# Leber's Hereditary Optic Neuropathy (LHON) with Mitochondrial ND4 Gene Mutation (11778) in a Thai Patient

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**Abstract**

Leber's hereditary optic neuropathy (LHON) is a maternally transmitted disease, characterized by bilateral optic atrophy predominantly in healthy young males. This disorder has shown to be associated with DNA mutation in mitochondrial genome of the patients. We report here a young man who came to the hospital with subacute visual loss in one eye, followed by the other eye within two months. His echocardiogram was normal. A G→A base substitution at nucleotide position 11778 which changes a conserved arginine to histidine at amino acid position 340 of ND4, a protein subunit of respiratory chain enzyme complex I in oxidative phosphorylation system, was detected in his leucocyte mitochondrial genome.

**Key word :** Leber's Hereditary Optic Neuropathy - Mitochondrial ND4 Gene Mutation - Thai

Leber's hereditary optic neuropathy (LHON) is characterized by an acute or subacute bilateral central visual loss caused by severe bilateral optic atrophy(1). About 80 per cent of affected individuals are male and LHON can be considered as the most common cause of blindness in young and other healthy males(2-4). LHON is maternally inherited and the mitochondrial transmission is confirmed when the mitochondrial DNA (mtDNA) mutation at ND4 gene is observed to be associated with the disease(5).

Human mitochondrial genome, an extrachromosomal DNA, is a closed circular double stranded polynucleotide of 16,569 bp and located in the matrix of mitochondria(6). The genome encodes 37 genes: 2 for rRNAs, 22 for tRNAs and 13 for polypeptide subunits of mitochondrial respiratory system (ND1, ND2, ND3, ND4, ND4L, ND5, ND6, COI, COII, COIII, Cyt B, ATP6, and ATP8). All 13 polypeptides are protein subunits of enzyme complexes in oxidative phosphorylation, seven of which are subunits of complex I (NADH

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ubiquinone oxidoreductase), one is of complex III (ubiquinol-cytochrome $\bar{c}$ oxidoreductase), three are of complex IV (cytochrome $\bar{c}$ oxidase) and two are of complex V (ATP synthase). Thus, these protein subunits are essential to normal energy production of the cell.

Several point mutations in mtDNA have been reported to be associated with LHON. The most prevalent mutation, covering over 50 per cent of the LHON cases, is a G→A at the nt11778 which converts an evolutionary conserved arginine to histidine at position 340 in subunit ND4 of NADH ubiquinone oxidoreductase, complex I of respiratory chain enzymes[7]. Other mtDNA point mutations have also been proposed to be associated with LHON, G→A at the nt3460, T→C at nt14484, G→A at nt9483 and nt9804, A→G at nt4216 and several others[8-11]. Some of these mutations seem to be sufficient in themselves to cause the disease, so called primary mutations. The secondary mutations appear to have a synergistic deleterious effect when present simultaneously with other mtDNA mutations[12].

In this report, we present the first case of a patient who suffered from subacute bilateral central visual loss. The diagnosis of Leber's hereditary optic neuropathy was made and the underlying molecular lesion associated with LHON, a G→A at nt 11778 in mitochondrial genome, was identified.

## MATERIAL AND METHOD

### Subject

The patient, SR 94-012, was a male of 19 years old and a university student. He was studied clinically at the Department of Ophthalmology, Faculty of Medicine Siriraj Hospital in Bangkok. He was healthy and had no family history of blurred vision. He came to the hospital with blurred vision in both eyes for two years, beginning at the right eye, followed by the left eye two months later. His visual acuity was finger count in both eyes. He had no pain on eye movement and anterior segment of both eyes were normal. Fundoscopic examination revealed pale optic disc in both eyes. The visual fields of both eyes demonstrated central scotoma, computerized tomography and MRI of brain were normal. He had no history of smoking, alcohol drinking or using any chemicals. He was treated as optic neuritis with intravenous methylprednisolone before transferred to Siriraj Hospital. The visual evoked potential revealed normal latency with delay amplitude of P100, not compatible with optic neuritis. His echocardiogram was normal and all neurological examinations were within normal limits. For molecular analysis, his peripheral blood was collected using EDTA as an anticoagulant.

