Hypertrophic Cardiomyopathy Due to the Mitochondrial DNA Mutation m.3303C>T Diagnosed in an Adult Male

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Summary

Mitochondrial disorders comprise a heterogeneous group of diseases with multisystem involvement including myocardium. Most cases of mitochondrial cardiomyopathy are associated with myopathy and encephalopathy and are generally present in infancy or childhood. The disease often exhibits a rapid downward course with death frequently occurring within the first year of life. We describe a unique case of hypertrophic cardiomyopathy due to mitochondrial DNA mutation m.3303C>T in the MT-TL1 gene, diagnosed accidentally in a 35-year-old male. The patient initially presented with stroke of assumed cardioembolic origin due to the presence of two interatrial communications associated with mobile aneurysm of the interatrial septum. No other extracardiac manifestations of mitochondrial disorder were observed. (Int Heart J 2012; 53: 383-387)

Key words: Mitochondrial disease, Short PR interval

Mitochondrial diseases are a heterogeneous group of disorders that result from the structural, biochemical, or genetic derangement of mitochondria.1) These syndromes are multisystemic and virtually every organ system can be affected. Cardiac involvement is frequently observed as an initial symptom of mitochondrial diseases,2,3) however in the majority of cases it is combined with impairment of other organs. On the other hand, cardiac involvement rarely causes presenting symptoms in adulthood.4) We describe a unique case of hypertrophic cardiomyopathy due to mitochondrial DNA mutation m.3303C>T in the MT-TL1 gene, diagnosed accidentally in an adult male. The patient initially presented with stroke of assumed cardiembolic origin due to the presence of patent foramen ovale and an atrial septal defect associated with mobile aneurysm of the interatrial septum. No other extracardiac manifestations of mitochondrial disorder were present.

Case Report

A 35-year-old man with no medical history was initially referred to the neurological department with sudden onset of mixed aphasia, right-sided hemiparesis, and central paresis of the facial nerve lasting for 4 hours. On admission, physical examination revealed no other abnormalities, blood pressure was 110/70 mmHg and pulse rate 100/minute. The ECG showed sinus rhythm with no apparent abnormalities as interpreted by the attending neurologist. A CT scan demonstrated left-sided basal ganglia hemorrhage combined with trace subarachnoidal hemorrhage in the left sylvian region. Subsequent CT examination performed during the first week of hospitalization confirmed the presence of an atypical putaminal hemorrhage that was interpreted as a hemorrhagical transformation of the original ischemic lesion in the territory of the left middle cerebral artery. Duplex ultrasound imaging did not reveal any atherosclerotic plaques or congenital pathology of carotid arteries. After 3 weeks of intensive rehabilitation, when complete recovery of the neurological status was achieved, the patient was referred to the cardiology department for further evaluation regarding a possible cardioembolic origin of the stroke.

On admission, physical examination performed by a cardiologist revealed only a soft systolic murmur at the left lower sternal border. All other parameters were within normal limits. The ECG showed regular sinus rhythm with a heart rate of 66 bpm. The PR interval was shortened to 106 ms and the duration of QRS complex was 102 ms with signs of incomplete right bundle branch block in lead V1. There were no voltage signs of left ventricular (LV) hypertrophy and slight ST-T depressions were present in leads II, III, and aVF (Figure 1). Transthoracic echocardiography demonstrated concentric hypertrophy of the nondilated left ventricle, with an interven-
tricular septal wall thickness of 14 mm and LV end-diastolic
diameter of 50 mm (Figure 2). No regional wall motion abnor-
malities were noted and global LV systolic function was pre-
served with an ejection fraction of 58%. The analysis of LV di-
astolic function revealed impaired relaxation (the ratio of early
to late LV filling velocities (E/A ratio) of 0.74, early diastolic
mitral septal annular velocity (e') of 8 cm.s\(^{-1}\), and E/e' ratio of
7.0 indicating normal left atrial pressure). The left atrium was
mildly dilated (left atrial diameter of 44 mm). Right heart
chambers were not enlarged with normal wall thickness and
systolic function of the right ventricle. The estimated pulmo-
nary artery systolic pressure of 28 mmHg was within normal
limits. All valves were without apparent morphologic abnor-
malities and only mild mitral and tricuspid regurgitation were
noted. A mobile aneurysm of the interatrial septum was detect-
ed. On transesophageal echocardiography, an atrial septal de-
fect (5 mm diameter) and patent foramen ovale were found. As
the neurologist identified the brain lesion as resulting from
ischemic infarction with secondary hemorrhagic transforma-
tion, we assumed a cardioembolic origin of the stroke related
to the interatrial communications described above. Both les-
ions were subsequently closed with 2 Amplatzer atrial septal
occlusion devices.

