Mitochondria are double membrane organelles present in the cytosol of eukaryotic cells. The main function of mitochondria is to produce energy in the form of ATP by utilizing oxygen and the byproducts of the oxidation of nutrients. The phenomenon of oxidative phosphorylation (OXPHOS) is carried out by five multi-subunit enzyme complexes located in the inner mitochondrial membrane.

Mitochondrial encephalomyopathies are a heterogeneous group of clinical disorders caused by defects in mitochondrial respiratory chain. The incidence of such disorders is about 1:5000 and mostly occurring in early years after birth. Organs such as the brain, heart and skeletal muscle are highly energy dependent and thus vulnerable to defects in energy metabolism.

Mitochondrial dysfunction is common cause of neuromuscular disorders in pediatric patients. In the present study, a total of 23 children diagnosed with possible encephalomyopathies were analyzed for mitochondrial dysfunction. Biochemical analysis of mitochondrial respiratory chain enzymes showed that 64% of the patients had complex I deficiency, 7.1% had complex IV and 14.2% had combined complex I plus III deficiency. Among the complex I defective patients, 22.2% had defects in complex assembly. A significant loss of mitochondrial membrane potential by patient lymphoblasts and a declined ATP synthesis by complex I-dependent substrates was also evident. But, an increased ATP synthesis by complex II dependent substrates was observed in patient derived cell lines. Increase in ATP synthesis by complex II dependent substrates suggests a compensatory mechanism to use complex II as a secondary route for ATP production. Expression analysis of respiratory chain protein subunits by western blot showed a decline in complex I protein expression and increased expression of other respiratory chain complexes particularly complex IV and V, again suggesting a compensatory mechanism.

The genetic cause of biochemical defect can be mutations in either mitochondrial DNA (mtDNA) or nuclear DNA. Analysis of mtDNA sequences from the 23 patients showed that a number of known and novel mtDNA variants were associated with the disease. Most of the non-synonymous
variants were heteroplasmic (A4136G, A9194G and T11916A) suggesting their possibility of being pathogenic in nature. Some of the missense variants although homoplasmic were showing changes in highly conserved amino acids (T3394C, T3866C, and G9804A) and were previously identified with diseased conditions. Similarly, two other variants found in tRNA genes (G5783A and C8309T) altered the secondary structure of cysteine-tRNA and lysine-tRNA. Most of the variants occurred in single cases; however, a few occurred in more than one case (e.g. G5783A and A10149T).

It has been suggested that dysfunction of electron transport chain (ETC) results in increased levels of reactive oxygen species (ROS), which in turn plays an important role in the pathogenic mechanism of mitochondrial encephalomyopathies. Secondly, the mechanism of free radical production by complex I deficiency is ill defined, and is of significant contemporary interest. We have studied the ROS production and antioxidant defenses in patients with mitochondrial complex I deficiency. ROS production has remained significantly elevated in patients ($P< 0.05$ or $P< 0.01$) compared to controls. The expression of all antioxidant enzymes significantly increased at mRNA level. However, the enzyme activities did not correlate with high mRNA or protein expression. Only the activity of superoxide dismutase (SOD) was found to correlate with higher mRNA and protein expression in patients. The activities of other antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT) and glutathione-S-transferase (GST) were significantly reduced in patient derived cell lines ($P< 0.05$ or $P< 0.01$). Glutathione reductase (GR) activity and intracellular glutathione (GSH) levels were not changed. Decreased enzyme activities could be due to post-translational or oxidative modification of these ROS scavenging enzymes.

Mitochondria have been found to play a leading role in triggering and mediating the apoptotic process. A defective oxidative phosphorylation (OXPHOS) caused changes in cellular ROS, antioxidant defenses and declined ATP pool, the further consequences of which can result in apoptosis or necrosis. To discover the status of apoptosis in patients displaying complex I deficiency, we investigated the apoptosis in lymphoblast cell lines established
from these patients in both glucose and galactose medium. No significant differences in percentage apoptotic cells were evident in glucose medium between control and patient group. In galactose medium, a 2-fold increase in apoptotic cell death was observed for complex I defective cell lines after 72 hours of culturing. The apoptotic cell death in galactose medium was probably due to energy deficit and not by oxidative stress. The oxidative stress in terms of hydrogen peroxide (H$_2$O$_2$) and superoxide (O$_2^•−$) was decreased in galactose medium. ATP levels of OXPHOS deficient cells were significantly ($P<0.01$) reduced in galactose medium.

The results from this study reveal that mitochondrial complex I deficiency is the major cause of disease in the studied pediatric population. The further consequences of this deficiency resulted in loss of mitochondrial membrane potential and declined ATP synthesis. The mtDNA variants identified in this study could be the possible cause of mitochondrial encephalomyopathies with childhood onset. Our study strengthens the pathogenic score of known variants previously reported as provisionally pathogenic in mitochondrial diseases. The novel variants found in the present study can be potential candidates for further investigations to establish the relationship between their incidence and role in expressing the disease phenotype. This study will be useful in genetic diagnosis and counselling of mitochondrial diseases in India as well as worldwide. The information on the status of ROS and marking the alteration of ROS scavenging enzymes in peripheral lymphocytes or lymphoblast cell lines will provide a better way to design antioxidant therapies for such disorders. The observations from our findings could also have profound implications for therapy of mitochondrial diseases by using anti-apoptotic drugs, in addition to most common therapies, which are focused on the use of antioxidant vitamins. But more in vivo studies are required to support these results.