NTNU), Trondheim, Norway; IRCCS Casa Sollievo della Sofferenza Hospital (E.M.V., S.P.), Mendel Laboratory, San Giovanni Rotondo; Institute of Molecular Bioimaging and Physiology (G.A., A.Q.), National Research Council, Section of Germaneto (CZ); Institute of Neurology (A.Q.), Department of Medical Sciences, University Magna Graecia, Catanzaro; Department of Neuroscience (C.F., L.B.), Section of Neurology, University of Milano-Bicocca, San Gerardo Hospital, Monza, Italy; Department of Neurology (A.D.), Max Planck Institute of Psychiatry, Munich, Germany; Department of Clinical Sciences (A.P.), Section of Neurology, Lund University; Department of Neurology (A.P.), Lund, Skåne University Hospital; Department of Clinical Sciences (C.N.), Clinical Memory Research Unit, Lund University, Sweden; Human Genetic Centre (G.G.), University Hospital of Liège, Belgium; Department of Neurology (M.S.L., R.F.P.), University of Tennessee Health Science Center, Memphis; Department of Neurology (M.B.-J., G.O.), Medical University of Silesia, Katowice, Poland; and Department of Neurology (D.M.M.), North-Shore University HealthSystem, Evanston, IL.

AUTHOR CONTRIBUTIONS

Jessie Theuns: drafting and revising the manuscript for content, study concept and design, analysis and interpretation of data, acquisition of data, statistical analysis, study supervision and coordination, obtaining funding. Aline Verstraeten: revising the manuscript for content, study design, analysis and interpretation of data, acquisition of data, statistical analysis, study coordination. Kristel Sleepers: drafting and revising the manuscript for content, study concept, analysis and interpretation of data, statistical analysis, obtaining funding. Eline Wauters: revising the manuscript for content, analysis and interpretation of data, acquisition of data. Ilse Gijssels: revising the manuscript for content, analysis and interpretation of data, acquisition of data. Stefanie Smolders: revising

the manuscript for content, analysis and interpretation of data, acquisition of data. David Crosiers: revising the manuscript for content, analysis and interpretation of clinical data, acquisition of clinical data, contribution of vital reagents. Ellen Corsmit: revising the manuscript for content, analysis and interpretation of data, acquisition of data. Ellen Elinck: revising the manuscript for content, analysis and interpretation of data, acquisition of data. Manu Sharma: revising the manuscript for content, analysis and interpretation of data, acquisition of data. Rejko Krüger: revising the manuscript for content, analysis and interpretation of data, acquisition of data. Suzanne Lesage: revising the manuscript for content, acquisition of data, analysis and interpretation of data, contribution of vital reagents, local study coordination. Alexis Brice: revising the manuscript for content, acquisition of clinical data, contribution of vital reagents, local study supervision. Sun Ju Chung: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents, local study supervision. Mi-Jung Kim: revising the manuscript for content, acquisition of clinical data, contribution of vital reagents. Young Jin Kim: revising the manuscript for content, acquisition of clinical data, contribution of vital reagents. Owen A. Ross: revising the manuscript for content, analysis and interpretation of data, acquisition of data, contribution of vital reagents. Zbigniew K. Wszolek: revising the manuscript for content, analysis and interpretation of clinical data, acquisition of clinical data, contribution of vital reagents. Ekaterina Rogaeva: revising the manuscript for content, analysis and interpretation of data, acquisition of data, contribution of vital reagents. Zhengrui Xi: revising the manuscript for content, analysis and interpretation of data, acquisition of data. Anthony E. Lang: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Christine Klein: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Anne Weissbach: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. George D. Mellick: revising the manuscript for content, acquisition and interpretation of clinical data, contribution of vital reagents. Peter A. Silburn: revising the manuscript for content, acquisition and interpretation of clinical data, contribution of vital reagents. Georgios M. Hadjigeorgiou: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Efsthimios Dardiotis: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Nobutaka Hattori: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Kotaro Ogaki: revising the manuscript for content, acquisition of clinical data, contribution of vital reagents. Eng-King Tan: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Yi Zhao: revising the manuscript for content, acquisition of clinical data, contribution of vital reagents. Jan Aasly: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Enza Maria Valente: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Simona Petrucci: revising the manuscript for content, acquisition of clinical data, contribution of vital reagents. Grazia Annesi: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Aldo Quattrone: revising the manuscript for content, acquisition of clinical data, contribution of vital reagents. Carlo Ferrarese: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Laura Brighina: revising the manuscript for content, acquisition of clinical data, contribution of vital reagents. Angela Deuschländer: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Andreas Puschmann: revising the manuscript for content, acquisition of clinical data and biospecimen, contribution of vital reagents, local study design, obtaining funding for local site. Christer Nilsson: revising the manuscript for content, local study design, obtaining funding for local site. Gaëtan Garraux: revising the manuscript for content, acquisition of clinical data, contribution of vital reagents. Mark S. LeDoux: revising the manuscript for content, acquisition and interpretation of clinical data and

