Mutation of DNAJC19, a human homolog of yeast inner mitochondrial membrane co-chaperones, causes DCMA syndrome, a novel autosomal recessive Barth syndrome-like condition.

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ABSTRACT

**Background:** We have identified a novel autosomal recessive condition, Dilated Cardiomyopathy with Ataxia (DCMA) Syndrome, in the Canadian Dariusleut Hutterite population characterized by early-onset dilated cardiomyopathy with conduction defects, non-progressive cerebellar ataxia, testicular dysgenesis, growth failure and 3-methylglutaconic aciduria. The aim of this study is to map DCMA syndrome, and identify the mutation underlying this condition.

**Methods:** A genome wide scan was performed on consanguineous Hutterite families using a homozygosity mapping approach in order to identify the DCMA associated chromosomal region. Mutation analysis was performed on positional candidate genes in this region by sequencing. RT-PCR and bioinformatics analyses were then used to characterize the mutation and determine its effect on the protein product.

**Results:** We report the association of DCMA syndrome with a 2.2 Mb region of chromosome 3q26.33, and the identification of a disease-associated mutation, IVS3-1 G>C, in the DNAJC19 gene, encoding a DNAJ domain-containing protein of previously unknown function (Entrez Gene ID 131118).

**Conclusions:** The DNAJC19 protein was previously localized to the mitochondria in cardiac myocytes, and shares sequence and organizational similarity with proteins from several species including two yeast mitochondrial inner membrane proteins, Mdj2p and Tim14. Tim14 is a component of the yeast inner mitochondrial membrane presequence translocase, suggesting that the unique phenotype of DCMA may be the result of defective mitochondrial protein import, and only the second human disorder due to defects in this pathway.
INTRODUCTION
Dilated cardiomyopathy with ataxia (DCMA) syndrome is a novel autosomal recessive disorder found in the Dariusleut Hutterite population, an endogamous population of the great plains region of Canada and the northern United States. The major clinical features of DCMA include a severe, early onset dilated cardiomyopathy (DCM), sometimes accompanied by Long QT syndrome (LQTS). Prenatal and/or postnatal growth failure is universally seen, as is a cerebellar syndrome with ataxia causing significant motor delays. Male genital anomalies are also frequently seen, and range from isolated cryptorchidism to severe perineal hypospadias. In addition, many patients have significant increases in urine organic acids particularly of 3-methylglutaconic (3-MGC) and 3-methylglutaric (3-MGA) acids. Additional features include optic atrophy, mild elevations of hepatic enzymes with microvesicular hepatic steatosis, a normochromic, microcytic anemia and mild to borderline non-progressive mental retardation. The combination of clinical features varies among patients, however cardiac involvement including DCM and LQTS, cerebellar ataxia and elevated levels of 3-MGC and 3-MGA are the hallmarks of DCMA syndrome.

Initially, DCMA syndrome presents in a very similar manner to Barth syndrome (Type II 3-methylglutaconic aciduria), which is an X-linked disorder caused by mutations in the TAZ gene and consists of DCM, elevated 3-MGC and 3-MGA levels as well as skeletal myopathy, neutropenia and growth failure. The lack of skeletal myopathy and immunological features however, as well as the cerebellar features and the autosomal recessive inheritance clearly distinguishes DCMA from Barth syndrome. DCMA syndrome also shares some resemblance with the other two defined classes of 3-methylglutaconic acidurias, however the levels of 3-MGC and 3-MGA are too low in DCMA to suggest a primary block in the 3-methylglutaconyl-CoA hydratase enzyme as in Type I 3-methylglutaconic aciduria, which is the result of mutations in the AUH gene. Cerebellar ataxia is a prominent feature in Costeff Optic Atrophy syndrome (Type III 3-methylglutaconic aciduria), a disorder of the Iraqi Jewish population resulting from mutations in the OPA3 gene. As the name suggests, early-onset bilateral optic atrophy is the most prominent feature of this disorder and none of the cardiac features of DCMA syndrome have been noted in this disorder. Optic atrophy has been seen in a few DCMA syndrome patients; however it is not considered one of the major features of DCMA. Type IV 3-methylglutaconic aciduria includes all other cases of 3-methylglutaconic aciduria that cannot be included in types I, II or III. Neurological, motor and sensory defects are common within this category, as well as cases of DCM and liver dysfunction. Cases included within this category likely result from a number of different causes and defects, as elevations in both 3-MCG and 3-MGA have been reported in several inborn errors of metabolism and also during pregnancy. All cases of DCMA that have been identified thus far are from the Dariusleut Hutterite population, however, it is possible that other cases of DCMA syndrome have been included within this category for lack of a better classification, and thus DCMA syndrome represents an additional category of the 3-methylglutaconic acidurias.

