repeat expansions in Parkinson disease

Lisa Wang, MSc
Jan O. Aasly, MD
Grazia Annesi, PhD
Soraya Bardien, MD
Maria Bozi, MD
Alexis Brice, MD
Jonathan Carr, MD
Sun J. Chung, MD
Carl Clarke, MD
David Crosiers, MD
Angela Deutschländer, MD
Gertrud Eckstein, PhD
Matthew J. Farrer, PhD
Stefano Goldwurm, PhD
Gaetan Garraux, MD
Georgios M. Hadjigeorgiou, MD
Andrew A. Hicks, PhD
Nobutaka Hattori, MD, PhD
Christine Klein, MD
Beom Jeon, MD
Yun J. Kim, MD, PhD
Suzanne Lesage, PhD
Juei-Jueng Lin, MD
Timothy Lynch, MD
Peter Lichtner, PhD
Anthony E. Lang, MD
Vincent Mok, MD
Barbara Jasinska-Myga, MD
George D. Mellick, PhD
Karen E. Morrison, MD
Grzegorz Opala, MD
Lasse Pihlstrøm, MD
Peter P. Pramstaller, MD
Sung S. Park, MD
Aldo Quattrone, MD
Ekaterina Rogaeva, PhD
Owen A. Ross, PhD
Leonidas Stefanis, MD

Author list continued on next page

ABSTRACT

Objectives: We aim to clarify the pathogenic role of intermediate size repeat expansions of SCA2, SCA3, SCA6, and SCA17 as risk factors for idiopathic Parkinson disease (PD).

Methods: We invited researchers from the Genetic Epidemiology of Parkinson's Disease Consortium to participate in the study. There were 12,346 cases and 8,164 controls genotyped, for a total of 4 repeats within the SCA2, SCA3, SCA6, and SCA17 genes. Fixed- and random-effects models were used to estimate the summary risk estimates for the genes. We investigated between-study heterogeneity and heterogeneity between different ethnic populations.

Results: We did not observe any definite pathogenic repeat expansions for SCA2, SCA3, SCA6, and SCA17 genes in patients with idiopathic PD from Caucasian and Asian populations. Furthermore, overall analysis did not reveal any significant association between intermediate repeats and PD. The effect estimates (odds ratio) ranged from 0.93 to 1.01 in the overall cohort for the SCA2, SCA3, SCA6, and SCA17 loci.

Conclusions: Our study did not support a major role for definite pathogenic repeat expansions in SCA2, SCA3, SCA6, and SCA17 genes for idiopathic PD. Thus, results of this large study do not support diagnostic screening of SCA2, SCA3, SCA6, and SCA17 gene repeats in the common idiopathic form of PD. Likewise, this largest multicentered study performed to date excludes the role of intermediate repeats of these genes as a risk factor for PD. ***Neurology*® 2015;85:1283–1292**

GLOSSARY

AAO = age at onset; **CI** = confidence interval; **GEO-PD** = Genetic Epidemiology of Parkinson's Disease; **PD** = Parkinson disease; **SCA** = spinocerebellar ataxia.

Spinocerebellar ataxias (SCAs) represent a clinically and genetically diverse group of neurodegenerative diseases, which share degeneration of the cerebellum and its afferent and efferent connections, besides variable degeneration of multiple neurologic systems.[1] Expansions of trinucleotide repeats in the coding or untranslated regions of various genes cause several SCAs; these expansions also account for most of the clinical and genetic heterogeneity.[2] Emerging evidence provides tangible support to the growing consensus that clinically heterogeneous yet biologically overlapping late-onset neurodegenerative disorders may have common genetic risk factors that might change predisposition to the diseases.[2,3]

Whether polyglutamine repeat expansions in SCA genes such as SCA2, SCA3, SCA6, and SCA17 wield a similar effect in idiopathic Parkinson disease (PD) needs to be determined. Previous clinical and pathologic findings emphasize the need to evaluate the significance of polyglutamine repeat expansions of these genes in PD worldwide.[4–9] Most studies performed to date, including this study, are biased by case selection at specialist movement disorders clinics. However, to get a better estimate of the frequency of repeat expansions in such a setting, their relative contribution to disease worldwide, we performed a large multicenter study with members of the Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium.

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.

Joanne D. Stockton, MD
Peter A. Silburn, MD
Jessie Theuns, PhD
Eng K. Tan, MD
Hiroyuki Tomiyama, MD, PhD
Mathias Toft, MD
Christine Van Broeckhoven, PhD
Ryan J. Uitti, MD
Karin Wirdefeldt, MD, PhD
Zbigniew Wszolek, MD
Georgia Xiromerisiou, MD
Kuo-Chu Yueh, MD
Yi Zhao, MD
Thomas Gasser, MD
Demetrius M. Maraganore, MD
Rejko Krüger, MD
Manu Sharma, PhD
On behalf of the GEO-PD Consortium

Correspondence to Dr. Sharma: manu.sharma@uni-tuebingen.de

Supplemental data at Neurology.org

METHODS

Participants and samples. The GEO-PD Consortium includes researchers from 59 investigative sites, across 30 countries and 6 continents (http://www.geopd.org/about/); we invited all sites to participate in the study. Twenty-five sites from 20 countries and 4 continents contributed DNA samples and clinical data, resulting in 20,528 participants. Patients were diagnosed with PD by a movement disorders specialist using the standard criteria.[10–12] Controls at the date of examination were neurologically healthy, unrelated individuals free of PD or another associated movement disorder. Local sites collected demographically similar and sex-, age-matched neurologically healthy individuals as controls. Not all controls were given a detailed neurologic examination, but all were questioned about previous diagnoses or familial history of a neurologic disease. After quality control of data, a total of 20,510 samples were included (12,346 cases, 8,164 controls). The Caucasian series consisted of 16,819 (10,204 cases and 6,615 controls), and the Asian series consisted of 3,691 patients (2,142 cases and 1,549 controls). Patients with missing data were excluded from the relevant analysis. There were a total of 508 patients missing SCA2 genotype information, 445 missing SCA3, 861 missing SCA6, and 608 missing SCA17.

Genotyping. The SCA2, SCA3, SCA6, and SCA17 loci containing the CAG repeats were amplified with PCR using fluorescently labeled primers (primer sequences are available upon request). PCRs for SCA2, SCA3, and SCA17 were performed in one multiplex assay, SCA6 in a singleplex. All amplicons of one individual were pooled and separated by size using capillary electrophoresis on an ABI3730 sequencer. Data analysis was performed with GeneMapper 4.0 software. This included automatic sizing and allele calling. A total of 8 individuals (2 for each locus) were Sanger sequenced and the number of triplet repeats was counted. This information was used to convert amplicon lengths to repeat numbers.