biospecimen, contribution of vital reagents. Ronald F. Pfeiffer: revising the manuscript for content, acquisition of clinical data, contribution of vital reagents. Magdalena Boczarska-Jedynak: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Grzegorz Opala: revising the manuscript for content, acquisition of clinical data, contribution of vital reagents, local study coordination. Demetrius Maraganore: revising the manuscript for content, acquisition of clinical data, executive approval and sponsorship of the study, leadership and coordination of the GEO-PD Consortium that participated in the study. Sebastiaan Engelborghs: revising the manuscript for content, acquisition of clinical data, analysis and interpretation of clinical data, contribution of vital reagents. Peter Paul De Deyn: revising the manuscript for content, local study design, acquisition of clinical data, interpretation of clinical data, contribution of vital reagents, local study supervision. Patrick Cras: revising the manuscript for content, local study design, acquisition of clinical data, interpretation of clinical data, contribution of vital reagents, local study supervision. Marc Cruts: drafting and revising the manuscript for content, study concept and design, analysis and interpretation of data, study supervision or coordination, obtaining funding. Christine Van Broeckhoven: drafting and revising the manuscript for content, study concept and design, analysis and interpretation of data, contribution of vital reagents/tools/patents, statistical analysis, study supervision or coordination, obtaining funding.

ACKNOWLEDGMENT

Belgium: The authors thank the VIB DMG Genetic Service Facility (<http://www.vibgeneticservicefacility.be/>) for technical assistance. Italy-Cosenza: The authors thank Patrizia Tarantino, Institute of Molecular Bioimaging and Physiology, National Research Council, Section of Germaneto (CZ), Italy and Monica Gagliardi, Institute of Molecular Bioimaging and Physiology, National Research Council, Section of Germaneto (CZ), Italy and Institute of Neurology, Department of Medical Sciences, University Magna Graecia, Catanzaro, Italy.

STUDY FUNDING

Belgium-Antwerp: The research is in part funded by the Belgian Science Policy Office Interuniversity Attraction Poles (IAP) program; the European Initiative on Centers of Excellence in Neurodegeneration (CoEN); the Flemish Government initiated Methusalem Excellence Program; the Alzheimer Research Foundation (SAO/FRA); the Queen Elisabeth Medical Foundation (QEMF); the Research Foundation Flanders (FWO); the Agency for Innovation by Science and Technology Flanders (IWT); the University of Antwerp Research Fund, Belgium; and the MetLife Foundation for Medical Research Award to C.V.B. The IWT provided a PhD fellowship to A.V. and E.W. and the FWO a post-doctoral fellowship to I.G. The authors acknowledge the personnel of the Genetic Service Facility of VIB (<http://www.vibgeneticservicefacility.be/>) and the Antwerp Biobank of the Institute Born-Bunge for their expert support. France: The authors thank the French Parkinson's Disease Genetics Study Group: Y. Agid, M. Anheim, A.-M. Bonnet, M. Borg, A. Brice, E. Broussolle, J.-C. Corvol, P. Damier, A. Destée, A. Dürr, F. Durif, S. Klebe, P. Krack, E. Lohmann, M. Martinez, P. Pollak, O. Rascol, F. Tison, C. Tranchant, M. Vérin, F. Viallet, and M. Vidailhet; France-Parkinson Association and the French program "Investissements d'avenir" funding (ANR-10-IAHU-06). USA-Florida: Mayo Clinic Florida is a Morris K. Udall Center of Excellence in PD Research NIH/NINDS P50 NS072187, and is supported by NINDS R01 NS078086, the Michael J. Fox Foundation, Mayo Clinic Center for Regenerative Medicine, and a gift from Carl Edward Bolch, Jr., and Susan Bass Bolch. Canada: W. Garfield Weston Foundation and Ontario Research Fund (E.R.). Germany-Luebeck: C.K. is supported by a career development award from the Hermann and Lilly Schilling Foundation. Germany-Tuebingen: The KORA (Cooperative Research in the Region of Augsburg) research platform was started and financed by the Forschungszentrum für Umwelt und Gesundheit, which is funded by the German Federal Ministry of Education, Science, Research, and Technology and by the State of Bavaria. This work has also been made possible by the kind support of the Michael J. Fox Foundation (MJFF) for Parkinson's Research (to Manu Sharma). This study was also funded by the German National Genome Network

