Mutations in Iron-Sulfur Cluster Scaffold Genes NFU1 and BOLA3 Cause a Fatal Deficiency of Multiple Respiratory Chain and 2-Oxoacid Dehydrogenase Enzymes

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Severe combined deficiency of the 2-oxoacid dehydrogenases, associated with a defect in lipoate synthesis and accompanied by defects in complexes I, II, and III of the mitochondrial respiratory chain, is a rare autosomal recessive syndrome with no obvious causative gene defect. A candidate locus for this syndrome was mapped to chromosomal region 2p14 by microcell-mediated chromosome transfer in two unrelated families. Unexpectedly, analysis of genes in this area identified mutations in two different genes, both of which are involved in [Fe-S] cluster biogenesis. A homozygous missense mutation, c.545G>A, near the splice donor of exon 6 in NFU1 predicting a p.Arg182Gln substitution was found in one of the families. The mutation results in abnormal mRNA splicing of exon 6, and no mature protein could be detected in fibroblast mitochondria. A single base-pair duplication c.123dupA was identified in BOLA3 in the second family, causing a frame shift that produces a premature stop codon (p.Glu42Argfs*13). Transduction of fibroblast lines with retroviral vectors expressing the mitochondrial, but not the cytosolic isoform of NFU1 and with isoform 1, but not isoform 2 of BOLA3 restored both respiratory chain function and oxoacid dehydrogenase complexes. NFU1 was previously proposed to be an alternative scaffold to ISCU for the biogenesis of [Fe-S] centers in mitochondria, and the function of BOLA3 was previously unknown. Our results demonstrate that both play essential roles in the production of [Fe-S] centers for the normal maturation of lipoate-containing 2-oxoacid dehydrogenases, and for the assembly of the respiratory chain complexes.

Introduction

Pyruvate dehydrogenase complex (PDHc) deficiency most often occurs as an isolated enzyme defect caused by mutations in X-linked PDHA1 (MIM 300502) and produces a spectrum of clinical presentations ranging from fatal infantile lactic acidosis to mild psychomotor retardation.1,2 A smaller number of PDHc deficient individuals have mutations in PDHB (MIM 179060), PDHX (MIM 608769), dihydrolipoyl transacetylase (DLAT, MIM 608770), dihydrolipoyl dehydrogenase (DLD, MIM 238331), or PDPI (MIM 605993), which controls the reactivation of phosphorylated PDHc.3,5 We have described two unrelated families with neonatal, severe deficiencies of PDHc associated with defects in oxoglutarate dehydrogenase complex (OGDHc) and with complexes I, II, and III of the respiratory chain, and we called this multiple mitochondrial dysfunction syndrome (MIM 605711, Table 1). Using the technique of microcell-mediated chromosome transfer, we defined a candidate locus on chromosome 2p14 for both families.6 The locus covers 10 Mb of sequence in a region containing more than 50 genes, many of which have no defined function. We sequenced a number of genes as candidates for this syndrome and focused on genes with a high probability of targeting to the mitochondria (determined with Mitoprot1). Two mitochondrial genes involved in iron-sulfur ([Fe-S]) cluster assembly were likely candidates for containing mutations that could cause the multi-enzyme deficiency. This deduction was based on the presence of [Fe-S] clusters in protein components of all the affected respiratory chain enzyme complexes.

In this study, we show that two genes involved in the biogenesis of [Fe-S] clusters have profound effects on the respiratory chain and 2-oxoacid dehydrogenase complexes (Figure 1).

Materials and Methods

Families

Both families have been described in a previous publication.6 Briefly, the family with the NFU1 mutation comprises two affected males, one affected female, and one unaffected female (Figure 2). The parents are nonconsanguineous and of Mexican descent. The family with the BOLA3 mutation comprises one affected male and one unaffected male (Figure 3). The parents are first cousins and of East Indian descent. Enzymatic deficiencies of the respiratory chain and 2-oxoacid dehydrogenases are summarized in Table 1. All procedures were carried out with approval from The Hospital for Sick Children’s research ethics board.

