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Report

Missense Mutation in Pseudouridine Synthase 1 (*PUS1*) Causes Mitochondrial Myopathy and Sideroblastic Anemia (MLASA)

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Mitochondrial myopathy and sideroblastic anemia (MLASA) is a rare, autosomal recessive oxidative phosphorylation disorder specific to skeletal muscle and bone marrow. Linkage analysis and homozygosity testing of two families with MLASA localized the candidate region to 1.2 Mb on 12q24.33. Sequence analysis of each of the six known genes in this region, as well as four putative genes with expression in bone marrow or muscle, identified a homozygous missense mutation in the pseudouridine synthase 1 gene (*PUS1*) in all patients with MLASA from these families. The mutation is the only amino acid coding change in these 10 genes that is not a known polymorphism, and it is not found in 934 controls. The amino acid change affects a highly conserved amino acid, and appears to be in the catalytic center of the protein, PUS1p. *PUS1* is widely expressed, and quantitative expression analysis of RNAs from liver, brain, heart, bone marrow, and skeletal muscle showed elevated levels of expression in skeletal muscle and brain. We propose deficient pseudouridylation of mitochondrial tRNAs as an etiology of MLASA. Identification of the pathophysiologic pathways of the mutation in these families may shed light on the tissue specificity of oxidative phosphorylation disorders.

Mitochondrial myopathy and sideroblastic anemia (MLASA [MIM 600462]) is a rare autosomal recessive disorder of oxidative phosphorylation and iron metabolism. Elsewhere, we have described a Jewish Iranian family living in the United States with four individuals affected with MLASA (Casas and Fischel-Ghodsian 2004). Linkage analysis and homozygosity testing in this family and in an elsewhere described family from Israel, which originated from the same Iranian town as the U.S. family (Inbal et al. 1995), localized a candidate region within 1.2 Mb of chromosome 12q24.33 (Casas et al., in press).

Analysis of the electronically available expression information in OMIM, UniGene, EST (at NCBI), Ensembl, and TIGR databases showed evidence for expression in at least one of the affected tissues (bone marrow and muscle) in 10 genes in the 1.2-Mb candidate region. Complete coding regions of the candidate genes, including 5′ UTRs and splice sites (intron-exon junctions), were sequenced directly in patients and obligate carriers (parents) from family 1 (fig. 1). We identified a total of 10 nucleotide substitutions in the coding regions of six genes (table 1). Six nucleotide substitions led to amino acid substitutions. Analysis of the electronic databases showed that five are common polymorphisms. Only one sequence change, a C→T mutation at mRNA position 656 in exon 3 of the *PUS1* gene (fig. 1*A*), results in a nonconservative amino acid change (R116W) and constitutes a novel change not present in SNP and/or EST databases. Direct sequencing of exon 3 of *PUS1*, as well as RFLP analysis using restriction endonuclease *Nci*I, in 16 members of the two families revealed all affected individuals to be homozygous, all parents to be heterozygous, and all unaffected siblings to be either heterozygous carriers or negative for the C656T mutation (fig. 1*B*). C656T results in a nonconservative amino acid change from arginine to tryptophan at position 116 of

