C9orf72 Hexanucleotide Repeat Expansion and Guam Amyotrophic Lateral Sclerosis–Parkinsonism-Dementia Complex

Beth A. Dombroski, PhD; Douglas R. Galasko, MD; Ignacio F. Mata, PhD; Cyrus P. Zabetian, MD, MS; Ulla-Katrina Craig, PhD; Ralph M. Garruto, PhD; Kiyomitsu Oyanagi, MD, PhD; Gerard D. Schellenberg, PhD

Importance: High-prevalence foci of amyotrophic lateral sclerosis (ALS) and Parkinsonism-dementia complex (PDC) exist in Japanese on the Kii Peninsula of Japan and in the Chamorros of Guam. Clinical and neuropathologic similarities suggest that the disease in these 2 populations may be related. Recent findings showed that some of the Kii Peninsula ALS cases had pathogenic C9orf72 repeat expansions, a genotype that causes ALS in Western populations.

Objectives: To perform genotyping among Guam residents to determine if the C9orf72 expanded repeat allele contributes to ALS-PDC in this population and to evaluate LRRK2 for mutations in the same population.

Design and Setting: Case-control series from neurodegenerative disease research programs on Guam that screened residents for ALS, PDC, and dementia.

Participants: Study participants included 24 with ALS and 22 with PDC and 43 older control subjects with normal cognition ascertained between 1956 and 2006. All but one participant were Chamorro, the indigenous people of Guam. A single individual of white race/ethnicity with ALS was ascertained on Guam during the study.

Main Outcomes and Measures: Participants were screened for C9orf72 hexanucleotide repeat length. Participants with repeat numbers in great excess of 30 were considered to have pathogenic repeat expansions. LRRK2 was screened for point mutations by DNA sequencing.

Results: We found a single individual with an expanded pathogenic hexanucleotide repeat. This individual of white race/ethnicity with ALS was living on Guam at the time of ascertainment but had been born in the United States. All Chamorro participants with ALS and PDC and control subjects had normal repeats, ranging from 2 to 17 copies. No pathogenic LRRK2 mutations were found.

Conclusions and Relevance: Unlike participants with ALS from the Kii Peninsula, C9orf72 expansions do not cause Guam ALS-PDC. Likewise, LRRK2 mutations do not cause Guam ALS-PDC.
found that about 20% of these patients carry a C9orf72 expansion. Because of the similarities between Kii Peninsula ALS cases and Guam ALS-PDC and because Western cases with the expansion are associated with ALS and parkinsonism, we investigated the role of expanded C9orf72 repeat alleles in ALS-PDC cases among Chamorros. We also investigated LRRK2 (OMIM 609007) as a candidate gene for Guam ALS-PDC because of its proximity to the chromosome 12p12.1 linkage signal for this disorder and because mutations in this gene cause autosomal dominant Parkinson disease.

### METHODS

#### PARTICIPANTS

Recruitment and diagnostic assessment procedures were described previously. The ALS-PDC cases and healthy control subjects were sampled on Guam between 1956 and 2006. Archival samples and clinical data were from the National Institute of Neurological and Communicative Disorders Research intramural program (1956 to 1983). Diagnoses were confirmed by examining clinical records and autopsy reports available for many of the participants (Table). Other samples were obtained 1995 to 2006, from a neurologically screening program. Controls were Chamorros who were cognitively and neurologically healthy. The diagnosis of PDC required gradual onset and progression of primary parkinsonism and dementia. The diagnosis of ALS was based on El Escorial criteria. All individuals (or proxies) provided written consent to participate in the study. Race/ethnicity was by self-report.

#### MUTATION SCREENING

The C9orf72 hexanucleotide repeat was genotyped using repeat-primed polymerase chain reaction as described. The size of the hexanucleotide repeat-containing region was determined by allele fragment length analysis as previously described. Only alleles with normal repeat lengths (range, 2-27) can be amplified by this procedure (Figure 1 insets). Participants with repeat numbers in great excess of 30 were considered to have pathogenic repeat expansions. All 51 exons of LRRK2 were screened by Sanger sequencing as previously described. Our results show that neither LRRK2 nor C9orf72 is the major gene responsible for ALS-PDC on Guam. We screened Guam residents with ALS and PDC and older control subjects with normal cognition (Table) for the C9orf72 pathogenic expanded hexanucleotide repeat that causes ALS in persons of white race/ethnicity. Some individuals with ALS and PDC and control subjects were from Umatac, a village with a higher disease incidence than that of other Guamanian villages. All participants were Chamorros except for a single individual of white race/ethnicity with ALS who was born in the United States, moved to Guam as a young adult, and developed ALS at age 53 years. He had no family history of ALS. We observed a single case of an expanded pathogenic hexanucleotide repeat among individuals of white race/ethnicity. The genotype was comparable to that of a previously described individual with a repeat expansion (ND06769*8B1) from the Coriell Institute for Medical Research (Camden, New Jersey) cell repository and was similar to that of other individuals with ALS evaluated in the laboratory of one of us (G.D.S.). This individual had a second normal repeat length allele (Figure 1A-inset).