Because of the lack of history of arterial hypertension and
the absence of aortic stenosis, we considered concentric LV
hypertrophy to be a phenotypic expression of hypertrophic car-
diomyopathy (HCM). The combination of unexplained LV
hypertrophy with short PR interval on ECG suggested a non-
sarcomeric form of HCM, possibly due to storage disease.
However, an examination of family history did not provide any
additional information, and the patient’s personal history was
also unremarkable with no complaints regarding physical fit-
ness (the patient was a recreational ice-hockey player). The
mental status of the patient was normal. Laboratory examina-
tions did not show any abnormalities in liver enzymes or renal
functional tests, and the plasma activity of creatinine kinase
was normal (0.55 \(\mu\)kat/L; normal value < 2.8 \(\mu\)kat/L). Howev-
er, serum levels of brain natriuretic peptide and troponin I were
elevated (352 ng/L and 0.27 \(\mu\)g/L, respectively; normal values
< 100 ng/L and < 0.03 \(\mu\)g/L, respectively). The plasmatic level
of lactic acid was elevated (4.94 mmol/L; normal values < 2.3
mmol/L). Fasting blood glucose level was repeatedly normal
(range 4.4-5.6 mmol/L, control 3.9-5.6 mmol/L). Plasma con-
centrations of monoclonal free kappa and free lambda chains and the concentration ratio were within the normal ranges. Blood leukocyte α-galactosidase A activity was also within normal limits excluding Fabry disease. Cardiac magnetic resonance imaging confirmed the presence of concentric LV hypertrophy with a maximal interventricular septal wall thickness of 16 mm. With late gadolinium enhancement imaging, transmural hyperenhancement was observed along the entire width of the interventricular septum (Figure 3). In order to further elucidate the etiology of LV hypertrophy, endomyocardial biopsy via the right jugular vein was performed with specimens taken from the interventricular septum. Portions of the biopsies were examined in an unfixed state by enzyme histochemistry, while others were fixed in 4% buffered paraformaldehyde for examination by histology and electron microscopy. The sections were stained by standard histological techniques (hematoxylin-eosin, periodic acid-Schiff), and examined in ultraviolet light. Standard methods were employed to examine the lysosomal system, and the activities of dehydrogenases of α-glycerophosphate, succinate, nicotinamide adenine dinucleotide (NADH) and cytochrome C oxidase (COX) as described in our in previous publications. Histological examinations showed hypertrophy and partial disarray of cardiomyocytes with disperse interstitial fibrosis (Figure 4). Cardiomyocytes exhibited focal accumulation of glycogen. There were borderline signs of steatosis. The lysosomal system accumulated lipofuscin and exhibited occasional autophagocytosis. Lysosomal markers showed moderate irregular expansion of the lysosomal system that was attributed to autophagocytosis. Lysosomal-associated membrane proteins 1 and 2 were both expressed. Electron microscopy demonstrated prominent focal accumulation of mitochondria between and within myofibrils (Figure 5). The size of individual mitochondria varied considerably, as did the amount and organization of the internal mitochondrial membranes. Mitochondria frequently exhibited cytosolic inclusions, most of which contained glycogen β granules. This culminated in complete obliteration of the mitochondrial structure by glycogen. Evaluation of the oxidative phosphorylation system in cryostat sections revealed a mosaic COX activity in cardiomyocytes (Figure 6A). Considerable succinate dehydrogenase activity was observed. It was further elevated in COX-deficient cardiomyocytes. NADH dehydrogenase activity was decreased. These findings strongly suggested that a primary mitochondrial disorder was the underlying cause of myocardial pathology and subsequent tests were focused in this direction. Histological and histochemical evaluation of a skeletal muscle biopsy revealed almost normal pattern without signs of neurogenic or myogenic rearrangement. Physiological mosaic COX and NADH dehydrogenase activities were detected (Figure 6B). COX-deficient fibres were exceptional and no “ragged blue fibres” were found in corresponding areas in parallel sections stained for SDH activ-
ity. Electron microscopy of skeletal muscle showed only a borderline increase in the number of mitochondria between myofibrils.