Standard protocol approvals, registrations, and patient consents. The local ethics committee approved the study. All participants signed an informed consent.

Statistical analysis. We first generated distribution plots for SCA2, SCA3, SCA6, and SCA17 genes (see figure e-1 on the *Neurology*® Web site at Neurology.org) to estimate the repeats' cutoffs in our cohort. Based on our observation, expanding repeats for each gene were categorized into short, intermediate, and long repeats. Using the Monte Carlo simulation method (1,000 simulations) as implemented in the CLUMP, we compared the distribution of allele length of SCA genes to determine the significance of departure from the expected values between cases and controls.[13] Because CLUMP uses the Monte Carlo simulation method, all significances should be unbiased and robust to small expected values or continuity corrections.[13] We also assessed the correlation between age at onset (AAO) (5,310 cases) and polyglutamine repeats in our cohort. Likewise, in a subset of the data with the age at study available (14 sites, 4,400 controls, 5,310 cases), we analyzed the data with age at study as a covariate in the models. Finally, the association between SCA CAG expansion repeats and PD was evaluated using a logistic regression model with sex included as a covariate. Datasets from countries that included only cases were not included in the modeling because they lacked a proper control set, and thus could not be modeled using logistic regression. Fixed- and random-effects models estimated the odds ratios. Fixed-effect models assume that populations from different sites have the same risk effect from the repeat expansions and that observed differences are due to random chance. For datasets containing between-study heterogeneity, fixed-effect estimates provide smaller confidence intervals (CIs) and p values, relative to random-effects models.[14–16] If, however, heterogeneity exists, the effects may diverge substantially across the populations. Random-effects models allow for random variation between the sites, therefore adjusting for genuine heterogeneity that may exist across different sites. We used the inverse variance method for fixed-effects models and the DerSimonian and Laird method for random-effects models.[17] To evaluate the between-site heterogeneity, we used the Cochran Q test of homogeneity and the I^2 metric. The I^2 parameter is bounded by 0 and 1 and estimates the proportion of heterogeneity that is highly unlikely due to random variation. A larger I^2 value implies more heterogeneity, with I^2 more than 0.75 or 75% indicating large heterogeneity. However, given that there exists significant imprecision in the estimation of I^2, particularly for variants with low minor allele frequency, we also provided the 95% CI of I^2.[16] The overall analysis considered all sites and populations regardless of ancestry. We then separately modeled the Caucasian and Asian sites. All statistical analyses were performed using R version 3.0.2, with package "metafor" for the random-effects logistic regression models. The p values are 2-tailed.

RESULTS

A total of 25 sites contributed 12,346 patients with PD and 8,164 neurologically normal controls. Table 1 displays the characteristics of all participating sites. Nineteen sites contributed patients of Caucasian descent; 6 sites were from countries of Asian descent. The proportion of males ranged from 46% to 63% over the participating sites (table 1). The mean AAO of PD in this investigated population was 60 years. We excluded 2 sites that contributed only cases to avoid the influence of population substructuring (Japan and South Africa, 519 patients). Nevertheless, these 2 sites were analyzed independently to assess the expanded repeats. One German site contributed only cases, and allelic repeat density analysis did not show differences in repeat length between different German sites. Therefore, we decided to merge German sites into one data site titled "Germany" for further analyses, thus combining data from Deutschländer, Klein, and Gasser sites.

Expanding repeats of SCA genes in PD. Of 20,528 participants who were successfully genotyped, we did not observe any definite pathogenic repeat expansion for SCA2 (>32), SCA3 (>61), SCA6 (>19), and SCA17 (>47) genes in our cohort, thus excluding the role of definite pathogenic repeat expansion of these genes in PD.

Intermediate repeats and PD. The distribution of the cutoff repeat length of SCA genes as observed in the density distribution plots in our study is in agreement with previously published studies.[2,18–20] Furthermore, the histogram plots showed that the distribution of intermediate repeat length are similar for SCA2, SCA3, SCA6, and SCA17 genes independent of ethnicities.[20]

Using CLUMP, we did not observe differences in allele length distribution between cases and controls

Table 1 Characterization of sites and overall database

Site	Country	Total	Cases	Controls	Male (%)	Mean AAO	Diagnostic criteria
Annesi	Italy	394	197	197	204 (51.8)	61.5	UKPDBB
Bardien/Carr	South Africa	398	398	0	246 (61.2)		UKPDBB
Bozi	Greece	218	114	104	105 (46.1)	69.9	UKPDBB
Brice	France	504	272	232	301 (59.7)	47.6	UKPDBB
Chung	Korea	1,900	1,200	700	876 (46.1)		UKPDBB
Deutschländer	Germany	140	70	70	80 (57.1)	69.7	UKPDBB
Garraux	Belgium	77	64	13	40 (51.9)	62.1	UKPDBB
Goldwurm	Italy	3,798	2,795	1,003	1,992 (52.4)		UKPDBB
Hadjigeorgiou	Greece	641	313	328	339 (52.9)	63.4	UKPDBB
Hattori	Japan	121	121	0	62 (51.2)		UKPDBB
Jeon	Korea	737	397	340	427 (57.9)		UKPDBB
Klein	Germany	320	317	3	185 (59.3)		UKPDBB
Krüger/Sharma/Gasser	Germany	1,909	1,219	690	1,149 (60.4)		UKPDBB
Lin	Taiwan	320	160	160	160 (50.0)	62.0	UKPDBB
Lynch/Ross	Ireland	700	339	361	322 (46.0)	50.5	UKPDBB
Mellick	Australia	1,809	893	916	929 (51.4)	59.0	Bower
Mok	China	390	214	176	232 (60.1)		UKPDBB
Morrison	United Kingdom	1,072	723	349	577 (53.9)	66.1	UKPDBB
Opala/Ross	Poland	614	352	262	358 (58.3)	50.2	UKPDBB
Rogaeva	Canada	562	391	171	296 (53.7)	49.7	UKPDBB
Tan	Singapore	344	171	173	217 (63.1)	59.7	UKPDBB
Toft	Norway	816	364	452	484 (59.3)		UKPBDD
Van Broeckhoven	Belgium	1,011	501	510	500 (49.6)	60.5	Pals/Gelb
Wirdefeldt	Sweden	260	67	193	128 (49.2)	65.8	Gelb
Wszolek/Ross	United States	1,455	69,4	761	764 (52.5)	64.4	UKPDBB
Total		20,510	12,346	8,164			

Abbreviations: AAO = age at onset; UKPDBB = United Kingdom Parkinson's Disease Brain Bank.

in the overall cohort (table e-1). Likewise, stratifying the analysis by ethnicity did not reveal associations; this suggests that intermediate repeats in SCA2, SCA3, SCA6, and SCA17 genes are not a major risk factor for PD (table e-2A).