(NGFNplus number 01GS08134, German Ministry for Education and Research); by the German Federal Ministry of Education and Research (NGFN 01GR0468, PopGen); and 01EW0908 in the frame of ERA-NET NEURON and Helmholtz Alliance Mental Health in an Ageing Society (HA-215), which was funded by the Initiative and Networking Fund of the Helmholtz Association. Australia: Geriatric Medical Foundation of Queensland. Greece: This work was supported in part by the Research Committee of University of Thessaly (code: 2845 for G.M.H.). Singapore: National Medical Research Council and Singapore Millennium Foundation. Italy-San Giovanni Rotondo: Italian Ministry of Health (Ricerca Corrente 2013). Sweden: Andreas Puschmann received funding from The Swedish Parkinson Foundation (Parkinsonfonden) and governmental funding for clinical research within the Swedish National Health Services (ALF-YF). USA-Tennessee: research was funded, in part, by grants from the NIH (R01NS069936; R01NS058850) to M.S.L. Korea: This work was supported by a grant of the Korea Healthcare technology R & D Project, Ministry of Health & Welfare, Republic of Korea (A092042) and Asan Institute for Life Sciences (2010-0416). USA-Evanston: Research was funded, in part, by a grant from the NIH (R01ES10751) to D.M.M.

DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received May 8, 2014. Accepted in final form August 13, 2014.

