

## Review

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# NUBPL: a Mitochondrial Complex I deficiency disorder

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## Abstract

**Mitochondrial ailments are diverse and devastating. Defects in mitochondrial DNA or its products lead to wide range of deficiencies in the mitochondrial electron transfer system and its ensuing energy production. Accessory proteins required for the assembly and function of the respiratory complexes are also required for healthy, coupled, and energy-producing mitochondria. Recently, the protein nucleotide binding protein like (NUBPL or IND1) was identified as an iron-sulfur cluster transfer protein specifically for Complex I. Since the presence of multiple iron-sulfur clusters in Complex I is necessary for its activity, deficiency in NUBPL leads to severely dysfunctional mitochondria, with upregulated compensatory Complex II activity. Here we present a short review of the debilitating disease related to NUBPL deficiency.**

## 1. Definition

In humans, as in most other organisms, the oxidative phosphorylation (OXPHOS) process involves five multimeric complexes embedded in the mitochondrial inner membrane (mtIM), Complex I through Complex IV and ATP synthase. These complexes work consecutively to transfer the electrons donated from the reduced forms of the electron carriers, Nicotinamide Adenine Dinucleotide (NADH) and Flavin Adenine Dinucleotide (FADH<sub>2</sub>), to the terminal electron acceptor, oxygen. This series of exergonic redox reactions produce enough energy to create an electrochemical gradient across the mtIM, namely the proton motive force, driving the synthesis of ATP via ATP synthase (Ohnishi et al 2018; Tang et al 2020).

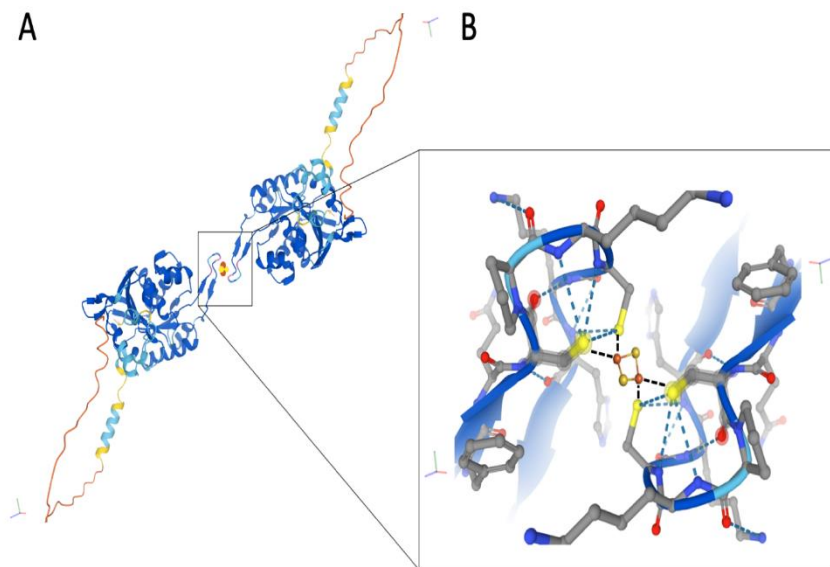
NADH:ubiquinone oxidoreductase (Complex I) is the largest component of the OXPHOS system. It comprises 45 protein subunits encoded by nuclear and mitochondrial

genes. Along with the apoprotein subunits, Complex I contains stably bound prosthetic groups, including flavin mononucleotide and eight to ten iron-sulfur (Fe-S) clusters (Ohnishi et al 2018). Thus, even in the minimalistic Complex I from *E. coli*, the proper assembly of this composite protein is an essential factor determining its function (Schimpf et al 2022). The assembly of Complex I is governed by a set of assembly factors responsible for the insertion of the prosthetic groups into either the monomeric forms of the complex subunits or the fully assembled apoenzyme (Sheftel et al 2009).

The Fe-S cofactors are ancient accessory parts of proteins that serve in a wide range of functions, from OXPHOS to cellular and nucleic acid metabolism (Khodour et al 2019; Stiban et al 2016). Despite the chemical spontaneity of Fe-S cluster assembly, the biogenesis and maturation of Fe-S-cluster-containing proteins in mammals is an enzymatic process. Briefly, this process begins in the mitochondria with the formation of sulfide from desulfurized cysteine by a cysteine desulfurase complex Nfs1-Isd11 (Sheftel et al 2009). It is then combined with a ferrous ion by the scaffold protein Isu1. The cluster is then transferred to an apoprotein by a monothiol glutaredoxin, among other chaperone proteins (Sheftel et al 2009). The mitochondrial scaffold is then exported by an Fe-S cluster export machinery (ISC) to construct cytosolic and nuclear Fe-S proteins. Then, the Fe-S-containing protein biogenesis resumes by two cytosolic NTPase proteins, Nbp35 and Cfd1, which function as scaffolds for Fe-S cluster assembly (Protasoni et al 2020).

