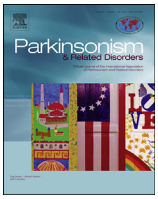
ARTICLE IN PRESS

Parkinsonism and Related Disorders xxx (2015) 1–5

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis



Genetic analysis of ten common degenerative hereditary ataxia loci in patients with essential tremor

L.N. Clark [a], [b], [*], X. Ye [a], X. Liu [a], K. Mirzozoda [a], E.D. Louis [c], [d]

[a] *Department of Pathology and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY, USA*
[b] *Taub Institute for Research on Alzheimer's Disease and the Aging Brain, College of Physicians and Surgeons, Columbia University, New York, NY, USA*
[c] *Department of Neurology, Yale School of Medicine, Yale University, New Haven, CT, USA*
[d] *Department of Chronic Disease Epidemiology, Yale School of Public Health, Yale University, New Haven, CT, USA*

ARTICLE INFO

Article history:
Received 25 February 2015
Received in revised form
1 May 2015
Accepted 3 June 2015

Keywords:
Essential tremor
Spinocerebellar ataxia loci
CAG repeat expansions
Genetics

ABSTRACT

Background: To investigate the association of repeat expansion size in 10 common degenerative hereditary ataxia genes with essential tremor. These genes were spinocerebellar ataxia (SCA)-1 (*ATXN1*), SCA-2 (*ATXN2*), SCA-3 (*ATXN3*), SCA-6 (*CACNA1A*), SCA-7 (*ATXN7*), SCA-8 (*ATXN8OS*), SCA-10 (*ATXN10*), SCA-12 (*PPP2R2B*), SCA-17 (*TBP*) and dentatorubral-pallidolysian atrophy (DRPLA) (*ATN1*).
Methods: Genetic analysis of repeat size in 10 degenerative hereditary ataxia loci was performed in 323 essential tremor patients and 299 controls enrolled at Columbia University. To test for differences in the allele distribution between patients and controls, a CLUMP analysis was performed.
Results: None of the essential tremor patients had a repeat expansion in the intermediate or pathogenic range. Significant differences in the distribution of repeats in the 'normal' range for SCA2 and SCA8 (both $p \leq 0.02$) were observed between essential tremor patients and controls.
Conclusions: Our study suggests that pathogenic repeat expansions in SCA loci are not associated with essential tremor.

1. Introduction

Essential tremor (ET), a disease whose hallmark feature is kinetic tremor (i.e., tremor during voluntary movements), is the most common cause of abnormal tremor in humans [1]. Prevalence increases with age, reaching ~20% during the 9th–10th decade of life [1].There are >30 prevalence studies from around the world, with an estimated 7 million affected individuals in the US alone (i.e., approximately 2.2% of the population). The disease is usually progressive and there is no cure.

Family studies and twin studies [2] provide strong evidence for a genetic contribution to ET, with heritability estimates ranging from 45 to 90% in twin studies [2]. Previously, we and others reported that ET aggregates in families, with many families containing multiple members with ET.

Patients with ET often have cerebellar signs, including intention tremor and mild gait ataxia, and recent postmortem studies are revealing degenerative changes in the cerebellum, including but not limited to Purkinje cell loss in some studies [3]. Hence, there is clinical-pathological overlap with the degenerative hereditary ataxias, including the spinocerebellar ataxias (SCAs). The autosomal dominant SCAs are neurodegenerative disorders that are clinically and genetically heterogeneous and neuropathologically characterized by degenerative changes in the cerebellum [4]. Currently, 31 SCA types are known (SCA1-36), with a prevalence (in Europe) of 3 per 100,000, with SCA1, SCA2, SCA3 and SCA6 the most common SCAs worldwide. Different types of mutations have been described at each SCA locus, including point mutations (SCA13 and 27), frameshift (SCA11), deletions (SCA15) and duplications (SCA20), CAG coding repeat expansions (SCA1-3, 6, 7, 17) and noncoding triplet, quintuplet or hexaplet repeat expansions (SCA8, 19, 12, 31, 36).

---

[*] Corresponding author. College of Physicians and Surgeons Building, Room 420A, Department of Pathology and Cell Biology, 650 West 168th Street, New York, NY, 10032, USA.

E-mail addresses: lc654@cumc.columbia.edu (L.N. Clark), xy2177@cumc.columbia.edu (X. Ye), xl2269@cumc.columbia.edu (X. Liu), km2825@columbia.edu (K. Mirzozoda), elan.louis@yale.edu (E.D. Louis).

http://dx.doi.org/10.1016/j.parkreldis.2015.06.004

Please cite this article in press as: L.N. Clark, et al., Genetic analysis of ten common degenerative hereditary ataxia loci in patients with essential tremor, Parkinsonism and Related Disorders (2015), http://dx.doi.org/10.1016/j.parkreldis.2015.06.004

There is prominent genetic anticipation in the SCAs that involve triplet repeat expansions. The presence of genetic anticipation has been suggested in some ET families as well [5].

In the current study we investigated an association of repeat expansion size in 10 common hereditary degenerative ataxia (esp., SCA) genes: SCA-1 (*ATXN1*), SCA-2 (*ATXN2*), SCA-3 (*ATXN3*), SCA-6 (*CACNA1A*), SCA-7 (*ATXN7*), SCA-8 (*ATXN8OS*), SCA-10 (*ATXN10*), SCA-12 (*PPP2R2B*), SCA-17 (*TBP*) and DRPLA (*ATN1*) in a clinical-epidemiological study of ET at Columbia University.