REFERENCES

1. Gijselink I, Van Langenhove T, van der Zee J, et al. A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration–amyotrophic lateral sclerosis spectrum: a gene identification study. *Lancet Neurol* 2012;11:54–65.
2. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 2011;72:245–256.
3. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 2011;72:257–268.
4. Cruts M, Gijselink I, Van Langenhove T, van der Zee J, Van Broeckhoven C. Current insights into the C9orf72 repeat expansion diseases of the FTL/ALS spectrum. *Trends Neurosci* 2013;36:450–459.
5. Takada LT, Pimentel ML, DeJesus-Hernandez M, et al. Frontotemporal dementia in a Brazilian kindred with the C9orf72 mutation. *Arch Neurol* 2012;69:1149–1153.
6. Savica R, Adeli A, Vemuri P, et al. Characterization of a family with c9FTD/ALS associated with the GGGGCC repeat expansion in C9ORF72. *Arch Neurol* 2012;69:1164–1169.
7. Luigetti M, Quaranta D, Conte A, et al. Frontotemporal dementia, parkinsonism and lower motor neuron involvement in a patient with C9ORF72 expansion. *Amyotroph Lateral Scler Frontotemporal Degener* 2013;14:66–69.
8. Hsiung GY, DeJesus-Hernandez M, Feldman HH, et al. Clinical and pathological features of familial frontotemporal dementia caused by C9ORF72 mutation on chromosome 9p. *Brain* 2012;135:709–722.
9. Van Langenhove T, van der Zee J, Gijselink I, et al. Distinct clinical characteristics of C9orf72 expansion carriers compared with GRN, MAPT, and nonmutation carriers in a Flanders-Belgian FTL/ALS cohort. *JAMA Neurol* 2013;70:1–9.
10. Harms MB, Neumann D, Benitez BA, et al. Parkinson disease is not associated with C9ORF72 repeat expansions. *Neurobiol Aging* 2013;34:1519.e1–1519.e2.
11. DeJesus-Hernandez M, Rayaprolu S, Soto-Ortolaza AI, et al. Analysis of the C9orf72 repeat in Parkinson's disease, essential tremor and restless legs syndrome. *Parkinsonism Relat Disord* 2013;19:198–201.
12. Xi Z, Zinman L, Grinberg Y, et al. Investigation of C9orf72 in 4 neurodegenerative disorders. *Arch Neurol* 2012;69:1583–1590.
13. Majounie E, Abramzon Y, Renton AE, Keller MF, Traynor BJ, Singleton AB. Large C9orf72 repeat expansions are not a common cause of Parkinson's disease. *Neurobiol Aging* 2012;33:2527.e1–2527.e2.
14. Daoud H, Noreau A, Rochefort D, et al. Investigation of C9orf72 repeat expansions in Parkinson's disease. *Neurobiol Aging* 2013;34:1710–1719.
15. Nuytemans K, Bademci G, Kohli MM, et al. C9ORF72 intermediate repeat copies are a significant risk factor for Parkinson disease. *Ann Hum Genet* 2013;77:351–363.
16. Jiao B, Guo JF, Wang YQ, et al. C9orf72 mutation is rare in Alzheimer's disease, Parkinson's disease, and essential tremor in China. *Front Cell Neurosci* 2013;7:164.
17. Lesage S, Le Ber I, Condroyer C, et al. C9orf72 repeat expansions are a rare genetic cause of parkinsonism. *Brain* 2013;136:385–391.
18. Yeh TH, Lai SC, Weng YH, et al. Screening for C9orf72 repeat expansions in parkinsonian syndromes. *Neurobiol Aging* 2013;34:1311–1314.
19. Akimoto C, Forsgren L, Linder J, et al. No GGGGCC-hexanucleotide repeat expansion in C9ORF72 in parkinsonism patients in Sweden. *Amyotroph Lateral Scler Frontotemporal Degener* 2013;14:26–29.
20. van der Zee J, Gijselink I, Dillen L, et al. A pan-European study of the C9orf72 repeat associated with FTL/ALS: geographic prevalence, genomic instability, and intermediate repeats. *Hum Mutat* 2013;34:363–373.
21. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177–188.
22. Beck J, Poulter M, Hensman D, et al. Large C9orf72 hexanucleotide repeat expansions are seen in multiple neurodegenerative syndromes and are more frequent than expected in the UK population. *Am J Hum Genet* 2013;92:345–353.
23. Nuytemans K, Inchausti V, Beecham GW, et al. Absence of C9ORF72 expanded or intermediate repeats in autopsy-confirmed Parkinson's disease. *Mov Disord* 2014;29:827–830.
24. Cooper-Knock J, Frolov A, Highley JR, et al. C9ORF72 expansions, parkinsonism, and Parkinson disease: a clinicopathologic study. *Neurology* 2013;81:808–811.
25. Lill CM, Roehr JT, McQueen MB, et al. Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: the PDGene database. *PLoS Genet* 2012;8:e1002548.
26. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. *Arch Neurol* 1999;56:33–39.
27. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. *Neurology* 1967;17:427–442.
28. Hughes AJ, Daniel SE, Lees AJ. Improved accuracy of clinical diagnosis of Lewy body Parkinson's disease. *Neurology* 2001;57:1497–1499.
29. Bower JH, Maraganore DM, McDonnell SK, Rocca WA. Incidence and distribution of parkinsonism in Olmsted County, Minnesota, 1976–1990. *Neurology* 1999;52:1214–1220.
30. Ward CD, Gibb WR. Research diagnostic criteria for Parkinson's disease. *Adv Neurol* 1990;53:245–249.

Neurology®

Global investigation and meta-analysis of the *C9orf72* (G₄C₂)_n repeat in Parkinson disease

Jessie Theuns, Aline Verstraeten, Kristel Slegers, et al.

Neurology 2014;83:1906-1913 Published Online before print October 17, 2014

DOI 10.1212/WNL.0000000000001012

This information is current as of October 17, 2014

Updated Information & Services	including high resolution figures, can be found at: http://n.neurology.org/content/83/21/1906.full
Supplementary Material	Supplementary material can be found at: http://n.neurology.org/content/suppl/2014/10/17/WNL.000000000001012.DC1 http://n.neurology.org/content/suppl/2014/10/17/WNL.000000000001012.DC2
References	This article cites 30 articles, 4 of which you can access for free at: http://n.neurology.org/content/83/21/1906.full#ref-list-1
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): All Genetics http://n.neurology.org/cgi/collection/all_genetics Parkinson's disease/Parkinsonism http://n.neurology.org/cgi/collection/parkinsons_disease_parkinsonism
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://www.neurology.org/about/about_the_journal#permissions
Reprints	Information about ordering reprints can be found online: http://n.neurology.org/subscribers/advertise

Neurology® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright © 2014 American Academy of Neurology. All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.