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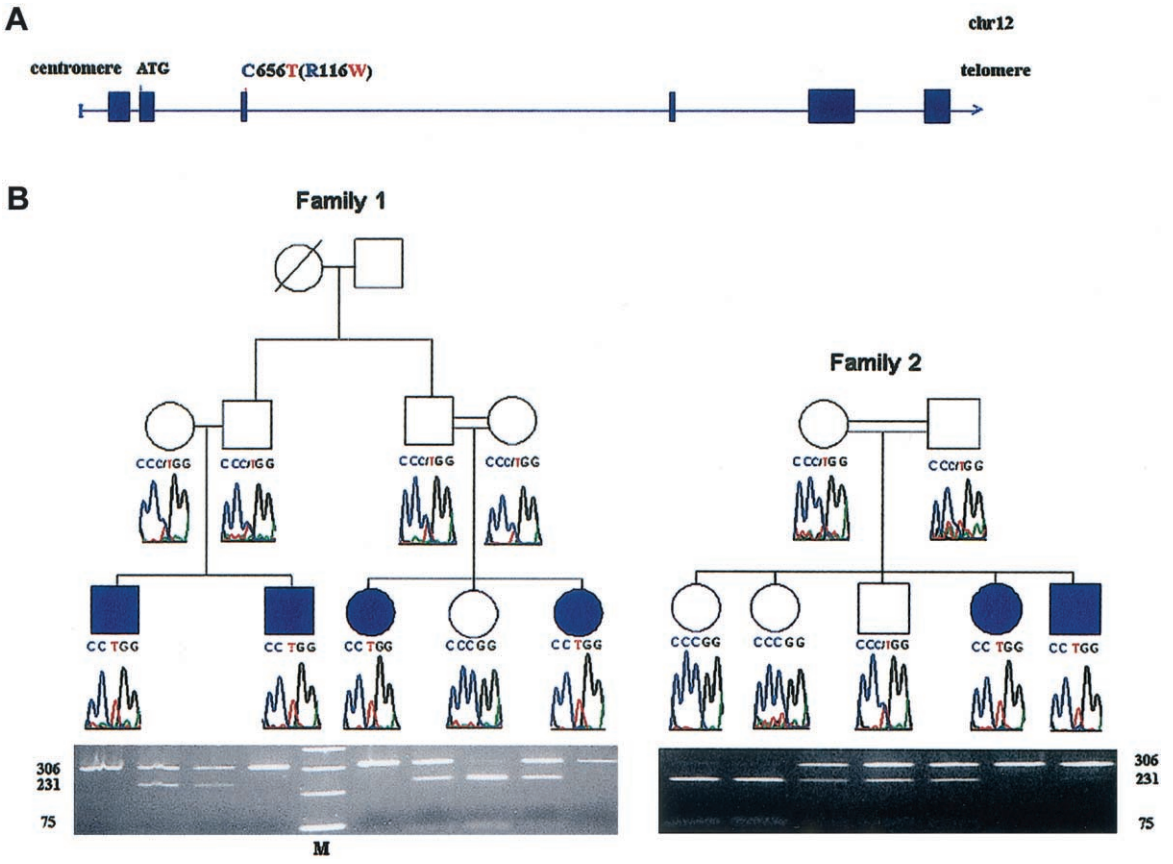

Figure 1 Homozygous mutation in *PUS1* causes MLASA. *A,* Map of *PUS1* with exons in dark blue. The start codon and mutated position 656 in exon3 are identified. *B,* Two pedigrees of MLASA families shown with their sequencing and RFLP results for the mutation at position 656 in the *PUS1* gene. Affected individuals are indicated by dark blue symbols. The mutation abolishes the restriction site for *Nci*I.

the protein. Arginine and tryptophan differ greatly in their physical qualities, and the two databases focused on amino acid properties, EMBL (Betts and Russel 2003) and the Centre for Molecular and Biomolecular Informatics, do not consider this substitution to be a benign polymorphic change. The mutation was not present in 204 DNA samples from Arab Israeli and Spanish/Italian families with mitochondrial deafness or in 730 Jewish and non-Jewish individuals from families with inflammatory bowel disease; all 934 controls contained a normal nucleotide in the homozygous state.

Arginine 116 (116R) is located in the highly conserved domain RTDKGV and is preserved perfectly in the eukaryotic and prokaryotic pus1 proteins (fig. 2). The RTDKGV sequence motif appears to be the catalytic center of the protein. It is conserved in all pseudouridine synthases of the TruA, RluA, RsuA, and TruB protein families (Koonin 1996) and contains the invariant aspartate residue (D) necessary for catalysis in the members of TruA, TruB, and RluA pseudouridine synthases (Huang et al. 1998; Gu et al. 1999; Ramamurthy et al. 1999; Ansmant et al. 2000). In the *E. coli* pseudouridine synthase 1, arginine residue homologous to the human 116R has been shown to interact through hydrogen bonds with the aspartate in the catalytic center (Foster et al. 2000).