2. Patients and methods

As described, ET patients (n = 323) were enrolled in an clinical-epidemiological study at the Neurological Institute of New York, Columbia University, New York (2000–2007) [6]. Controls (n = 299) were ascertained from the same set of zip codes as ET patients, and were recruited using random-digit telephone dialing, and frequency-matched on age (5-year strata), gender and race categories. Each control was initially screened for tremor using a screening questionnaire and later underwent the same detailed videotaped neurological examination as the patients to ensure that they did not have ET. All participants underwent a demographic and medical history questionnaire, a family history questionnaire (any first- or second-degree relative with nonspecific tremor, ET or Parkinson's disease [PD]), and a videotaped neurological examination. Self-reported information on race and ethnic group was obtained. Beginning in 2002, self-reported information on Ashkenazi Jewish (AJ) ancestry was also collected. Data on age of onset of tremor, which we have shown to be reliable, were by self-report. On the basis of previous data on the distribution of age of onset in ET, early age of onset was designated as ≤40 years of age.

After review of the history and videotaped examinations, the diagnosis of ET was then reconfirmed by a senior neurologist specializing in movement disorders (E.D.L) using published research criteria for possible, probable or definite ET, which all required moderate amplitude or greater kinetic tremor on several tasks. Definite ET required both a moderate or greater amplitude postural tremor and moderate or greater amplitude kinetic tremor on 4 or more tasks, in the setting of no other tremor etiology. The presence of bradykinesia or any other sign of parkinsonism (except isolated rest tremor) was an exclusionary criterion for ET. No patients or controls had a history of amyotrophic lateral sclerosis (ALS) or evidence of ALS on neurological examination.

The study was approved by the Institutional Review Board at Columbia University and signed informed consent was obtained from all enrollees.

3. Genotyping/SCA repeat analysis

PCR was performed using fluorescently-labeled primers flanking the respective repeats in SCA-1 (*ATXN1*), SCA-2 (*ATXN2*), SCA-3 (*ATXN3*), SCA-6 (*CACNA1A*), SCA-7 (*ATXN7*), SCA-8 (*ATXN8OS*), SCA-10 (*ATXN10*), SCA-12 (*PPP2R2B*), SCA-17 (*TBP*) and DRPLA (*ATN1*) (primers and PCR conditions are available upon request). Following amplification, the PCR products were separated and allele sizes determined on an ABI3730xl genetic analyzer. Data were captured using Peak Scanner software v1.0 (Applied Biosystems, Inc.) and allele size was defined based on comparison with the molecular weight standard Gene Scan 500 LIZ (Applied Biosystems, Inc.). The following positive controls, with repeat sizes determined by sequencing, were also used to accurately determine repeat size. For SCA2, a 22 repeat (19CAG + 3CAA) gene scan and genemer control DNA was used (Gene Link Inc, Cat#40-2038-01). For other loci, the following controls with known CAG repeat size were obtained from the Coriell Institute for Medical Research (Camden, NJ): SCA1 (NA06926, 29/52 CAG repeats; NA13537, 32/60 CAG repeats), SCA2 (NA14982, 24/42 CAG repeats), SCA7 (NA03561, 8/62 CAG repeats). For SCA3 and SCA6 patient samples with known repeat sizes were used as positive controls (SCA3, 29/32 CAG repeats and SCA6, 11/13 repeats).

4. Statistical analysis

Allele frequencies were calculated from observed genotypes. CLUMP analysis [7], used for association testing when markers produce sparse contingency tables, was used to test differences in allele distribution between ET patients and controls. The stratified analyses involved a separate clump analysis. In stratified clump analyses, matching was accounted for by variable (e.g. ethnicity or gender). In the stratified analyses, based on age at ET onset (≤40 years vs. >40 years), ET cases (≤40 years or >40 years) were compared to all controls.

5. Results

5.1. Demographic and clinical characteristics of subjects

A total of 622 subjects (323 ET patients and 299 controls), were analyzed for repeat expansion size in ten gene loci (Table 1). The

Table 1
Demographic and clinical characteristic of genotyped subjects.

	ET cases (N = 323)	Controls (N = 299)	Statistical test	p-value
% Male (n)	48.3 (156)	41.8 (125)	$X^2 = 2.39$	0.1221
Mean age at tremor onset (years) (SD)	44.2 (21.9)	NA	NA	NA
% with family history of ET (n)	29.4 (95)	NA	NA	NA
% Ashkenazi Jewish ancestry (n)*	38.7 (125)	19.7 (59)	$X^2 = 25.91$	<0.0001
% Age at onset ≤ 40 yr (n)[a]	39.6 (128)	NA	NA	NA
% Age at onset > 40 yr (n)[a]	56.0 (181)	NA	NA	NA
% Non-hispanic White (n)[b]	93.8 (303)	86.0 (257)	$X^2 = 9.82$	0.0017
% Non-hispanic Black (n)[b]	2.2 (7)	5.7 (17)	$X^2 = 4.28$	0.0386
% Hispanic (n)[b]	2.5 (8)	4.0 (12)	$X^2 = 0.74$	0.3897
% Non-hispanic White-AJ (n)*	38.1 (123)	19.1 (57)	$X^2 = 26.39$	<0.0001

*Statistical significance P < 0.0001.

[a] Age at onset data not available for 14 ET cases.

[b] Ethnicity data not available on 5 ET cases and 13 controls; NA, Not applicable.

Please cite this article in press as: L.N. Clark, et al., Genetic analysis of ten common degenerative hereditary ataxia loci in patients with essential tremor, Parkinsonism and Related Disorders (2015), http://dx.doi.org/10.1016/j.parkreldis.2015.06.004