### Restriction enzyme analysis of LHON mutation

Total leucocyte DNA of the patient was extracted from the whole blood sample according to the standard protocol[13]. For restriction analysis of the 11778 mutation, a 214 bp segment spanning from nt11728 to nt11942 was amplified by the polymerase chain reaction (PCR)[14] using a pair of primer as follows: L11728 5' CTCATTACTAT TCTGCCTAGCAAACTCAAACTACGAACGCACTCA TGATC 3' and H11942 5' GTAGGAGAGTGATA TTTGATCAGG 3'. The mismatch was designed to create a restriction site for restriction enzyme *BclI* if the G→A at nt11778 is presented. The PCR product was then digested with *BclI* and electrophoresed in 2 per cent agarose gel. The gel was stained with ethidium bromide and photographed under UV light. Since this PCR segment contains one site of *BclI*, the fragment of 193 and 21 bp were obtained in normal sample. If there is a G→A at nt11778, the fragment of 193 bp would be digested into 143 and 50 bp respectively. For the analysis of the other primary point mutation of LHON, a G→A at nt3460, a 2657 bp segment from nt2826 to nt5482 was amplified using a pair of primer complementary to the heavy strand from nt5459 to nt5482 and complementary to the light strand from nt2826 to nt2849. The G→A at nt3460 was detected by the absence of *Bsa*HI restriction site. The normal mtDNA would be digested into the fragment of 2022 and 635 bp whereas the mutant mtDNA contained G→A at nt3460 would not be digested.

### Sequence analysis of G→A at nt11778

The G→A at nt11778 was confirmed in this patient by "Thermal PCR direct sequencing method" (*fmol* sequencing kit, Promega, U.S.A.). The 214 bp PCR segments was used and purified with "Wizard PCR Preps" (Promega, U.S.A) according to the instruction manual. The four microcentrifuge tubes were labeled (A,G,C,T) and added 200 μM of each d/ddNTP Mix. Each reaction tube was consisted of the amplification buffer (25 μM Tris-HCl pH 9.0 and 1mM $MgCl_2$), 1.25 μCi/μl

α-$^{32}$S dATP, 10 nmol of the purified template, 0.75 pmol of the oligonucleotide primer, and 1.25 u of Sequencing grade *Taq* DNA Polymerase. The oligonucleotide primer is H11942 which is complementary to the light strand from nt11919 to nt 11942. After one drop of mineral oil was added, the reaction was carried out for 30 cycles of denaturation (30 sec at 95°C) and annealing-extension (30 sec at 70°C). After amplification, 3 μl of *fmol* sequencing stop solution was added to each tube. The sequencing reactions were run in 6 per cent polyacrylamide gel. The gel was dried and exposed with X-ray film at room temperature for 24 hour.

## Results and Discussion

The restriction enzyme analysis of the mitochondrial genome in this patient revealed a G→A at nt11778 (Fig. 1). The mtDNA of this patient seems to be homoplasmy for G→A at nt11778. Further analysis of the G→A base substitution at nt3460 and the common point mutations at nt8344 and nt3243 was negative (data not shown). The G→A at nt11778 was confirmed by nucleotide sequence analysis (Fig. 2).

