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

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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 ALS-PDC in Chamorros. Likewise, *LRRK2* mutations do not cause Guam ALS-PDC.

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A FOCUS OF HIGH-PREVALENCE amyotrophic lateral sclerosis (ALS) was identified during World War II among the indigenous Chamorros of Guam.[1] Soon afterward, the related disorder parkinsonism-dementia complex (PDC) was characterized in the same population.[2] Neuropathologic findings in ALS-PDC include tangles, which occur in the hippocampus and neocortex and sometimes in anterior horn cells.[3] Environmental[4] and genetic[5] risk factors for ALS-PDC have been reported. Results of genetic studies show that polymorphisms in *MAPT* (Online Mendelian Inheritance in Man [OMIM] 157140), which encodes for tau increase risk,[5] but no mutations in *MAPT* or other genes are known to cause Guam ALS-PDC.[6-8] Linkage analysis of families with ALS-PDC suggest chromosome 12 loci.[9]

Both ALS and PDC are also prevalent among Japanese living on the Kii Peninsula of Japan.[10] The results of clinical and neuropathology studies[11] suggest that ALS and PDC cases from the Kii Peninsula are similar to those observed on Guam and indicate that these 2 sites may represent foci of the same disorder.

The findings of recent work showed that expansion of a hexanucleotide repeat sequence in an intron of *C9orf72* (OMIM 614260) causes autosomal dominant ALS and frontotemporal dementia.[12,13] Parkinsonism is also observed in some cases of *C9orf72* expansion.[14] A study[15] of Kii Peninsula families with ALS

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Table. Study Participants

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

Abbreviations: ALS, amyotrophic lateral sclerosis; PDC, parkinsonism-dementia complex.
[a]Age at onset for cases and age at the last examination for controls.

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[9] and because mutations in this gene cause autosomal dominant Parkinson disease.

METHODS

PARTICIPANTS

Recruitment and diagnostic assessment procedures were described previously.[5,16] 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 neurological screening program.[17] 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.[18] 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.[12] The size of the hexanucleotide repeat–containing region was determined by allele fragment length analysis as previously described.[13] 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.[19]

RESULTS

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.[1] 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*B1)[12] 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 in-

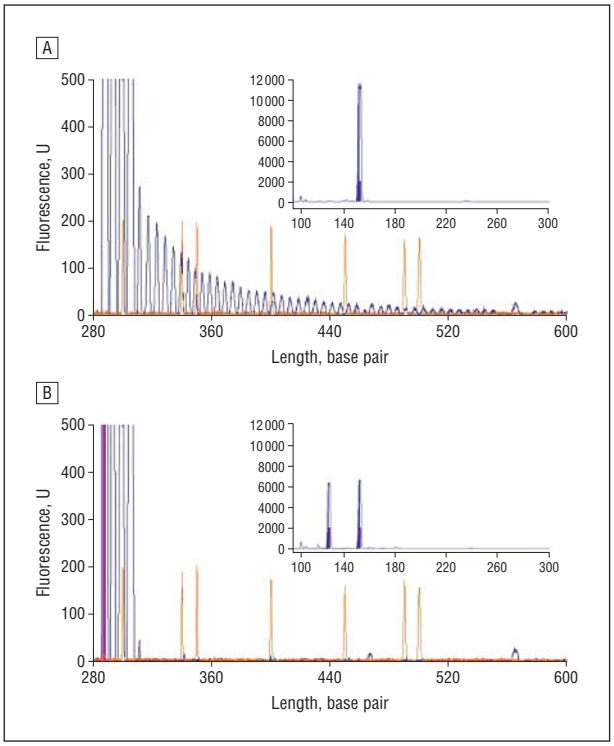

Figure 1. *C9orf72* repeat genotyping. The traces are repeat-primed polymerase chain reaction products. The blue traces are DNA products, and the red peaks are size standards. Insets are traces from fragment length analysis polymerase chain reaction and indicate the size of the entire hexanucleotide repeat region. A, The single individual of white race/ethnicity has 1 normal allele (inset) and 1 expanded allele (main trace). B, A healthy control subject has 2 normal alleles (inset).

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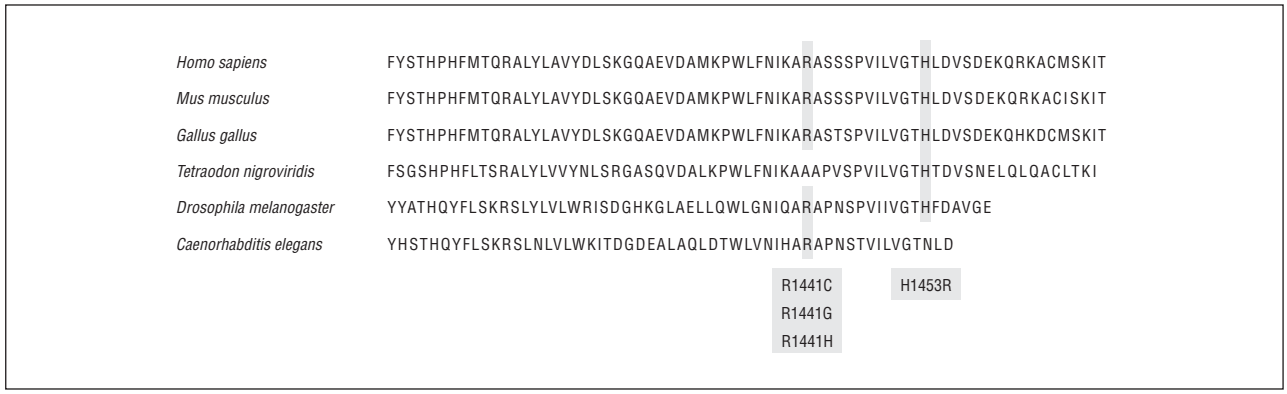

Figure 2. Alignment of the *LRRK2* amino acid sequence corresponding to the human sequence of 1401 to 1470. H1453 occurs near 3 known pathogenic mutations (R1441C, R1441G, and R1441H).