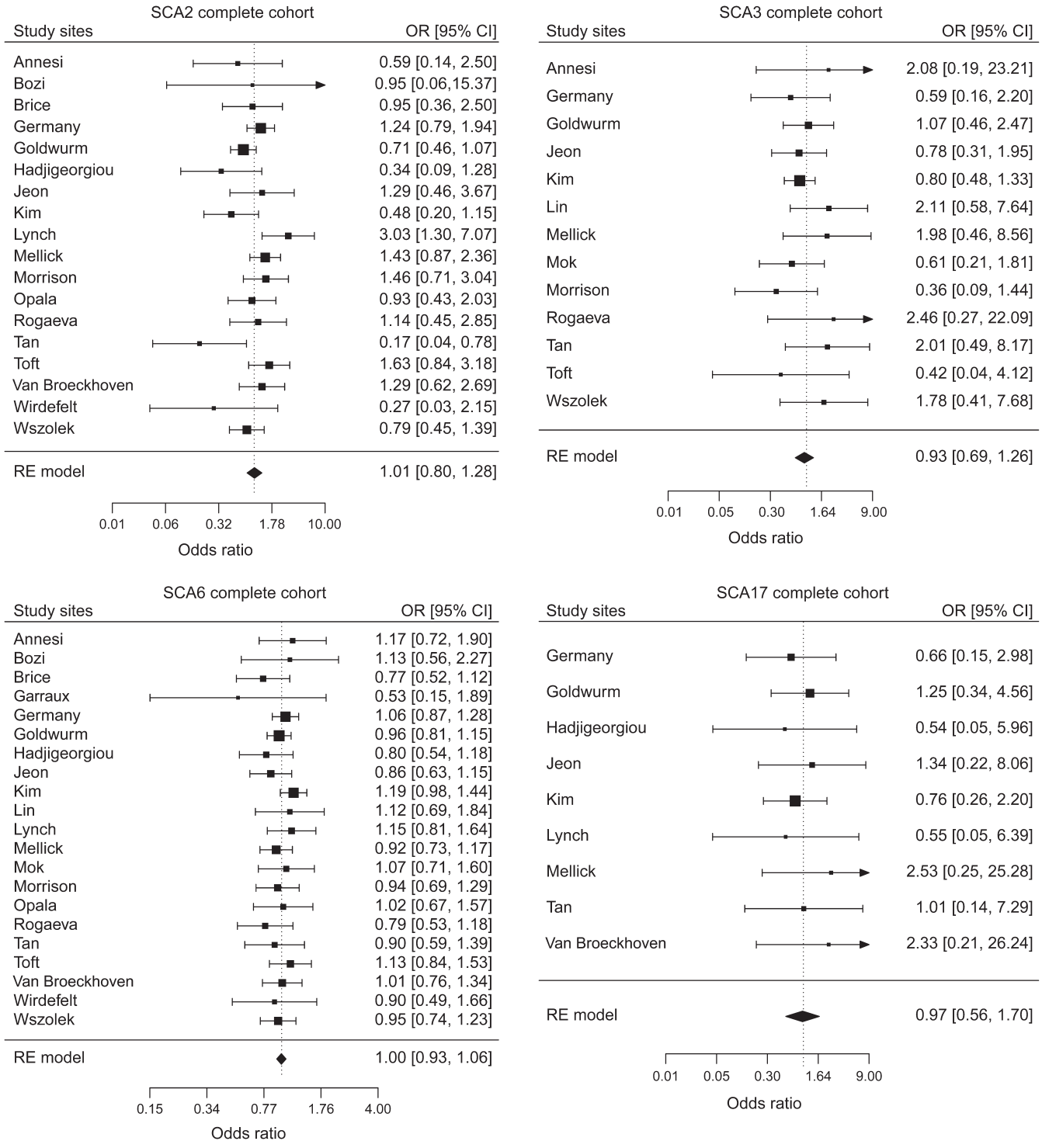
Overall analysis. In the overall cohort, we observed no statistically significant associations between PD and intermediate repeat length for the SCA2, SCA3, SCA6, and SCA17 genes. The odds ratio ranged from 0.93 to 1.01 in the overall cohort (table 2, figure 1). We observed no heterogeneity for SCA3, SCA6, and SCA17 loci in our cohort, while SCA2 showed moderate heterogeneity; however, all heterogeneity 95% CIs contained 0 (table 2). Of note, we observed a p value of 0.013 (uncorrected) in our CLUMP

Table 2 Overall analysis irrespective of ethnicity and influence of between-study heterogeneity

Locus	Gene name	Q test p value	OR (95% CI)	I^2	RE p value	FE p value
SCA2	*ATXN2*	0.03	1.01 (0.78, 1.28)	37 (0, 82)	0.93	0.76
SCA3	*ATXN3*	0.66	0.93 (0.69, 1.26)	0 (0, 63)	0.64	0.64
SCA6	*CACNA1A*	0.87	1.00 (0.93, 1.06)	0 (0, 26)	0.90	0.90
SCA17	*TBP*	0.97	0.97 (0.56, 1.70)	0 (0, 6)	0.92	0.92

Abbreviations: *ATXN2* = ataxin 2; *ATXN3* = ataxin 3; *CACAN1A* = calcium channel, voltage dependent, P/Q type, alpha 1A subunit; CI = confidence interval; FE = fixed effects; OR = odds ratio; RE = random effects; *TBP* = TATA box binding protein.

