Machado-Joseph disease/spinocerebellar ataxia 3: a clinical, imaging and genetic study in three Chinese families

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Abstract

Machado-Joseph disease (MJD) is an autosomal dominant inherited disorder which was recently determined to be the same disease as spinocerebellar ataxia type 3 (SCA3). It has been reported in various ethnic populations round the world. We describe clinical, imaging and genetic findings in 5 individuals from 3 Chinese families who have MJD/SCA 3. All 5 patients were females, with age between 21 to 45 years and duration of disease ranging 3 to 5 years. All had cerebellar ataxia, pyramidal signs and ophthalmoplegia. One had prominent extrapyramidal signs and severe dementia. Their CAG repeat size ranged from 59 to 69 and normal allele size ranged from 7 to 28. Magnetic Resonance Imaging revealed mild to moderate cerebellar atrophy and mild cerebral atrophy in all 5 patients. MJD/SCA 3 is present amongst the Singaporean Chinese population.

Key words: Machado-Joseph disease, Spinocerebellar ataxia 3, Chinese, Singaporeans

INTRODUCTION

Machado Joseph Disease (MJD) belongs to a group of autosomal dominant cerebellar ataxias (ADCAs) that are genotypically and phenotypically heterogeneous. ADCAs have been classified into types I, II and III based on phenotypic expression.1 ADCA I is clinically and genetically the most heterogeneous group, characterised by signs of cerebellar dysfunction, extrapyramidal signs, dementia, ophthalmoplegia and myoclonus. Subdivision of ADCA I into spinocerebellar ataxia (SCA) types 1 to 7 is based on genetic abnormalities. Genetic linkage studies have isolated at least 7 gene loci for the SCAs. Of these, the genes for SCA 1, SCA 2, SCA 3, SCA 6, SCA 7 have been identified with unstable CAG repeat expansion in their respective coding region (2,3,4). MJD and SCA 3 are now thought to be the same disease with the gene mutation localised in chromosome 14q.5-7

MJD was originally described in two ethnic families from the Azorean islands.8 Subsequently it has been reported in various ethnic populations around the world.19,10

This study is the first report on SCA3/MJD in Singapore. We describe the clinical, genetic and Magnetic Resonance Imaging (MRI) findings of five individuals from three separate families who had the MJD CAG trinucleotide repeat expansion.

MATERIALS AND METHODS

Patients

Between September 1997 to December 1997, five patients (all female) from 3 separate families seen in Singapore General Hospital with clinical suspicion of hereditary spinocerebellar ataxia had blood samples taken for MJD/CAG repeat expansion and also underwent MRI examination. These patients had cerebellar ataxia and a strong family history of similar disorder. Another 4 asymptomatic individuals from one of the families were also screened for MJD/SCA repeats.

Genetic Analysis

Genomic DNA was extracted from nuclei collected from lysed peripheral blood using standard phenol/chloroform method after proteinase K digestion. Two to five hundred nanograms of genomic DNA were amplified by polymerase chain reaction (PCR) using 3 MJD primers in two separate reactions (MJD 52/25, MJD 52/70).11 Amplification was performed


**TABLE 1: Demographic data, clinical characteristics and CAG repeat size of the patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset (years)</td>
<td>26</td>
<td>33</td>
<td>39</td>
<td>18</td>
<td>27</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Dysarthria</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Nystagmus</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Ophthalmoplegia</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Optic atrophy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tongue fascication</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dysmetria</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Gait ataxia</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Tone upper limbs</td>
<td>Spastic</td>
<td>Spastic</td>
<td>Spastic</td>
<td>Normal</td>
<td>Spastic</td>
</tr>
<tr>
<td>lower limbs</td>
<td>Spastic</td>
<td>Spastic</td>
<td>Spastic</td>
<td>Spastic</td>
<td>Spastic</td>
</tr>
<tr>
<td>Reflexes upper limbs</td>
<td>Hypereflexic</td>
<td>Hypereflexic</td>
<td>Hypereflexic</td>
<td>Normal</td>
<td>Hypereflexic</td>
</tr>
<tr>
<td>lower limbs</td>
<td>Hypereflexic</td>
<td>Hypereflexic</td>
<td>Hypereflexic</td>
<td>Hypereflexic</td>
<td>Hypereflexic</td>
</tr>
<tr>
<td>Dystonia</td>
<td>+++</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chorea</td>
<td>+++</td>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Amyotrophy</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Dementia</td>
<td>+++</td>
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<tr>
<td>Scoliosis</td>
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<td>0</td>
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<tr>
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<tr>
<td>CAG repeat size</td>
<td>69</td>
<td>63</td>
<td>59</td>
<td>61</td>
<td>63</td>
</tr>
</tbody>
</table>

