Mitochondrial Complex III Deficiency Associated with a Homozygous Mutation in *UQCRQ*

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A consanguineous Israeli Bedouin kindred presented with an autosomal-recessive nonlethal phenotype of severe psychomotor retardation and extrapyramidal signs, dystonia, athetosis and ataxia, mild axial hypotonia, and marked global dementia with defects in verbal and expressive communication skills. Metabolic workup was normal except for mildly elevated blood lactate levels. Brain magnetic resonance imaging (MRI) showed increased density in the putamen, with decreased density and size of the caudate and lentiform nuclei. Reduced activity specifically of mitochondrial complex III and variable decrease in complex I activity were evident in muscle biopsies. Homozygosity of affected individuals to *UQCRB* and to *BCSIL*, previously associated with isolated complex III deficiency, was ruled out. Genome-wide linkage analysis identified a homozygosity locus of approximately 9 cM on chromosome 5q31 that was further narrowed down to 2.14 cM, harboring 30 genes (logarithm of the odds [LOD] score 8.82 at $\theta = 0$). All 30 genes were sequenced, revealing a single missense (p.Ser45Phe) mutation in *UQCRQ* (encoding ubiquinol-cytochrome c reductase, complex III subunit VII, 9.5 kDa), one of the ten nuclear genes encoding proteins of mitochondrial complex III.

Mitochondrial complex III, located within the mitochondrial inner membrane, catalyzes the transfer of electrons from succinate and nicotinamide adenine dinucleotide-linked dehydrogenases to mitochondrially encoded cytochrome b (*MT-CYB* [MIM 516020]) and utilizes the energy to translocate protons from the mitochondrial matrix to its exterior.[1] Complex III is composed of ten nuclear-encoded subunits and one mitochondrial-encoded subunit (*MT-CYB*).[1] Although rare, isolated complex III deficiency has been detected in a heterogeneous group of neuromuscular and nonneuromuscular disorders in children and adults.[1–9] Different pathogenic mutations in *MT-CYB* have been described,[2–5] whereas nuclear mutations have so far been identified only in two genes: the BCS1-like (*BCS1L* [MIM 603647]) gene, encoding a complex III assembly factor on chromosome 2q33,[6–8] and the *UQCRB* gene (ubiquinol-cytochrome c reductase binding protein [MIM 191330]), encoding subunit VI (chromosome 8q22) of the complex.[9]

MT-CYB is a highly evolutionarily conserved hydrophobic protein containing eight or nine transmembrane domains and two heme groups. To date, about 27 different mutations have been identified in cytochrome *b*, mostly in patients with skeletal muscle weakness and exercise intolerance.[1,4] Most patients with *MTCYB* mutations have sporadic myopathy-rhabdomyolysis associated with ragged-red fibers. Some of these patients have isolated complex III defects, whereas other presentations can be associated with a defect involving complexes I and III because of the structural interdependence of these complexes.[10] Mutations in the autosomal *BCS1L* gene had previously been found to cause mitochondrial complex III deficiency manifested by neonatal renal tubulopathy, encephalopathy, and liver failure,[7] as well as the syndromes GRACILE[8] (a severe disorder of intrauterine growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death; MIM 603358) and Bjornstad[9] (an autosomal-recessive disorder characterized by sensorineural hearing loss and pili torti; MIM 262000). A single patient with a homozygous deletion in the autosomal gene *UQCRB* was shown to have isolated defective complex III function with clinical hypoglycemia and lactic acidosis[11] (MIM 191330). We now describe a severe novel autosomal-recessive neurological phenotype of a complex III defect associated with a mutation in *UQCRQ*, encoding ubiquinol-cytochrome c reductase, complex III subunit VII, 9.5 kDa.