The characteristics of endogamous populations make it possible to map genetic conditions using a homozygosity mapping approach. By this approach, we aimed to map DCMA
syndrome by identifying regions of the genome that are identical by descent between affected patients from different consanguineous families. Here we report the association of DCMA syndrome with a 2.2 Mb segment of 3q26.33 and the identification of a splice mutation in a novel gene DNAJC19 in DCMA patients. The DNAJC19 protein shares similarity with the yeast TIM14 protein, a component of the mitochondrial protein import system, and DCMA syndrome may represent only the second human disorder associated with defects in mitochondrial protein import.

METHODS
Patients and Controls
This study was approved by the Conjoint Health Research Ethics Board at the University of Calgary. Informed consent was obtained from patients and family members, and genomic DNA was extracted from either cultured fibroblasts or peripheral blood leukocytes by standard phenol chloroform extraction. Fibroblast cells were cultured in MEM Medium (Gibco), with 10-12% fetal bovine serum at 37°C and 5% CO2. A set of 250 genomic DNA samples of various ethnicities were used to check whether the DNAJC19 IVS3-1 G>C mutation exists as a polymorphism in the general population. Genomic DNA from these controls was extracted from peripheral blood lymphocytes also by standard phenol chloroform procedure.

Genome Scan
Using a homozygosity mapping approach, a complete genome scan was performed by genotyping 400 microsatellite markers covering the entire genome to an average density of 10 cM using the ABI PRISM MD-10 Linkage Mapping Set, Version 2 (Applied Biosystems). The genome scan was performed on 5 severely affected Hutterite patients meeting inclusion criteria defined by at least two of dilated cardiomyopathy, 3-methylglutaconic aciduria and genital anomalies. Fine mapping around potential candidate regions was done by selecting additional microsatellite markers from the ABI PRISM HD-5 Linkage Mapping Set (Applied Biosystems), and NCBI STS map, and by genotyping additional patients and unaffected family members. UniSTS accession numbers for these markers are provided in the Electronic-Database Information section below.

Mutation Screen
Mutation analysis of candidate genes was performed by bi-directional sequencing of predicted exons and splice junctions from genomic DNA from an affected patient, an obligate carrier and a non-carrier as determined by the pattern of inheritance of the disease associated haplotype. Sequence data was analyzed by subtraction analysis using the Staden Package. Entrez Gene entries for positional candidate genes are provided in the Electronic-Database Information section.

The DNAJC19 gene was PCR amplified in 3 segments using the following primers (letters in brackets indicate primer positions shown in figure 1b): Segment 1 corresponding to exon 6, 1263 bp, forward (P) 5’-GAAGTGTAGACGCTAGGTAGTATAA, reverse (I) 5’-GCTAAATCTCCCTCAGATAAG; Segment 2 corresponding to exons 4 and 5, 1298 bp,
forward (H) 5'-ATTATCCCATTAATAACTATTGGTC, reverse (E) 5'-CTAAATACCTCGGGAATTTG; Segment 3 corresponding to exons 1, 2 and 3, 1955 bp, forward (D) 5'-CAGGAGAATGGGTCACAAGCAATCA, reverse (A) 5'-CCGCCACCCCTTCAACCAAC. Amplifications were carried out using the Platinum Taq buffer system (Invitrogen) with 2.5 mM magnesium chloride, and an annealing temperature of 60°C.

Sequencing reactions were performed at 60°C using ABI BigDye v.3.1 (Applied Biosystems) and the following primers: Exon 1 forward (primer A, sequence shown above), Exon 1 reverse (b) 5' -AGCTGAGGTTGAGGCCTGGG; Exons 2 and 3 forward (c) 5'-ATGGAAGAATGGGCAAAATG, Exons 2 and 3 reverse (primer D, sequence shown above); Exon 4 forward (primer E, sequence shown above), Exon 4 reverse (f) 5' -AAGGAGAAGGTTGCTTTTCT; Exon 5 forward (g) 5' -GTCTTTAATTGGGCTTTTATG, Exon 5 reverse (primer H, sequence shown above); Exon 6 forward-1 (primer I, sequence shown above), Exon 6 forward-2 (j) 5' -AAATGCTCCAGAGCTACAAT, Exon 6 forward-3 (l) 5' -TCTTTATCGGTGAAAGGCT, Exon 6 forward-4 (n) 5' -TGGGATTACAGGCATGAGTA, Exon 6 reverse-1 (k) 5' -ATCCCCAATTTAAACAAGA, Exon 6 reverse-2 (m) 5' -TCCAAGTGTGCCAGCCAGTT, Exon 6 reverse-3 (o) 5' -AGAAATAGGCAACTGAAGGA, Exon 6 reverse-4 (primer P, sequence shown above).

