ORIGINAL ARTICLE

Homozygous mutations in MFN2 cause multiple symmetric lipomatosis associated with neuropathy

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Abstract

Multiple symmetric lipomatosis (MSL) is a mitochondrial disorder with impaired brown fat metabolism that has been associated with MERRF mutations in some, but not all, patients. We studied a sibling pair and an unrelated individual who presented with MSL and neuropathy to determine the genetic etiology of this disorder in patients who did not carry the MSL-associated MERRF mutation. Whole-exome sequencing was performed on the siblings, and a rare, shared homozygous mutation in MFN2 (c.2119C>T: p.R707W) was identified. The mutation was not present in their healthy siblings. In silico programs predict it to be pathogenic, and heterozygous carriers of the MFN2 p.R707W substitution are known to have Charcot-Marie-Tooth (CMT) disease. A third, unrelated patient with multiple symmetrical lipomatosis and neuropathy also harbored the same homozygous mutation and had been previously diagnosed with CMT. Functional studies in patient fibroblasts demonstrate that the p.R707W substitution impairs homotypic (MFN2–MFN2) protein interactions required for normal activity and renders mitochondria prone to perinuclear aggregation. These findings show that homozygous mutations at p.R707W in MFN2 are a novel cause of multiple symmetrical lipomatosis.

Introduction

Multiple symmetric lipomatosis (MSL, OMIM 151800) is a rare mitochondrial disorder of brown fat. Patients in their 20–40s present with cervical and thoracic lipomatosis with progressive distal weakness and neuropathy. Patients often develop diabetes mellitus (DM) later in the course of disease. A mitochondrial etiology was first suggested in 1991, when ragged red fibers (RRFs) were identified on muscle biopsies of patients with MSL (1). Since then, the m.8344A>G mutation associated with MERRF (2,3) and mitochondrial deletions have been identified in a subset of individuals with MSL (4), and conversely, multiple lipomatosis/lipomas have been described in 15–25% of the patients with...
MERRF syndrome (5). Familial recurrences consistent with matrilineal transmission of mitochondrial mutations have been reported. There remain, however, a large number of patients with clinically diagnosed MSL without identifiable MERRF-associated mutations, suggesting that there may be other genetic etiologies of MSL. Here we report three patients with MSL, two brothers and an unrelated individual, who harbor a recurrent homozygous mutation in MFN2 and propose that this mutation in MFN2 is a novel cause of MSL.

Results
Patients 1 and 2
Patients 1 and 2 are brothers (Fig. 1) who developed cervical and thoracic lipomatosis in their mid-20–40s. Patient 1 presented with two small lipomas on the back of his neck in his 20s. Over the course of the following 25 years, he developed striking unencapsulated cervical and thoracic lipomatosis and progressive tongue hypertrophy. He has undergone multiple liposuction surgeries, which were only of temporary benefit before the lesions returned. He has swallowing difficulties due to the bulk of the cervical lesion and tongue hypertrophy. At the age of 53 years, he presented with significant peripheral neuropathy, paresthesias and weakness and required a cane to ambulate by 58 years. Nerve conduction studies and EMG demonstrated length-dependent axonal polyneuropathy with evidence of denervation/reinnervation. He is on pregabalin for neuropathic pain. He was diagnosed with DM in his early 50s and is well controlled by oral hypoglycemic medication. His glucose control was improved after liposuction; fasting glucose and HbA1C were within the normal range a few weeks post-liposuction on his medications. Genetic testing for the MERRF mutation (m.8344A>G) was negative in a peripheral blood sample, as were other common mitochondrial mutations (m.3243A>G, m.3260A>G, m.3303C>T and m.8993T>G/C). Biochemical studies have repeatedly demonstrated an elevated lactate (5.02 and 4.7 mmol/l, upper limit of normal 2.2 mmol/l). His leptin was low at <0.6 ng/ml (0.7–5.3 ng/ml normal range),