A large consanguineous inbred Israeli Bedouin kindred presented with a previously unreported autosomal-recessive syndrome. The severe neuromuscular phenotype affected a total of 25 individuals (Figure 1). Twenty of the affected individuals were available for detailed clinical and molecular analyses. The study was approved by the Soroka University Medical Center Institutional Review Board and by the Israel National Helsinki Committee in Genetics, and informed consent was obtained from all individuals tested. The abnormal phenotype was markedly uniform and consisted of severe psychomotor retardation and extrapyramidal signs. Affected individuals seemed normal at birth without any dysmorphic features. During the first few months of life, the prominent clinical feature was failure to achieve normal developmental milestones. By age 2–3 years, extrapyramidal signs were evident in all patients, presenting with dystonic postures, athetoid movements, and ataxia. All affected individuals had severe mental retardation. Neurological examination

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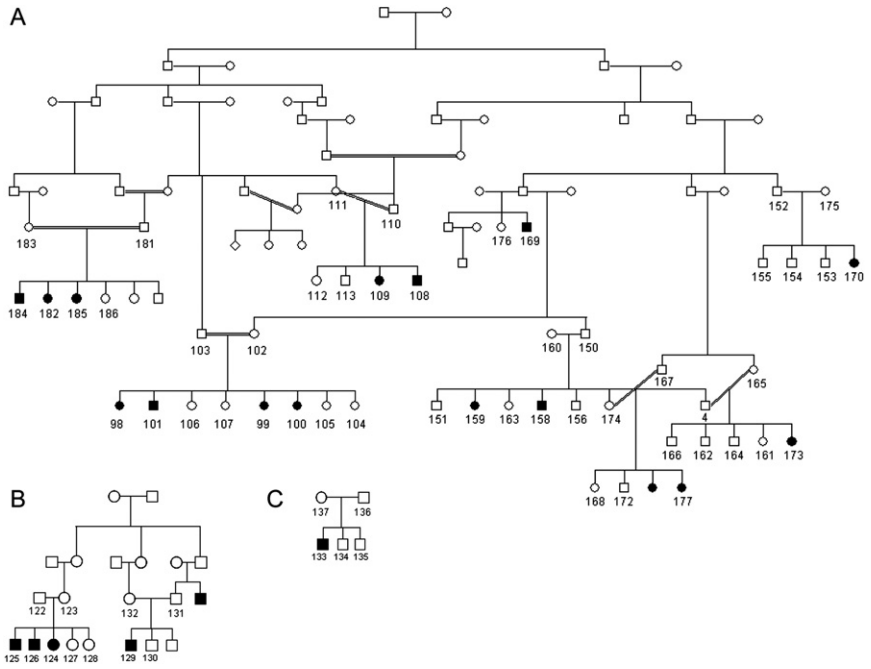

Figure 1. Pedigree of Affected Israeli-Bedouin Kindred

Solid and open symbols represent affected and unaffected individuals, respectively. All affected individuals are offspring of consanguineous marriages. The numbers denote individuals whose DNA samples were analyzed. (A), (B), and (C) are branches of a single extended kindred, remotely related (not first or second degree).