Interestingly, in 2008, a mitochondrial protein with sequence similarity to the cytosolic NTPases was identified and initially termed IND1 for an Fe-S protein required for NADH dehydrogenase. This protein harbors a conserved C-terminal CXXC motif that is thought to be the site where Fe-S clusters bind during the biogenesis of Fe-S-cluster-containing proteins of respiratory Complex I (Bych et al 2008). Therefore, a defect in IND1, now known as nucleotide-binding protein-like (NUBPL), is considered a

subclass of primary mitochondrial diseases that cause mitochondrial Complex I deficiency, nuclear type 21; MC1DN21 (Kimonis et al 2021). As an Fe-S transfer protein,



**Figure 1. Possible structure of a dimeric human NUBPL (Q8TB37).** The full protein structure (A) was modeled using AlphaFold protein structure database (Jumper et al 2021; Varadi et al 2022). The presence of a 2Fe-2S cluster is modeled in the midst of the two monomers. A zoomed in structure showing the interacting CXXC motifs from both monomers and the interacting Fe-S structure is shown in (B).

NUBPL has only one CXXC motif and, therefore, is likely to be a dimer with the 2Fe-2S cluster bridging the two monomers (Figure 2). It is worth noting that the *Drosophila* homolog of the mammalian NUBPL was recently found to alleviate mitochondrial DNA (mtDNA) replication stalling in a similar fashion to overexpression of mtDNA helicase, indicating a possibility of interaction between the helicase and NUBPL (So et al 2021). This finding in fruit flies may have implications as NUBPL may transfer an Fe-S cluster to the N-terminal domain of mtDNA helicase (Stiban et al 2014) initiating its activity. How this relates to the human mtDNA helicase which lacks an Fe-S cluster is currently under investigation.

## 2. Etiology

In 2009, Sheftel et al. accentuated that RNA-interference-mediated-IND1 knockdown in HeLa cells resulted in mitochondrial ultrastructural changes. These changes included massive remodeling of the respiratory supercomplexes, loss of crista membranes, and increased lactate production, likely due to a higher dependency on fermentation in the absence of proper electron transfer system (ETS) function. They also revealed that IND1 depletion resulted in significant remodeling of the membrane arm of respiratory Complex I, as an abnormal subcomplex comprising parts of the original membrane complex appear upon IND1 absence (Sheftel et al 2009).

A year later, the first clinical genetic evidence was brought to attention by Calvo et al., who reported a compound heterozygous patient with two different mutations in the NUBPL gene. The first allele, inherited from the patient's father, consisted of c.166G>A/p.G56R and another splice site mutation (c.815-27T>C). The former resulted in a missense mutation in a glycine conserved across 36 vertebrate species, whereas the latter caused skipping of exon 10, and thus aberrant splice products. On the other hand, the maternal allele had a complex rearrangement comprising of exon 7 duplication along with a deletion in exons 1-4. As a result, this patient suffered developmental delay, leukodystrophy, and elevated cerebrospinal fluid lactate due to Complex I deficiency (Calvo et al 2010).

Disease	Gene Defect	Other Names
★ NUBPL	★ NUBPL/IND1	★ Mitochondrial Complex I Deficiency, nuclear type 21; MC1DN21
★ OMIM: 618242	★ OMIM: 613621	★ Nucleotide-binding protein-like gene (NUBPL) Deficiency
		★ Iron-sulfur protein required for NADH dehydrogenase (IND1) Deficiency

**Figure 2. NUBPL disease card.** Online Mendelian Inheritance in Man (OMIM) entries of NUBPL deficiency along with deficient genes and other names of the disease (obtained from <https://www.omim.org/entry/613621?search=nubpl&highlight=nubpl>)

In 2013, Kevelam and colleagues identified six unrelated patients with Complex I deficiency. All patients shared at least one copy of the previously identified haplotype G56R/c.815-27T-C in the NUBPL gene. Consequently, all patients had a characteristic leukoencephalopathic MRI pattern, including white matter lesions, abnormally swelled corpus callosum, motor ataxia, and other cortical and brainstem abnormalities (Kevelam et al 2013). In the same year, Wydro and Balk investigated the impact of the c.815-27T>C mutation on Complex I expression and function. They concluded that this mutation could introduce changes in Complex I, eventually leading to protein misfolding and instability (Wydro et al 2013). Thus, a disease was associated with NUBPL deficiency (Figure 2).