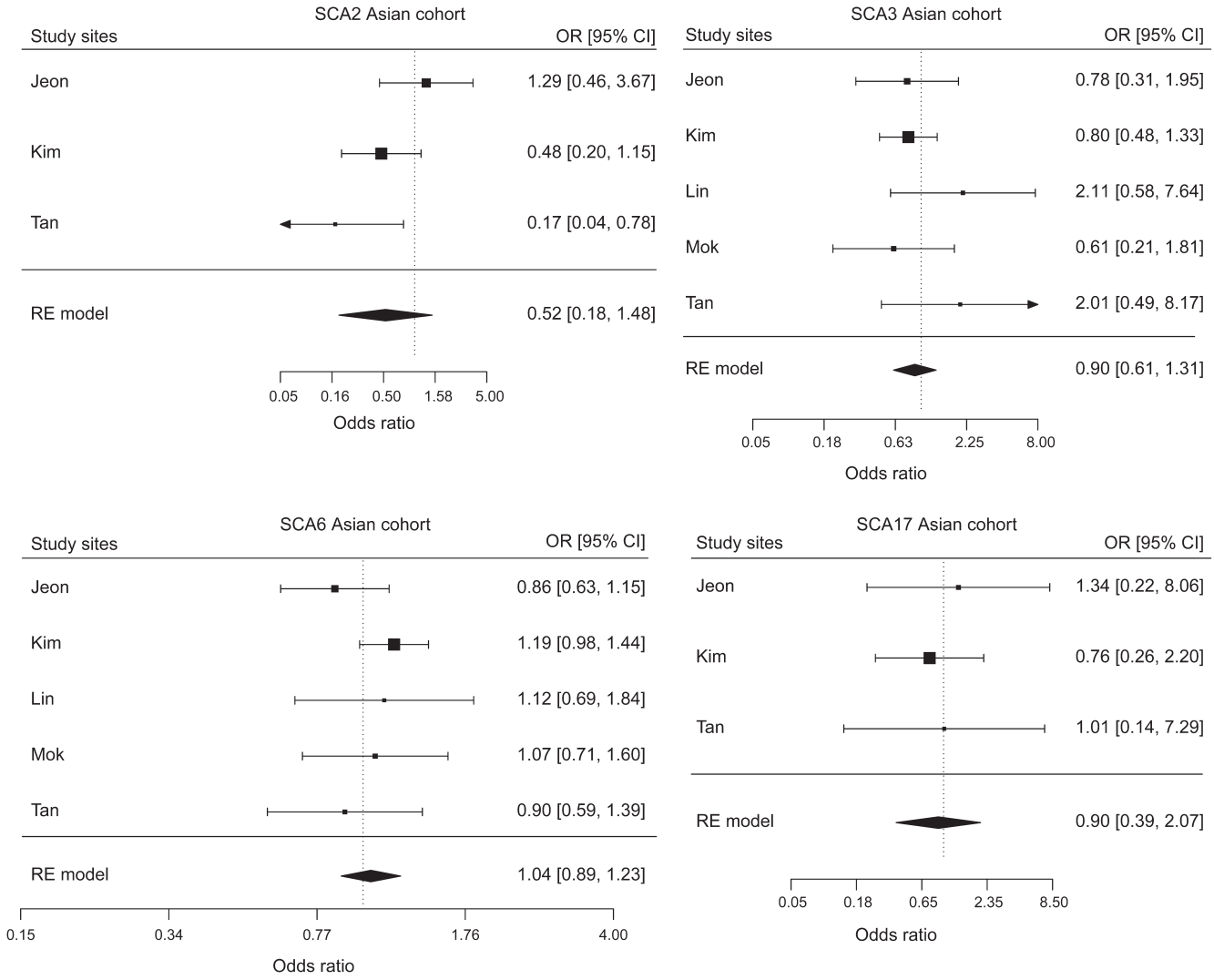
Figure 1 **Forest plot of effect sizes of SCA2, SCA3, SCA6, and SCA17 loci in the overall cohort**



Boxes indicate the summary effect estimate. Germany site is a combination of Deutschländer, Klein, and Gasser sites. Axis scaled in relation to CIs. CI = confidence interval; OR = odds ratio; RE = random effects.

analysis for SCA6 in the overall cohort, but it was not significant after correcting for multiple testing (table e-1). The I^2 estimates ranged from 0% to 37% in the overall cohort. The Q test was not statistically significant for all SCA loci (table 2). Restricting the analysis to the Caucasian and Asian populations did not reveal an association between PD and intermediate repeat length. The odds ratio ranged from 0.97 to 1.09 for the Caucasian population, while for the Asian population, effect estimates ranged from 0.52 to 1.04 for SCA loci (figures 2 and 3 and table e-2A). We observed a trend for association for the SCA2 locus

Figure 2 Forest plot of SCA2, SCA3, SCA6, and SCA17 loci in the Asian population



Boxes indicate the summary effect estimate. Germany site is a combination of Deutschländer, Klein, and Gasser sites. Axis scaled in relation to CIs. CI = confidence interval; OR = odds ratio; RE = random effects.

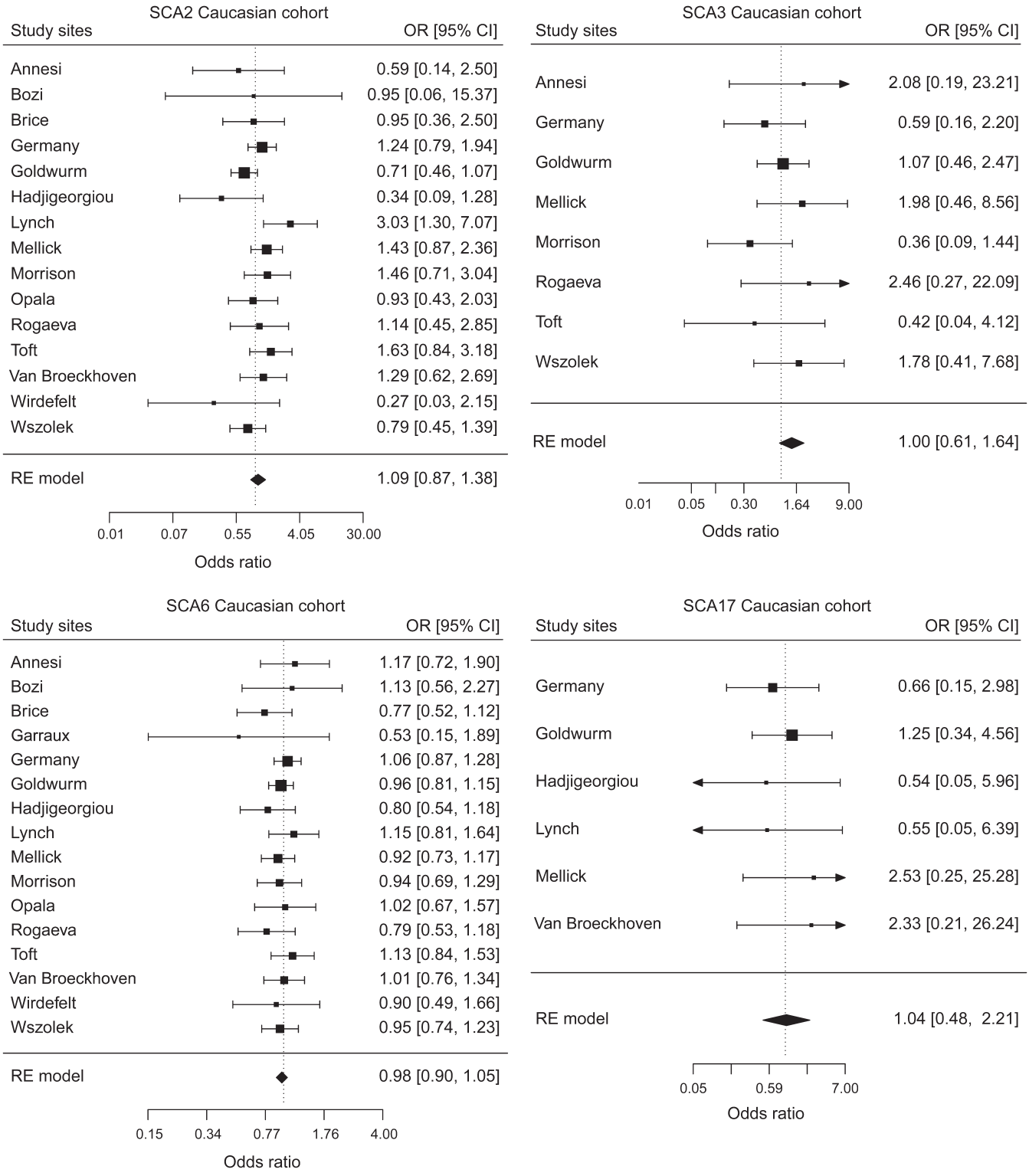
only in the Asian population with large heterogeneity, but this was not significant (table e-2A).