Cell Culture

Cultured skin fibroblasts were grown from forearm skin biopsy (taken with informed consent) in α-MEM culture medium (11 mM glucose) and 20% fetal calf serum (Wisent, Saint-Jean-Baptiste de Rouville, Quebec, Canada). Lymphoblasts were grown in

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10% fetal calf serum/RPMI. Primary human skin fibroblasts were immortalized with a retrovirus expressing the E7 gene of type-16 human papilloma virus and a retroviral vector expressing the protein component (htert) of human telomerase. These cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

Molecular Genetic Techniques
Genomic DNA was isolated from fibroblasts or blood with the Puregene genomic DNA isolation kit (Inter Medico). RNA was isolated with Trizol, and full-length NFU1 cDNA sequence was reverse transcribed with Superscript II reverse transcriptase and amplified with Platinum Hi-Fi Taq polymerase (all from Invitrogen). Oligonucleotide primers used are shown in Table S1, available online. All PCR products were sequenced directly by fluorescent sequencing methods (ACGT Corporation, Toronto, Canada).

Retroviral vectors containing the HA-tagged cDNA sequence of the cytosolic and mitochondrial versions of NFU1 and isoforms 1 and 2 of BOLA3 were created with the Gateway cloning system (Invitrogen) as previously described. We amplified cDNAs from these genes by using RNA isolated from fibroblasts with the RNeasy kit (QIAGEN), by OneStep RT-PCR (QIAGEN) with specific primers modified for cloning into Gateway vectors (flanked with attB1 and attB2-HA sequences). The PCR constructs were cloned into a Gateway-modified retroviral expression vector pLXSH. The fidelity of cDNA clones was confirmed by automated DNA sequencing. Retroviral constructs were used to transiently transfect a Phoenix packaging cell line with the HBS/Ca3(PO4)2 method (see Web Resources). Affected and control fibroblasts were infected 48 hr later by exposure to virus-containing medium in the presence of 4 μg/ml of polybrene.

Immunoblotting and Blue Native Gel Electrophoresis
For immunoblot analysis, fibroblasts were used to prepare mitochondria. Twenty-five micrograms of mitochondria was resolved through a 12.5% stacking SDS/PAGE gel. The SDS/PAGE gel was electrobotted onto polyvinylidene fluoride membrane, blocked with 5% skim milk/Tris buffered saline with Tween (TBST), and probed with antibodies. Anti-human citrate synthase and anti-holoCOX were prepared by immunizing rabbits with purified citrate synthase or bovine heart cytochrome oxidase. The anti-NFU1 was a gift from Tracey Rouault, National Institutes of Health. Antibodies to complex I subunits 75 kDa (NDUFS1), 51 kDa (NDUFV1), and 49 kDa (NDUFS2) were prepared by immunizing rabbits with protein peptides, and complex I 39 kDa (NDUFA9) and ASHI (NDUFB8), complex II SDHA, complex III UQCRFS1, and UQCRC1 antibodies were purchased from Mitosciences (Eugene, OR, USA). CYTB antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Lipote antibody was purchased from Cedarlane (Burlington, ON, Canada) and anti-GAPDH from Abcam (Cambridge, MA, USA). Immunoreactive proteins were visualized with Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life and Analytical Sciences). Blue native gel electrophoresis was carried out as previously described. We carried out densitometry measurements by using ImageJ software.

Immunofluorescence
Cells were rinsed two times with PBS, fixed with PBS 4% PFA for 20 min, and rinsed three times with PBS. Permeabilization was performed with PBS 0.1% Triton for 15 min followed by three rinses with PBS. Nonspecific antigenic sites were blocked with PBS 10% ND. The secondary antibody was applied in blocking solution for 2 hr at room temperature. After three rinses in PBS, the primary antibody was applied in blocking solution for 1 hr followed by three rinses with PBS. Nuclei were counterstained with DAPI, and cover glasses were mounted in Fluoromount. The following antibodies were used: rabbit anti-NFU1 (1:1000), mouse anti-SDHA (1:1000), mouse anti-HA (1:5000), goat anti-rabbit Alexa488 (1:2000), goat anti-mouse Alexa594 (1:2000), and goat anti-mouse Alexa594 (1:2000).