We evaluated intracellular localization of PUS1p with the use of publicly available prediction algorithms. Neither PSORTII or MITOPROT found evidence of a mitochondrial targeting sequence. However, the determination of possible subcellular localization on the basis of the global amino acid composition of proteins (Reinhardt and Hubbard 1998), with the use of the Neural Networks Protein Subcellular Localization (NNPSL), classified PUS1p as a likely mitochondrial enzyme. NNPSL was used successfully to determine subcellular localization of yeast mitochondrial pseudouridine synthase (pus5p), which catalyzes formation of a single pseudouridine in the yeast 21S mt rRNA (Ansmant et al. 2000). Yeast pus5p also had no evidence of a mitochondrial targeting sequence (Ansmant et al. 2000).

We evaluated *PUS1* expression by RT-PCR, using

Table 1

Summary of Sequence Changes in the Candidate Genes for MLASA in the 1.2-Mb Region of Chromosome 12q24.33

Gene	GenBank Accession Number	Exons	Protein Length (aa)	Variation(s) from GenBank Sequence	Heterozygous in Carrier, Homozygous in Affected	Amino Acid Change	Polymorphic in Human EST Database or Known SNP[a]
SFRS8	NM004592	19	951	T1402C	No[b]	Leu→Pro	SNP rs1982528
MMP17	NM016155	10	606	None			
ULK1	NM003565	26	1,050	C2539A	Yes	No	No
PUS1	***NM025215***	***6***	***399***	***C656T***	***Yes***	***Arg→Trp***	***No (0/48)***
EP400	NM015409	53	3,124	G9319A	Yes	Ala→ Thr	ESTs (2/19)
FLJ33915	NM182613	9	430	A588G	Yes	Asp→Gly	ESTs (3/11)
				G973A	Yes	No	No
				C1199T	Yes	His→Tyr	ESTs (4/8)
DDX51	NM175066	13	666	G896A	Yes	Arg→Gly	SNP rs7958174
				G977A	Yes	Ala→Val	ESTs (11/21)
				T1823C	Yes	No	No
MCC3162	NM024078	15	516	None			
hCG1820940[c]	None	3	127	None			
GALNT9	NM021808	7	237	None			

NOTE.—Genes are arranged in order from centromere to telomere. The disease-causing mutation in the *PUS1* gene is in bold italics.

[a] Number of ESTs containing variant sequence and total number of ESTs are shown in parentheses for sequence changes identified in the EST database.

[b] SNP is homozygous in both.

[c] Celera database only.

RNA from lymphoblastoid cell lines from our patients with MLASA and from controls, as well as RNA from normal liver, brain, heart, bone marrow, and skeletal muscle. A single PCR product of expected size in all samples was obtained (data not shown). Quantitative expression analysis, with the use of Assay-On-Demand (Applied Biosystems) gene expression product, showed elevated levels of expression in skeletal muscle and brain (~14 and ~6 times more than in bone marrow, respectively; see also fig. 3). Although our electronic BLAST search analysis, as well as RT-PCR analysis, of the ESTs of multiple normal human tissues indicated existence of a single transcript, expression studies of the mouse *pus1* gene showed that several differentially spliced shorter isoforms, none catalytically active on tRNA substrates, exist (Chen and Patton 2000). The function of these isoforms is unknown, but they are speculated to be involved in the dimerization of the active mouse pus1p. The existence of similar isoforms in normal human tissues has not been identified at this time.

Mutations in another pseudouridine synthase, coded by the dyskerin gene (*DKC1* [accession number NM001363]), have been implicated in two human diseases associated with bone marrow dysfunction, X-linked dyskeratosis congenita (MIM 305000) (Heiss et al. 1998; Luzzatto and Karadimitris 1998; Knight et al. 1999*b*, 2001) and Hoyeraal-Hreidarsson (MIM 300240) syndrome (Knight et al. 1999*a*; Yaghmai et al. 2000). Reduced pseudouridylation with 10%–40% reduction in pseudouridines in the 28S and 18S mouse rRNA have been shown in a mouse model for dyskeratosis congenita (Ruggero et al. 2003).

