Medicinski podmladak

Medical Youth

Mini review article

LEBER HEREDITARY OPTIC NEUROPATHY GENOTYPE, PHENOTYPE, AND BIOCHEMICAL CHARACTERISTICS

GENOTIPSKE, FENOTPISKE I BIOHEMIJSKE KARAKTERISTIKE LEBEROVE HEREDITARNE OPTIČKE NEUROPATIJE

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Abstract

Leber hereditary optic neuropathy (LHON) is a mitochondrial neurodegenerative disease presented as a painless, acute, or subacute, usually sequential, loss of central visual acuity. The other eye is typically affected in a few weeks or months (on average in 6 to 8 weeks), while in about 25% of cases, the disease is simultaneously bilateral. Although individual unilateral cases have been reported, in 97% of cases, both eyes are affected within a year. Detailed genetic analysis is crucial in the process of diagnosing LHON. Between 75 - 90% of all the recorded LHON cases occur due to one of the three common mutations in mitochondrial DNA (mtDNA), located on nucleotides 11778, 14484, and 3460, although other, rare mutations with familial occurrence have been associated with LHON as well. More recently, the so-called autosomal recessive LHON has been described, which occurs due to a mutation in the *DNAJC30* gene and presents with identical clinical features, but a better visual prognosis compared to mtLHON. This short review aims to present relevant information on the phenotype, genotype, and biochemical characteristics of LHON.

Keywords:
LHON,
diagnostic process,
bilateral optic neuropathy,
LHON phenotype,
LHON genetics, OCT,
electrophysiology

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Sažetak

Leberova hereditarna optička neuropatija (LHON) mitohondrijsko je neurodegenerativno oboljenje koje se manifestuje kao bezbolni, akutni ili subakutni gubitak centralnog vida. Drugo oko obično biva zahvaćeno za nekoliko nedelja ili meseci (u proseku za 6 do 8 nedelja), dok je u oko 25% slučajeva oboljenje simultano bilateralno. Iako su opisani pojedinačni unilateralni slučajevi, kod 97% obolelih unutar godinu dana zahvaćena su oba oka. Neophodna je detaljna genska analiza u procesu dijagnostikovanja LHON. Između 75% i 90% svih LHON slučajeva dešava se zbog jedne od tri mutacije u mitohondrijskoj DNK (mtDNK), koje su locirane na nukleotidima 11778, 14484 i 3460, mada i takozvane retke mutacije mogu da prouzrokuju nastanak LHON. Retke mutacije javljaju se u okviru pojedinih familija i uglavnom su heteroplazmične. U skorije vreme opisan je i takozvani autozomno recesivni LHON, koji nastaje kao posledica mutacije u *DNAJC30* genu sa identičnim osobinama, ali boljom prognozom u odnosu na mtLHON. Cilj ovog mini revijskog rada je da prezentuje relevantne fenotipske, genotipske i biohemijske karakteristike LHON.

Ključne reči:
LHON,
dijagnostika LHON,
bilateralna optična neuropatija,
LHON fenotip,
LHON genotip,
OCT,
elektrofiziologija

Introduction

Leber Hereditary Optic Neuropathy (LHON) is a neurodegenerative disease characterized by the appearance of a large central scotoma, dyschromatopsia and profound visual acuity (VA) loss, mostly affecting young males (1). In the majority of cases, the disease is bilateral with simultaneous, or sequential loss of visual function (1). Most patients become legally blind already in the subacute phase of the disease (2). A small number of patients experience a modest spontaneous recovery of visual function (2), with reduction or fenestration of the central scotoma, resulting in VA improvement. A higher percentage of visual function recovery was observed in *ND6* m.14484T > C and *DNAJC30* mutations which have the best prognosis. Likewise, the earlier age at the onset of the disease, especially if the disease occurs in childhood, has a better prognosis regardless of the mutation type (3). The disease most often appears in a young age somewhat later in women, but cases of early onset in childhood (4), as well as in older age (5) have been described.

Patients without confirmed mutations either in mtDNA or in the genome, but with a typical LHON phenotype, represent a clinical diagnostic challenge. This review focuses on the main genotypic, phenotypic and biochemical characteristics of LHON.