Results
In the locus defined by chromosome transfer at 2p14, we searched for a gene that might have effects on both

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>[Fe-S] Clusters</th>
<th>NFU1 Mutant Individuals</th>
<th>BOLA3 Mutant Individual</th>
<th>ISCU Mutant Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I (I+III)</td>
<td>[2Fe-2S] (×2), [4Fe-4S] (×6)</td>
<td>~30%</td>
<td>~30%</td>
<td>~70%</td>
</tr>
<tr>
<td>Complex II</td>
<td>[2Fe-2S], [3Fe-4S], [4Fe-4S]</td>
<td>50%</td>
<td>50%</td>
<td>20%</td>
</tr>
<tr>
<td>Complex III</td>
<td>[2Fe-2S]</td>
<td>~60%</td>
<td>~60%</td>
<td>~40%</td>
</tr>
<tr>
<td>Complex IV</td>
<td>~50% in 2 sibs, normal in 1 sib</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Mitochondrial Aconitase</td>
<td>[4Fe-4S]</td>
<td>normal</td>
<td>normal</td>
<td>~30%</td>
</tr>
<tr>
<td>PDHc</td>
<td>requires covalently attached lipote on E2</td>
<td>~5–10%</td>
<td>~5–10%</td>
<td>ND</td>
</tr>
<tr>
<td>E2</td>
<td>requires covalently attached lipote on E2, but assay works with added lipote acid</td>
<td>normal</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>E3</td>
<td>normal</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>OGDHc</td>
<td>lipote requiring</td>
<td>~10%</td>
<td>~30%</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND is used as an abbreviation for not determined.

Table 1. Relative Mitochondrial Enzyme Activities for NFU1, BOLA3, and ISCU Mutant Fibroblasts
2-oxoacid dehydrogenase complexes and respiratory chain complexes. Because the metabolic pathways involving [Fe-S] cluster assembly are a likely common cause for such defects, we sequenced NFU1 (MIM 608100), an essential protein in one of the two pathways for [Fe-S] assembly in both families (Figures 1 and 2A). [Fe-S] clusters function as the prosthetic groups of many proteins essential for intermediary metabolism and oxidative phosphorylation. They are constructed and assembled into proteins in a series of complex biochemical reactions that appear to run in two parallel pathways (Figure 1). Both ISC U (MIM 611911) and NFU1 can assemble [2Fe-2S] and [4Fe-4S] clusters, although only a human defect in ISC U has been noted before; this defect causes Swedish myopathy with exercise intolerance (MIM 255125). In family 1, a homozygous missense mutation, c.545G>A (p.Arg182Gln), was identified in exon 6 of NFU1 in the three affected siblings but not in their unaffected sibling, P4 (II-4). The mutation was not identified in seven different control samples or in the expressed sequence tag (EST) database (99 NFU1 ESTs).

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sequence coded in exon 1A. Isoform 1 (NM_015700.2) contains exon 1B, and translation is predicted to start from a downstream AUG start codon in exon 2 and produce a protein that remains in the cytosol. The c.545G>A mutation thus affects both the mitochondrial and cytosolic isoforms of the protein, and is predicted to result in an p.Arg182Gln substitution, but only if exon 6 is spliced correctly to exon 7. It is, however, adjacent to the GU 5′ splice donor in intron 6, and RT-PCR analysis in Figure 2C shows that exon 6 is spliced out from the majority of transcripts. In fact the full-length mitochondrial transcript is not detected by this analysis. We have sequenced separate cloned RT-PCR transcripts of NFU1 from individuals P1–P3 (II-1, II-2, and II-3 in Figure 2) representing cNFU1a (cytosolic transcript missing exon 6), mNFU1 (mitochondrial transcript with c.545G>A mutation), mNFU1a (missing exon 6), and mNFU1b (missing exons 6 and 7). The only transcript that was not confirmed by sequencing was cNFU1 containing the c.545G>A mutation. Mutations in the coding regions of NFU1, the promoter, or the intron/exon boundaries of the gene were not identified in the single affected individual from the second family (individual P5, II-1 in Figure 3), suggesting another gene defect in the same chromosomal region. Several candidate genes were tested and were chosen on the basis of chromosomal location, a high probability of mitochondrial import (determined with Mitoprot 7), and tentative roles in metabolism or gene regulation (genes sequenced were AHSA2, BCS1L [MIM 603647], SMEK2 [MIM 610352], CCT4 [MIM 605142], SLC1A4 [MIM 600229], SPR [MIM 182125], SFXN5, FLJ30838, FAM161A [MIM 613596], LOC647074, MDH1 [MIM 154200], RAB1A [MIM 179508], and LOC388955). A homozygous single-base-pair duplication (c.123dupA; p.Glu42Argfs*13) was identified in exon 2 of BOLA3 (MIM 613183, 4.7 Mb upstream of NFU1) in individual P5 (II-1 in Figure 3), resulting in a frameshift and predicting a premature stop codon (p.Glu42Argfs*13). The parents and an unaffected...
sibling (individual P6, II-2 in Figure 3), were all heterozygous carriers for the mutation. The mutation was not identified in seven different control samples or in the EST database (68 BOLA3 ESTs).