Biochemical examination of a skeletal muscle biopsy demonstrated mildly decreased activity of respiratory chain complexes I (101.1 nmol/minute/mg of protein; normal values 110-290 nmol/minute/mg of protein) and IV (COX; 522.5 nmol/minute/mg of protein; normal values 658-1552 nmol/minute/mg of protein) and control enzyme citrate synthase were comparable with controls. A reduced amount of complex V was also revealed by blue-native electrophoresis. In skeletal muscle, sequencing of whole mitochondrial DNA (mtDNA) revealed a pathogenic mutation m.3303C>T in the MT-TL1 gene coding for mitochondrial tRNA^Leu^ (UUR). The level of heteroplasmy of the mtDNA m.3303C>T mutation in skeletal muscle was very high (98%) as determined by [α-32P]-dCTP PCR-RFLP analysis (Figure 7). Similar heteroplasmy of this mutation was found in hair follicles and urinary epithelial cells. Lower mutation load was observed in cultured skin fibroblasts (95%), leukocytes (92%), and buccal swab cells (82%). Cardiac cells from the patient were not evaluated for heteroplasmy of the mutation because of a shortage of samples. Maternal DNA was not available for analysis, therefore maternal inheritance is not clarified in this case. The patient is currently under follow up at our outpatient cardiomyopathy clinic and his treatment consists of acetylsalicyl acid and dietary supplements of vitamins and antioxidant drugs.

**Discussion**

Mitochondrial diseases result from abnormalities in the mitochondrion, the essential organelle that provides energy in the form of adenosine triphosphate. Mitochondrial diseases may be caused by mutations in more than 150 nuclear genes or by mitochondrial DNA (mtDNA) mutations. 

Mitochondria and the mitochondrial genome are exclusively maternally inherited. Each mitochondrion contains 2 to 10 DNA molecules, and each cell contains multiple mitochondria. Normal and mutant mtDNA can thus coexist within the same cell or tissue. The proportion of mutant mtDNA, ie, a level of heteroplasmy, required for the occurrence of a deleterious phenotype, known as a threshold effect, varies among organ systems, within a given tissue and is mutation-specific. The threshold effect depends on the balance between oxidative supply and demand. Because the heart, the central nervous system and the skeletal muscles are highly dependent on the energy generated by oxidative metabolism, these tissues are more vulnerable to mitochondrial defects. Cardiac involvement is common and sometimes even the first presenting symptom of mitochondrial diseases, however, it is often preceded or accompanied by impairment of the nervous system and skeletal muscle.  

Mitochondrial cardiomyopathy with neonatal onset might present as an isolated symptom and usually has a rapid downhill course with early death during infancy with some exceptions. Mutations in several mitochondrial tRNA genes result in cardiomyopathy. The most common mtDNA mutations related to cardiomyopathy are the m.3243A>G mutation in the MT-TL1 gene, which is observed in 83% of MELAS cases (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes), and the m.8344A>G point mutation in the MF-TK gene, that is found in 79% of MERRF cases (Myoclonus Epilepsy with Ragged-red Fibres). When neurological or peripheral muscle symptoms are not present, it is extremely difficult to distinguish mitochondrial cardiomyopathy from other types of hypertrophic or dilated cardiomyopathy. Concentric LV hypertrophy seems to be most commonly associated with mitochondrial disease, usually with progressive decline in systolic function due to global ventricular hypokinesia and dilatation of the LV cavity. Creatinine kinase and troponin levels are frequently elevated. Atrioventricular conduction abnormalities, high voltage of the QRS complex, and changes in ST-T segment may be observed.