The DNAJC19 IVS3-1 G>C mutation abolishes a StuI cut site. Controls were tested for the presence of this mutation by PCR amplification of DNAJC19 segment 2 (primers and conditions given above). 10 ul of PCR product was digested for 2 hours in 3 units of StuI enzyme (Roche). The normal G allele when cut gives 1160 bp and 138 bp bands. The mutant C allele remains uncut and is a 1298 bp band. The G/C heterozygote gives 1298 bp, 1160 bp and 138 bp bands (data not shown).

**RT-PCR and Tissue Expression**

RT-PCR was performed on total RNA isolated from fibroblasts using the Qiagen RNeasy midi kit (Qiagen). DNAJC19 specific primers were designed to amplify a 525 bp cDNA product corresponding to the full length coding sequence and the 5’ and 3’ UTR boundaries: RT forward primer (RTF) 5’-GGTAAAGGCGTGCTGATCAATT, RT reverse primer (RTR) 5’-AAAATTTGCTCCTGAGGCAATT. RT-PCR was performed using the Superscript One-Step RT-PCR kit (Gibco) with an initial incubation at 46°C for 20 minutes followed by 38 cycles at an annealing temperature of 55°C. cDNA RT-PCR products were sequenced using the RTF and RTR primers. Gene expression studies used cDNA panels containing a number of fetal and adult tissue types (BD Clontech Multiple Tissue cDNA Panels I, II and Fetal Multiple Tissue cDNA panel). The cDNA panels were amplified using Platinum Taq buffer (Invitrogen) and the above RTF and RTR primers with an annealing temperature of 60°C, and run on 1.5% agarose.

**Informatics**

Map positions of markers and positional candidate gene models were identified using NCBI Map Viewer. All primers were designed using Oligo 6. Protein domain
predictions were made using the Conserved Domain Architecture Retrieval Tool (CDART), and similarity searches based on this domain architecture were performed using the reverse position specific BLAST algorithm. Other predictions were made using proteomics tools available from the expert protein analysis system (expasy). Multiple alignments were constructed using ClustalW, and alignments were visualized using Genedoc. URLs for these tools and GenBank identification numbers for aligned sequences are provided in the Electronic-Database Information section below.

RESULTS

Clinical Histories

Clinical features of our 18 study patients are summarized in Table 1.

**TABLE 1 Summary of the clinical features of DMCA syndrome**

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Sex</th>
<th>Alive at (deceased at)</th>
<th>Dilated cardiomyopathy</th>
<th>Long QT</th>
<th>IUGR</th>
<th>FTI/Growth Failure</th>
<th>Cerebellar signs/Ataxia</th>
<th>Microcytic anemia</th>
<th>Elevated AST/ALT</th>
<th>Hepatic Steatosis</th>
<th>Male Genitalia</th>
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<th>Mild/Mightline MR</th>
<th>Optic atrophy</th>
<th>Malformations</th>
<th>Medial Other</th>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>(13 yrs)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>C</td>
<td>+</td>
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<td>5</td>
<td>M</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>C,H</td>
<td>D</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>C,H</td>
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<td>C,H</td>
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<td>Arachnoid Cyst</td>
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<tr>
<td>25</td>
<td>M</td>
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<td>+</td>
<td>+</td>
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<td>C</td>
<td>+</td>
<td>Seizures</td>
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M, Male; F, Female; MR, Mental Retardation; C, Cryptorchidism; D, Diaphragmatic eventration; H, Hypospadias; A, Atrial Septal Defect
Eleven of these patients had either echocardiographic or pathological features at autopsy consistent with a diagnosis of a dilated cardiomyopathy (DCM). The onset of the dilated cardiomyopathy was always prior to the age of 3 years and, unfortunately, over 70% of patients with the DCM died from either progressive cardiac failure or sudden cardiac death. Some patients improved with standard medical therapy, and complete resolution of the DCM was seen in two patients with normalization of the echocardiogram, even following withdrawal of cardiac medications. Long QT syndrome was frequently seen in the patients with DCM, however, it was also seen in patients without clinical or echocardiographic evidence of DCM. Prenatal and/or postnatal growth failure was universally seen. All patients over the age of 2 years had a cerebellar syndrome with ataxia resulting in significant motor delays, although most patients ambulate independently. Male genital anomalies ranging from isolated cryptorchidism to severe perineal hypospadias were common and were secondary to testicular dysgenesis based on the frequent findings of small atrophic testes and incomplete testosterone rise after hCG stimulation. A few male patients also had Mullerian remnants and therefore both androgen and anti-Mullerian hormone synthesis was impaired, consistent with testicular dysgenesis. Additional features included optic atrophy, mild elevations of hepatic enzymes with microvesicular hepatic steatosis, a normochromic, microcytic anemia and mild to borderline non-progressive mental retardation.

