Neurobiology of Aging xxx (2014) 1–7

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Linkage analysis and whole-exome sequencing exclude extra mutations responsible for the parkinsonian phenotype of spinocerebellar ataxia-2

Chaodong Wang [a,b,c,d,1], Yanming Xu [e,1], Xiuli Feng [f], Jinghong Ma [a,b,c], Shu Xie [f], Yanli Zhang [a], Bei-Sha Tang [g], Piu Chan [a,b,c,h,i,*]

[a] Department of Neurobiology, Xuanwu Hospital of Capital Medical University, Beijing, China
[b] Department of Neurology, Beijing Institute of Geriatrics, Xuanwu Hospital of Capital Medical University, Beijing, China
[c] Beijing Institute for Brain Disorders Parkinson's Disease Center, Beijing, China
[d] Department of Neurology, The Affiliated Sanming First Hospital of Fujian Medical University, Sanming, Fujian, China
[e] Department of Neurology, West China Hospital of Sichuan University, Chengdu, China
[f] National Human Genome Center in Beijing, Beijing, China
[g] Department of Neurology, Xiangya Hospital of Central South University, Changsha, China
[h] Key Laboratory on Neurodegenerative Disease of Ministry of Education, Beijing, China
[i] Key Laboratory on Parkinson's Disease of Beijing, Beijing, China

ARTICLE INFO

Article history:
Received 23 July 2014
Accepted 27 July 2014

Keywords:
Spinocerebellar ataxia-2
Parkinsonism
Linkage analysis
Whole-exome sequencing
Mutation

ABSTRACT

CAG expansion within the exon 1 of ataxin-2 (*ATXN2*) gene responsible for spinocerebellar ataxia-2 (SCA2) has been reported to cause pure parkinsonism and other neurodegenerative disorders. However, it remains unclear whether CAG expansion is the only cause for SCA2 and its clinical alternatives, and whether extra mutations exist to modify the phenotypic diversity. To address this, we have conducted fine genetic mapping and exome sequencing for a large Chinese SCA2 pedigree predominantly manifesting parkinsonism (called SCA2-P). In addition, we compared the CAG expansions between the SCA2-P and 16 SCA2 families presenting as pure ataxia (SCA2-A). As a result, CAG repeat expansions, ranging from 37 to 40 copies, were detected among 10 affected and 8 nonsymptomatic members of the SCA2-P family. The CAG repeats in the diseased alleles were interrupted by CAA in the 3′-end. In contrast, CAG expansion ranging from 36 to 54 without CAA interruption was detected in all probands of the SCA2-A families. Genetic mapping located the SCA2-P pedigree on 12q24.21, which spans the *ATXN2* gene. Exome sequencing for 3 patients and 1 normal member revealed no extra mutations in this family. In addition, by genotyping single-nucleotide polymorphisms around SCA2 locus, we have excluded the existence of haplotypes predisposing different patterns of CAG expansion. These results demonstrate that the ATXN2 CAG expansion is the sole causative mutation responsible for SCA2-P, and that genetic modifiers may not be the major cause of the phenotypic diversity of SCA2.

1. Introduction

Mutations in several genes (*SNCA*, *LRRK2*, *VPS35*, *EIF4G1*, and so forth) have been identified in patients with autosomal dominant Parkisonsim (ADP) (Chartier-Harlin et al., 2011; Paisan-Ruiz et al., 2004; Polymeropoulos et al., 1997; Zimprich et al., 2011). However, such mutations have been proven to be rare in Chinese (Chan et al., 2000, 2013; Lin et al., 2008; Zhang et al., 2012). Although other mutations (G2385R and K616R) in *LRRK2* gene have been reported in Chinese ADP (Guo et al., 2013; Wang et al., 2010), these reports have not been verified extensively, raising the need to screen other causative mutations in this population.

Over the recent years, trinucleotide expansion, (CAG)n, responsible for a number of autosomal dominant cerebellar ataxias (also called spinocerebellar ataxias, SCAs), has been recognized as a cause of parkinsonism in some families, especially in those with spinocerebellar ataxia-2 (SCA2) and SCA3 (Furtado et al., 2004; Lim et al., 2006; Wang et al., 2009; Wu et al., 2004). Genetic analyses revealed that SCA2-related Parkinsonism

* Corresponding author at: Department of Neurobiology, Xuanwu Hospital of Capital Medical University, #45 Changchun Street, 100053 Beijing, China. Tel.: +86 10 83198677; fax: +86 10 83161294.
E-mail address: pbchan90@gmail.com (P. Chan).
[1] These authors contributed equally to this work.

0197-4580/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2014.07.039

(SCA2-P) is associated with relatively mild CAG repeat expansions ranging from 33 to 43 in the exon 1 of the ataxin-2 (*ATXN2*) gene, which are interrupted by a CAA interruption, whereas those presenting as pure ataxia (SCA2-A) patients harbor a high-range expansion from 32 to over 200 without the CAA interruption (Charles et al., 2007). More recent studies have demonstrated that moderate expanded ATXN2 repeats are also a genetic risk factor for multiple other neurodegenerative diseases such as amyotrophic lateral sclerosis, frontotemporal lobar degeneration, Alzheimer's disease, progressive supranuclear palsy, and multisystem atrophy, and, more importantly, the expanded repeat alleles in a selection of such patients can also show CAA interruptions (Liu et al., 2013; Ross et al., 2011; Van Langenhove et al., 2012). However, it is unclear why CAG mutations in the same gene that encode the identical mutant protein can lead to considerably variable clinical manifestations.

