Is 8860 variation a rare polymorphism or associated as a secondary effect in HCM disease?

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Abstract

Introduction: mtDNA defects, both deletions and point mutations, have been associated with hypertrophic cardiomyopathies. The aim of this study was to establish a spectrum for mtDNA mutations in Iranian hypertrophic cardiomyopathy (HCM) patients.
Material and methods: The control group was chosen among the special medical centre visitors who did not have hypertrophic cardiomyopathy or any related heart disease. Hypertrophic cardiomyopathy (HCM) is widely accepted as a pluricausal or multifactorial disease. Because of the linkage between energy metabolism in the mitochondria and cardiac muscle contraction, it is reasonable to assume that mitochondrial abnormalities may be responsible for some forms of HCM. Point mutations and deletions in the two hot spot regions of mtDNA were investigated by PCR and sequencing methods.
Results: Some unreported point mutations have been found in this study but no deletion was detected. Meanwhile some of these point mutations have been investigated among HCM patients for the first time.
Conclusions: A8860G transition was detected in a high proportion, raising the question whether this rare polymorphism is associated as a secondary effect in HCM disease.

Key words: hypertrophic cardiomyopathy, mtDNA mutation disease.

Introduction

The cardiomyopathies are a clinically and genetically heterogeneous group of cardiac diseases in which the myocardium is primarily involved. Three distinct categories of cardiomyopathy can be classified by functional and pathological criteria: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) and restrictive cardiomyopathy. It has been proposed that approximately 45-50% of familial hypertrophic cardiomyopathy cases are due to mutations in genes encoding contractile proteins of the sarcomere including the *á*-cardiac myosin heavy chain gene, the cardiac troponin T gene and the *á*-tropomyosin gene.

It is now known that hypertrophic cardiomyopathy can occur as a result of point mutations in the highly conserved transfer RNA genes of mitochondrial DNA. The mitochondrial genome, exclusively inherited through the maternal line, is a 16.5 kilobase (kb), circular, double stranded

molecule that encodes two ribosomal RNAs, 22 transfer RNAs and 13 proteins involved in the mitochondrial respiratory chain and oxidative phosphorylation system. The oxidative phosphorylation system is fundamental to the synthesis of cellular ATP and consequently pathogenic mutations of mitochondrial DNA are believed to cause disease by impairing the function of the mitochondrial respiratory chain and oxidative phosphorylation system, thereby decreasing cellular ATP synthesis.

Mitochondrial DNA point mutations have been identified in a broad spectrum of clinical phenotypes. The majority of these are associated with neurological disorders such as the mitochondrial myopathies and encephalomyopathies, although a number of mitochondrial DNA point mutations have been reported in patients with hypertrophic cardiomyopathy, either alone or as part of a multisystem disorder [1]. In this study we investigated point mutation diversities in the two hot spot regions of mitochondrial DNA among Iranian HCM patients.

Material and methods

Patients

A total of 31 hypertrophic cardiomyopathic adult patients of unrelated families were selected based on their medical examination, electrocardiogram, Holter monitoring, and angiography [2]. All of the patients were informed regarding the aims of the study and gave their consent for genetic analysis. The control group consisted of 100 visitors of the special medical centre who were chosen among the visitors without hypertrophic cardiomyopathic disease or any related heart disease and with no family history of this disease.

DNA extraction

Genomic DNA was extracted from 5 ml of peripheral blood using a DNA extraction kit (Diatom DNA Extraction Kit, Genefanavaran, Tehran, Iran).

mtDNA deletion analysis

We screened the 31 HCM patients for mtDNA deletions according to previous work carried out by Houshmand *et al.* The PCRs were performed in 35 cycles composed of the following steps: 94°C for 1 min, 55°C for 1 min and 72°C for 35 s. The deletion-prone region between nt5461 of the light strand and nt 15000 of the heavy strand was investigated in all patients using the primers ONP 86, ONP 89, ONP10, ONP 74, ONP 25 and ONP 99 (Table I). The distances between the primers were long enough to allow amplification only if a part of the DNA between the respective primers was deleted. The primer pair ONP 86/ONP 89 was used to amplify a normal internal mtDNA fragment in a region which is seldom afflicted by deletions, thus serving as a control in the PCR analysis. We categorized mtDNA deletions into 4 classes of A, B, C, and D corresponding to 8.7-, 7.5-, 5- and 7.5-kb deletions, respectively (Table II) [3].

PCR sequencing

The first mtDNA region from nt 1441 to nt 6001 including RNR2, TL1, ND1, TQ, ND2 and the second region (1,635 bp) from nt 7377 to nt 9031 that includes CO2 and ATP6 were amplified according to the method by Ahari *et al.* [4].