Although no enzyme for mitochondrial pseudouridylation in humans has been described, indirect evidence suggests that pseudouridylation of human mitochondrial tRNAs requires PUS1p. Yeast and mouse homologs of PUS1p have been shown to catalyze the formation of pseudouridines at specific positions in cytoplasmic tRNAs (Hellmuth et al. 2000; Huang et al. 1998; Motorin et al. 1998; Chen and Patton 1999, 2000; Foster et al. 2000). Also, plant mitochondrial tRNA has been shown to contain a pseudouridine at the corresponding site to the cytoplasmic tRNA (Fey et al. 2002). We know that at least one pseudouridine is known to be universally present in almost all mitochondrial tRNAs from all three kingdoms (Sprinzl et al. 1998). Interestingly, the T8356C mutation associated with the mitochondrial syndromes MERRF (MIM 545000) and MELAS (MIM 540000) (Silvestri et al. 1992; Zeviani et al. 1993) is adjacent to the universally present pseudouridine in mitochondrial tRNA Lys. It has been shown that yeast pseudouridine synthase 4 (pus4p) is responsible for the catalysis of the uridine at this position in cytoplasmic and mitochondrial tRNAs (Becker et al. 1997) and that yeast pus1p is indispensable for cell viability in the presence of pus4p mutations (Grosshans et al. 2001).

In summary, the assertion that the homozygous C656T mutation in the *PUS1* gene is the pathogenic mutation in two families with MLASA is supported by the following points: (1) within the 1.2-Mb candidate

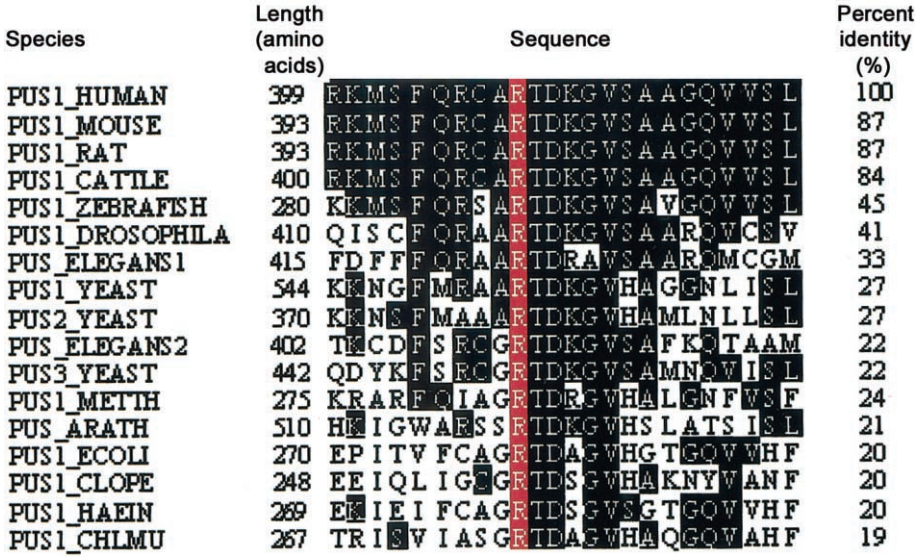

Figure 2 Evolutionary conservation of the human 116R (arginine) residue in the eukaryotic and prokaryotic homologues of PUS1p. Percent identity was calculated by ALIGN (Pearson et al. 1997). GenBank accession numbers of the sequences, in the order of appearance *(top to bottom)*: *Homo sapiens* NP079491, *Mus musculus* NM019700, *Rattus norvegicus* XM222267, *Bos taurus* CB440130, *Danio rerio* BC050502, *Drosophila melanogaster* NM142642, *Caenorhabditis elegans* T26253, *Saccharomyces cerevisiae* Q12211, *S. cerevisiae* P53167, *C. elegans* Q09524, *S. cerevisiae* P31115, *Methanothermobacter thermautotrophicus* O26928, *Arabidopsis thaliana* O22928, *Escherichia coli* P07649, *Clostridium perfringens* Q8xlq0, *Haemophilus influenzae* p45291, *Clostridium muridarum* Q9PJT0.