To resolve the mechanisms underlying the SCA2 phenotypic diversity, especially parkinsonism, 2 fundamental questions need to be but have never been answered: whether ATXN2 CAG expansion is the only cause for SCA2 and its clinical alternatives, are there extra mutations modifying the phenotype? What is the molecular basis of selective expression of expanded polyQ in different pathologically involved tissues? To address these questions, we have conducted fine genetic mapping and whole-exome sequencing in a large Chinese pedigree with autosomal dominant parkinsonism carrying the ATXN2 CAG expansion. We also compared the DNA sequence of the core promoter of ATXN2 between SCA2-P and SCA2-A, which usually determines the tissue-specific expression of the gene.

2. Methods

2.1. Study subjects

A large pedigree (Fig. 1) predominantly manifesting parkinsonian symptoms with autosomal dominant inheritance residing in Hubei Province in Central China was initially visited in 2007 and reported by another group (Sun et al., 2011) and revisited in 2011 by neurologists from Xuanwu Hospital, Capital Medical University. Clinical assessment was performed by specialists in movement disorders. Diagnosis of parkinsonism was established according to the UK Parkinson's Disease Society Brain Bank criteria. In addition to the SCA2-P families, 16 SCA2-P families presenting with pure ataxic symptoms and classified by molecular analysis as SCA2 were collected by collaborators from Xiangya Hospital of Central South University. Peripheral blood was collected, and DNA was extracted for genetic analysis. Written informed consent was obtained from each participating subjects, and the study was approved by Institutional Ethics Committee at both Xuanwu Hospital and Xiangya Hospital.

2.2. Analysis of trinucleotide expansions within the ATXN2 gene

Amplification of the CAG repeat region was performed with the following primers selected from the flanking sequence of the repeat region. Forward: 5′-GGGCCCCTCACCATGTCG-3′; reverse: 5′-CGGGCTTGCGGACATTGG-3′. The amplified fragment covers both the CAG repeats and the 2 single-nucleotide polymorphisms (SNPs), rs695871 and rs695872, which were used for SNP haplotype analysis. For sizing of the CAG repeats, fluorescence-polymerase chain reaction products were analyzed on an ABI-Prism 3100 automatic sequencer (PE Applied Biosystems, Foster City, CA, USA) using the GS500 size standard. For sequence analysis of the *ATXN2* alleles, TA cloning strategy were used as previously reported. The rs695871-rs695872 haplotype and its linkage to the CAG repeats were determined according to the sequence.

2.3. Whole-genome scan and fine mapping by 2-point linkage analysis

Whole-genome screening for the disease locus for the large SCA2-P pedigree was performed using 382 microsatellite markers from the ABI PRISM Linkage Mapping Set Version 2 (Applied Biosystems). Microsatellites for fine-mapping were chosen from the Genethon linkage map and the Marshfield sex-average linkage map (http://research.marshfieldclinic.org/genetics) and fluorescence-labeled with 6-FAM or HEX. Polymerase chain reaction (PCR) was performed using 20 ng of DNA, 5 pmol of each primers, 0.25 mM each of dNTPs, 15 mM Tris-HCL (pH 8.0), 2.5 mM $MgCl_2$, and 0.5 units of DNA polymerase (AmpliTaq Gold; Applied Biosystem). Amplification conditions were as follows: preincubation at 95 °C for 12 minutes, 10 cycles of denaturing at 94 °C for 15 seconds, annealing at 55 °C for 15 seconds, and extension at 72 °C for 30 seconds, and final extenteion at 72 °C for 30 seconds. The PCR products were mixed with a gel-loading cocktail containing molecular weight standards (Genescan 400HDROX) and analyzed on 4% polyacrylamide denaturing gels with ABI PRISM 377 DNA sequencer (Applied Biosystems). Microsatellite alleles were determined with GeneScan 3.0 and Genotyper 2.1 softwares.

Log10 of-odds scores were calculated using the MLINK program of the LINKAGE package. The parameters used in linkage analysis were autosomal dominant inheritance, complete penetrance, a mutation rate of zero, equal male-female recombination rate, equal microsatellite-allele frequency, and a disease-allele frequency of 1 in 10,000.

2.4. Haplotype analysis of microsatellite markers around the ATXN2 locus

Haplotype analysis of the SCA2-P family consisting of affected and unaffected members was carried out using microsatellite

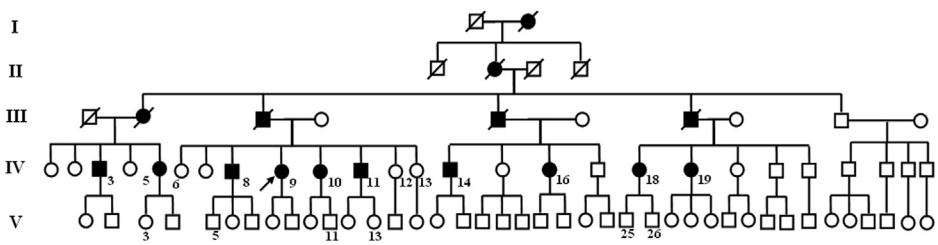

Fig. 1. The large Chinese pedigree of SCA2-Parkinsonism. The family consists of 82 members in 5 generations, with 16 affected members (arrow indicates the proband). The inheritance pattern is autosomal dominant. Age at onset varied from 32 to 45 years and disease duration ranged from 1 to 15 years. All the patients showed predominantly parkinsonian symptoms including resting tremor, bradykinesia, rigidity, and postural instability, which were almost symmetric. Only IV-10 presented mild cerebellar signs such as gait and limb ataxias and ataxic dysarthria, which occurred 4 years later after parkinsonian symptoms.