Severity grading: 0 nil, + mild, ++ moderate, +++ severe

for 35 cycles with denaturing at 95°C for 3 minutes, annealing at 56°C for 1 minute and extension at 72°C for 2.5 minutes. The PCR products were electrophoresed in 2% agarose gel and 6% denaturing polyacrylamide gel. The product sizes were estimated by comparison with DNA size markers. PhiX174 Hae III digest and 100bp ladder was used as markers in agarose gel. STR markers (Promega) were used for polyacrylamide gel. The expected size of the PCR product for 21 repeats is 239bp (176bp + number of CAG repeats X3) using MJD 52/25, and 278bp (215bp + number of CAG repeats X3) using MJD 52/70. Confirmation of the CAG repeat nature of PCR products were carried out by hybridization using a pentamer CAG as probe.

**RESULTS**

**Clinical Characteristics**

The clinical characteristics is as in Table 1. All 5 patients were ethnic Chinese and female. They had dysarthria, nystagmus, limb and gait ataxia, increased deep tendon reflexes and ophthalmoplegia. In addition, Patient 1 had myopathic facies, limb dystonia, choreoathetosis and dementia.

**Molecular Analysis**

The molecular analysis is as in Fig. 1. CAG repeat expansion at the MJD locus was detected in 5 of the 9 individuals analysed. All 5 patients had signs of neurologic dysfunction. The 4 asymptomatic individuals had no repeat expansion at the MJD locus. Each of the 5 affected patients had an allele containing between 7 to 20 repeats and another allele between 59 to 69 repeats. These fall within the ranges of normal and expanded alleles reported for individuals with SCA3/MJD.12-15

Patients 1 and 2 were siblings from Family A (Fig. 2a). Their onset of neurologic dysfunction was at 26 and 33 years of age, and CAG repeats were 69 and 63 respectively. Patients 3 and 4 were mother and daughter from Family B (Fig. 2b). Age of onset were at 39 and 18 years old and repeats were 59 and 60 respectively. Patient 5 from Family C (Fig. 2c) had age of onset at 27 years old and repeats of 63. The 4 asymptomatic individuals were from Family B. The CAG
repeat normal alleles ranged from 7 to 22.

**MRI**

The results of the MRI study is as in Table 2. Spin echo T1 and T2 weighted scans of the brain in three orthogonal planes, as well as sagittal and axial scans of the cervical cord were acquired on a 1.0 or 1.5 Tesla MR system. Abnormal features were tabulated.

**DISCUSSION**

MJD, once thought to be of Azorean origin, has been widely described amongst various ethnic populations around the globe.\(^1\) It appears to be one of the commonest autosomal dominant spinocerebellar ataxia\(^{16}\) and a member of a growing list of neurodegenerative diseases (eg Huntington's disease, Kennedy syndrome) with expansion of a CAG repeat in the coding sequence of a gene resulting in an elongated polyglutamine tract in the translated protein. How the resultant expanded glutamine gives rise to the pathophysiological consequences remains an enigma. Recently, the MJD gene product has been identified to be a cytoplasmic protein widely expressed in various brain tissues.\(^{17}\)

In the past, SCA3 and MJD (both subtypes of ADCA I) were thought to be clinically distinct entities. SCA3 was believed to differ from MJD by less common occurrence of amyotrophy, dystonia and tongue fasciculation.\(^{13}\) But it has been shown that SCA3 and MJD have the same mutation, a trinucleotide expansion in chromosome 14q32.1.\(^{8,7}\) It seems likely that

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**FIG. 2:** Pedigrees of family A (a), family B (b) and family C (c) demonstrating autosomal dominant pattern of disease spanning three generations. Shaded and clear symbols represent affected and unaffected status respectively. A diagonal line indicates that the subject is deceased, and Pat = Patient.
TABLE 2: MRI findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Brainstem</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cervical cord</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Atrophy grading: 0 nil, + mild, ++ moderate, +++ severe

SCA 3 and MJD represent a clinical spectrum of the same disease.

In our present study, we analyzed the clinical, molecular and radiological features of 5 patients with ataxia, dysarthria, hyperreflexia and a positive family history suggestive of hereditary spinocerebellar ataxia.