demonstrated restlessness, marked global dementia, and severe defects in verbal receptive communication and near total absence of expressive communication skills, with inability to express any words at any age. Mild axial hypotonia, increased tone in the upper and lower limbs, and increased deep tendon reflexes in all four extremities with inability to walk unsupported were evident in all patients. Cranial nerves were intact and ophthalmologic exam was normal. The phenotype is not lethal, with some affected individuals surviving well into their thirties. Electroencephalogram (EEG) demonstrated nonspecific mild generalized slowing. Brain magnetic resonance imaging (MRI) done in five patients showed bilateral symmetric abnormal findings in the basal ganglia (Figure 2), with increased density in the putamen and decreased density and size of the caudate and Globus pallidum nuclei. There was no involvement of the brain-stem area. Audiograms and karyotypes were normal. A thorough metabolic work up was done: All laboratory values were within normal limits except plasma lactate, which ranged from slightly to significantly increased levels (1.7–5.2 mM, mean value 3.3 mM; control range $<$ 1.55 mM). Cerbrospinal fluid lactate was measured in a single patient (individual 173, Figure 1) and was slightly elevated (2.8 mM; control range $<$ 2.2 mM). Muscle biopsies were done in three of the affected individuals—patients 99, 109, and 177 (Figure 1), ages 3, 15 and 2 years, respectively. Microscopic examination of muscle biopsies was nonspecific: The overall architecture of the skeletal muscle was preserved. There were some myopathic changes reflected by a borderline mild increase in variation in fiber size with few sporadic fibers demonstrating internal nuclei, as well as scattered regenerating fibers demonstrated by alpha-fetal myosin stain (positive in some 10% of the muscle fibers). There was no evidence of ragged-red fibers or mitochondrial aggregates, nor of group atrophy of muscle fibers, inflammation, accumulation of lipid or glycogen, or replacement of muscle fibers by fibrous or adipose tissue. ATPase stains demonstrated preserved fiber type distribution. Immunohistochemical stains for spectrin, 50-DAG, merosin, and dystrophin 1,2,3 were positive. Electron microscopy (EM) studies done on one of the biopsies demonstrated no mitochondrial structural abnormality. As demonstrated in Table 1, activities of the mitochondrial respiratory chain enzymatic complexes in muscle biopsies of these three patients showed 34%–56% reduction of antimycin-sensitive ubiquinol-cytochrome c reductase (complex III) with variable decreases in rotenone-sensitive NADH-cytochrome c reductase (complex I+III) and rotenone-sensitive NADH-CoQ reductase (complex I) (assays done as previously described[12,13]).

On the basis of the consanguinity of the families studied, we assumed that the phenotype was a consequence of a founder effect. Homozygosity of affected individuals at the genomic loci of *BCSIL*[7–9] and *UQCRB*,[11] previously associated with isolated complex III deficiency, was ruled out (data not shown). We thus went on to study 11 affected individuals and five healthy individuals by genome-wide linkage analysis with 400 fluorescent end-labeled polymorphic markers (ABI PRISM Linkage Mapping Set MD10 [Applied Biosystems]) as previously described.[14] Noninformative regions were excluded with additional adjacent microsatellite markers (data not shown). We identified only a single locus in which most affected individuals demonstrated increased homozygosity, whereas all obligatory carriers tested were shown to be heterozygous (data not shown). The locus, spanning approximately 9 cM (corresponding to 15.84 Mb) on chromosome 5q31, resides between marker D5S471 (homozygosity in five of seven affected individuals) and D5S2115 (homozygosity in eight of 11 affected individuals). Fine mapping of the locus was done, testing DNA samples of the 62 available family members (20 affected and 42 nonaffected) with additional polymorphic markers adjacent to the interval between D5S471 and D5S2115: D5S471, D5S503, D5S1712, D5S1975, D5S2059, D5S804, D5S2078, D5S642, D5S2057, D5S1984, D5S2002, D5S_2, D5S2497,

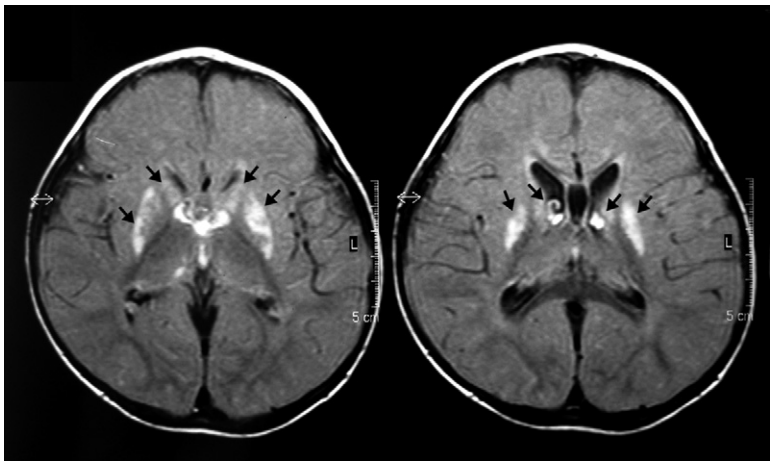

Figure 2. MRI Studies of Affected Individual 133 at Age 2 Years
Bilateral symmetrical abnormal findings in the basal ganglia, with increased density in the putamen and decreased density and size of the caudate and globus pallidum nuclei.

