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Screening of mtDNA Mutations in Mitochondrial Cytopathies

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The mitochondrial cytopathies are used to describe a number of diseases which have as their root cause a disturbance in on or more of the mitochondrial metabolic pathways. Many fundmental metabolic pathways occur in mitochondria, including the respiratory chain, fatty acid beta-oxidation and the tricarboxylic acid (TCA) cycle. Mitochondrial cytopathies show marked clinical heterogeneity, involving a range of different symptoms which are exercise intolvery wide range of different symptoms, which are exercise intolerance, hypotonia, proximal myopathy, external ophthalmoparesis, cardiac dysrhythmia, optic atrophy, pigmentary retinopathy, dementia, epileptic seizures, deafness, ataxia, stroke-like episodes, mental disorders, peripheral neuropathy, diabetes and hypoparathyroidism. This clinical heterogeneity in part reflects the complex genetics underlying these disorders. Mitochondria are unique among human cellular organelles in that they contain their own genome. Every mitochondrion has several copies of circular 16,569 bp DNA, which encodes 13 essential proteins of the respiratory chain enzyme complexes I, III, IV and V, 22 tRNAs, and 2 rRNAs. MtDNA is inherited only from the mother, thus, mtDNA disease can only be transmitted down the maternal line. There are multiple copies of mtDNA in each cell. A cell or individual is said to be homoplasmic if each of these copies is identical. If two or more sequence variants exist in a cell or individual, the condition is referred to as heteroplasmy. If mutant and normal mtDNA co-exist in the same cell, however, the metabolic functions will not be impaired as long as there is sufficient normal mtDNA to overcome the effects of the mutant DNA. To date, more than 200 different mtDNA changes in different diseases have been described. These are divided into two groups: point mutations in protein, tRNA or rRNA encoding regions, which are often maternally inherited, and structural rearrangements such as duplications and deletions, which are usually sporadic. Many diverse pathogenic mtDNA mutations have been described since 1988. However, genetic defects for Korean mitochondrial patients were not much analyzed. We have collected a total of 48 patient samples from 33 families having the mitochondrial cytopathy, which include MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes), MERRF (myoclonic epilepsy with ragged-red fibers), CPEO (chronic progressive external ophthalmoplegia, KSS (Kearns-Sayre syndrome) and LS (Leigh syndrome). Blood samples were collected from mitochondrial cytopathy-diagnosed patients of Korean origin at Yonsei University Hospital and Ewha Womans University Hospital. Total DNA was extracted using the genomic DNA purification kit. For the screen of point mutation, mtDNA was amplified to 6 overlapping fragments as approximate sizes of 3 kb and then directly sequenced using automatic sequencing analyzer. The long template PCR was performed for the detection of long deletions. If any smaller bands were observed, the specific deletion site was determined by the sequencing method. From the screen of point mutations, 5 causative mutations were observed from 6 families. The A3243G, T3271C and T3290C point mutations in tRNA-Leu1 gene were found from MELAS patients. The A3243G mutation was found from 3 families. As a very rare case, a severe MELAS family (family ID: MT-24) showed two causative mutations, A3243G and T3290C. The T3290C was determined as a novel mutation. A8344G mutation in tRNA-Lys gene was found from 2 MERRF families. One of MERRF family (MT-6) having A8344G also showed C3497T mutation in NADH Dehydrogenase subunit 1 gene. The C3497T mutation is usually found in LHON patients, however, the MT-6 family did not showed any LHON phenotypes. Most point mutations were showed restriction fragment length polymorphisms (RFLP). The A3243G, T3290C, C3497T and A8344G mutations showed Apa I, Tsp509 I, Mae III and Ban II RFLPs, respectively. From the screen of large deletions, two kinds of deletions were observed in the commom deletion region. The 5kb deletion (8482-13460) was found from two KSS families. A CPEO patient showed two kinds of deletions, 5kb(8482-13460) and 8kb(5739-13758). Besides mitochondrial cytopathy-causative mutations, several polymorphic variations were found: A3426G, C8414T, A8440G, A8563G, T13602C, T13818C and 9 bp deletion (8271-8279). Of them. the A8440G, T13602C and T13818C were unreported variants, which implies regional (or ethnic) specific SNP distribution in mtDNA. Most observed mtDNA mutations were present as heteroplasmy, thus, further study should be performed to analyze the corelation of the ratios of mutant to normal mtDNA and clinical phenotypes (Supported by the grant (R05-2003-000-11496-0) from KOSEF).