region delineated by genetic mapping, it is the only mutation in the coding region of all appropriately expressed genes that changes an amino acid and is not a previously described polymorphism; (2) the mutation is present in homozygous form in all patients, and in heterozygous form in all obligate carriers (parents); (3) the mutation was not found in 934 controls from different ethnic backgrounds, including Jewish, and was not present in any of the 48 available ESTs; (4) the mutation constitutes a nonconservative change; (5) the mutation affects an amino acid that has been conserved throughout evolution, including in prokaryotes; (6) the mutation appears to be in the catalytic center of the protein; (7) the intracellular localization of the protein is predicted to be in the mitochondria on the basis of its amino acid composition; (8) a defect in pseudouridylation has been demonstrated in a mouse model for dyskeratosis congenita, another tissue-restricted syndrome that affects the bone marrow; (9) indirect evidence suggests that pseudouridylation of human mitochondrial tRNAs requires the PUS1p; and (10) the gene is expressed in both skeletal muscle and bone marrow. Though these points do not completely rule out the possibility that an as-yet-unrecognized gene in the region harbors the actual disease mutation, the weight of the evidence makes such a possibility highly unlikely. Further investigation of the role of PUS1p and pseudouridylation in different tissues is expected to lead to a better understanding of the pathophysiology and tissue specificity of MLASA as well as other oxidative phosphorylation disorders.

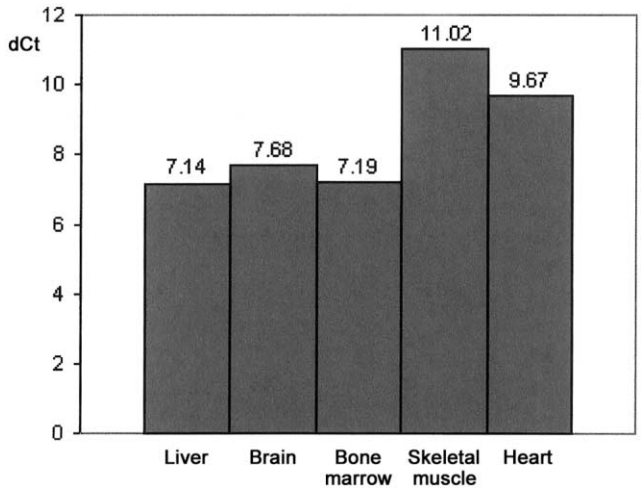

Figure 3 Quantitative analysis of *PUS1* expression in different tissues. dCt indicates the difference between Ct (*PUS1*) and Ct (*GAPDH*).

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

BLAST, http://www.ncbi.nlm.nih.gov/blast/index.html
Celera, http://myscience.appliedbiosystems.com/navigation/cdsLogin.jsp
Centre for Molecular and Biomolecular Informatics, BIOcomputing unit, http://www.cmbi.kun.nl/swift/future/aainfo/
EMBL, database of amino acid properties, http://www.russell.embl-heidelberg.de/aas/aas.html
Ensembl Genome Browser, http://www.ensembl.org/
MITOPROT, http://mips.gsf.de/cgi-bin/proj/medgen/mitofilter
NCBI, http://www.ncbi.nlm.nih.gov/mapview/
NNPSL, http://www.doe-mbi.ucla.edu/cgi/astrid/nnpsl_mult.cgi
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim
PSORTII, http://psort.nibb.ac.jp/form2.html
TIGR, http://www.tigr.org/tdb/tgi/hgi/
UniGene, http://www.ncbi.nlm.nih.gov/UniGene

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