D5S2117, D5S2053, D5S1995, and D5S2115; D5S_2 is polymorphic marker designed with Tandem Repeats Finder[15] (sequences available on request).

As seen in Figure 3A, an approximately 2.14 cM interval of homozygosity common to all affected individuals in the family lies between markers D5S2057 and D5S2497. Three affected siblings (184, 182, and 185) in the family presented with a crossing-over event at D5S2057, setting the upper limit of the homozygosity interval at D5S2057 (Figure 3A). The affected individual 129 presented with a crossing-over event at D5S2497, setting the lower limit of the homozygosity interval at D5S2497. Linkage was demonstrated by two-point analysis with *SUPERLINK*,[16] with a logarithm of the odds [LOD] score of 8.82 at $\theta = 0$ for marker D5S_2 (data not shown).

The common haplotype indicated a minimal candidate interval: a 2 Mb region that contains 30 known or predicted genes. Sequencing of the coding regions of all 30 genes within the 5q31 locus (other than *UQCRQ*) revealed no mutations (data not shown). As shown in Figure 4, sequence analysis of *UQCRQ* (GenBank accession number NM_014402.4) revealed a c.208C → T mutation in exon 2, replacing serine at position 45 by phenylalanine (p.Ser45Phe) in the encoded protein, *UQCRQ*, complex III subunit VII, 9.5 kDa. The mutation abolishes a recognition site of the restriction enzyme HinfI, allowing easy analysis of the entire kindred and controls (polymerase chain reaction [PCR] amplification primers: 5′- CCGCACGT CTTCACTAAAGG-3′ and 5′-CTTAAGGGCGGTCAGGAA TA-3′). Analysis of all 62 DNA samples of the kindred was compatible with the mutation being associated with the disease phenotype, implying full penetrance (data not shown; PCR primer sequences and amplification procedures used for sequencing are available on request). The mutation was not found in any of 600 chromosomes from ethnically matched controls tested by restriction analysis, or in any of 470 chromosomes from unrelated non-Bedouin individuals of various ethnic backgrounds. It should be noted that the serine at position 45 of *UQCRQ* is replaced in some species by glutamine or arginine but is not replaced by phenylalanine in any of the homologs of this gene in all organisms sequenced to date. Whereas serine, glutamine and arginine have aliphatic side chains, phenylalanine has an aromatic side chain, leading to a significantly different three-dimensional structure. Three-dimensional modeling of the Bovine Mitochondrial bc1 Complex[17] (which contains the *UQCRQ* homolog) suggests that the Ser45 lies at a critical hinge position likely determining the orientation of the extramembrane domain of the molecule.[18] Thus, we assume that an amino acid that is significantly structurally different at this position alters the orientation of this extramembrane domain. This is in line with the significant yet relatively mild effect on complex III activity and the changes seen in complex I activity in affected individuals, probably mediated by the mutation altering the supramolecular structure of the mitochondrial respiratory chain. It has been demonstrated that a fully assembled complex III is essential for the stability and activity of complex I.[19,20] Thus, our results specifically confirm the structural dependency between complexes I and III that was previously demonstrated in patients carrying cytochrome b mutations, associated with decreased complex I activity.[10,19] It is noteworthy that no significant changes were found in other complexes (in contrast with some other mitochondrial syndromes, such as the recently described ubiquinone deficiency).[21]

Only minor morphological changes were seen in muscle biopsies of affected individuals. This is not surprising: Although *MTCYB* mutations are associated with ragged-red