Table I. Primers used for mtDNA deletion analysis

ONP 86 (LF): 5461–5480 (ND2)	5'-CCC TTA CCA CGC TAC TCC TA-3'
ONP 89 (HB): 5740–5721 (OL)	5'-GGC GGG AGA AGT TTG AA-3'
ONP 10 (HB): 15000–14981 (Cytb)	5'-TTG GCG TGA AGG TAG CGG AT-3'
ONP 74 (HB): 13640–13621 (ND50)	5'-GGT TGA CCT GTT AGG GTG AG-3'
ONP 25 (LF): 8161–8180 (COII)	5'-CTA CGG TCA ATG CTC TGA AA-3'
ONP 99 (HB): 16150–16131 (D-loop)	5'-GTG GTC AAG TAT TTA TGG TA-3'

LF – forward primer, HB – reverse primer

Table II. Primers used for detection of 4 deletions

Primers	Starting point	Ending point	Deletion length [Kb]	PCR product length [bp]	Deletion
ONP 86, ONP 10	5461	15000	8.7	500	A
ONP 86, ONP 74	5461	13640	7.5	680	B
ONP 25, ONP 74	8180	13640	5	420	C
ONP 25, ONP 99	8180	16150	7.5	970	D
ONP 86, ONP 89	5461	5740	–	279	Internal control

Table III. Primers used for mtDNA sequencing

F60	1441-1460	5'-AGT AGA GTG CTT AGT TGA GC-3'
R63	3140-3121	5'-TCT CTT GTC CTT TCG TAC AG-3'
F62	3001-3020	5'-GGA CAT CCC GAT GGT GCA GC-3'
R65	4650-4631	5'-GGA AAT ACT TGA TGG CAG CT-3
F64	4491-4540	5'-GTC ATC TAC TCT ACC TAC TT-3'
R67	6020-6001	5'-GGC TCG AAT AAG GAG GCT TA-3'
F21	7377-7396	5'-CTG GAG TGA CTA TAT GGA TG-3'
R56	9050-9031	5'-CCA AAT AGG TGC ATG AGT AG-3'

PCR products were checked on 1% agarose gel. MtDNA sequencing was done by using forward and reverse primers (Table III) to detect novel mutations that might impair mitochondrial protein synthesis in the two hot spot regions by a 3700 ABI sequencer (Macrogene Korea). The obtained mtDNA sequences were aligned with a multiple sequence alignment interface CLUSTAL_X and then compared with the reference sequence [5]. MITOMAP: MtDNA Coding Region Sequence Polymorphisms web site was used for investigation and comparison of detected mutations for final conclusions [6].

Results

In the first step no mtDNA deletion was found among the cases. After that samples from all 31 patients were forwarded for sequencing analysis of the two hot spot regions in mtDNA that included RNR2, TL1, ND1, TQ, ND2, CO2 and ATP6 segments.

Altogether 43 point mutational polymorphisms were found in this study; 10 unpublished polymorphisms were found among them. This finding demonstrates the high variability of human mtDNA. The rest of the polymorphisms have been reported before. No previously reported disease-causing mutations of mtDNA were found within the sequenced regions of participants (Table IV).

Some of these novel base substitutions do not lead to amino acid exchange and are consequently defined as polymorphisms, such as T1700C. The second group of sequence alterations can be divided into two classes: 1) conservative exchanges, leading to substitution by a homologous amino acid, such as A5480G, and 2) non-homologous substitutions, such as G5466A.

An apparently homoplasmic substitution within tRNA genes was found: T4384C, which affects the T_C loop of tRNA glutamine. No other point mutation was found among the studied mitochondrial tRNA genes.

Except for 3 remarkable mutations (A8860G, A2706G and N3107C) most of these polymorphisms were observed among fewer than 5 patients (Table IV).

Discussion

During the past decade, more than 100 mutations in 11 causal genes coding for sarcomeric proteins, the γ subunit of AMP-activated protein kinase and triplet-repeat syndromes and in mitochondrial DNA, have been identified in patients with hypertrophic cardiomyopathy (HCM). Genotype-phenotype correlation studies show significant variability in the phenotype expression of HCM among affected individuals with identical causal mutations. Overall, causal mutations account for a fraction of the variability of phenotypes, and the genetic background, referred to as the modifier genes, plays a significant role. The final phenotype is the result of interactions between the causal genes, the genetic background (modifier genes), and probably environmental factors. The individual modifier genes for HCM remain largely unknown, so a large-scale genome-wide approach and candidate gene analysis are needed [7].