Figure 1. (A–C) Patient 1 at 61 years of age with cervical and thoracic lipodystrophy with decreased subcutaneous fat on arms and lower thorax down to his legs. (D) Patient 2, brother of Patient 1, shown at age 63 years: post-liposuction surgery. (E and F) Patient 3 at 60 years with cervical and thoracic lipodystrophy with decreased subcutaneous fat on legs.
Patient 3

This patient has been previously described as individual 3 in Australian CMT40 family, described in Table 2 of (6). She presented with bilateral club feet at birth and had corrective surgery at 7 years of age, which was unsuccessful. Hearing loss was also noted in early childhood. At the age of 12 years, she had muscle wasting and weakness in her legs, and she could not feel heat or pain in her feet. At 34 years, she was fitted with hinged ankle-foot orthoses and at 48 years of age she required a cane to ambulate and a wheelchair for longer distances. She developed pronounced cervical and thoracic lipomatosis in her late 20s, as well as fatty nodules on her forearms and chest (Fig. 1). She had progressive muscle wasting of her lower extremities with distal weakness (one out of five at the ankles) and deltoid weakness at four of five by age 38 years. The distal and lower limb reflexes were absent, and at age 38 years, she had no detectable sensory action potentials, no lower limb motor responses and a median conduction velocity of 44 m/s with a latency of 3.3 ms and a distal latency of 3.3 ms. Magnetic resonance imaging (MRI) of the neck showed an accumulation of subcutaneous, unencapsulated fat posterior to the cervical spine folded to a depth of 10.5 cm extending down the lumbar spine with additional significant subcutaneous fat in the thorax, chest, abdomen pelvis and upper thigh (Fig. 2). No discrete lipomata were obvious. There was diffuse atrophy and fatty marbling of most of the musculature with some sparing of the iliopsoas muscles. There was a raised blood lactate at 3.1 mmol/l (upper limit of normal 2.1 mmol/l), and apolipoproteins A1 and B, cholesterol and triglycerides were normal range. A random blood cholesterol and triglycerides were normal range. A random blood glucose was 7.2 mmol/l. Sanger sequencing identified a homozygous substitution c.2119C>T;p.R707W (NM_001127660) in MFN2. Her parents and two daughters all had minor symptoms and signs suggestive of CMT including slow running, recurrent ankle sprains, difficulty heel walking and slight pes cavus or flat feet, but had normal neurophysiology and thus were not diagnosed with CMT.

Whole-exome sequencing

Given that the brothers had the same rare disease and there was a remote history of consanguinity, the variants were first filtered for shared rare variants carried in the homozygous state. WES identified a rare homozygous variant shared by both brothers in exon 17 of MFN2 at c.2119C>T;p.R707W (NM_001127660), which has been reported to be associated with CMT. The gene was in an extended region of homozygosity seen in the brothers that spanned 6.4 Mb in length, consistent with the remote history of consanguinity. This mutation was not present in either of the two unaffected siblings in a homozygous or heterozygous state. No other rare homozygous variants were identified that were shared by the brothers. The mutation was predicted to be pathogenic by SIFT, Polyphen2 and MutationTaster. The mutation is as adiponectin at 3 mcg/ml (normal range 4–20 mcg/ml). His creatine kinase (CK), cholesterol, triglycerides, low-density lipoprotein, homocysteine, apolipoprotein B and lipoprotein A were normal. His high-density lipoprotein was slightly low. A skin biopsy was sent for electron microscopy, and mitochondrial structure was unremarkable. He has no history of alcohol abuse.

