Clinical Study

The MFN2 V705I Variant Is Not a Disease-Causing Mutation: A Segregation Analysis in a CMT2 Family

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Charcot-Marie-Tooth (CMT) disease is a clinically and genetically heterogeneous group of disorders affecting both motor and sensory neurons in the peripheral nervous system. Mutations in the MFN2 gene cause an axonal form of CMT, CMT2A. The V705I variant in MFN2 has been previously reported as a disease-causing mutation in families with CMT2. We identified an affected index patient from an Australian multigenerational family with the V705I variant. Segregation analysis showed that the V705I variant did not segregate with the disease phenotype and was present in control individuals with an allele frequency of 4.4%. We, therefore, propose that the V705I variant is a polymorphism and not a disease-causing mutation as previously reported.

1. Introduction

Charcot-Marie-Tooth (CMT), also known as hereditary motor and sensory neuropathy, is classified into two major categories: type 1 and type 2 based on the value of motor median nerve conduction velocity (NCV) [1]. CMT1, also known as the demyelinating form of CMT, is clinically characterized by slow NCVs due to myelin sheath abnormalities. CMT2 is an axonal degeneration and is characterized by the reduction of the amplitudes of motor and sensory nerve action potentials with relatively normal conduction velocities [2]. The most common subtype of CMT2 is CMT2A, an autosomal dominant axonal degeneration of motor and sensory nerves caused by mutations in the mitochondrial mitofusin-2 (MFN2) gene (MIM 608507) [3]. Mutations in the MFN2 gene are the most common cause for CMT2 [2–6]. MFN2 is involved in mitochondrial fusion and the maintenance of mitochondrial morphology [7–9]. Numerous studies have reported mutations in the MFN2 gene; the majority are point mutations [10]. We have identified the single base change c.2113G>A changing valine to isoleucine (V705I) of the MFN2 gene in a patient from a multigenerational Australian family (CMT105) with CMT2 and pyramidal signs. The V705I variant of MFN2 has been previously reported as a disease causing mutation in single individuals with CMT2 [11, 12]. To determine if this variant causes CMT2 in the family, we performed segregation analysis and tested the variant in a cohort of ethnically matched controls of English descent.

2. Methods

2.1. Subjects. Thirty-two individuals from a large, multigenerational Australian CMT2 family (CMT105) were tested (Figure 1). Sixteen individuals were clinically affected. Informed consent was obtained from all participants according to protocols approved by the Sydney Local Health District Human Ethics Committee. Genomic DNA was extracted from peripheral blood using standard methods by the Molecular Medicine Laboratory, Concord Hospital, Australia.

2.2. High Resolution Melt (HRM) Analysis of MFN2. The coding regions of the MFN2 gene were screened in the index
Table 1: Clinical motor and sensory electrophysiology study performed in the index patient IV-6 and individual III-2.

| Patient | Age | Motor MAP | Ulnar MAP | Sensory SAP | Ulnar CV |
|---------|-----|-----------|-----------|-------------|---------|
| IV-6    | 41y | 1.5       | 49        | 59          | 0       |
| III-2   | 66y | —         | 56        | 58          | 0       |

MAP: motor action potential (mV), CV: conduction velocity (m/s), SAP: sensory action potential (μV), NO: not obtainable, (—): not done. Normal motor values were as follows: median MAP > 4.2 mV, median CV > 49 m/s, ulnar MAP > 5.6 mV, and ulnar CV > 47 m/s. Normal sensory values were as follows: median SAP > 9.0 μV, median sensory CV > 56 m/s, ulnar SAP > 8 μV, and ulnar sensory CV > 55 m/s.

3. Results

3.1. Clinical Findings. Detailed clinical features of this large Australian family with CMT2 and pyramidal signs have been previously published [14, 15]. The family pedigree (Figure 1) shows autosomal dominant inheritance with the disease phenotype segregating in five generations. Affected individuals in the family showed a reduction of compound amplitudes of motor and sensory nerve action potentials. Nerve conduction velocities were within the normal range. The disease was slowly progressive. Two individuals (IV-6 and III-2) were wheelchair bound with severe CMT2. Electrophysiological findings of these two patients are shown (Table 1).