The human protein BOLA3 is predicted to have a mitochondrial targeting sequence, and a 95% probability of import into the mitochondria. Human BOLA3 has two isoforms (NM_001035505.1 and NM_212552.2); isoform 2 lacks exon 3, which is present in isoform 1, resulting in different stop codons in the two transcripts (Figure 3B). Both isoforms are affected by the mutation in exon 2 in P5 (II-1 in Figure 3), and the amount of full-length transcript for either isoform does not appear to be reduced (data not shown). Respiratory chain complexes I, II, and III all have components that contain [Fe-S] clusters. For both families, several subunits of complex I (NDUFS1, which contains [Fe-S]; NDUFV1, which contains [Fe-S]; NDUFS2; NDUFA9; and NDUFB8) were analyzed and all showed a complete loss of protein except for NDUFS1, which had a 20% reduction in the NFU1 mutant compared to citrate synthase and 40% reduction in the BOLA3 mutant (Figure 4). Because NFU1 and ISCU have the same proposed role in [Fe-S] cluster synthesis, albeit in parallel pathways, we thought it prudent to compare the biochemical and clinical consequences of both defects. In contrast to the NFU1 and BOLA3 mutant fibroblasts, the ISCU mutant individual has normal NFU1; complex I NDUFS2, NDUFA9, and NDUFB8; and complex III UQCRCl protein levels. However, complex III UQCRFS1, which contains an [Fe-S] cluster, was severely reduced in this individual (60% compared to citrate synthase). In the NFU1 and BOLA3 mutant individuals, complex II subunit SDHA was reduced by 69% and 73%, respectively, as well as UQCRFS1 (70% and 76% reduction) and UQCRCl subunits of complex III (22% and 60% reduction), whereas cytochrome b expression was affected differently in the two individuals (13% increase and 30% reduction) (Figure 4).

In contrast PDHc and OGDHc constituent subunit proteins do not contain [Fe-S] clusters, but those complexes show profound enzyme deficiency. The common feature of these enzymes is the requirement for covalently attached lipoate on the E2 subunits of PDHc (dihydrolipoamide transacetylase [DLAT]) and OGDHc (dihydrolipoyl transsuccinylase [DLST] [MIM 126063]), and the E3BP subunit of PDHc.

We used an antibody to lipoate to identify the lipoate containing subunits of both PDHc and OGDHc complexes in fibroblast cell mitochondria. Anti-lipoate failed to detect the expected lipoylated E2 proteins of PDHc and OGDHc in both families with multiple mitochondrial dysfunctions syndrome (Figure 4). The individual with the NFU1 mutation has a single weak band at a different molecular weight, which could represent a modified form of lipoate. P5 (II-1 in Figure 3) showed no detectable lipoate labeling. We attempted to improve the defective enzyme activities in the affected fibroblasts by adding exogenous lipoate, but no effect was seen on PDHc activity after three days of culture in media supplemented with 30 μM lipoic acid.

To confirm the pathogenicity of the NFU1 and the BOLA3 mutations, we constructed retroviral expression vectors for both isoforms of each gene, transduced fibroblast cell lines, and tested for complementation of the biochemical defect by immunoblot, BN-PAGE electrophoresis...
We have identified a family with a mutation in NFU1, which encodes an [Fe-S] cluster biogenesis protein. The c.545G>A mutation causes splice site instability leading to the production of several truncated transcripts and no mitochondrial NFU1 protein product. We have also identified a family with a mutation in BOLA3, a previously uncharacterized gene that the present results suggest is also involved in [Fe-S] cluster synthesis. How do these findings fit with our knowledge of [Fe-S] cluster biogenesis?