AAO analysis. In a subset of data with AAO available, we did not find any significance correlation between the SCA2, SCA3, SCA6, or SCA17 repeats and the AAO of PD (table e-2D). Likewise, stratifying by ethnicity, we did not observe any association between intermediate repeats. The effect estimates of SCA loci on AAO ranged from −0.79 to 2.13 for the overall cohort, and for the Caucasian population, effect estimates ranged from −0.13 to 4.76 (table e-2D). In addition, the age-adjusted analysis did not yield any significant association between SCA repeats and PD (table e-2C). We also performed random-effects models with the Student *t* test comparing the mean repeat length between cases and controls, and logistic regression models using repeat length as a quantitative trait. We did not observe a significant association between disease and repeat length ($p > 0.05$).

DISCUSSION The expansion of trinucleotide repeats has provided mechanistic explanations for human disorders. Besides defining autosomal dominantly inherited disease genes, variability in the distribution of repeat length as well as composition has remarkable influence on the disease phenotype; the longer the expansion, the earlier the AAO and the more aggressive the disease course.[1] Therefore, we performed a large-scale multicenter evaluation to assess the role of SCA2, SCA3, SCA6, and SCA17 gene repeats in PD. Our study excluded a major role of poly-(Q) repeat expansions for these genes in the causation of PD, at least in typical PD.

So far, there is no clear consensus on the appropriate threshold to understand the influence of

Figure 3 Forest plot showing the comparison of effect of SCA2, SCA3, SCA6, and SCA17 loci in the Caucasian population



Boxes indicate the summary effect estimate. Germany site is a combination of Deutschländer, Klein, and Gasser sites. Axis scaled in relation to CIs. CI = confidence interval; OR = odds ratio; RE = random effects.

intermediate repeat expansions in PD. We used our large cohort to estimate the global distribution of repeat length for SCA genes in PD. The allelic density as well as histogram distribution plots showed the threshold for intermediate repeats ranges from 24 to 32 for SCA2, 36 to 61 for SCA3, 11 to 19 for SCA6, and 42 to 47 for SCA17 in the PD cases. The intermediate range as observed in our study is in agreement with previously published studies.[21–26]

In contrast, a recently published study from a Japanese population suggested that a population-specific SCA2 intermediate repeat cutoff length could influence the PD outcome.[27] Using a cutoff of 25, the authors observed a significant association for the autosomal dominant form of PD in their population.[27] By using this repeat length cutoff, as suggested by Yamashita et al.,[27] we did not observe significant association for the SCA2 locus, and thus our study did not support the notion that variability in cutoff repeat length varies from population to population (table e-2B). Likewise, our study excluded the role of population-specific intermediate repeat length variability on the risk of PD, at least in sporadic forms of PD. Of note, using the cutoff as observed in our study, we observed a trend (nonsignificant) for SCA2 locus in the Asian population. The proportion of intermediate carriers for SCA2 in our Asian population cohort is small (1.5%) and thus these results need to be interpreted cautiously.

Most, if not all, studies that have been published so far screened the SCA2, SCA3, SCA6, and SCA17 genes only in cohorts of autosomal dominant forms of PD,[21–26] and identified carriers for SCA2, SCA3, SCA6, and SCA17 repeats in different ethnic populations, which suggests that intermediate repeat structure influenced the clinical variability in autosomal dominant forms of PD and autosomal dominant cerebellar ataxia. For example, a previous French study identified 9 patients with PD who are carriers for SCA2 repeats.[28] They observed interrupted repeats for SCA2 as compared to the patients with autosomal dominant cerebellar ataxia who carry pure CAG repeats suggesting that differences in the repeat structure may lead to different phenotypes. Likewise, a study in Asian patients identified 7 SCA2 carriers that showed overlapping phenotype with ataxia such as dysarthria and postural instability.[7] However, such patients would not have been included in this study because the inclusion criterion was diagnosis of PD. Our study also did not investigate the role of interruptions in the repeats on PD, thus we cannot draw any conclusions for this subcategory of patients. It is worthwhile to mention that most of the participants in our cohort showed intermediate repeats in the normal range, and hence it will be unlikely that intermediate repeats will have an important role in PD. Nevertheless, deep sequencing of intermediate repeats should be pursued to resolve the role of intermediate repeats in PD, as emerging evidence has shown that genetic variations in these regions have an important role in explaining the missing heritability.[29,30]

Taken together, we examined the role of poly-(Q) repeats in PD using the largest sample size until now, and our results unequivocally show that polyglutamine repeats in SCA2, SCA3, SCA6, and SCA17 are unlikely to be clinically important risk factors for typical, idiopathic PD, without evidence of a family history of neurodegenerative disease (parkinsonism) or atypical signs (e.g., ataxia). Nevertheless, emerging genetic and functional evidence suggest that further studies of these genes in the context of other neurodegenerative diseases are justified.