### RESULTS

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Male Sex, %</th>
<th>Age at Risk, Mean (SD), y</th>
<th>From Umatac, %</th>
<th>Autopsy Performed, %</th>
<th>Race/Ethnicity</th>
<th>C9orf72 Expansion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS (n = 23)</td>
<td>56.5</td>
<td>44.6 (10.7)</td>
<td>26.1</td>
<td>100.0</td>
<td>Chamorro</td>
<td>No</td>
</tr>
<tr>
<td>ALS (n = 1)</td>
<td>Yes</td>
<td>53</td>
<td>No</td>
<td>Yes</td>
<td>White</td>
<td>Yes</td>
</tr>
<tr>
<td>PDC (n = 22)</td>
<td>59.1</td>
<td>51.1 (9.3)</td>
<td>54.5</td>
<td>86.4</td>
<td>Chamorro</td>
<td>No</td>
</tr>
<tr>
<td>Healthy controls (n = 43)</td>
<td>30.2</td>
<td>63.6 (13.5)</td>
<td>48.8</td>
<td>0.0</td>
<td>Chamorro</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: ALS, amyotrophic lateral sclerosis; PDC, parkinsonism-dementia complex.

*Age at onset for cases and age at the last examination for controls.
set). Therefore, ALS in this case was caused by a highly penetrant mutation and not by exposure to an environmental factor on Guam. All Chamorro participants had normal repeat lengths, with copy numbers ranging from 2 to 17 (Figure 1B).

For LRRK2 screening, we screened 47 unrelated individuals with ALS and 92 unrelated individuals with PDC for mutations in exons 31, 35, and 41. These exons harbor the known pathogenic mutations. In 2 individuals with ALS, we found a new missense variant in exon 31 (H1453R). We then sequenced exon 31 in 341 Chamorro control subjects and identified one individual who was heterozygous for the variant. Finally, we sequenced all 51 LRRK2 exons in 16 affected members of a large Guamanian ALS-PDC pedigree from the village of Umatac with evidence of linkage to the LRRK2 region and detected no missense variants.

The genetic origin of Guamanian ALS-PDC remains largely unknown. MAPT alleles contribute to disease susceptibility but do not cause ALS-PDC.\textsuperscript{39} Findings from our work show that ALS-PDC in Chamorros is not caused by C9orf72 expansions or by LRRK2 mutations. Therefore, the primary genetic change responsible for this disorder requires further investigation.

In contrast, C9orf72 expansions seem to cause a subset of ALS cases from the southern Kii Peninsula. Also, some ALS cases from this region carry a low-penetrance SOD1 (OMIM 147450) mutation.\textsuperscript{38} Still, most ALS and PDC cases from this region of Japan have no known genetic mutation. Therefore, while ALS on the Kii Peninsula is genetically heterogeneous, the genetic origin of unexplained cases may be similar to that of the Chamorro ALS-PDC cases.

LRRK2 is located on chromosome 12 and is close to the ALS-PDC linkage signal obtained in an extended family from the village of Umatac.\textsuperscript{9} However, the H1453R variant was not observed in the Umatac cases and does not account for the chromosome 12 linkage signal. The H1453R variant was observed in 2 cases with ALS herein (with onset at age 42 years and age 43 years) and in a 69-year-old healthy Chamorro control subject. Therefore, although the histidine at position 1453 is conserved among vertebrates and in Drosophila (Figure 2), it is unlikely pathogenic or at least is not fully penetrant.

Accepted for Publication: November 15, 2012. Published Online: April 15, 2013. doi:10.1001/jamaneurol.2013.1817

Author Affiliations: Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia (Drs Dombroski and Schellenberg); Department of Neurosciences, University of California–San Diego, La Jolla (Dr Galasko); Veterans Affairs Puget Sound Health Care System and Department of Neurology, University of Washington, Seattle (Drs Mata and Zabetian); Laboratory of Biomedical Anthropology and Neurosciences, Graduate Program in Biomedical Anthropology, Binghamton University, Binghamton, New York (Dr Garruto); and Department of Neuropathology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan (Dr Oyanagi). Dr Craig is retired from practice.

Correspondence: Gerard D. Schellenberg, PhD, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Room 609B, Stellar-Chance Laboratories, 422 Curie Blvd, Philadelphia, PA 19104 (gerardsc@mail.med.upenn.edu).

Author Contributions: Study concept and design: Dombroski and Schellenberg. Acquisition of data: Dombroski, Galasko, Mata, Zabetian, Craig, Oyanagi, and Schellenberg. Analysis and interpretation of data: Dombroski, Galasko, Zabetian, Garruto, and Schellenberg. Drafting of the manuscript: Dombroski, Galasko, and Schellenberg. Critical revision of the manuscript for important intellectual content: Dombroski, Mata, Zabetian, Craig, Garruto, Oyanagi, and Schellenberg. Obtained funding: Zabetian and Schellenberg. Administrative, technical, and material support: Craig, Garruto, Oyanagi, and Schellenberg.

Conflict of Interest Disclosures: None reported.

Funding/Support: This work was supported by grants AG14382, P01-AG-017586, and R37 AG11762 from the National Institute on Aging and by grant R01 NS065070 from the National Institute of Neurological Disorders and Stroke.

Additional Contributions: We thank the individuals who participated in this study.
REFERENCES