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.

COMMENT

The genetic origin of Guamanian ALS-PDC remains largely unknown. *MAPT* alleles contribute to disease susceptibility but do not cause ALS-PDC.[5,6] 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.[20] 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.[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.

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REFERENCES

1. Kurland LT, Mulder DW. Epidemiologic investigations of amyotrophic lateral sclerosis, I: preliminary report on geographic distribution, with special reference to the Mariana Islands, including clinical and pathologic observations. *Neurology*. 1954;4(5):355-378.
2. Hirano A, Kurland LT, Krooth RS, Lessell S. Parkinsonism-dementia complex, an endemic disease on the island of Guam, I: clinical features. *Brain*. 1961; 84:642-661.
3. Buee-Scherrer V, Buee L, Hof PR, et al. Neurofibrillary degeneration in amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam: immunochemical characterization of tau proteins. *Am J Pathol*. 1995;146(4):924-932.
4. Borenstein AR, Mortimer JA, Schofield E, et al. Cycad exposure and risk of dementia, MCI, and PDC in the Chamorro population of Guam. *Neurology*. 2007; 68(21):1764-1771.
5. Sundar PD, Yu CE, Sieh W, et al. Two sites in the *MAPT* region confer genetic risk for Guam ALS/PDC and dementia. *Hum Mol Genet*. 2007;16(3):295-306.
6. Poorkaj P, Tsuang D, Wijsman EM, et al. *TAU* as a susceptibility gene for amyotropic lateral sclerosis–parkinsonism dementia complex of Guam. *Arch Neurol*. 2001;58(11):1871-1878.
7. Yu CE, Bird TD, Bekris LM, et al. The spectrum of mutations in progranulin: a collaborative study screening 545 cases of neurodegeneration. *Arch Neurol*. 2010; 67(2):161-170.
8. Harlin MC, Crawford F, Perl DP, Steele J, Hardy J. Sequencing of exons 16 and 17 of the β-amyloid precursor protein gene reveals the β-amyloid sequence to be normal in cases of the Parkinson dementia complex of Guam. *J Neural Transm Park Dis Dement Sect*. 1993;5(1):63-65.
9. Sieh W, Choi Y, Chapman NH, et al. Identification of novel susceptibility loci for Guam neurodegenerative disease: challenges of genome scans in genetic isolates. *Hum Mol Genet*. 2009;18(19):3725-3738.
10. Kuzuhara S, Kokubo Y, Sasaki R, et al. Familial amyotrophic lateral sclerosis and parkinsonism-dementia complex of the Kii Peninsula of Japan: clinical and neuropathological study and tau analysis. *Ann Neurol*. 2001;49(4):501-511.
11. Itoh N, Ishiguro K, Arai H, et al. Biochemical and ultrastructural study of neurofibrillary tangles in amyotrophic lateral sclerosis/parkinsonism–dementia complex in the Kii peninsula of Japan. *J Neuropathol Exp Neurol*. 2003;62(7):791-798.
12. Renton AE, Majounie E, Waite A, et al. ITALSGEN Consortium. A hexanucleotide repeat expansion in *C9ORF72* is the cause of chromosome 9p21–linked ALS-FTD. *Neuron*. 2011;72(2):257-268.
13. 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(2):245-256.
14. O'Dowd S, Curtin D, Waite AJ, et al. *C9ORF72* expansion in amyotrophic lateral sclerosis/frontotemporal dementia also causes parkinsonism. *Mov Disord*. 2012; 27(8):1072-1074.
15. Ishiura H, Takahashi Y, Mitsui J, et al. *C9ORF72* repeat expansion in amyotrophic lateral sclerosis in the Kii Peninsula of Japan. *Arch Neurol*. 2012;69(9):1154-1158.
16. Hardy J, Gwinn-Hardy K, McGeer P, Morris H, Perez-Tur J, Steele J. Clinical features and changing patterns of neurodegenerative disorders on Guam, 1997-2000. *Neurology*. 2002;59(7):1121.
17. Galasko D, Salmon D, Gamst A, et al. Prevalence of dementia in Chamorros on Guam: relationship to age, gender, education, and APOE. *Neurology*. 2007;68 (21):1772-1781.
18. Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2000;1(5):293-299.
19. Zabetian CP, Yamamoto M, Lopez AN, et al. *LRRK2* mutations and risk variants in Japanese patients with Parkinson's disease. *Mov Disord*. 2009;24(7):1034-1041.
20. Kikugawa K, Nakano R, Inuzuka T, et al. A missense mutation in the *SOD1* gene in patients with amyotrophic lateral sclerosis from the Kii Peninsula and its vicinity, Japan. *Neurogenetics*. 1997;1(2):113-115.

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