AUTHOR AFFILIATIONS

From the Centre for Genetic Epidemiology (L.W., M.S.), Institute for Clinical Epidemiology and Applied Biometry, University of Tubingen, Germany; Department of Neurology (J.O.A.), St. Olavs Hospital and NTNU, Trondheim, Norway; Institute of Molecular Bioimaging and Physiology (G.A.), National Research Council, Section of Catanzaro, Italy; Division of Molecular Biology and Human Genetics (S.B., J.C.), University of Stellenbosch, Cape Town, South Africa; General Hospital of Syros (M.B.); Hygeia Hospital (M.B.), Clinic of Neurodegenerative Disorders, Athens; 2nd Neurology Clinic (M.B., L.S.), University of Athens, Attikon Hospital, Greece; INSERM U 1127 (A.B., S.L.), CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, Paris, France; Department of Neurology (S.J.C.), Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; School of Clinical and Experimental Medicine (C.C., K.E.M.), College of Medical and Dental Sciences, University of Birmingham and City Hospital, Birmingham, UK; Neurodegenerative Brain Diseases Group (D.C., J.T., C.V.B.), Department of Molecular Genetics, VIB, Antwerpen; Institute Born-Bunge (D.C., J.T., C.V.B.), University of Antwerp; Department of Neurology (D.C.), University Hospital Antwerpen, Belgium; Department of Neurology (A.D.), Max Planck Institute for Psychiatry, Munich; Helmholtz Zentrum München (G.E., P.L.), German Research Centre for Environmental Health (Gmbh), Neuherberg, Germany; Djavad Mowafhagian Centre for Brain (M.J.F.), University of British Columbia, Vancouver, Canada; Parkinson Institute (S.G.), Istituti Clinici di Perfezionamento, Milano, Italy; Cyclotron Research Centre (G.G.), Department of Neurology, University of Liège, Belgium; Department of Neurology (G.M.H., G.X.), Faculty of Medicine, University of Thessaly and Institute of Biomedical Research and Technology, CERETETH, Larissa, Greece; Center for Biomedicine (A.A.H., P.P.P.), EURAC Bolzano, Italy; Department of Neurology (N.H., H.T.), Juntendo University School of Medicine, Tokyo, Japan; Institute of Neurogenetics (C.K.), University of Luebeck, Germany; Department of Neurology (B.J., S.S.P.), Seoul National University Hospital; ILSONG Institute of Life Science (Y.J.K., K.-C.Y.), Hallym University, Hallym Institute of Translational Genomics & Bioinformatics, Department of Neurology, Hallym University Sacred Heart Hospital, Korea; Department of Neurology (J.-J.L.), Chushang Show-Chwan Hospital, Nantou and Chung-Shan Medical University Hospital, Taichung, Taiwan; The Dublin Neurological Institute at the Mater Misericordiae University Hospital (T.L.), and Conway Institute, University College Dublin, Ireland; Movement Disorders Centre (A.E.L.), Toronto Western Hospital, University of Toronto, Canada; Clinical Neurology Research Centre (V.M.), Department of Medicine & Therapeutics, The Chinese University of Hong Kong, 10/F, Clinical Sciences Building, Prince of Wales Hospital, Shatin, New Territories, Hong Kong; Department of Neurology (B.J.-M., G.O.), Medical University of Silesia, Katowice, Poland; Eskitis Institute for Cell and Molecular Therapies (G.D.M.), Griffith University, Australia; Neurosciences Department (K.E.M., J.D.S.), Queen Elizabeth Hospital Birmingham, University Hospitals Birmingham NHS Foundation Trust; Department of Neurology (L.P., M.T.), Oslo University Hospital, Norway; Institute of Neurology (A.Q.), Department of Medical Sciences, University Magna Graecia, Catanzaro; Institute of Molecular Bioimaging and Physiology (A.Q.), National Research Council, Section of Catanzaro, Italy; Tanz Centre for Research in Neurodegenerative Diseases (E.R.), Department of Medicine, University of Toronto, Canada; Departments of Neuroscience (O.A.R.) and Neurology (R.J.U., Z.W.), Mayo Clinic, Jacksonville, FL; Divisions of Basic Neurosciences & Cell Biology (L.S.), Biomedical Research Foundation of Academy of Athens, Greece; University of Queensland Centre for Clinical Research (P.A.S.), Herston, Australia; Department of

Neurology (E.K.T., Y.Z.), Singapore General Hospital, National Neuroscience Institute, and Duke NUS Graduate Medical School, Singapore; Department of Medical Epidemiology and Biostatistics (K.W.), Karolinska Institute, Stockholm, Sweden; Department of Neurodegenerative Diseases (T.G., R.K., M.S.), Hertie Institute for Clinical Brain Science Research, University of Tubingen, Germany; Department of Neurology (D.M.M.), North Shore University Health System, Evanston, IL; and Clinical and Experimental Neuroscience (R.K.), Luxembourg Centre for Systems Biomedicine, University of Luxembourg, and Centre Hospitalier de Luxembourg, Luxembourg.

AUTHOR CONTRIBUTIONS

Lisa Wang, MSc: Ms. Wang performed statistical analysis and wrote the draft. Jan O. Aasly, MD: Dr. Aasly contributed samples and collected phenotypic data. Grazia Annesi, MD: Dr. Annesi contributed samples and collected phenotypic data. Soraya Bardien, PhD: Dr. Bardien contributed samples and collected phenotypic data. Maria Bozi, MD: Dr. Bozi contributed samples and collected phenotypic data. Alexis Brice, MD: Prof. Brice contributed to sample collection and collected phenotypic data. Jonathan Carr, MD: Dr. Carr contributed samples and collected phenotypic data. Sun Ju Chung, MD: Dr. Chung contributed samples and collected phenotypic data. Carl Clarke, MD: Dr. Clarke contributed samples and collected phenotypic data. David Crosiers, MD: Dr. Crosiers contributed samples and collected phenotypic data. Angela Deutschländer, MD: Dr. Deutschländer contributed samples and collected phenotypic data. Gertrud Eckstein, PhD: Dr. Eckstein contributed analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval and acquisition of data. Matthew J. Farrer, PhD: Dr. Farrer contributed samples and collected phenotypic data. Stefano Goldwurm, PhD: Dr. Goldwurm contributed samples and collected phenotypic data. Gaetan Garraux, MD, PhD: Dr. Garraux contributed samples and collected phenotypic data. Georgios M. Hadjigeorgiou, MD: Dr. Hadjigeorgiou contributed samples and collected phenotypic data. Andrew A. Hicks, PhD: Dr. Hicks contributed samples and collected phenotypic data. Nobutaka Hattori, MD: Dr. Hattori contributed samples and collected phenotypic data. Christine Klein, MD: Dr. Klein contributed samples and collected phenotypic data. Beom Jeon, MD: Dr. Jeon contributed samples and collected phenotypic data. Yun Joong Kim, MD: Dr. Kim contributed samples and collected phenotypic data. Suzanne Lesage, MD: Dr. Lesage contributed samples and collected phenotypic data. Juei-Jueng Lin, MD: Dr. Lin contributed samples and collected phenotypic data. Timothy Lynch, MD: Dr. Lynch contributed samples and collected phenotypic data. Peter Lichtner, PhD: Dr. Lichtner contributed samples and collected phenotypic data. Anthony E. Lang, MD: Dr. Lang contributed samples and collected phenotypic data. Vincent Mok, MD: Dr. Mok contributed samples and collected phenotypic data. Barbara Jasinska-Myga, MD, PhD: Dr. Jasinska-Myga contributed samples and collected phenotypic data. George D. Mellick, PhD: Dr. Mellick contributed samples and collected phenotypic data and critically reviewed the manuscript. Karen E. Morrison, MD: Dr. Morrison contributed samples and collected phenotypic data. Grzegorz Opala, MD: Dr. Opala contributed samples and collected phenotypic data. Lasse Pihlstrøm, MD: Dr. Pihlstrøm contributed samples and collected phenotypic data. Peter P. Pramstaller: Dr. Pramstaller contributed samples and collected phenotypic data. Sung Sup Park, MD: Dr. Park contributed samples and collected phenotypic data. Aldo Quattrone, MD: Dr. Quattrone contributed samples and collected phenotypic data. Ekaterina Rogaeva, PhD: Dr. Rogaeva contributed samples and collected phenotypic data and participated in writing of the manuscript. Owen A. Ross, PhD: Dr. Ross contributed samples and collected phenotypic data. Leonidas Stefanis, MD: Dr. Stefanis contributed samples and collected phenotypic data. Joanne D. Stockton, PhD: Dr. Stockton contributed samples and collected phenotypic data. Peter A. Silburn, PhD: Dr. Silburn contributed samples and collected phenotypic data. Jessie Theuns, PhD: Dr. Theuns contributed samples and collected phenotypic data. Eng King Tan, MD: Dr. Tan contributed samples and collected phenotypic data. Mathias Toft, MD, PhD: Dr. Toft contributed samples and collected phenotypic data. Dr. Hiroyuki Tomiyama, MD, PhD: Dr. Tomiyama contributed samples and collected phenotypic data. Christine Van Broeckhoven, PhD: Dr. Van Broeckhoven contributed samples and collected phenotypic data. Ryan J. Uitti, MD: Dr. Uitti contributed samples and collected phenotypic data. Karin Wirdefeldt, MD: Dr. Wirdefeldt contributed samples and collected phenotypic data. Zbigniew Wszolek, MD: Dr. Wszolek contributed samples and collected phenotypic data. Georgia Xiromerisiou, MD: Dr. Xiromerisiou contributed samples and collected phenotypic data. Kuo-Chu Yueh, MD: Dr. Yueh contributed samples and collected phenotypic data. Yi Zhao, MD: Dr. Zhao contributed samples and collected phenotypic data. Thomas Gasser, MD: Dr. Gasser contributed samples and collected phenotypic data and participated in writing of the manuscript. Demetrius M. Maraganore, MD: Dr. Maraganore contributed to the administrative leadership and coordination of this study as the overall principal investigator of the Genetic Epidemiology of Parkinson's Disease Consortium and he provided critical review of the manuscript. Dr. Maraganore contributed samples and collected phenotypic data. Rejko Krüger, MD: Dr. Krüger contributed samples and collected phenotypic data and participated in writing of the manuscript. Manu Sharma, PhD: Dr. Sharma contributed to 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, and obtained funding.