Patient 2 had a history of ‘bowed limbs’ requiring casting in infancy; however, he was very healthy and physically active throughout early and mid-adulthood. He noted a change in his physique around age 45, with evidence of lipomatosis by his early 50s. He has a history of multiple encapsulated cervical and thoracic lipomas, with frequent surgical interventions. He has an approximately 15-year history of progressive neuropathy and normal HbA1C. He had elevated lactate (3.0 mmol/l, upper limit of normal 2.2 mmol/l). He also had an elevated CK as high as 550 U/l. He had a muscle biopsy as part of his workup, which reported marked variation in myofiber size and grouping by type, as well as diffuse expression of cd56 and esterase-positive myofibers, which together is suggestive of chronic denervation. The biopsy also identified rare vacuoles, increased internalized nuclei and rare RRFs, which are subtle myopathic features, though can also be seen secondary to denervation.

The brothers have two unaffected siblings. The parents were of Irish descent and are fifth-degree cousins. Both parents are deceased, and neither was diagnosed with Charcot–Marie–Tooth (CMT) disease nor MSL. Both Patients 1 and 2 have children in their early 20s, none of whom has apparent MSL or gait abnormalities.

Whole-exome sequencing

Figure 2. MRI findings in Patient 3. (A) MRI image showing homogeneous unencapsulated cervico-thoracic subcutaneous fat distribution. (B) MRI image showing normal image of the liver; there is no evidence of visceral fat deposition in the patient.
present in the heterozygous state in the ExAC database of 60,000 exomes, with a frequency of 0.0003. A broader analysis under different possible modes of inheritance (shared compound heterozygous variants, dominant or X-linked) was unrevealing.

Functional analysis of p.R707W

We performed cell-based experiments to probe for functional deficits conferred by the R707W mutation and compared results with the severe CMT2A mutation R94W. We observed no difference in mitochondrial morphology in fibroblasts from the index patient compared with normal subjects (Supplementary Material, Fig. S1), consistent with the normal morphology in fibroblasts from patients with severe CMT2A (7). Next, we assessed the capacity of MFN2 R707W to support mitochondrial fusion by monitoring mitochondrial morphology in cells depleted of endogenous MFN1 and MFN2 using RNA interference. Compared with wild-type MFN2, the R707W mutant showed reduced capacity to tubulate mitochondria (Fig. 3A and B). In addition, mitochondria were more prone to aggregation in cells expressing an MFN2 R707W cDNA tagged with the V5 epitope to facilitate detection (Fig. 3C). Co-immunoprecipitation (Co-IP) experiments in cells expressing V5 and flag-tagged MFN2 revealed a defect of MFN2 R707W to form homo-oligomers, whereas hetero-oligomer formation with MFN1 was unaffected (Fig. 3D). When cells expressing V5-tagged MFN2 mutants were subjected to the crosslinking agent bismaleimidohexane (BMH), we observed that MFN2 R707W formed smaller oligomeric complexes compared with wild-type MFN2 (Fig. 3E).

Discussion

We have identified a recurrent homozygous MFN2 mutation in patients from two unrelated families with MSL associated with neuropathy, thereby identifying a second disease gene responsible for this rare condition. Heterozygous mutations in the MFN2 gene cause CMT2A, and the p.R707W is a recognized CMT2A mutation (6); lipomatosis appears to be unique to individuals carrying this mutation in a homozygous state. There are three siblings described in the literature who are compound heterozygous carriers of the p.R707W and a p.G108R mutation associated with CMT2A and ‘lipodystrophy’ (8), although it is unclear whether MSL was a feature. Patients with compound heterozygote mutations in MFN2, which do not include the p.R707W mutation, have also been reported (6) without MSL or lipodystrophy. Thus, it appears that the p.R707W mutation, at least in the homozygous recessive form, is specific to the etiology of the MSL.
phenotype. Obligate carriers of the p.R707W mutation in these two families had not been diagnosed with CMT2, despite clinical assessment of the children of Patient 3, which may be secondary to a mild presentation, late onset or other modifying risk factors.