Genetics of LHON

One of the three-point mutations in mtDNA, located at nucleotides 11778 (70%), 14484 (14%), and 3460 (13%) affecting the NADH dehydrogenase (ND) subunits of complex I (ND1, ND4 and ND6) of the electron transport system (ETS) are responsible for more than 90% of LHON cases (6,7). These primary pathogenic LHON mutations encode proteins of the membrane sector of mitochondrial complex I. The *ND1*: 3460G > A mutation results in the substitution of moderately conserved alanine 52 with threonine (A52T). The *ND4*: 11778G > A mutation results in the change of highly conserved arginine 340 to histidine (R340H), while the *ND6*: 14484T > C mutation changes the weakly conserved methionine 64 to valine (M64V). Primary mutations are mostly homoplasmic (all mtDNA is mutated, there is no wild type mtDNA), even though heteroplasmic cases whereby various mutated mtDNA percentages have also been described. However, in some geographical regions, the percentage of typical mutations may be lower, such as in Slovenia where only 74% of cases were reported to be caused by typical mutations, and their frequency is different (8).

Both LHON and LHON-plus phenotypes can also be associated with other mtDNA mutations - mostly in the so-called mutational "hotspots" - *ND1* and *ND6* genes (9). These mutations are regarded as rare mutations (10), and they are considered pathogenic if mitochondrial function tests confirm impaired mitochondrial function. There are presently more than 30 different point mutations that are associated with LHON. They can be classified into two distinct groups: the "top-14" (including the three primary mutations), as well as a group of possibly pathogenic variants or their combinations, for which there is still no firm evidence of their functional impact (11). Such mutations usually occur within individual families or individuals and are mostly heteroplasmic. However, genetically unconfirmed cases with a typical LHON phenotype are a diagnostic challenge (12).

Recently, LHON phenotype has also been associated with autosomal recessive traits related to nuclear-encoded components or mildly pathogenic variants with incomplete penetrance in oxidative phosphorylation (OXPHOS)-related genes such as Tyr51Cys in the *DNAJC30* gene, clinically indistinguishable from mtDNA-related disease (12-14).

Mitochondrial LHON (mtLHON) is developed as a consequence of a mitochondrial DNA mutation. It is inherited via a maternal genetic line with an incomplete penetrance and a predilection for the male sex. The mutation can be transmitted from mother to both male and female offspring, but approximately half of male and only one in every ten female patients were reported to develop vision loss (14). Recent studies in Australia show that this percentage may be far lower: 17.5% in male and 5.4% in

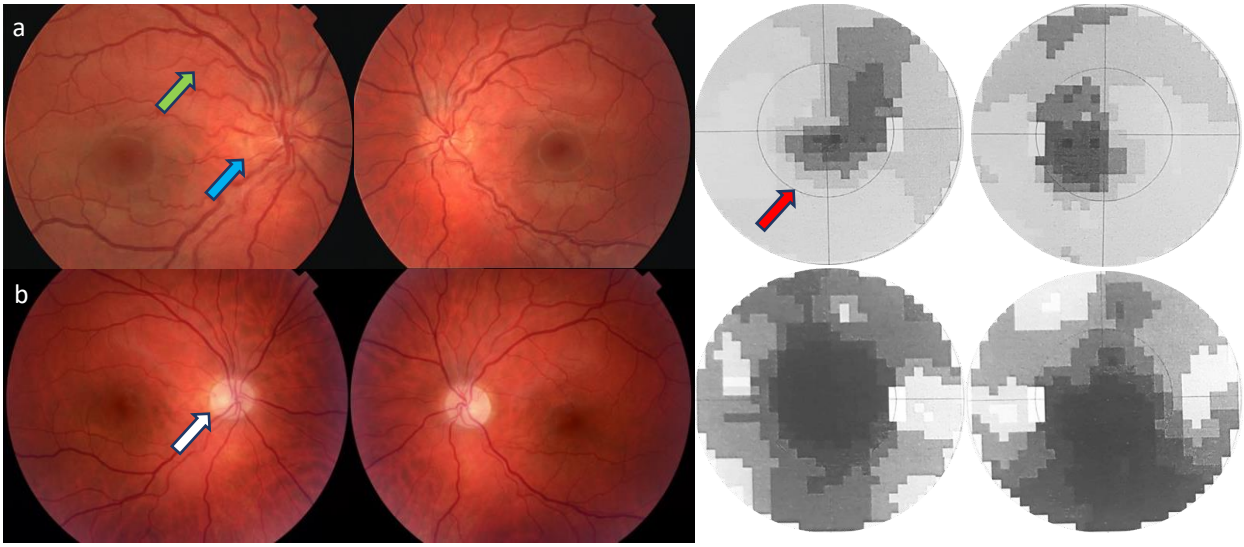

Figure 1. Patient's* fundoscopy and a visual field a) in the acute phase with tortuous blood vessels (green arrow), hyperemic optic discs and pseudoedema (blue arrow), and central scotoma in the visual field (red arrow); b) in the chronic phase with pale and atrophic optic disc (white arrow) and large scotoma in the visual field.