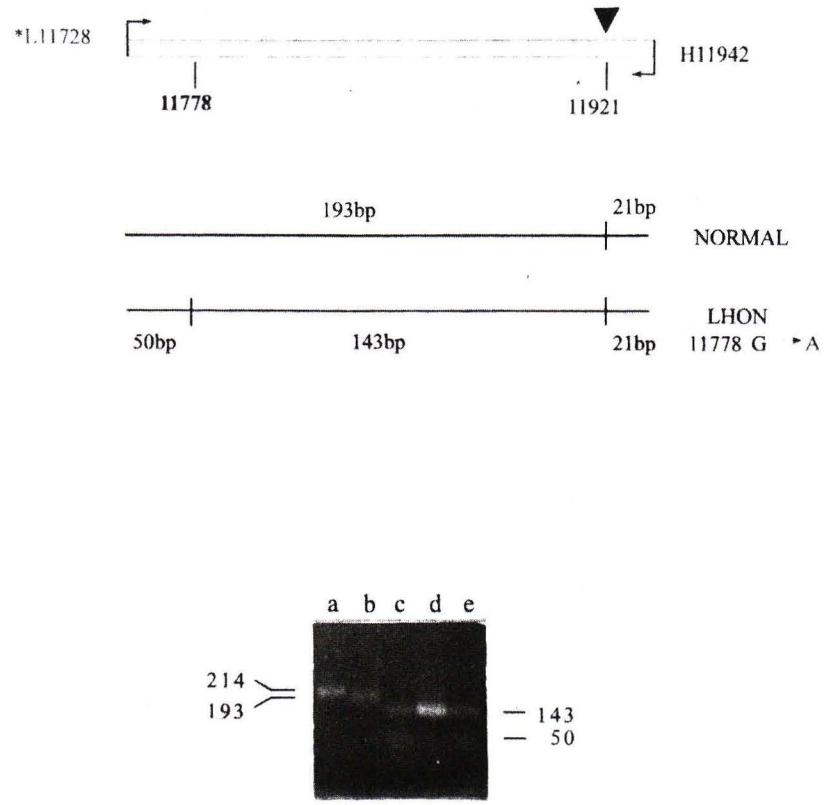


**Fig. 1. Restriction enzyme analysis of a G→A at nt11778 of patient SR 94-012. MtDNA was amplified from nt11778 to nt11942 (214 bp in size). In this amplified segment, there is one *BclI* cleavage site at nt11921. With the G→A mutation at nt11778 and together with the alteration in the modified primer (L11728), an additional cleavage site of restriction enzyme *BclI* would be created. Thus *BclI* would digest the PCR product into three fragments (143, 50, and 21 bp) in the patient carrying the G→A mutation at nt11778. a) ϕ 174 *Hae*III, b) PCR product, c) negative control, d) positive control, and e) patient SR-94-012 (B) where B = blood.**