Table 1. Enzymatic Activities of the Mitochondrial Respiratory Chain

	Sample			
Assay	Control, n = 50	99	109	177
Citrate synthase[a]	2520 ± 760	3300	1800	2500
Complex I[a]	227 ± 70	128 (45%)	157 (96%)	118 (52%)
Complex I+III[a]	608 ± 213	326 (43%)	306 (70%)	450 (74%)
Complex II+III[a]	295 ± 101	318 (86%)	190 (89%)	340 (114%)
Complex II[a]	210 ± 60	208 (79%)	145 (94%)	196 (92%)
Complex III[a]	2500 ± 1400	2100 (62%)	1290 (66%)	1200 (44%)
Complex IV[b]	17.4 ± 6.9	21.0 (95%)	12.1 (94%)	14.2 (80%)
Complex V[a]	536 ± 193	869 (135%)	350 (94%)	425 (82%)

Enzymatic activities of the five mitochondrial respiratory-chain complexes were measured in isolated muscle mitochondria. Values in parentheses present activities as a percentage of the control mean, normalized for citrate synthase activity.
[a] Expressed as nmol/min/mg.
[b] Expressed as velocity constant.

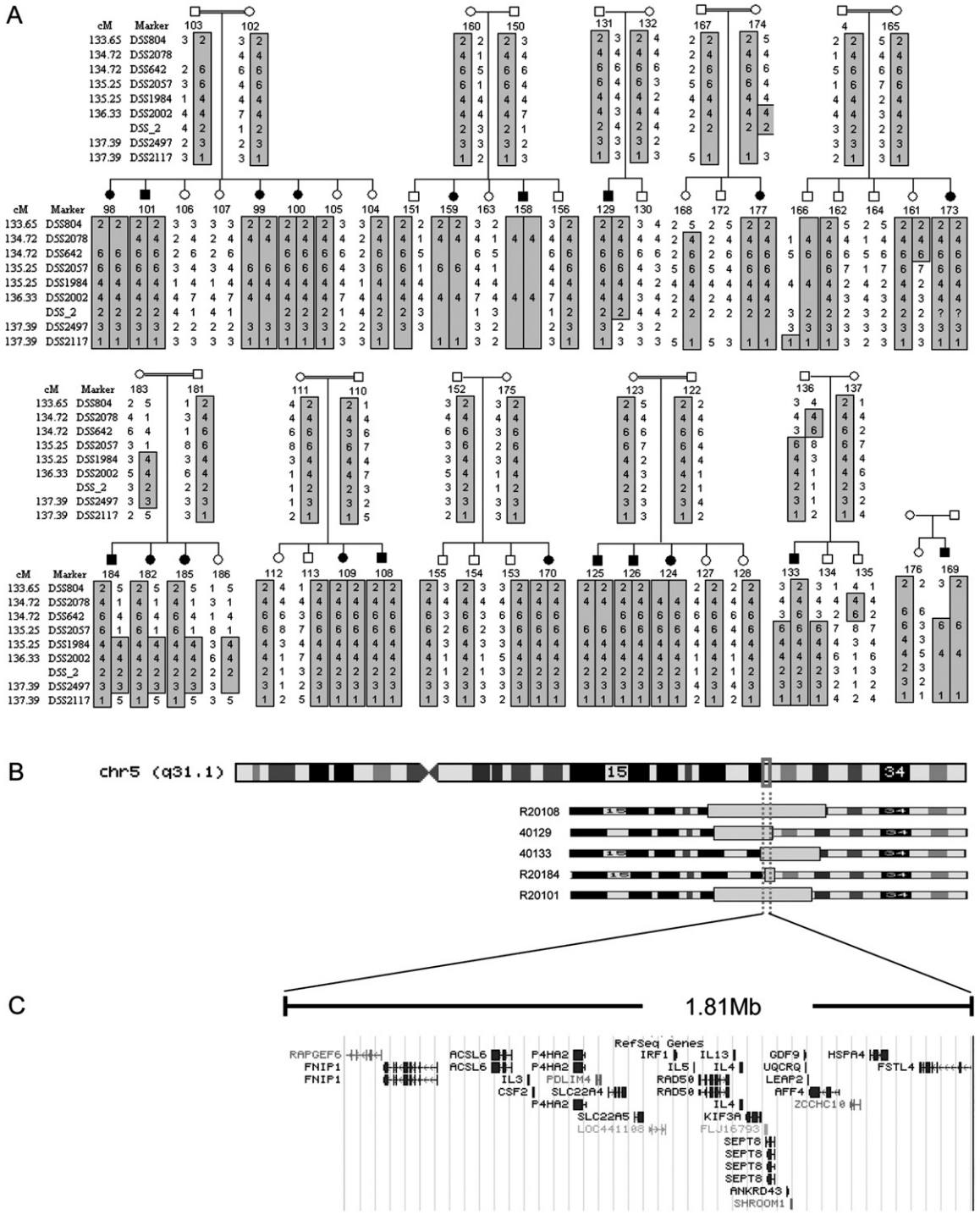

Figure 3. Defining the Disease-Associated Locus

(A) Partial pedigree of the large Israeli-Bedouin kindred. The affected haplotype is shaded. Physical distances between the markers are shown.

(B and C) Fine mapping of the 5q31 locus. In (B), the disease-associated haplotype is boxed. Minimal homozygosity locus associated with the disease is defined between the markers *D5S2057* and *D5S2497* (gray box). (C) shows a schematic presentation of the defined locus.