STUDY FUNDING

The samples were obtained from the Parkinson Institute Biobank (http://www.parkinsonbiobank.com), member of the Telethon Network of Genetic Biobank (project GTB12001) funded by TELETHON Italy, and supported by Fondazione Grigioni per il Morbo di Parkinson.

DISCLOSURE

L. Wang reports no disclosures relevant to the manuscript. J. Aasly is supported by the Norwegian Research Council and Reberg's Legacy. G. Annesi, S. Bardien, M. Bozi, A. Brice, J. Carr, S. Chung, C. Clarke, D. Crosiers, A. Deutschländer, and G. Eckstein report no disclosures relevant to the manuscript. M. Farrer reports grants from the Canadian Federal Government, Cunhill Foundation, and BC Leading Edge Endowment, during the conduct of the study; also personal fees from Gentech and Teva, outside the submitted work; In addition, Dr. Farrer has a patent on genetic variability in *LRRK2* and Parkinson disease (US8409809, US8455243B2) and on *LRRK2* mouse models subsequently developed with royalties paid. S. Goldwurm, G. Garraux, G. Hadjigeorgiou, and A. Hicks report no disclosures relevant to the manuscript. N. Nobutaka Hattori has been serving as an advisory board member for Boehringer Ingelheim and as a result of attending advisory board meetings he received personal compensation. Nobutaka Hattori also has been serving as an advisory board member for FP Pharmaceutical Company and by attending these advisory meetings he received personal compensation. He has been consulting with Otsuka Pharmaceutical Company, Kyowa Hakko Kirin Pharmaceutical Company, GlaxoSmithKline, Novartis, and Schering-Plough, and when he attended these advisory board meetings, he received personal compensation. C. Klein reports no disclosures relevant to the manuscript. B. Jeon has received funding for travel from GlaxoSmithKline Korea and Novartis Korea, and has received research support as principal investigator from Novartis, Boehringer Ingelheim, Ipsen Korea, the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A030001), ABRC (Advanced Biometric Research Center), KOSEF (Korea Science and Engineering Foundation), Seoul National University Hospital, the Mr. Chung Suk-Gyoo and Sinyang Cultural Foundation, and the Song Foundation. Y. Kim, S. Lesage, and J. Lin report no disclosures relevant to the manuscript. T. Lynch has served on an advisory board for Biogen and Novartis and has received honoraria from Lundbeck, Biogen, and Boehringer Ingelheim. P. Lichtner reports no disclosures relevant to the manuscript. A. Lang has served as an advisor for Abbott, Allon Therapeutics, AstraZeneca, Biovail, Boehringer Ingelheim, Cephalon, Ceregene, Eisai, Medtronic, Lundbeck A/S, Novartis, Merck Serono, Solvay, and Teva, and received grants from Canadian Institutes of Health Research, Dystonia Medical Research Foundation, Michael J. Fox Foundation, National Parkinson Foundation, and Ontario Problem Gambling Research Centre and has served as an expert witness in cases related to the welding industry. V. Mok reports no disclosures relevant to the manuscript. B. Jasinska-Myga was partially supported by the Robert