Many patients were found to have significant increases in urine organic acids particularly of 3-methylglutaconic (3MGC) and 3-methylglutaric acid (3MGA). Quantitative measurement of the 3MGC and 3MGA was performed in affected patients, controls and unaffected relatives. Affected patients consistently showed 5-10 fold elevations in both plasma and urine 3MGC and 3MGA levels (data not shown).

**Genome Scan**

In order to identify the chromosomal region associated with DCMA syndrome we performed a genome scan using a homozygosity mapping approach in 5 severely affected patients (Figure 2A, individuals 1, 7, 9, 10 and 11). Following the initial scan, none of the markers were homozygous in all 5 patients. Homozygosity was suggested, however, at 9 loci with either 4 of 5, or 3 of 5 patients homozygous for the same allele and the non-homozygotes sharing the same allele on one chromosome (D2S2211, D2S325, D3S1565, D5S408, D12S1723, D12S310, D14S74, D22S420 and D22S315). Fine mapping around these candidate regions ruled out evidence of an ancestral homozygous haplotype in all but the chromosome 3 locus (D3S1565). Additional saturating markers enabled the identification of an extended disease associated haplotype of 23.9 cM on chromosome 3q26.2-q27.3, between markers D3S1282 and D3S1262 (Fig 1A). An additional ten affected patients as well as any available unaffected family members were genotyped reducing this to a core region of 2.2 megabases between markers D3S3603 and D3S2314 on 3q26.33 (Fig. 1A, 2A).

**Mutation Screening**

To identify the DCMA syndrome mutation in our patients, all predicted exons and splice junctions were bidirectionally sequenced for all 12 positional candidate genes in the minimal region. We identified a G>C transversion in the conserved AG splice acceptor
site of intron 3 of the DNAJC19 gene (IVS3-1G>C) (Fig. 2B). DNAJC19 was previously uncharacterized, and comprises 6 exons spanning 5.2 kb of genomic sequence (Fig. 1B). All affected patients included in this study for whom DNA samples were available (N=16) were homozygous for this mutation, while unaffected parents showed a pattern of inheritance of this mutation consistent with their carrier status of the affected haplotype (N=8). No unaffected siblings were homozygous for this haplotye (N=3) (Fig. 2A). The IVS3-1 G>C mutation eliminates a StuI restriction site. Mutation screening of 236 unaffected controls by StuI digest revealed only the normal G/G genotype (data not shown). No mutations were identified in any of the other 11 positional candidates in this region.

**DNAJC19 Gene Expression**

The DNAJC19 IVS3-1G>C mutation was predicted to prevent splicing of the exon 4 coding sequence into the DNAJC19 mRNA (Δex4). To confirm this effect, DNAJC19 specific RT-PCR analysis was performed on total fibroblast RNA from an affected patient and an unaffected control. A single 445 bp product was detected in the affected patient, while two products, a dominant 525 bp band and a minor 445 bp band were detected in the control sample (Fig. 3A).

In order to look at expression of the DNAJC19 gene in various tissue types, we PCR amplified the DNAJC19 cDNA using multiple tissue first strand cDNA panels. The DNAJC19 transcript is ubiquitously expressed, as we detected a dominant 525 bp product corresponding to the full length DNAJC19 cDNA as well as a minor product of 445 bp in all tissue types tested (Fig. 3B). To confirm the identity of these products, we gel purified and sequenced the 445 bp band from the patient RT-PCR sample, as well as both the 445 bp and 525 bp bands from the adult leukocyte sample from the cDNA panel. The 445 bp products from the affected patient and the wildtype adult leukocyte sample are missing coding sequence corresponding to exon 4 (Δex4), while the predominant 525 bp product from the leukocyte sample corresponds to the predicted full length mRNA sequence for the DNAJC19 gene (Fig. 3C).