Other cases of compound heterozygosity were identified by Friederich et al. in 2020, where they reported a novel missense M117I mutation in the NUBPL gene (Friederich et al 2020). In 2021, Kimonis et al. made the recognition that cerebellar dysfunction was prevalent in patients carrying splice-site mutation c.815-27T>C alone or as part of the c.815-27T-C/G46R haplotype and was absent in patients carrying other NUBPL mutations (Kimonis et al 2021).

To conclude, Complex I deficiency is inherited in an autosomal recessive pattern of mutations/pathogenic haplotypes in the NUBPL gene mapped to chromosome 14q12, leading to a range of neurodegenerative manifestations.

### 3. Clinical Manifestations

**Table 1. Clinical manifestations of NUBPL deficiency** (El-Hattab et al 2016; Kimonis et al 2021)

Systems	Major (Signs/Symptoms)	Minor (Signs/Symptoms)
Brain	Onset of neurological symptoms at 3-18 months Global developmental delay Cerebellar dysfunction (including ataxia, dysarthria, nystagmus and tremor) Some forms of Parkinson's disease Seizures Leukoencephalopathy Macrocephaly Sensorineural deafness	-
Heart	Cardiomyopathy	-
Muscle	Spasticity Muscle atrophy	Hypotonia
Liver	Hepatic dysfunction	-
Plasma	-	Lactic acidosis Hypoglycemia

## 4. Diagnosis

The presence of a plethora of rare childhood leukoencephalopathies causes the specific diagnosis to be an extremely challenging process. However, elevated lactate levels in the plasma and cerebrospinal fluid are the first diagnostic markers for identifying mitochondrial leukoencephalopathies. This is usually followed by a ETS function analysis of the patient's muscle biopsy. Nevertheless, these diagnostic tests are shared among multiple mitochondrial leukoencephalopathies. Consequently, for a specific diagnosis of NUBPL, a DNA analysis (e.g., whole-exome sequencing) guided by the previous results usually follows. An MRI pattern analysis can also support this molecular diagnostic test since patients with NUBPL variants were shown to possess a unique combination of T2-hyperintense signal of the cerebellar cortex bilaterally and supratentorial white matter abnormalities (Roosendaal et al 2021). Moreover, there is a distinct MRI progressive pattern among NUBPL patients carrying the splice-site mutation. This pattern begins with cerebellar, deep white matter, and corpus callosum abnormalities at the early stages. At later stages, the white matter and corpus callosum abnormalities improve. In contrast, new brainstem abnormalities develop (Kevelam et al 2013). Recently, in five new patients, brain MRI showed cerebellar atrophy (Kimonis et al 2021).

## 5. Management

Despite the lack of management and treatment approaches, a pre-clinical investigation of candidate therapeutic strategies for NUBPL disease is currently being conducted at The Children's Hospital of Philadelphia. To achieve this goal, researchers are developing three evolutionarily distinct NUBPL knockout models to help in the optimization of the therapeutic regimen (Mathew 2022).

## 6. Conclusion and future directions

Being a debilitating rare disease that affects a miniscule percentage of the world's population is challenging on several fronts. There is limited basic research on this topic and hence treatment options are lacking. Nevertheless, several questions pertaining this disease can be articulated and they merit scientific investigation. 1) What is the bona fide function of NUBPL/IND1? 2) What is the structure of NUBPL/IND1? 3) What are the molecular bioenergetics ramifications of NUBPL/IND1 deficiency (especially with the enhanced Complex II function)? 4) How is NUBPL/IND1 related to Complex I and does it actually transfer some or all Fe-S clusters to it? 5) Does human NUBPL/IND1 interfere positively or negatively with mtDNA helicase? 6) What is the mechanism of Fe-S transfer from NUBPL/IND1 to Complex I? 7) Is the protein actually a dimer and if so, how stable is the Fe-S in the dimer? 8) Are there any drugs that alleviate the symptoms of NUBPL disease? Answering these general questions will certainly move along this field and provide some hope for the known patients who have this disease (Ohnishi et al 2018).

## Abbreviations

OXPHOS	oxidative phosphorylation	Fe-S	iron-sulfur
Complex I	NADH:ubiquinone oxidoreductase	mtIM	mitochondrial inner membrane
ISC	iron-sulfur cluster export machinery	mtDNA	mitochondrial DNA
ETS	electron transfer system	NUBPL	nucleotide-binding protein-like

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