female patients (15). Vision loss is influenced by haplogroups, heteroplasmy and environmental factors (smoking, alcohol, nutritional deficiency, head trauma, industrial toxins, antiretroviral drugs, ethambutol, certain antibiotics, low levels of vitamin B12, etc.) (16–18).

From a pathological basis, *in vitro* studies on polymorphonuclears, fibroblasts, and cytoplasmic hybrid (cybrid) cells from LHON patients show that mtDNA mutation damages the complex I of the respiratory chain. This reduces bioenergetic efficiency (19) and increases the reactive oxygen species (ROS) production (20), which, in the end, increases the likelihood of cell death by apoptosis (20).

LHON phenotypic characteristics (clinical features)

Decreased central visual acuity, dyschromatopsia, and centrocecal scotoma are the earliest signs of the disease, while the pupillary reflex to light is usually preserved even though asymmetric bilateral or unilateral cases might be characterized by a relative afferent pupillary defect (6). The disease can be divided into 2 stages (21). The preclinical stage can last a lifetime without activating the disease and refers to non-diseased mutation carriers in whom various fundus abnormalities may be observed (22). Varying degrees of retinal nerve fiber layer (RNFL) swelling, as well as microangiopathic features, such as vessel tortuosity and arteriovenous shunts, have also been described in asymptomatic carriers, however, no biomarker predictive of conversion has been identified (23). When conversion to an active disease occurs, it is divided into subacute, dynamic and chronic stages. Central vision loss is characteristic of the subacute stage and occurs as a consequence of a progressive enlargement of the central scotoma in the visual field. In this phase, fundoscopy shows enhancement of all features described in the preclinical stage (23) with hyperemia of the optic nerve head, peripapillary telangiectasia, vascular tortuosity, and edema of the RNFL around the optic nerve disc, usually without leakage on fluorescein angiography (pseudo-edema) (**figures 1 and 2**).

On average, after 6 weeks, the optic disc atrophy becomes apparent with the presence of the temporal pallor (24, 25). Once the disease reaches the acute phase, optical coherence tomography (OCT) can demonstrate the thickening of the RNFL. This will firstly occur in the temporal and lower, subsequently moving to the upper and nasal quadrants, and finally thinning of RNFL in the chronic stage can be observed (24). The microangiopathy seen in the first weeks of disease evolution generally disappears with the progression of optic nerve atrophy. This stage can last from several weeks to 6 months. Subsequently, the disease will progress into the commonly known dynamic stage. The dynamic stages will generally last from 6 months to a year from the onset of the disease when the chronic stage begins. This represents the final active disease phase. In the dynamic stage, so-called NADIR visual acuity (the lowest visual acuity) is reached, which is the poorest visual acuity the patient achieves, but the RNFL can still be swollen and appear normal on OCT, but later on, profound axonal loss becomes apparent with optic atrophy as the final stage (26).

Characteristics of OCT and peripapillary RNFL changes in LHON patients and carriers have been described in several longitudinal studies. Significant thickening of the temporal RNFL and a trend of the inferior RNFL thickening was observed in asymptomatic LHON mutation carriers compared to controls (22). The early thickening is considered to be a consequence of axoplasmic transport disorders and probably reflects early basic stress on nerve fibers. Thickening of the superior and inferior parts of the peripapillary RNFL is usually present at the disease onset, probably due to axoplasmic transport disorders which reflect early basic stress on the retinal nerve fiber layer and retinal ganglial cell layer (27). At this stage, the temporal quadrant is of normal thickness due to the onset of atrophy (27). This corresponds to pseudoedema and hyperemia of

