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Clinical Study

Analysis of mitochondrial DNA variations in a Chinese family with spinocerebellar ataxia

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ABSTRACT

Mitochondrial dysfunction and mitochondrial DNA (mtDNA) variations have been shown to have a role in several neurological diseases. To determine whether there is an association between mtDNA variations and spinocerebellar ataxia (SCA), we analyzed the mtDNA main control region (D-loop), as well as mtDNA content and the prevalence of the common deletion, in blood samples from members of a large Chinese family (14 individuals with SCA and 13 healthy individuals). All 14 individuals with SCA were genotyped as SCA3. Thirteen mtDNA haplotypes were identified among the 27 subjects. We detected no mutations in the mtDNA D-loop region and found no significant differences in mtDNA copy number or common deletion level between patients and their healthy relatives. Contrary to some previous reports, our study showed that mtDNA variations seem to be a rare event in individuals with SCA3.

1. Introduction

Autosomal dominant spinocerebellar ataxia (SCA) is a late-onset genetic disorder that can cause various slowly progressive clinical symptoms resulting from cerebellar dysfunction, including poor coordination of limbs, speech and eye movements.[1,2] The major cause of SCA is an expansion of CAG repeats in the coding region of an SCA-related gene, among which SCA1, SCA2, SCA3, SCA6, SCA7, SCA17 and DRPLA are the most commonly affected.[2] Proteins encoded by these genes are inactivated when the number of CAG repeats exceeds 40.[3] The inactivated polyglutamine proteins encoded by the defective genes eventually evoke neuronal cell dysfunction by altering calcium homeostasis and generation of reactive oxygen species.[4,5]

The mitochondria generate approximately 90% of ATP in most organs. Human mitochondrial DNA (mtDNA) encode the subunits of respiration chain complexes I, III, IV and V.[6,7] Mitochondrial dysfunction and mtDNA mutations have been associated with several neurological diseases, including Friedreich's ataxia,[8] Huntingon's disease[9] and myoclonic epilepsy with ragged red fiber disease.[10,11] Interestingly, one of the major clinical manifestations of all of these disorders is cerebellar ataxia, which is also a major symptom of SCA.

To explore whether mtDNA variations play a role in SCA, we analyzed mtDNA variations in a large Chinese family with SCA. We focused on the main control region (the 4977-base pair [bp] D-loop), and assessed common deletion level and mtDNA copy number, as these alterations are frequently found among patients with mitochondrial dysfunction.

2. Materials and methods

2.1. Sample collection

We reconstructed five generations of a large Chinese family, based on family members' recollections, as all members of the first and second generations were deceased at the time of the study. From the third and fifth generations, 14 individuals with SCA (average age 49.7 ± 12.44 years) and 13 healthy individuals (average age 47.8 ± 16.3 years) were recruited (Fig. 1). The informed consent of all subjects was obtained under protocols approved by the Ethical Committee of Wenzhou Medical College. Diagnosis, neurologic examinations (assessments of motor and sensory function, reflexes, and coordination of limbs) and MRI studies were carried out at the Affiliated Wenling Hospital of Wenzhou Medical College between April and May 2006. All patients had limb ataxia and an

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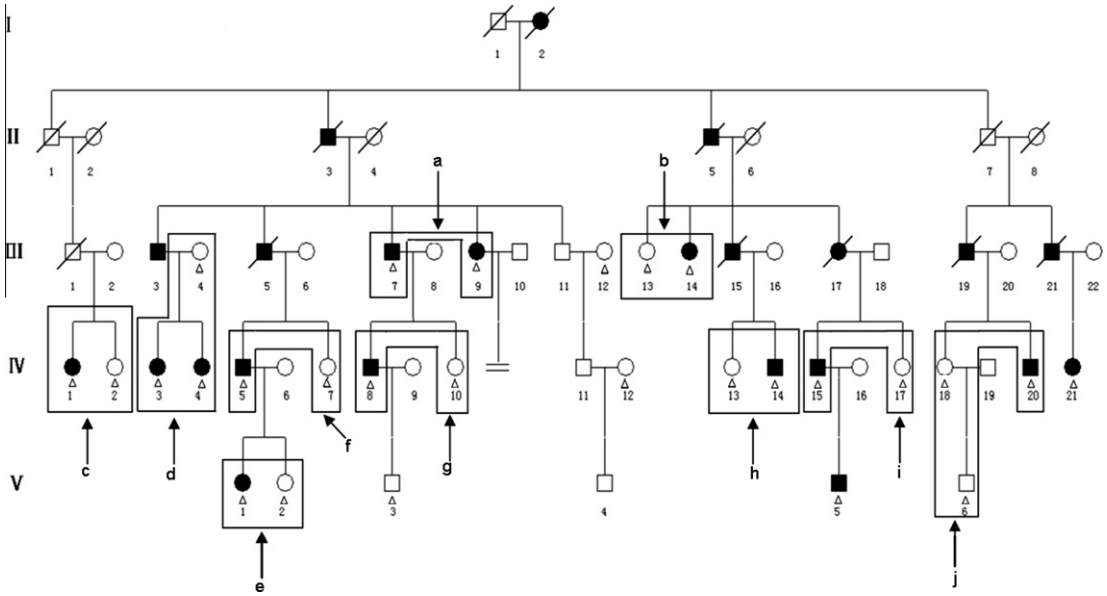