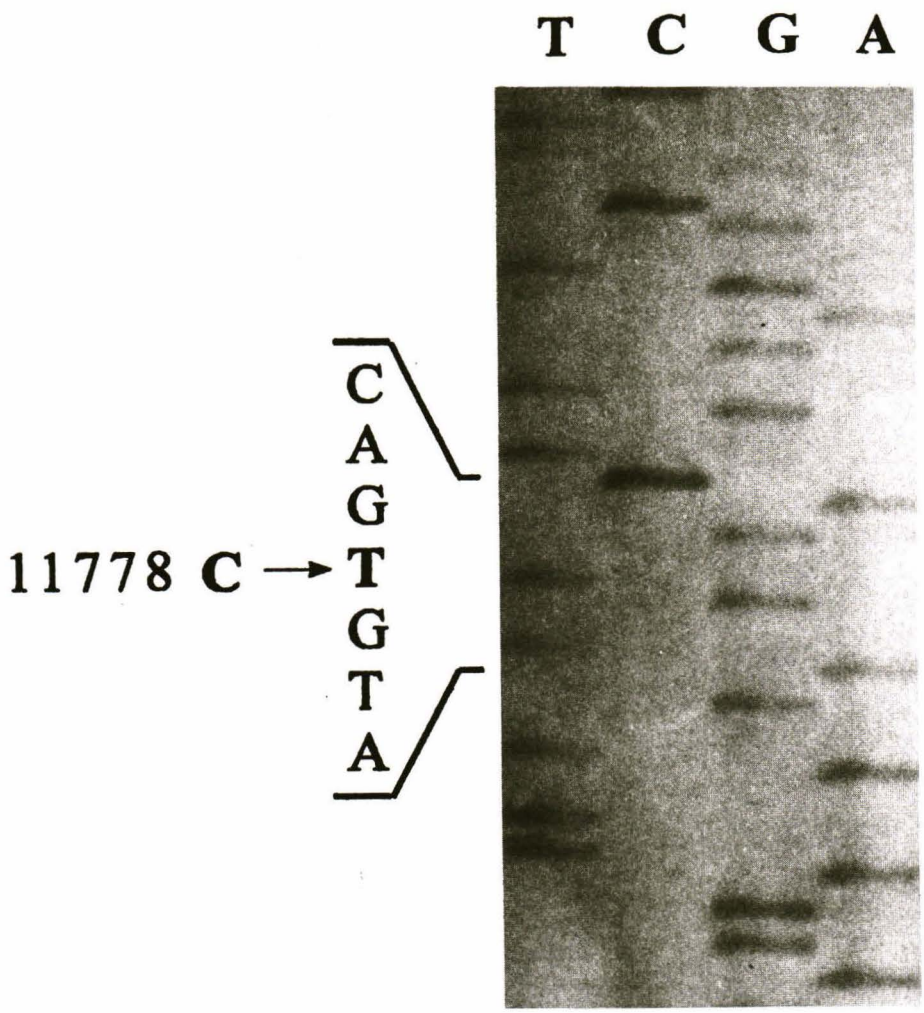


**Fig. 2. Sequencing analysis of a G→A at nt 11778 of patient SR 94-012. PCR amplified product (214 bp) from nt11778 to nt11942 was used as the template. The primer used in the direct sequencing is H11942 which is complementary to the light strand. The sequencing autoradiogram demonstrates the C→T point mutation of the mtDNA at nt11778 (complementary to the G→A of the light strand at the same position).**

The G→A at nt11778 presented in this patient was the first report of the underlying molecular lesion in this disorder in a Thai patient. An accurate diagnosis of LHON is often missed out since diagnosis of LHON was previously based on clinical findings only. This may explain the low frequency of LHON in Thai patients. MtDNA analysis can be useful for the diagnosis especially in the case where clinical diagnosis and family history cannot be verified. Ophthalmologists should be aware of this disorder especially in the case of acute or subacute visual loss of both eyes with optic atrophy in young male. Molecular analysis of mtDNA can be performed conveniently from blood samples. However, absence of this mutation does not exclude LHON since other mtDNA point mutations have also been proposed to be associated with LHON, such as G→A at the nt3460, T→C at nt14484, G→A at nt9483 and nt9804, A→G at nt4216 and several others.

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# ความผิดปกติของยีนไมโตคอนเดรียที่นูคลีโอไทด์ตำแหน่ง 11778 ในผู้ป่วยโรค Leber's hereditary optic neuropathy (LHON) คนไทย

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โรค Leber hereditary optic neuropathy (LHON) เป็นโรคไมโตคอนเดรียโรคหนึ่ง มีลักษณะอาการที่สำคัญคือ มีตาบอดจากประสาทตาเสื่อมเฉียบพลัน และจะเกิดขึ้นกับประสาทตาทั้งสองข้าง โดยจะเกิดขึ้นพร้อมกันหรือเกิดทีละข้างก็ได้ โรคนี้มีการถ่ายทอดทางพันธุกรรมจากมารดาไปสู่บุตร และพบในผู้ชายมากกว่าผู้หญิง และส่วนใหญ่จะมีอาการก่อนอายุ 30 ปี พบว่าโรคนี้เกิดเนื่องมาจากการกลายพันธุ์ของยีนไมโตคอนเดรียของผู้ป่วย คณะผู้วิจัย รายงานโรคนี้ในผู้ป่วยชายชาวไทย มาพบแพทย์ด้วยอาการมองไม่เห็นด้วยตาข้างขวา และตามด้วยข้างซ้ายภายใน 2 เดือน การตรวจยีนไมโตคอนเดรียพบการกลายพันธุ์ของยีนไมโตคอนเดรียที่ตำแหน่ง 11778 ในเม็ดเลือดขาวของผู้ป่วย

**คำสำคัญ** : เลเบอร์ส เฮเรดิทารีย์ อ็อพติคนิวโรพาธีย์ – ความผิดปกติของยีนไมโตคอนเดรีย ND4 – คนไทย

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