In our patient, mtDNA mutation analysis revealed a m.3303C>T mutation in the MT-TL1 gene coding for mitochondrial tRNA^Leu^ (UUR). To the best of our knowledge, there are only three reports on this mutation-related cardiomyopathy currently available in the literature. So far only 15 patients have been reported. The mutation was first reported by Silvestri, et al, who described 7 patients from one family. Three subjects presented with fatal infantile cardiomyopathy while the remainder suffered from sudden cardiac death or cardiomyopathy and myopathy which developed later in life. Another 8 patients from 4 families were described by Bruno, et al and Goldstein, et al. Summarizing data published to date, we can conclude that a total of 6 patients with the m.3303C>T mutation manifested as fatal infantile cardiomyopathy have been reported, and another 6 subjects have been described with combination of cardiomyopathy and myopathy developing during the first decade of life. In two patients the disease presented as isolated myopathy at the age of 20 and 27 years, respectively. One patient suffered from sudden cardiac death. None of these patients manifested stroke-like episodes. The relationship of heteroplasmy levels and age of onset of the first clinical symptoms is not clear. As we found the mutation m.3303C>T only in one patient, we can only speculate about factors affecting the onset of first clinical symptoms. There is a broad heterogeneity in clinical manifestation of mtDNA mutations. In particular, point mutations affecting mitochondrial tRNA genes display huge variability in age of onset of first clinical symptoms that cannot be simply explained by their heteroplasmy levels. Mutation m.4300A>G in MTTI (coding for mitochondrial tRNA^Leu^ (UUR) results in onset of hypertrophic cardiomyopathy in early infancy. On the contrary, the first symptoms of m.7512T>C in MTTS1 (coding for mitochondrial tRNA^Leu^ (UUR) usually appears in adulthood.

The dominant histopathological finding in the heart of our patient was alterations in mitochondria, namely the invasion of cytosol with glycogen into the mitochondrial interior, which eventually resulted in obliteration of their internal structure. Such intramitochondrial accumulation of glycogen has been repeatedly described but never explained. We suggest that the barrier between mitochondrial membranes and cytosol may be altered, allowing the glycogen synthesizing apparatus to penetrate the mitochondria.

We believe that the case described here is unique, as the first reported case of mitochondrial hypertrophic cardiomyopathy due to m.3303C>T mutation, diagnosed in an adult patient. Importantly, no extracardiac manifestations of mitochondrial
disorder including encephalopathy or obvious skeletal myopathy were observed. Although stroke may be considered a symptom of MELAS syndrome, the lesion in our subject was located in basal ganglia, which are not usually affected in MELAS patients.22-27 Because the observed interatrial communications are typical pathologies in young adults with stroke of cardioembolic origin, we assume the stroke was not a manifestation of MELAS syndrome.

Our case illustrates the importance of considering nonsarcomeric forms of hypertrophic cardiomyopathy in the differential diagnosis of unexplained LV hypertrophy associated with short PR interval. In these cases, storage disorders like Fabry disease, PRKAG2 cardiomyopathy, Danon disease, and mitochondrial abnormality should be excluded.28-30 As shown in our case, mitochondrial cardiomyopathy may be present even without extracardiac symptoms. The elevated blood level of lactic acid is consistent with mitochondrial abnormality. The final diagnosis should be based on findings from endomyocardial biopsies and genetic analysis, where complete mtDNA genome analysis should be performed to identify the causal mutation.

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REFERENCES