The phenotypic features of MSL show some overlap with those of familial partial lipodystrophy type 2 (FPLD2). Patients with FPLD2 present with late onset progressive lipatrophy in a similar pattern of distribution to our MSL patients and occasionally also demonstrate lipomas (9). FPLD2 is caused by mutations in LMNA, and interestingly, bi-allelic mutations in LMNA cause CMT disease type 2, which presents with axonal polyneuropathy.

The identification of MFN2 as an MSL disease gene contributes to our understanding of the role of mitochondria in brown fat metabolism. MFN2 encodes a GTPase of the dynamin family that is involved in homotypic interaction and fusion of apposed mitochondria (10). The p.R707W alteration is found within the heptad repeat 2 domain located at the C-terminal of MFN2, which is thought to permit homotypic interactions as well as heterotypic binding to MFN1. MFN2 homozygous p.R707W mutant cells demonstrate a reduced ability to form homotypic interactions in vitro and tubulate mitochondria. The presence of MFN1 has been proposed to mask the consequences of MFN2 disease alleles in most tissues (11). Studies looking at the relative amounts of MFN1 and MFN2 proteins in various tissues demonstrated that although MFN1 is enriched in the heart, in agreement with previous data (12), it was almost undetectable in brown fat (Fig. 3F), a tissue implicated in the pathogenesis of MSL. These data are in agreement with the notion that deleterious mutations in MFN2 can lead to mitochondrial dysfunction in brown fat (13) and support MFN2 as a novel gene causing MSL.

Materials and Methods
Whole-exome sequencing

Patients 1 and 2 underwent whole-exome sequencing as part of the Care4Rare Canada Consortium, with the objective to identify novel causes of rare disease. Institutional Research Ethics Board (Children’s Hospital of Eastern Ontario) approval of the study design was obtained, and informed consent was obtained. DNA was extracted from blood according to standard protocols. Targeted enrichment was performed using the Agilent SureSelect 50 Mb (V5) All Exon Kit. The Illumina HiSeq 2000 was used to sequence the captured product, and three samples were run per lane. Coverage of the exome was between 135 and 140×. WES data analysis was performed using our standard pipeline, as described previously (14). Variants were annotated with the functional type (e.g. nonsense versus missense), allele frequency data using an in-house control sample database (filtered to <3%) and frequency data from the Exome Variant Server (filtered to <3%) and from the 1000 genomes project (filtered to <5%). Candidate mutations were validated by Sanger sequencing and segregated in the siblings.

Functional analysis of p.R707W

Lentiviral preparation was performed as described previously (15). Lentiviral vector encoding MFN2 shRNA targeted the following sense sequence: GCAGGTTTACTGCGAGGAAAT. MFN1 siRNA was from Dharmacon (Lafayette, CO, USA) (L-010670-01). For lentiviral constructs expressing MFN2 and MFN1, MFN2 and MFN1 cDNA were gateway recombined into pLenti6.3 V5 DEST vectors (Invitrogen, Carlsbad, CA, USA). R94W, R707W and MFN2 shRNA-resistant cDNAs were generated by QuikChange site-directed mutagenesis. Antibodies to TOMM20 (FL-145) and mortalin were from Santa Cruz (Dallas, TX, USA). MFN1 antibody was obtained from Abcam (Cambridge, UK) (ab104274). MFN2 antibody was from Abnova (Taipei, Taiwan) (H00009927-M03). V5 antibody was from Invitrogen. For tissue blots, antibody to MFN1 was from Cell Signaling (Danvers, MA, USA). Flag (M2)-HRP antibody was from Sigma (St. Louis, MO, USA). Immunofluorescence, confocal microscopy and Co-IP were performed as described previously (16). Mitochondrial length measurements (aspect ratio) and BMM crosslinking experiments were performed, as described previously (17). Tissue for blots was harvested from C57BL/6 wild-type mice and subjected to homogenization using TissueLyser (Qiagen, Venlo, Limburg, the Netherlands). Insoluble material was removed by centrifugation prior to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and western blotting.

Supplementary Material

Supplementary Material is available at HMG online.

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