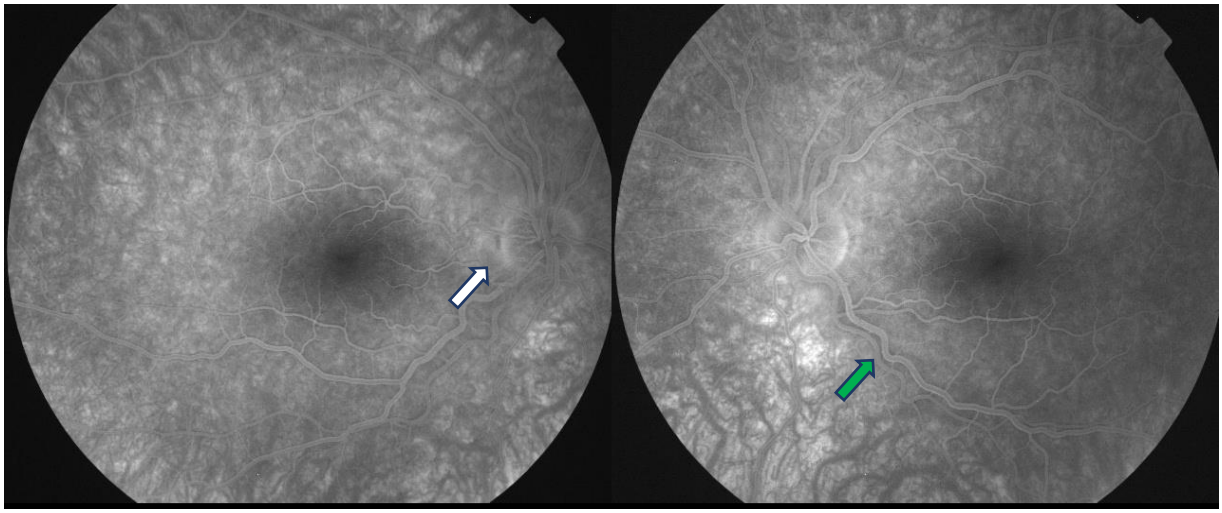

Figure 2. Fluorescein angiography of a patient* in the acute phase of bilateral LHON showing tortuosity and telangiectatic vessels (green arrow), but no leakage on the optic discs (white arrow).

the optic disc, and microvascular changes which are present on the fundoscopy. On average, three months after the disease onset, the thinning of the temporal part of the peripapillary RNFL is registered on the OCT, followed by the thinning of the superior and inferior parts (usually nine months after the disease onset) (24). On the other hand, the nasal part of the peripapillary RNFL is relatively preserved which is identified as one of the main characteristics of mitochondrial optic neuropathies (28, 29) (**figure 3**).

Patients with LHON also show more severe thinning of the superior temporal RNFL when compared to patients without identified causes of the optic atrophy and this might also be considered a biomarker of LHON (29).

Macular OCT shows thinning of the inner Early Treatment Diabetic Retinopathy Study (ETDRS) ring and outer nasal quadrant in the first three months of disease onset which progresses further to the outer temporal parts in the next three months, whilst by the 12th month from the onset of the disease, the diffuse thinning of the ganglion cell layer and inner plexiform layer in the macula is present (30). Ganglion cell-inner plexiform (GCL-IPL) layer thinning seems to be even more sensitive in the early stages of LHON. The thinning of GCL-IPL in the nasal quadrant was observed even before the onset of symptoms (23) progressing to the inferior, superior, and temporal quadrants in the first three months of the disease. This thinning continues up to 6 months after the onset and then stabilizes (23). In these studies, the chronic phase was observed, and relative preservation of the ganglion cell layer exists in the central ETDRS field in comparison to other optic neuropathies which might be considered as a potential novel biomarker of the disease and a structural basis for spontaneous recovery seen in some LHON patients (29).

The LHON patients show electrophysiological characteristics typical for severe ganglion cell loss. Electrophysiological signs of LHON are a decrease in the N95 wave amplitude and N95/P50 ratio of the pattern electroretinogram (PERG) and a prolongation of the latency of the visual evoked potentials (VEP) P100 wave, changes in the morphology of the VEP (31) (**figure 4**). In more than 50% of patients, an abnormal amplitude of the PERG N95 wave in the acute phase of LHON was seen (12), whilst, in the chronic phase, almost all patients have reduced amplitude of the N95 wave and abnormal or undetectable VEP (29, 32).