and Clarice Smith Fellowship Program and the Pacific Alzheimer Research Foundation grant C06-01. G. Mellick reports no disclosures relevant to the manuscript. K. Morrison serves on the editorial board of *Neurodegenerative Disease Management* and previously served on the editorial board of *Journal of Neurology* and *Neuromuscular Disorders*. She has received grant support from Parkinson's UK, The Medical Research Council UK, the Wellcome Trust, and the Midlands Neurological Teaching and Research Fund. G. Opala was partially supported by the Robert and Clarice Smith Fellowship Program and the Pacific Alzheimer Research Foundation grant C06-01. L. Pihlstrøm, P. Pramstaller, S. Park, A. Quattrone, and E. Rogaeva report no disclosures relevant to the manuscript. O. Ross serves on the editorial board of the *American Journal of Neurodegenerative Disease*, *PLoS One*, and *Parkinsonism and Related Disorders* journals. He is funded by NIH grant R01 NS078086 and the Mayo Clinic Udall Center of Excellence for Parkinson's Disease Research (P50 NS072187), and received research support from the Michael J. Fox Foundation. L. Stefanis has received a grant for genetic studies of PD from the Hellenic Secretariat of Research and Technology (PENED 2003), in which Novartis Hellas acted as a cosponsor. J. Stockton, P. Silburn, and J. Theuns report no disclosures relevant to the manuscript. E. Tan serves as an editor of *Parkinsonism and Related Disorders*, *European Journal of Neurology*, and *Basal Ganglia*, and is the chief editor of *Annals Academy of Medicine*. He is funded by the National Medical Research Council, Duke-NUS Graduate Medical School, and Singapore Millennium Foundation. He has received honoraria from Novartis, Boehringer Ingelheim, and GSK. M. Toft serves on the editorial board of *PLoS One* and is supported by research grants from the Research Council of Norway and South-Eastern Norway Regional Health Authority. H. Tomiyama received a grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Grants-in-Aid for Scientific Research (to H.T.: 21591098) and Grants-in-Aid from the Research Committee of CNS Degenerative Diseases and Perry syndrome (to HT: 22140901). C. Van Broeckhoven reports no disclosures relevant to the manuscript. R. Uitti, MD, has received research funding from the NIH, PARRF, PSG, Noscira, Inc., and Advanced Neuromodulation Systems, Inc. Dr. Uitti has served as a Continuing Medical Educator for the AAN. His institution has received annual royalties from the licensing of the technology related to PARK8/LRRK2 greater than the federal threshold for significant financial interest; Dr. Uitti has not received any royalties. Dr. Uitti receives an honorarium as associate editor of *Neurology®*. K. Wirdefeldt reports no disclosures relevant to the manuscript. Z. Wszolek serves as co–editor-in-chief of *Parkinsonism and Related Disorders*, associate editor of the *European Journal of Neurology*, and on the editorial boards of *Neurologia i Neurochirurgia Polska*, *Advances in Rehabilitation*, the *Medical Journal of the Rzeszow University*, and *Clinical and Experimental Medical Letters*. Dr. Wszolek holds and has contractual rights for receipt of future royalty payments from patents re: A novel polynucleotide involved in heritable Parkinson's disease; received royalties from publishing *Parkinsonism and Related Disorders* (Elsevier 2012, 2013, 2014) and the *European Journal of Neurology* (Wiley-Blackwell 2012, 2013, 2014); receives educational research support from Allergan, Inc., research support from NIH/NINDS P50NS072187, and the gift from Carl Edward Bolch, Jr., and Susan Bass Bolch (MCF 90052031/PAU 90052). G. Ximerisiou, K. Yueh, and Y. Zhao report no disclosures relevant to the manuscript. T. Gasser serves as an editorial board member of *Movement Disorders* and *Parkinsonism and Related Disorders* and is funded by Novartis Pharma, the Federal Ministry of Education and Research (BMBF) (NGFN-Plus and ERA-Net NEURON), the Helmholtz Association (HelMA, Helmholtz Alliance for Health in an Ageing Society), and the European Union (MeFoPa, Mendelian Forms of Parkinsonism). He received speakers honoraria from Novartis, Merck Serono, Schwarz Pharma, Boehringer Ingelheim, and Valeant Pharma and royalties for his consulting activities from Cephalon Pharma and Merck Serono. Dr. Gasser holds a patent concerning the *LRRK2* gene and neurodegenerative disorders. D. Maraganore has received research funding support from the NIH (R01-ES10751), the Michael J. Fox Foundation (LEAPS award, Edmond J. Safra Global Genetics Consortia award), Alnylam Pharmaceutical and Medtronic, Inc. (study of α-synuclein genotypes and outcomes in Parkinson disease), and GE Healthcare (imaging biomarker studies of delayed sequelae of mild traumatic brain injury). He is on the editorial board of *Parkinsonism and Related Disorders*. He has a patent filed for a method to treat Parkinson disease. It has been licensed to Alnylam Pharmaceuticals and he has received more than $10,000 in royalty payments. R. Krüger serves as editor of *European Journal of Clinical Investigation*, *Journal of Neural Transmission*, and *Parkinsonism and Related Disorders* and associate editor of *BMC Neurology*; has received research grants of the German Research Council (DFG; KR2119/8-1), the Michael J. Fox Foundation (USA), the Fritz Thyssen Foundation (Germany), and the Fond National de Recherche (Luxembourg), as well as speakers honoraria and/or travel grants from AbbVie, St. Jude, and Medtronic. M. Sharma received funding support from the Michael J. Fox Foundation USA and the EU-JPND program of neurodegenerative diseases. Dr. Sharma serves on the editorial board of the *American Journal of Neurodegenerative Disease* and *PLoS One*. Dr. Sharma reports no disclosures related to this study. Go to Neurology.org for full disclosures.

Received October 8, 2014. Accepted in final form May 21, 2015.

Comment: CAG repeats in idiopathic Parkinson disease—To screen or not to screen

In this large study within the Genetic Epidemiology of Parkinson's Disease Consortium, Wang et al.[1] examined the relationship between idiopathic Parkinson disease (PD) and CAG repeat expansions in ataxia genes. They examined, on a larger scale, an issue that has been examined by several authors. The rationale for the study is that parkinsonian phenotypes, and even L-dopa–responsive PD, occur in carriers of SCA mutations, mainly in Asians, and that intermediate poly-Q expansions are predisposing factors for dominant PD.[2,3] The authors studied 12,346 patients with PD from Caucasian and Asian populations and 8,164 controls, seeking CAG expansions in SCA2, SCA3, SCA6, and SCA17 genes. The study is "negative" since they did not identify causative mutations or increased risk of PD attributable to long normal repeat alleles.

The major strength of the study is that this is the largest screening of ataxia loci in idiopathic PD and takes advantage of large samples of cases and controls from multiple international sites. The study is technically well executed: all samples were tested in one facility; quality monitored; internal standards run; and some samples were sequenced to confirm repeat length. Limitations of the study are that a similar number of patients and controls from the different centers would have been advisable, and that the full range of disease-associated CAG expansions (i.e., Huntington disease and SCA1) was not investigated.

These results suggest that the previously reported association between these loci and parkinsonism probably refers to familial or atypical forms and not to typical idiopathic PD.[3,4] The study therefore does not support genetic screening of SCA2, SCA3, SCA6, and SCA17 in idiopathic PD and excludes the role of intermediate alleles of these genes as risk factors for PD; this may redirect the field away from association studies of CAG repeats in ataxia genes and PD, a valuable contribution in and of itself.

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Alessandro Filla, MD

From the Department of Neurosciences, Reproductive Sciences, and Odontostomatology, Federico II University, Napoli, Italy.

Study funding: No targeted funding reported.
Disclosure: The author reports no disclosures. Go to Neurology.org for full disclosures.

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