**Characterization of DNAJC19**

Since DNAJC19 is a previously uncharacterized gene, a number of informatics analyses were applied to examine the consequence of the IVS3-1 G>C mutation on the DNAJC19 protein, and identify any functional domains. The full length DNAJC19 gene encodes a predicted 116 amino acid protein with a molecular mass of 12.5 kDa. Translation of the Δex4 transcript is predicted to produce a truncated protein of 47 amino acids with a theoretical molecular weight of approximately 5 kDa. The first 43 residues encoded by exons 1-3 would be preserved; however, skipping of exon 4 produces a reading frame shift in the exon 5 coding sequence resulting in a predicted aberrant 4-amino acid substitution (PYCQ) followed by a termination codon.

CDART analysis of the 116 amino acid DNAJC19 protein indicates the presence of a conserved DNAJ domain from residues 66 to 116 at the C-terminus of the protein. A single transmembrane segment was identified using the TMpred program at the N-terminus of the protein from residues 4 to 23 (Fig. 4A). In addition, the region directly
following the transmembrane domain is predicted to form an amphipathic \( \alpha \)-helical structure that is rich in positively charged residues resembling a mitochondrial targeting sequence. If translated, the truncated protein encoded by the \( \Delta \)ex4 transcript would contain only a transmembrane domain, and would completely lack the DNAJ domain.

Similarity searches were performed using rpsBLAST to identify proteins in other species that have similar domain architecture to DNAJC19. A number of similar proteins were identified that contained a highly conserved DNAJ domain at the C-terminus and a predicted transmembrane domain positioned towards the N-terminus, a selection of these are shown in figure 4B.

**DISCUSSION**

DCMA syndrome is a novel autosomal recessive disorder that shares some clinical similarity with the X-linked Barth syndrome and the other 3-methylglutaconic acidurias. The three defined classes of 3-methylglutaconic aciduria, 3-methylglutaconyl-CoA hydratase deficiency (Type I), Barth syndrome (Type II) and Costeff Optic Atrophy syndrome (Type III) all are due to defects in known or predicted mitochondrial proteins. In addition, elevated 3-methylglutaconic acid is a common finding in respiratory chain disorders. The major clinical and biochemical features of DCMA syndrome are consistent with a mitochondrial cytopathy, however, this degree of testicular dysgenesis to our knowledge has never been reported in a mitochondrial disorder. The autosomal recessive pattern of inheritance, and the unique combination of clinical features in DCMA syndrome distinguishes it from the other 3-methylglutaconic acidurias, and suggests that DCMA syndrome is a fifth type of 3-methylglutaconic aciduria.

To date, DCMA syndrome has only been seen in patients from the Dariusleut Hutterite population. It is possible that additional DCMA patients may exist outside this population, potentially in European ancestral populations from which the Hutterites descended. If this is the case, these patients may have been misclassified within the Type IV 3-methylglutaconic aciduria category for lack of a better diagnosis, as cases of dilated cardiomyopathy not attributed to Barth syndrome have been included in this category.

Using a homozygosity mapping approach, we identified an ancestral haplotype on chromosome 3q26.2-3q27.3 segregating with DCMA syndrome. Mutation analysis of positional candidate genes identified a splice site mutation in a previously uncharacterized gene, DNAJC19, that encodes a mitochondrial DNAJ domain-containing protein. This mutation was predicted to cause aberrant splicing and result in the loss of the full length DNAJC19 transcript. RT-PCR analysis confirmed the splice defect (\( \Delta \)ex4), and in affected patients homozygous for the IVS3-1G>C mutation splicing is completely abnormal, and a full-length DNAJC19 transcript is not produced. The presence of the \( \Delta \)ex4 transcript in wildtype tissues in addition to the full length transcript may indicate that alternative splicing is common at this site.