3.2. High Resolution Melt (HRM) Analysis. The MFN2 gene was screened by HRM analysis in the index patient (IV-6) from CMT105 and a differential melt curve for exon 18 was observed when compared with the melt curves of control individuals (Figure 2). Dideoxy sequence analysis identified the following nucleotide transition c.2113G > A (V705I). We tested for segregation of the variant in the family. A unique differential melt curve corresponded to individual IV-6 was obtained while the melt curves of other affected individuals tightly grouped together with unaffected family members and controls (Figure 2). This demonstrated that the V705I variant was absent in other affected family members and, therefore, the V705I was not segregating with the disease phenotype. To confirm the HRM findings, the index patient IV-6, two additional affected individuals, and a healthy individual from the family were sequenced. The analysis showed that the heterozygous V705I variant was present only in the index patient IV-6 while it was absent in other two affected individuals and the normal individual (Figure 3). No homozygous V705I variant was identified. We examined the frequency of the V705I in 57 healthy and ethnically matched controls of English descent and identified the heterozygous variant in five control samples (5/57) with an allele frequency of 4.4%.

4. Discussion

The MFN2 V705I variant was found in an index patient (IV-6) from a large kindred with CMT2 and pyramidal signs. No other known or novel CMT mutations were found in the family. The V705I variant did not segregate with the disease in the family. The mother (III-20) and brother (IV-7) of the index patient are clinically affected but the variant was absent in both cases, indicating that the V705I variant was probably inherited from the father who was said to be unaffected and, unfortunately, was not available for testing. Furthermore, another individual (III-2) had similar clinical
severity to the index patient (Table 1) and did not carry the V705I variant. This suggests that the V705I variant has no role in modifying the disease phenotype.

To test the frequency of this variant in the population, we screened 57 unrelated controls (114 chromosomes) and detected the heterozygous V705I variant in 4.4% of these subjects. Our data, therefore, suggests that the variant, in its heterozygous state, is not a disease-causing mutation as previously reported [11].

Compound heterozygous mutations in MFN2 have been identified in early onset CMT2 where MFN2 mutations are not pathogenic unless co-inherited with another MFN2 mutation [16–18]. However, the MFN2 gene was excluded in our family by linkage analysis [15]. Until a homozygous V705I variant is identified, the pathogenicity of the homozygous state will remain unknown and will require further studies if identified.

The MFN2 variant, V705I, was first reported as a pathogenic mutation in 2006 [11] in a proband clinically diagnosed with CMT2. This change leads to the exchange of the nonpolar valine to the nonpolar isoleucine at position 705 (c.2113G > A) (Figures 2 and 3). In the initial report, the variant was not detected in 212 ethnically matched control chromosomes from Norway. DNA samples from family members of the proband in the study were not available and therefore segregation of the variant has not been tested. The V705I variant has also been reported in two probands from unrelated Norwegian patients with CMT2 [12]. However, segregation of the variant in the families was not reported.

Our thirty-two family members allowed further validation studies of the variant. The current dbSNP (Build 137, Jun 2012) database (http://www.ncbi.nlm.nih.gov/projects/SNP/) showed 690 SNP variants in the MFN2 gene. The Inherited Peripheral Neuropathies Mutation database (http://www.molgen.ua.ac.be/CMTMutations/Home/Default.cfm) shows about 46 pathogenic mutations (6.3%). Therefore, any newly found variant is more likely to be a polymorphism than pathogenic mutation.

Proof that a DNA variant is a disease-causing mutation requires rigorous validation when reporting novel sequence
alterations [19–21]. This approach has been shown to be necessary in other diseases. The P1148A substitution in FBN1 gene was initially thought to be a pathogenic mutation causing Marfan syndrome in the majority of patients of Asian descent [22]. This variant was subsequently described as a common polymorphism in Asian populations [23, 24].

We have now shown that the heterozygous MFN2 V705I variant does not segregate with the disease proving that it is not a disease-causing mutation in this family. The identification of V705I in 5/57 ethnically matched normal controls establishes that in the heterozygote state this is not pathogenic. Our observation is supported by a recent report in dbSNP 135 (rs142271930), where the V705I variant was found in normal controls with minor allele frequency of 0.4% indicating that it is a rare variant in that population, whereas the frequency in the Australian population may be higher. Furthermore, our findings emphasise the importance of segregation studies and the use of many healthy controls from a variety of ethnic groups when describing novel, potentially pathogenic mutations.

5. Conclusion

We have shown that the heterozygous MFN2 V705I variant is a polymorphism and not a disease-causing mutation in our family. We have also previously excluded MFN2 by linkage analysis. As this was previously reported as a disease-causing mutation, our study highlights the importance of variant validation by segregation studies and genotyping in ethnically matched controls. Further studies are needed to investigate the pathogenicity of homozygous MFN2 V705I variant if identified.

Conflict of Interests

All authors have no financial relation with the commercial identity mentioned in the paper. There is no conflict of interests for any of the authors.

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