In the subacute phase, the latency of the P100 wave becomes progressively longer (29, 33). In a small number of LHON patients, partial recovery of visual acuity and visual field in the chronic phase is seen. In some patients, there is a correlation with electrophysiological improvement (31, 34), while in some patients, no correlation was observed (12). Oscillatory potentials in dark-adapted flash ERG have also been severely affected in LHON patients and they show a significant negative correlation with the thickness of the outer plexiform layer (35) suggesting

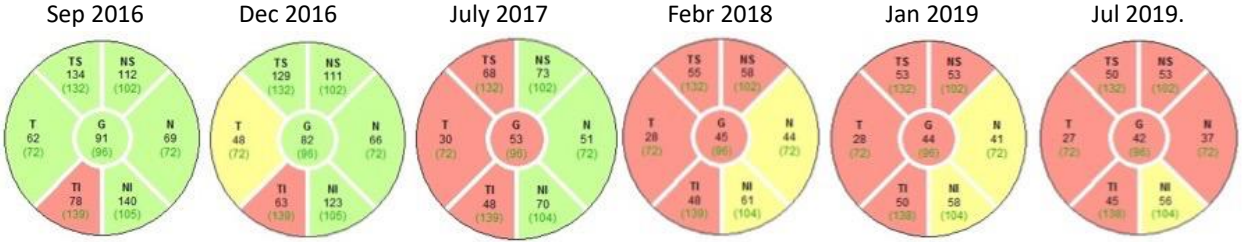

Figure 3. Pattern of progressive thinning of the peripapillary RNFL in an LHON patient* during a follow-up period of 22 months (RE).

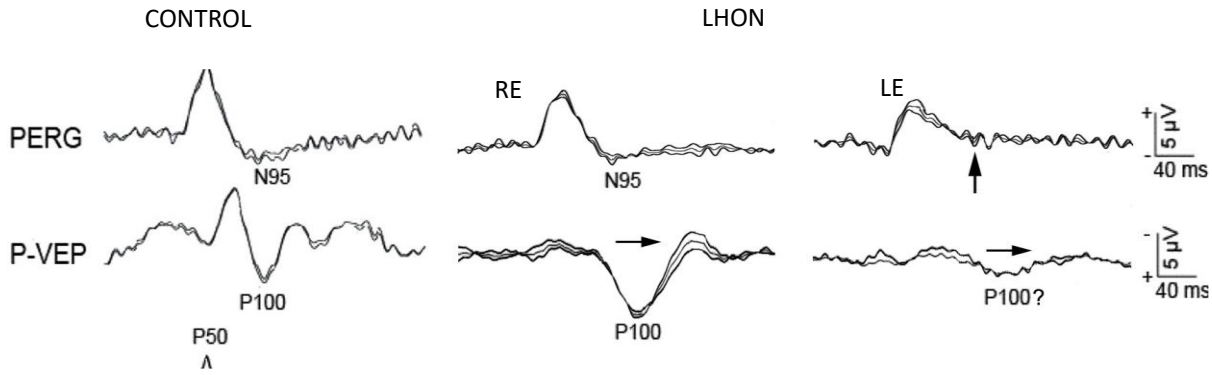

Figure 4. Pattern ERG and P-VEP in LHON patient* showing reduced PERG N95 and reduced and delayed VEP P100.

retinal remodeling in the chronic stage of LHON. Another electrophysiological method that originates from retinal ganglion cells are photopic negative responses (PhNR). As this is a flash ERG component, fixation is not needed, however, it is less sensitive than PERG as approximately 40% of LHON patients in the chronic phase were shown to have an amplitude of PhNR within the normal range (36).

There are presently no effective treatments available for the LHON disease. Idebenone is the only disease-specific medication available for the treatment of visual function impairment in LHON. It is a synthetic analog of ubiquinone (coenzyme Q) Its mechanism of action is based on its antioxidant properties. Idebenone can act as an electron carrier, directly transferring electrons to mitochondrial complex III, thus bypassing mitochondrial complex I respiratory chain deficiencies in patients with LHON. In this way, it restores the cellular adenosine triphosphate (ATP) production and re-activates inactive, but viable retinal ganglion cells (RGCs), aiming to prevent further vision loss and to promote the recovery of visual function (37). It is important to start with the Idebenone treatment as soon as possible. Also, specific gene therapy is in development and gives promising results, with moderate improvement of vision. However, it still fails to either avert or stop the disease development and it is not as yet available for clinical use (38–40).