Proteins containing the DNAJ domain are typically involved in molecular chaperone systems of the Hsp70/Hsp40 type. These chaperone systems aid in folding and assembly of newly synthesized proteins, as well as preventing abnormal folding and aggregation of
proteins during stress conditions. The Hsp70 protein binds unfolded hydrophobic domains of the substrate protein, while the Hsp40 co-chaperone aids in loading the substrate protein onto Hsp70 and stimulates Hsp70 binding activity via the DNAJ domain. The domain architecture of the DNAJC19 protein is unusual among members of the DNAJ protein family, in that the DNAJ domain is positioned at the C-terminus of the protein following a single transmembrane domain. This is in contrast with *E. coli* DNAJ, the classical example of a DNAJ co-chaperone, where the DNAJ domain is located at the N-terminus, and is followed by other conserved domains involved in its other functions. In addition, the classical DNAJ proteins are soluble whereas the predicted transmembrane domain of DNAJC19 suggests that it may be membrane bound. Similarity searches based on this domain architecture indicate that proteins similar to DNAJC19 are present in a number of species having a highly conserved C-terminal DNAJ domain and an upstream transmembrane domain. Two of these, the yeast Tim14 and Mdj2p proteins are components of the mitochondrial inner membrane.

The role of Tim14 has been recently elucidated in yeast, where it is a component of the TIM23 inner membrane translocase complex, and is an essential protein required for cell viability. The TIM23 complex facilitates the import of mitochondrial pre-sequence-containing proteins through the inner membrane. Tim14 is an essential subunit of the TIM23 complex, functioning with mitochondrial Hsp70, and the Tim16 and Zim17 proteins as a component of the import motor subcomplex. This import motor is an adaptation of the Hsp70/Hsp40 chaperone system utilizing a membrane bound DNAJ domain to stimulate binding of mitochondrial Hsp70 (mtHsp70) to the incoming peptide chain at its point of entry. This is thought to aid in import through the TIM23 translocase in two ways; first, ATP-driven conformational changes in mtHsp70 upon substrate binding are thought to pull the incoming peptide into the matrix, and second, binding of mtHsp70 to the unfolded chain prevents backsliding through the Tim23 import pore. This system couples the chaperone function of mtHsp70 with import, aiding in proper folding and assembly of newly imported matrix proteins.

The functions of the other DNAJC19-like proteins are as yet unknown, and it is unclear whether DNAJC19 is in fact the human ortholog of yeast Tim14. The yeast Mdj2p protein is a known component of the inner mitochondrial matrix, however, a defined function has yet to be identified for it. What is known is that Mdj2p is not an essential protein for viability, and it can partially compensate for the loss of Mdj1p, the major mitochondrial hsp40 homolog in yeast. A second human protein, DNAJC15 methylation-controlled J protein (MCJ), is also included within this group. A differential pattern of expression has been noted for *MCJ* in wildtype cell lines dependent on the methylation status of a CpG island in the first intron of the *MCJ* gene. Differences in expression of MCJ have been noted in ovarian cancers and may be associated with the cells’ response to chemotherapeutic drugs. A proteomic screen of the mitochondria of human cardiac myocytes identified DNAJC19 as a component of the mitochondrial proteome, however, MCJ was not identified in this screen. Like DNAJC19, MCJ shares sequence similarity with the yeast Tim14 and Mdj2p proteins. The differential pattern of expression of MCJ, however, argues against a housekeeping-type role as would be expected for a Tim14
ortholog. The identification of DNAJC19 in the mitochondrial proteome, along with its role in the DCMA syndrome phenotype are congruent with the DNAJC19 protein being orthologous with the yeast Tim14 or Mdj2 proteins.

The similarity of DNAJC19 with the yeast Tim14 protein suggests that the phenotype of DCMA syndrome might result from the defective import and assembly of presequence containing mitochondrial proteins via the TIM23 translocase pathway. Defects in mitochondrial import have been previously reported to cause a progressive neurodegenerative condition, Mohr-Tranebjaerg syndrome (MT syndrome), which includes sensorineural deafness, vision loss, mental retardation and a movement disorder. MT syndrome is caused by mutations in DDP1, the ortholog of yeast Tim8, which appears to be important in facilitating the transfer of Tim23, the core component of the TIM23 inner membrane translocase complex, from the translocase of the outer membrane (TOM) through the intermembrane space to the TIM22 translocase. The phenotype of MT syndrome is, therefore, thought to result from abnormal assembly of the TIM23 transporter. MT syndrome is the only known genetic disorder involving a defect in the mitochondrial protein import system.