fibers,[2–5] BCS1L mutations are not.[7–9] The only report on the *UQCRB* gene mutation is incomplete in this regard.[11] The high LOD score, the lack of mutations in any of the other genes in the defined genomic locus, and the absence of the p.Ser45Phe mutation in 1070 control chromosomes, together with the fact that UQCRQ is a component of complex III—consistent with the reduced complex III activity in affected individuals—heavily imply that the *UQCRQ* mutation described is the basis for this severe phenotype. We thus suggest that a homozygous mutation in *UQCRQ*

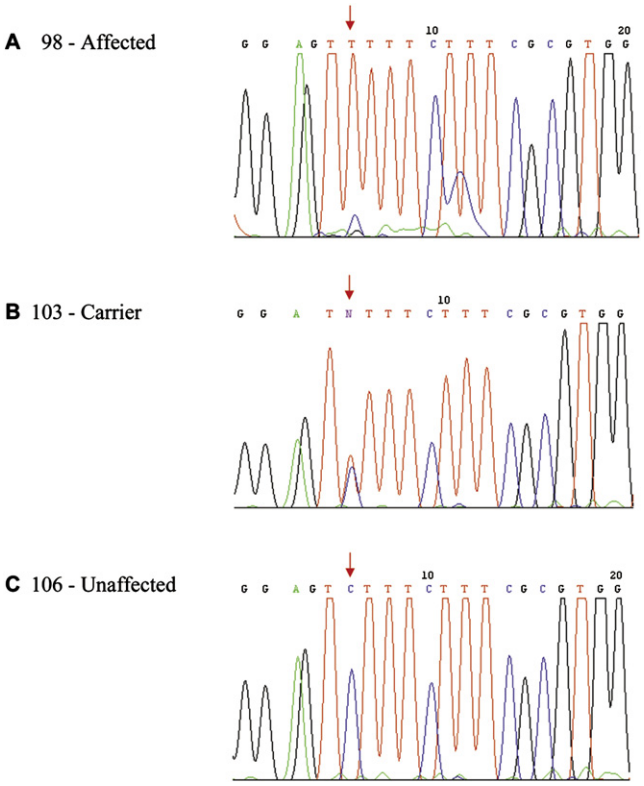

Figure 4. Analysis of the C208T Mutation in Exon 2 of *UQCRQ*
Sequence analysis is shown for an affected individual (A), an obligatory carrier (B), and an unaffected individual (C).

is associated with defective function of mitochondrial complex III, leading to a severe autosomal-recessive neurological phenotype.

Supplemental Data

A genome-wide scan is available at http://www.ajhg.org/.

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Web Resources

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

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