Recently, maternally inherited forms of HCM have been associated with defects in oxidative metabolism that are primarily due to alterations in mtDNA, such as deletions and various point mutations [8-10]. But in fact we did not find any deletion in our study.

Previously, a variety of neurodegenerative and neuromuscular diseases that demonstrate strict maternal inheritance have been attributed to single point mutations in mtDNA. The best characterized examples include MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) and MERRF (myoclonic epilepsy and ragged red fibres) [11-15].

Point mutations in tRNA genes have also been attributed to maternally inherited forms of sudden cardiopulmonary arrest and HC. Mutations in mtDNA that lead to HC include A-to-G and C-to-T transitions at nucleotide 3260 and nucleotide 3303, respectively, in tRNALeU(UUR); A-to-G transitions at nucleotide 4317 and nucleotide 4266 in tRNAIle; and, possibly, an A-to-G transition at nucleotide 15951 in tRNAThr. A novel point mutation (T to C) was found in the mitochondrial tRNAgly gene at

Table IV. Frequency of some polymorphisms among Iranian HCM patients

Locus	Nucleotide position	Frequency (31 patients)	Nucleotide change	Amino acid change	Reported in MitoMap references
RNR2	1700	1	T-C	Non-coding	+
	1718	1	A-AA	Non-coding	+
	1719	1	G-A	Non-coding	*+
	1810	1	A-G	Non-coding	Unpublished
	1811	2	A-G	Non-coding	+
	1888	2	G-A	Non-coding	+
	1977	1	T-C	Non-coding	*+
	2071	1	T-C	Non-coding	Unpublished
	2260	1	A-C	Non-coding	Unpublished
	2306	1	A-T	Non-coding	Unpublished
	2706	15	A-G	Non-coding	*+
	2707	1	A-C	Non-coding	+
	2778	1	T-C	Non-coding	Unpublished
	2805	1	A-T	Non-coding	Unpublished
	3010	3	G-A	Non-coding	*+
	3107	26	N-T	rCRcorrection	*+
	3170	1	C-A	Non-coding	Unpublished
	3197	2	T-C	Non-coding	+
	3202	1	T-C	Non-coding	+
TL1	3285	2	T-C	Non-coding	*+
ND1	3338	1	T-C	V-A	*+
	3392	1	G-C	G-A	+
	3480	2	A-G	Syn	+
TQ	4384	1	T-C	??????	Unpublished
ND2	4529	1	A-T	Syn	+
	4646	2	T-C	Syn	+
	4655	1	G-A	Syn	+
	4823	1	T-C	Syn	+
	4917	2	A-G	N-D	+
	5021	1	T-C	Syn	Unpublished
	5046	3	G-A	V-A	+
	5460	4	G-A	A-T	+
	5480	1	A-G	Syn	+
	5495	1	T-C	Syn	+
CO2	8251	1	G-A	Syn	+
ATP6	8614	3	T-C	Syn	+
	8682	1	A-G	Syn	Unpublished
	8648	1	G-A	R-Q	+
	8697	2	G-A	Syn	+
	8701	2	A-G	T-A	*+
	8705	1	T-C	M-T	+
	8860	30	A-G	T-A	*+
	8994	1	G-A	Syn	+
				S-N	

Reported among HCM patients before. rCRcorrection: http://www.mitomap.org/mitoseq.html

nucleotide 9997 in a maternally affected inherited HC [16]. Only T4384C mutation was found in tRNA glutamine that affects the T_C loop among our patients. As this mutation has not been reported before, more investigation is needed to specify it accurately.

Unreported base substitutions in mtDNA were found in this study. Whereas the "silent" ones, i.e., not affecting amino acid composition of the encoded polypeptide, are generally assumed to represent polymorphism, the role of the others is less obvious.

An A-to-G transition at nucleotides 8860, previously characterized, was present in a high proportion (30 of 31 patients) in our study with respect to the Cambridge Reference Sequence [17]. While this polymorphism was found among idiopathic cardiomyopathic patients, it was considered as a possible error in the Cambridge sequence but future studies showed that it is a rare polymorphism [18]. In fact none of our 60 controls showed this polymorphism. Some other studies have found this transition in a high proportion, such as all 79 patients with Alzheimer's disease [19], all 23 patients affected with bipolar affective disorder [20], and 13/14 patients with Leber's hereditary optic neuropathy [21]. These findings suggest that this polymorphism may a have close relation with these kinds of mitochondrial degenerative diseases.

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