Over 99% of mitochondrial proteins in humans are nuclear-encoded, thus the mechanisms for transfer and assembly of the cytoplasmically synthesized precursor proteins into or across both mitochondrial membranes are of critical importance. The potential association of DCMA syndrome with mitochondrial protein import defects would suggest that this may be an important cause of mitochondrial cytopathies. Mechanisms for protein transport and assembly in the mitochondria continue to be elucidated largely based on work in the yeast S. cerevisiae. The relevance of these studies to our understanding of the pathogenic mechanisms underlying human mitochondrial diseases, however, remains poorly understood and further development of this area will likely benefit from not only the identification of additional human diseases, but also from the development of mammalian model systems based on the studies already completed in simple eukaryotes. The present discovery of the association of DCMA syndrome with a mutation in the DNAJC19 gene will help to expand our insight into the role of mitochondrial import and trafficking in human cytopathies.

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The authors declare no competing interests.
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ELECTRONIC-DATABASE INFORMATION


Oligo 6 primer design software (http://www.oligo.net)

Entrez UniSTS entries for markers making up ancestral DCMA syndrome haplotype (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unists): D3S1282 [68930], D3S3523 [57600], D3S3725 [73565], D3S3520 [17983], D3S1565 [63165], D3S2425 [82628], D3S2421 [13287], D3S2427 [37149], D3S3676 [30955], D3S3715 [74051], D3S1754 [9710], D3S3699 [43474], D3S3603 [30527], D3S3662 [78965], D3S2314 [15487], D3S1618 [72781], D3S1571 [8133], D3S2399 [10726], D3S1617 [39046], D3S1262 [53008])

Entrez Gene entries for positional candidates in the region D3S3603 to D3S2314 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene): LOC131054 similar to RalA binding protein 1 (RalBP1) [131054], TTC14 tetratricopeptide repeat domain 14 [151613], Hypothetical protein DKFZp434A128 [339829], LOC389178 similar to RING finger Protein 13 [389178], LOC402151 similar to beta-actin [402151], FLYWCH-type zinc finger 1-like [391594], LOC391595 similar to 6OS ribosomal protein L32 [391595], Fragile X mental retardation, autosomal homolog 1 [8087], DNAJ (Hsp40) homolog, subfamily D, member 2 [131118], SOX2 overlapping transcript (non-coding RNA) [347689], SOX2 SRY(sex determining region Y)-box 2 [6657], LOC401102 similar to zinc finger, CCHC domain containing 10 [401102].

GenBank identification numbers for nucleotide and protein sequences (http://www.ncbi.nlm.nih.gov/Entrez/): Homo sapiens chromosome 3 genomic contig [GI: 37550867], Homo sapiens DNAJC19, DNAJ (Hsp40) homolog, subfamily C, member 19 mRNA [GI: 41152082], Homo sapiens DNAJC19, DNAJ (Hsp40) homolog, subfamily C, member 19 [GI: 21687102], Saccharomyces cerevisiae Tim14 [GI: 6323036], Caenorhabditis elegans DNAJ domain (prokaryotic heat shock protein) (11.7 kDa) (dnj-21) [GI: 17509089], Homo sapiens DNAJC15, Methylation-controlled DNAJ (MCJ) [GI: 66472920], Schizosaccharomyces pombe hypothetical protein SPAC824.06 [GI: 19114357], Saccharomyces cerevisiae protein of the mitochondrial inner membrane with similarity to E. coli DNAJ and other DNAJ-like proteins, function partially overlaps that of Mdj1p; Mdj2p [GI: 6324001], Mus musculus Unnamed Protein Product (Riken cDNA 1810055D05) [GI: 12841697], Mus musculus DNAJ (HSP40) Homolog, Subfamily D, Member 1[GI: 13384760], Drosophila melanogaster CG7394-PA [GI: 23093624], Escherichia coli DNAJ/HSP40 [GI: 118719])


Expert Protein Analysis System (ExPASy) Proteomics Tools for protein sequence prediction and analysis; includes Translate Tool for translation of DNA sequence, ClustalW for multiple
sequence alignments, PSORT for prediction of subcellular localization, TMpred for prediction of transmembrane regions and protein orientation (http://au.expasy.org/tools/).

Genedoc for visualization and editing of multiple sequence alignments (http://www.psc.edu/biomed/genedoc/)
REFERENCES


FIGURE CAPTIONS

Figure 1  Map of the chromosome 3q DCMA candidate region. (A) Refined map interval spanning 23.9 cM from 3q26.2-q27.3 between markers D3S1282 and D3S1262. The markers in bold represent the core disease-associated region of approximately 2.2 Mb (see Electronic-Database Information section for unistSTS accession numbers). (B) An expanded map of the genomic organization of the DNAJC19 gene. The six exons extend over 5.2 kb. The coding sequence is indicated in dark gray and the non-coding sequence is in light gray. The locations of primers used for mutation screening are indicated. Primers used for amplification of 3 genomic segments are indicated with capital letters. Additional primers used for sequencing are shown in lowercase. Primers used for RT-PCR analysis are also indicated.

