บทนำ

Leber's hereditary optic neuropathy (LHON) [OMIM 535 000] is one of the commonest mitochondrially inherited diseases, with a prevalence of ~ 1:31,000 – 50,000 in some parts of Europe (1). It is also one of the common causes of blindness in young men. More than 80% of LHON patients are male with varying degree of the male to female ratio (2). As a result of degeneration of retinal ganglion cell layers, the patients usually develop symptoms of acute or sub-acute painless loss of central vision, either in both eyes simultaneously or in one eye followed by the other eye within 6-12 weeks after the initial onset. They also have fading of colors and deterioration of visual acuity (3). Most affected persons suffer sudden monophasic visual loss but some present as a subclinical condition with a low-grade constant deterioration of retinal ganglion cells. The typical age of onset of the disease is between 15 – 35 years varying from 8 - 60 years (4-5).

The three missense mitochondrial DNA (mtDNA) mutations 11778G>A (p.R340H; ND4), 14484T>C (p.M64V; ND6), 3460G>A (p.A52T; ND1) are the primary mutations responsible for 95% of worldwide LHON cases (6). All of the LHON patients detected so far in Thailand carry 11778G>A (>90% of cases) or 14484T>C (7-8). Though the primary mutation is essential to develop the disease, the primary mutation per se cannot explain the distinctive features of LHON (6, 9). To have better understanding on the pathogenesis of the LHON, there are some issues that need to be addressed, especially in the context of male preference, incomplete penetrance, relatively later age of onset though mutation is present since birth and retinal ganglion cells being the only affected tissue. The level of heteroplasmy of mutant mtDNA, mtDNA background and haplogroups, nuclear DNA background, and environmental factors are supposed to influence the development of LHON for certain extent (5-6, 10-15).

Numerous efforts have been done to have a better understanding on the pathogenesis of LHON, covering from the single gene study (16-18) to global gene expression profile (19-21), especially to hunt for nuclear modifier, if any present. Given that OXPHOS subunits are encoded by both mitochondrial and nuclear genes and that there are numerous cross-talks between the mitochondria and the nucleus, consequently, differential expressions of not only the mitochondrial genes but also the nuclear genes are observed in various OXPHOS deficiency models (22). The

oligonucleotide microarrays of LHON using cybrids and lymphoblastoid cell line revealed that there are some LHON-specific transcriptional alterations of the metabolic proteins, chromosomal organization, regulation of cell proliferation and apoptosis in the cells bearing LHON mutations (19). Another study has found that mitochondrial diseases including LHON have up-regulation of multiple transcripts of the unfold protein response (UPR) and inhibition of transcripts involved in vesicular secretion, protein synthesis and oligodendrogenesis (21). Recent analysis of global gene expression profile in LHON lymphocytes reveals up-regulation of 137 genes and down-regulation of 152 genes - most were related to immune response, carbohydrate metabolism, lipid metabolism, amino acid phosphorylation, electron transport, and apoptosis (20).

In addition to the transcriptomic profiles, there are some reports for the proteomic profiles of the mitochondria in different mitochondrial disorders and different experimental settings (23-28). Proteome of mitochondria contains approximately 1,000 proteins and 99% of which are the products of nuclear genes (29). The coordinated expressions of imported nuclear encoded proteins as well as 13 mtDNA encoded proteins are crucial for the integrity of mitochondrial functions. In the case of various OXPHOS deficiency, nuclear genes respond in many ways (22). Interestingly, there is no general consensus for the direction (up or down regulation) of the nuclear response in mitochondrial disorders though there are some reports for the higher occurrences of down regulation than up regulation (21, 24, 30). Moreover, differential expression is usually observed with nuclear encoded mitochondrial proteins belonging to a variety of functional pathways in addition to the subunits of OXPHOS.

With these distinct characteristics of mitochondrial proteins in cellular homeostasis, there would be no surprising that alteration in mitochondrial proteins are found to be frequently associated with many diseases including neurodegenerative diseases such as Alzheimer's (31-32) and Parkinson's (33-34) diseases and aging processes (26, 35). There is a limited study on comprehensive expression profiles of mitochondrial proteins in LHON which is also a neurodegenerative disease. Therefore, in the present study, we explored the differential mitochondrial proteomic profiles of affected LHON, unaffected LHON and the control fibroblasts using 2 Dimensional polyacrylamide gel electrophoresis (2-DE) and mass spectrometry. This mitochondrial proteomic profile will provide more understanding of the consequences and the gene expression response in OXPHOS deficiency of LHON.