All five patients had clinical features of spinocerebellar ataxia, but it would be difficult to confidently classify them into the various SCA subtypes. Patient 1, however, had additional features of limb dystonia, choreoathetosis, lingual fasciculation and myopathic facies which strongly suggested MJD. She had the most severe clinical manifestations (Table 1) although her symptom onset was not the earliest among our patients.

Anticipation (increasing severity and symptom onset in succeeding generation), a characteristic of trinucleotide repeat diseases was suggested in Family B. Patient 4, daughter of Patient 3, had much earlier onset of illness at 18 years of age compared to her mother’s disease onset at 38 years. However, her CAG repeat size at 60 was not much larger than her mother’s (59 repeats). Various studies have shown that a larger number of extranucleotide repeats correlated with earlier symptom onset. We could not confirm anticipation in Families A and C because both parents of these tested patients had passed away. However, the history given by our patients did suggest possible anticipation. Patient 1, who had more severe clinical abnormalities and earlier disease onset than Patient 2 had repeat size of 69 compared to 63. Other than the age of onset, the number of repeat size has not been conclusively shown to correlate with the clinical differences observed. Maruyama et al. however noted in their study that the subtype of MJD with dystonia showed a larger CAG repeat size than the other subtypes. Presently, the mechanism behind the clinical variation is not entirely clear. Some felt that the narrow range of CAG repeat size is unlikely to cause the varied clinical pattern. Perhaps the existence of somatic mosaicism (different tissues have differing number of CAG repeats) and presence of a ‘modifier gene’ may play a contributory role. Furthermore, the duration of the disease also affects the clinical variability to some extent.

In SCA3/MJD, normal allele and abnormal allele size range have been reported to be between 14 to 34 and between 56 to 84 respectively. Unlike Huntington’s disease, there has been no overlap between the normal and abnormal alleles for CAG repeat length. Our results concur, for in our patients, normal alleles range from 7 to 20 and CAG repeats range 59 to 69. Whether the size of the normal allele has any influence on the clinical severity is presently unknown. While almost all cases of MJD reported in the literature, including our patients, are heterozygotes, it must be remembered that juvenile onset cases with severe neurologic dysfunction can turn out to be homozygotes.

From their history, all of our patients traced their likely ancestry to various parts of Southern China (Fujian Province for patients 1, 2, 5 and Guangdong Province for patient 3, 4). While we could not verify their history, it does suggest possible clustering of this disease in certain geographical areas.

Significant differences in the frequency of the various subtypes have been described to occur in different population. Reports from Taiwan and China suggest that SCA3/MJD is the most common SCA subtype amongst the Chinese in Asia. SCA 3/MJD and SCA 6 appear to be the two commonest SCA in Japan. SCA3/MJD has also been reported in Australia. In India, SCA 2 is the most common SCA subtype. In Europe, SCA3/MJD is more is far more prevalent in Germany, France and Portugal than in Italy or United Kingdom.

For MRI features on SCA 3/MJD, Blurrk et al. found that they had only mild cerebellar and brainstem atrophy compared to typical changes of olivopontocerebellar atrophy (OPCA) in SCA 2 patients. SCA 1 on the other hand, had milder OPCA features. The MRI findings in all our 5 patients with MJD were concordant with Blurrk’s data. They showed non specific mild to moderate cerebellar, and cerebral atrophy (Table 2). Taniwaki et al. have recently demonstrated using Positron Emission Tomography (PET) that abnormalities of glucose metabolism can also be seen in other apparently normal regions (on MRI) of the brain.
FIG. 3: T2-weighted MRI scans of Patient 1. (a) Sagittal image of the posterior fossa and cervical cord demonstrating moderate, and mild atrophy of the cerebellar vermis, and the brainstem & cervical cord respectively. (b) Mild dilatation of the lateral ventricles indicating mild cerebral volume loss.
In conclusion, our preliminary data showed that SCA3/MJD is present in Singapore. Supported by clinical and imaging features, identification of the molecular defect causing the various SCAs can help in their classification and in distinguishing the hereditary SCAs from other types of cerebellar syndromes. However, it must be borne in mind that genetic testing in asymptomatic individuals who are at risk of developing the ADCAs, raises many disturbing ethical questions, for there is no effective treatment at present. Clinicians need to work closely with the affected and their family members to search for a common pathway upon which hopefully Science and Humanity can walk hand in hand.

ACKNOWLEDGEMENTS

The authors would like to thank the staff from the Departments of Neurology, Clinical Research and Diagnostic Radiology, Singapore General Hospital for their contributions. This work was supported by grants from National Medical Research Council (Singapore) and Ministry of Health.

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