NFU1 as an Alternate Scaffold for [Fe-S] Cluster Biogenesis

[Fe-S] clusters function as the prosthetic groups of many proteins essential for intermediary metabolism and oxidative phosphorylation. They exist mainly as [2Fe-2S] and [4Fe-4S] clusters and participate in electron transfer reactions in which oxidation or reduction of oxygen or sulfur is desirable. [Fe-S] clusters are essential components of enzymes involved in the maturation of subunits of complexes I, II, and III as well as in the synthesis of the enzyme-bound cofactor lipoate present in the 2-oxoacid dehydrogenases. [Fe-S] clusters are constructed and assembled into proteins in a series of complex biochemical reactions that appear to run in two parallel pathways. The process occurs in the mitochondrial matrix with the export of some clusters to form cytosolic and nuclear proteins (Figure 1).

The role of NFU1 in [Fe-S] cluster biogenesis has been deduced from its orthology to the bacterial protein NifU. NFU1 and ISCU in eukaryotes both evolved from different domains of NifU, a protein present in nitrogen-fixing bacteria. ISCU is thought to function as a scaffold for the assembly of [Fe-S] clusters, but the role of NFU1 is less clear. Several roles for NFU1 have been hypothesized, two of which are involved in [Fe-S] cluster biosynthesis. By demonstrating the ability of NFU1 to assemble [4Fe-4S] clusters and transfer them to apoproteins, it has been suggested that NFU1 could function as an alternate scaffold to ISCU for assembly of [Fe-S] proteins, thus providing parallel pathways (Figure 1).17,19,20

The c.545G>A mutation results in truncated transcripts for both mitochondrial and cytosolic NFU1 isoforms, and therefore could affect both protein products. Mammalian NFU1 is one of several [Fe-S] cluster genes (NFS1 [MIM 603485], ISCU, and NFU1) reported to have mitochondrial and cytosolic isoforms. In the individuals reported here, the c.545G>A mutation appears to be spliced out of virtually all of the transcribed forms of mitochondrial forms of NFU1, resulting in barely detectable levels of the protein (Figure 5A). Transfection of the cytosolic transcript does result in a partial restoration of complex I NDUF9 and complex II SDHA subunit levels, but this appears to result from a low level of expression of the mitochondrial isoform from this construct, probably because of readthrough of the stop codon in exon 1B.
Inference of the Role of BOLA3 in [Fe-S] Cluster Synthesis

Mammalian BOLA3 belongs to a BolA-like family of proteins that is conserved from prokaryotes to eukaryotes. They were originally thought to be DNA-binding proteins (based on the presence of a helix-turn-helix motif), which create a round morphology in *Escherichia coli* cells when overexpressed. They have also been shown to be expressed under stress situations such as carbon starvation and oxidative stress.

Little is known about the function of human BOLA3, but predictions can be made from the roles of ortholog proteins. BolA family members have been postulated to act as reductases, interacting with the mono-thiol glutaredoxin family. Genome-wide yeast two-hybrid studies have shown a physical interaction between cytosolic monothiol glutaredoxins (Grx) and BolA-like proteins in *Saccharomyces cerevisiae* and *Drosophila melanogaster*. In several genomes, genes encoding Grx and BolA-like proteins lie physically next to each other, and the two protein families show co-occurrence in organisms because only those that express BolA proteins possess monothiol Grx proteins. It is possible that BOLA3 is interacting with glutaredoxin 5, which along with several chaperones is involved in inserting [2Fe-2S] and [4Fe-4S] clusters into apoproteins (Figure 1).

Transfection of both isoforms of BOLA3 into deficient fibroblasts showed that only isoform 1 can restore protein steady-state levels, respiratory chain complex assembly, and enzyme activities (Figures 5B, 5D, and 7). The role of the shorter isoform is unknown. Immunofluorescence results show this isoform, but not BOLA3-2, localizes to mitochondria (Figure 6B). What is the nature of the 2-oxoacid dehydrogenase enzyme c defect in the mutant NFU1 and BOLA3 individuals?

