Novel NMNAT1 mutations causing Leber congenital amaurosis identified

References


NMNAT1 mutations cause Leber congenital amaurosis
Falk et al. (2012)
Nature Genetics 44: 1040–1045
Mutations in NMNAT1 cause Leber congenital amaurosis and identify a new disease pathway for retinal degeneration
Koenekoop et al. (2012)
Nature Genetics 44: 1035–1039

Leber congenital amaurosis (LCA; MIM 204000) is a common form of incurable childhood blindness manifested by severe loss of vision. This severe dystrophy of the retina becomes evident in the first year of life and is characterized by poor visual function accompanied by nystagmus, photophobia, high hyperopia and keratoconus (1). LCA is generally an autosomal recessive disorder caused by abnormal development of the photoreceptor cells. Presentation of LCA can vary because it is associated with multiple genes. Mutations associated with LCA have been identified in over 17 genes involved in retinal function, but these account for only 70% of the patients with this inherited retinal dystrophy.

Using whole-exome sequencing, Falk et al. in their study identified a homozygous missense mutation (c.25G>A, p.Val9Met) in the nicotinamide adenine dinucleotide (NAD) synthase gene NMNAT1 encoding nicotinamide mononucleotide adenyllytransferase 1, an enzyme essential for NAD biosynthesis (Fig. 1). The mutation was identified in two affected consanguineous Pakistani siblings (from the Ophthalmology-Genetics Clinic at the Children’s Hospital of Philadelphia) for whom mutations in known LCA-causing genes were not found. It was shown to segregate with the disease in three other LCA affected cousins. The four unaffected parents were heterozygous for the mutation, while the unaffected siblings with normal vision had only the wild-type allele. Subsequently, additional homozygous or compound heterozygous mutations in NMNAT1 were identified in 13 unrelated families with LCA (from the Children’s Hospital of Philadelphia, Massachusetts Eye and Ear Infirmary, Institute de la Vision in Paris, LVPEI in India and University College London), while no variants were identified in the control samples.

The authors characterized three novel missense variants (p.Val9Met, p.Arg66Trp and p.Arg237Cys) of NMNAT1, predicted to damage the NMNAT1 protein structure and stability. Interestingly, enzymatic activity of NMNAT1 variants was significantly lower compared with the wild-type when measured using both recombinant proteins and fibroblast cell extracts from a proband. Furthermore, fibroblast cells from proband expressing p.Val9Met NMNAT1 had 16% less NAD+ content compared with control.

In the same issue of the journal, Koenekoop et al. identified 10 mutant alleles of NMNAT1 in eight families with LCA. Similar to Falk et al. they suggested that the variants would result in altered structure and function of the NMNAT1 protein and tested this by performing both in vivo and in vitro functional assays. They observed that an individual homozygous for p.Glu257Lys variant had significantly lower concentration of NAD+ in red blood cells compared with his heterozygous mother. In vitro assays using affinity purified wild-type and mutant NMNAT1 yielded synonymous results of reduced enzymatic activity of the mutant protein.

De Novo Pathway
Salvage Pathway

\[ \text{L-Tryptophan} \rightarrow \text{Nicotinic Acid} \rightarrow \text{Mononucleotide (NaMN)} \rightarrow \text{Nicotinamide Adenine Dinucleotide (NAD+)} \]

Mutations in NMNAT1 result in low levels of NAD+. Fig. 1. Mammalian nicotinamide adenine dinucleotide (NAD+) biosynthetic pathways: in mammals, NAD+ can be synthesized through two pathways- de Novo pathway and salvage pathway. NMNAT1 encoding nicotinamide mononucleotide adenyllytransferase 1 is an important enzyme for the production of NAD+ in both these pathways.
HotSpots

Immuno-histochemistry studies in HeLa cells transfected with constructs encoding wild-type or mutant NMNAT1 showed stronger staining outside of the nucleus along with positive staining for ubiquitin suggesting improper protein folding of mutant NMNAT1. This was in contrast to Falk et al. which showed correct nuclear localization and normal expression levels of p.Val9Met, p.Arg66Trp and p.Arg237Cys NMNAT1 variants.

Interestingly, they also reported that in patients exhibiting LCA, in addition to severe loss of vision, all individuals with biallelic NMNAT1 mutations had a peculiar ‘macular coloboma’, which is characterized by complete loss of neural tissue in the fovea, which includes photoreceptors, bipolar cells and ganglion cells. This provides a link between NMNAT1 dysfunction and neurodegeneration.

Furthermore, the authors identified p.Glu257Lys as a founder mutation and thus developed an amplification-refractory mutation system primer set to easily distinguish between wild-type and mutant alleles by polymerase chain reaction in order to facilitate rapid and inexpensive diagnosis of newborns with LCA carrying NMNAT1 mutations.

In summary, both Falk et al. and Koenekoop et al. have identified NMNAT1 as an LCA-causing gene. Although, the exact mechanism remains yet unknown, it appears that retinal degeneration in individuals harboring mutations in NMNAT1 may be attributed to low levels of NAD+. Future studies are needed to examine whether there are alternate mechanisms by which NMNAT1 mutations could cause this rare form of childhood blindness. Treatment in the form of gene therapy is available for patients affected by certain LCA-causing mutations (2). For the time being however, restoring NAD+ levels in the retinal cells appears to be the most promising therapeutic strategy for NMNAT1-related LCA.

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