Figure 2  The DNAJC19 IVS3-1G>C mutation is associated with a dilated cardiomyopathy syndrome in consanguineous Hutterite families. (A) Patients and families included in this study show an extended common haplotype from D3S1282 to D3S1262; alleles included in this haplotype are highlighted in gray with the core disease-associated region highlighted in dark gray. The genotypes for the DNAJC19 IVS3-1G>C mutation are also shown. Patient numbers correspond with numbers in Table 1. DNA was unavailable for patients 6 and 22, however these patients were included in the study cohort based on their clinical phenotypes. DNA for patient 25 was unavailable for haplotyping, but clinical diagnosis was confirmed by genotyping for the DNAJC19 IVS3-1G>C mutation at a later date. (B) Electropherograms showing DNAJC19 IVS3-1G>C genotypes from an affected patient (C/C), an obligate carrier (G/C) and a wildtype individual (G/G).

Figure 3  Expression analysis of DNAJC19. (A) RT-PCR analysis of DNAJC19 using gene specific primers and RNA extracted from cultured fibroblasts from an affected patient and a non-Hutterite normal control. Two bands were amplified from the normal control, one of 525 bp corresponding to the full-length cDNA and the second less abundant band was 445 bp. In the affected patient, only the 445 bp band was detected. The predicted size of the product from a DNAJC19 transcript missing the exon 4 coding sequence is 445bp. (B) Expression analysis of DNAJC19 was performed using gene specific primers and a number of normalized first strand cDNA preparations from fetal and adult tissues. Both cDNAs were found expressed in all tissues with the full-length cDNA more abundant in all tissues tested except for adult leukocytes. The tissue types tested include fb, fetal brain; fl, fetal lung; flv, fetal liver; fk, fetal kidney; fh, fetal heart; fsk, fetal skeletal muscle; fsp, fetal spleen; ft, fetal thymus; sp, adult spleen; ty, adult thymus; p, adult prostate; ts, adult testis; ov, adult ovary; si, adult small intestine; co, adult colon; lk, adult leukocytes; h, adult heart; bn, adult brain; pl, placenta; l, adult lung; lv, adult liver; sk, adult skeletal muscle; k, adult kidney; and pa, adult pancreas. Each set was run with G3PDH, glyceraldehyde-3-phosphate dehydrogenase control; C, positive control and b, water blank. (C) To determine the identity of the cDNA products, the 445 bp cDNA from the affected patient, the 445 bp and 525bp bands from adult leukocytes were sequenced. Both the cDNA from the affected individual, and the 445 bp band from leukocytes were found to have exons 3 and 5 spliced together and the absence of exon 4 coding sequence (Δex4). The 525 bp band corresponded to the full coding sequence of DNAJC19. The Δex4 cDNA detected in normal tissues and control are thought to be a normal splice variant.
Figure 4  DNAJC19 has an unusual domain architecture with respect to other DNAJ proteins and similar proteins are found in a number of species. (A) Graphical representation of the domain structure of *H. sapiens* DNAJC19 versus the E. coli DNAJ/HSP40 protein. The DNAJC19 protein contains only the DNAJ domain (pfam00226.11), which is located at the C-terminus following a predicted transmembrane domain. This is in contrast with the E. coli DNAJ/HSP40 protein which is the classical example of a DNAJ protein. The DNAJ domain (pfam00226.11) is located at the N-terminus of the protein followed by a glycine/phenylalanine-rich linker domain, a cysteine-rich domain (pfam00684.11) containing 4 copies of a zinc-finger-like motif (CXXCXXGXXG), and a C-terminal domain (pfam01556.11) of unknown function. (B) ClustalW multiple sequence alignment of DNAJC19 homologs (see Electronic-Database Information section for GenBank identification numbers). The conserved helix structures of the DNAJ domain are underlined, the DNAJ domains of these proteins only contain 3 of the helices that make up the classical tetrahedral DNAJ domain. They all also contain the absolutely conserved HPD motif which is indicated by (**). Highlighted regions indicate conserved blocks of sequence. Residues highlighted in black indicate positions with highest degree of conservation and those highlighted in gray indicate less highly conserved positions. Gaps introduced to optimize the alignment are indicated by dashes (-). Alignment was adjusted in Genedoc based on previously published alignments.