LHON biochemical characteristics

Although several mtDNA mutations were identified in families with LHON, three primary mutations in *ND1*, *ND4*, and *ND6* affecting the function of the complex I are responsible for most LHON cases (41). Overall, complex I dysfunction induced by LHON mutations is reflected in decreased efficiency of OXPHOS (18, 39) and increased ROS production (42, 43).

Due to changes in complex I activity, the production of ATP is reduced, leading to low cell energy levels, with a decreased ATP pool. Neurons depend on the supply of ATP for normal neurotransmission, as well as for the arrangement of mitochondria along the axon. In RGCs, similar to other neurons, mitochondrial biogenesis occurs in the soma (44). Mitochondria are afterward transported along the axon by a transport mechanism dependent on ATP (44). Unique characteristics of RGC axons, with long unmyelinated initial segments of their intra-retinal tract, make them very vulnerable, and impairment of ATP production is reflected in the abnormal distribution of mitochondria along the axon. This may explain why ganglion cells are affected first, although, in patients with LHON, defective ATP synthesis is present also in brain and skeletal muscle tissue (45).

In cellular lines derived from LHON patients, in metabolically stressful conditions (eg. in a medium with galactose instead of glucose), complex I-linked ATP synthesis and as total ATP level in the cells is reduced, compared to cells from control subjects (46). This suggests that the biochemical effect of the mutation becomes evident in conditions of high energy demand. However, the exact biochemical consequences of LHON pathogenic mutations are controversial, as there are likely multiple mechanisms that can help compensate for the complex I impairment. Variability in modulating genetic and environmental factors in adaptation to such conditions may influence different susceptibility to conversion from carrier stage to disease.

Studies of LHON cells grown in a galactose medium showed their higher susceptibility to mitochondria-dependent apoptotic death in comparison with the control (47). The occurrence of apoptotic cell death is abrupt, almost simultaneously affecting most of the retinal ganglion cells (RGC), ultimately leading to the atrophy of the optic nerve (48–50). The absence of signs of inflammation on histopathological examination, as well as biochemical analysis, were indicative of programmed cell death, but the trigger is still poorly understood (48–50).

As it has been established in various studies, the RGC degeneration in LHON occurs via the mitochondria-dependent apoptotic pathway, but the exact link between apoptosis and dysfunction of the complex I is unclear. The increased ROS production and oxidative stress are thought to be a key part of the degeneration of the optic nerve (49), as complex I, affected in LHON, is the main source of superoxide anion in mitochondria. The disruption of the function of complexes is associated with increased ROS production and the cell's redox status imbalance. The role of oxidative stress in LHON pathogenesis is further supported by the possibility that LHON mutations affect the interaction of complex I with coenzyme Q (CoQ), since partially reduced CoQ molecules are also a potential

source of ROS (50). Mouse LHON model revealed that decreased activity of the complex I and increased ROS production were not accompanied by diminished ATP production, (43) whereas LHON-affected cells (fibroblasts and cybrids) carrying pathogenic mutations presented increased ROS levels, providing evidence that increased ROS production is a prevalent pathological mechanism in RGC degeneration in LHON (51). Recent publications provide evidence that evaluation of mitochondrial function and homeostasis in peripheral blood mononuclear cells (PBMCs) reveal complex I dysfunction, combined with increased ROS levels, which leads to an increased susceptibility to apoptosis (47). This paves the way toward the use of mitochondrial function evaluation in PBMCs (measurement of the mitochondrial oxygen consumption, mitochondrial membrane potential, ROS production, respirometry) (52), as a minimally invasive, highly effective procedure that could be a valuable addition to the diagnosing process of patients with optic neuropathies (51, 53).

Conclusion

Leber hereditary optic neuropathy (LHON) is a mitochondrial neurodegenerative disease presented as a painless, acute or subacute, usually sequential, loss of central visual acuity, mostly affecting young males. Diagnosis may sometimes be delayed, however, treatment with Idebenone should be started as soon as possible. Despite detailed and extensive genotypic, phenotypic, and biochemical research many questions in LHON remain unanswered. Identification of the novel clinical biomarkers of the disease and disease conversion could lead to a better understanding of LHON and potentially to the novel treatment strategies' development.

*Presented patients are included in the Candidate's PhD thesis and photographs might have been used as a part of the Figures in the Candidate's thesis.

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