Mutation of NFU1 would be expected to interfere with the pathway of [Fe-S] cluster synthesis resulting in a decrease in the assembly and activity of complexes I, II, and III. This does not however explain the profound deficit in PDHc activity (<10% control), in the face of apparently normal assembly of the protein components of PDH. The lack of immunoreactive lipoate in the PDH or 2OGDH complex, however, is compelling and suggests that both NFU1 and BOLA3 are necessary for lipoate synthesis. That these are candidates for defects in lipoate synthesis is evident from work on yeast in which elimination of cysteine desulphurase (NFS1), iron sulfur assembly protein (ISA1), or IBA57 results in an inability to produce lipoate.
or lipoylated proteins. The E2 protein of PDHc, 2OGDH, and BCKADHc is initially octanoylated on lysine residues by lipoyl transferase 2 (LIPT2) in the biosynthetic pathway (Figure 1) or lipoyl transferase 1 (LIPT1) in the salvage pathway. This is subsequently transformed to lipoate by the addition of two sulfur atoms in a reaction catalyzed by lipoic acid synthase (LIAS), an enzyme possessing two [4Fe-4S] clusters with unusual cysteine-containing motifs CX₃CX₂C and CX₄CX₅C in the protein sequence. It is known that the E2 protein of the PDHc runs at a different mobility on SDS-PAGE gels when lipoylated (72 kDa) compared to its unlipoylated mobility (55 kDa). The mobility observed in individual P1 for the PDHc-E2 is less than 72 kDa, suggesting that the mobility has been altered by octanoylation. The intactness of the PDHc-E2 enzyme function is illustrated by the fact that fibroblasts from both affected individuals possess normal dihydrolipoyl transacetylase activity when measured by acetylation of reduced lipoic acid by acetyl CoA. However, because this assay is carried out with added reduced lipoic acid, the enzyme does not require enzyme-bound lipoate to function. Nevertheless, the activity of both PDHc and OGDHc in the unsupplemented complex is greatly reduced, and there is virtually no such activity in individual P5. We hypothesize that the loss of mNFU1 and BOLA3-1 in these individuals might be specifically affecting the maturation of lipoic acid synthase, an [Fe-S] cluster protein, which in turn will impact the enzyme activities of PDHc and OGDHc (Figure 7).

Why Do NFU1 and BOLA3 Mutations Have a Different Phenotype from that Seen with ISCU Mutations?
The most profound differences between the three classes of defect is a loss of aconitase protein and enzyme activity...
in ISCU mutants and lipoate abnormalities in NFU1/BOLA3 mutants; there is an indication of less affected complex I in the ISCU mutant. Mitochondrial aconitase enzyme levels in the NFU1/BOLA3 mutant individuals were normal (Table 1) as were protein levels (data not shown). This suggests a divergence between the roles of NFU1/BOLA3 and ISCU, with NFU1 and BOLA3 specializing in proteins destined to become involved in the lipoate pathway. It had previously been suggested that aconitase-like proteins and radical S-adenosyl methionine (SAM) proteins (such as lipoate synthase) are assembled in the same pathway. 26 Recent work shows that many more [Fe-S] cluster-containing proteins appear to have specific maturation or assembly factors, for example, human INDI for complex I proteins, 31,32 and E. coli ErpA, essential for an [Fe-S] protein involved in isoprenoid biosynthesis. 33 It is therefore likely that the roles of NFU1 and BOLA3 in eukaryotes are not as simple as has so far been hypothesized with more specialization than previously thought. ISCU assembled [Fe-S] clusters appear to be preferentially channeled to aconitase and complex II, whereas NFU1- (and BOLA3-) dependent assembled clusters are more directed to complex I and lipoate synthesis.

We conclude that in humans there are parallel pathways for synthesis of [Fe-S] clusters, and there is a bias in each pathway for maturation of certain target proteins. How this is achieved will be the subject of further research.

Supplemental Data

Supplemental Data include one table and can be found with this article online at http://www.cell.com/AJHG/.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org

References