Fig. 1. Pedigree for a large Chinese family (males, squares; females, circles) including many individuals with spinocerebellar ataxia (solid symbols). The subjects of this study are labeled with a triangle (n = 27). Groupings of individuals with spinocerebellar ataxia and a maternal relative control are labeled with lower-case letters. Deceased persons are indicated by a diagonal line through the symbol.

abnormal Romberg test. MRI revealed marked cerebellar atrophy in three patients, III3, III9 and IV15.[12]

To avoid potential errors in the analysis of somatic mtDNA,[13] each individual with SCA was grouped with a healthy control with the same maternal ancestry (i.e. a mother, brother or sister). We termed this type of control the "maternal relative control" (MRC). A mtDNA mutation was deemed to exist only when an individual with SCA and their MRC had a different mtDNA nucleotide.[14] As shown in Fig. 1 and Table 1, there were 10 such groups containing an MRC (labeled "a" to "j"). Two patients, IV-21 and V-5, had no MRC available. Three additional related controls, III-13, IV-12 and V-3, were also included in this study. Data from the additional three controls were only used in analysis of copy number and common deletion level.

2.2. Spinocerebellar ataxia genotyping

Genomic DNA was extracted from peripheral blood using a standard phenol/chloroform method.[15] The primers listed in Table 1 were used for polymerase chain reaction (PCR) genotyping of SCA1, SCA2, SCA3, SCA6, SCA7, SCA17 and DRPLA (TaKaRa PCR Amplification Kit; TaKaRa, Tokyo, Japan). The PCR conditions were as follows: predenaturation at 95 °C for 5 minutes; 35 cycles of 94 °C for 30 seconds, 58 °C for 30 seconds, and 72 °C for 1 minute; then final extension at 72 °C for 10 minutes. The SCA genotypes were identified by PCR-polyacrylamide gel electrophoresis and sequencing (ABI prism 3730; Applied Biosystems, Foster City, CA, USA).

2.3. Mutation analysis of the mtDNA D-loop region

Primers used in this study were designed using Primer Premier 5.0 (Biosoft International, Palo Alto, CA, USA) and synthesized by TaKaRa. The following primers were used to amplify and sequence the whole D-loop region: L15792F, 5′-TCATTGGACAAGTAGCATCC-3′; H794R, 5′-AGGCTAAGCGTTTTGAGCTG-3′. PCR was carried out using the same conditions described above. Amplified fragments were first purified (DNA Fragment Purification Kit; TaKaRa) and then sequenced (ABI 3730 DNA analyzer; Applied Biosystems). Another set of primers was designed to cross the poly-C tracts at the 16189 and 310 regions in the sequencing reaction: H16559R, 5′-TCTTATTTAAGGGGAACGTG-3′; L16517F, 5′-CATCTGGTTCC-TACTTCAGG-3′. Sequences were aligned using CodonCode Aligner (version 3.0.1). Single nucleotide variations (SNPs) were identified by comparison with the Revised Cambridge Reference Sequence.[16] mtDNA haplotypes were determined for all subjects in accordance with the reconstructed East Asian phylogenetic tree.[17–19] Mutation analysis was carried out as described previously.[13]

2.4. Analysis of the 4977-bp common deletion

Nested PCR analysis was used to detect the 4977-bp common deletion, with primers as follows: Out8192F, 5′-AACCACAGTTTCATGCCCATC-3′; Out13663R, 5′-TGTTAGTAAGGG TGGGGAAGC-3′; Inner8261F, 5′-ACCCTATTGCACCCCCTCTAC-3′; Inner13575R, 5′-CTTGTCAGGGAGGTAGCGATG-3′. As reported previ-

Table 1
Primers used for the screening of spinocerebellar ataxia (SCA) genotype

Gene locus	Primer sequence	
	Forward primer	Reverse primer
SCA1	5′-AACTGGAAATGTGGACGTA-3′	5′-CAACATGGGCAGTCTGAG-3′
SCA2	5′-GGGCCCCTCACCATGTCG-3′	5′-CGGGCTTGCGGACATTGG-3′
SCA3	5′-CCAGTGACTACTTTGATTCG-3′	5′-CTTACCTAGATCACTCCCAA-3′
SCA6	5′-CACGTGTCCTATTCCCCTGTGATCC-3′	5′-TGGGTACCTCCGAGGGCCGCTGGTG-3′
SCA7	5′-TGTTACATTGTAGGAGCGGAA-3′	5′-CACGACTGTCCCAGCATCACTT-3′
SCA17	5′-ATGCCTTATGGCACTGGACTG-3′	5′-CTGCTGGGACGTTGACTGCTG-3′
DRPLA	5′-TCAGAGACCCCAGGGAGGGAGACAT-3′	5′-TAGCCAACAGCAATGCCCATCCAG-3′