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Analysis of mtDNA A3243G mutation frequency in Hungary

Research Article

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Abstract: The A3243G mutation in the mitochondrial tRNA$^{Leu\ (UUR)}$ gene is one of the most common causes of mitochondrial DNA related disorders. Originally it was described in MELAS syndrome (Mitochondrial Encephalomyopathy, Lactic acidosis, Stroke-like episodes), later it had been found to be associated with various phenotypes. In our study the mutation frequency of the A3243G mtDNA mutation was investigated in patients with maternal sensoneural hearing loss, stroke-like episodes, ataxia and myopathy with undetermined etiology. We screened 631 Hungarian patients in North-East, South-West and Central Hungary between 1999 and 2008 for this mutation. The mtDNA analysis was performed from blood and/or muscle tissue. The A3243G substitution was present in 6 patients in heteroplasmic form. The segregation analysis detected 8 further cases. The frequency of the A3243G mutation was 2.22% in the investigated patients. The A3243G mutation frequency in Hungary does not differ significantly from other countries using similar patient selection criteria, however in Finland a higher mutation rate was found. In studies investigated the mutation frequency of this mutation in diabetes mellitus similarly wide variety was detected as well. We conclude that the study design has a huge impact on the result of the genetic epidemiological investigation analyzing the mutation frequency of the A3243G mutation due to the broad clinical phenotype and the different mutation load in different tissues.

Keywords: *Mitochondrial encephalomyopathy • Epidemiology • mtDNA • A3243G*

1. Introduction

The A3243G mutation in the mitochondrial tRNA$^{Leu(UUR)}$ gene is the most common cause of the MELAS syndrome (mitochondrial enchephalomyopathy lactic acidosis, stroke like episodes) [1]. Clinical evaluation of patients with A3243G mutation has shown various phenotypes, such as sensoneural hearing loss, hypertrophic cardiomyopathy, ataxia, basal ganglia calcifications, ophthalmoplegia, epilepsy, and diabetes mellitus [2]. Apart from the classic MELAS syndrome, some infants with A3243G mutation have been described with failure to thrive, hypotonia, seizures, cardiomyopathy, lactic acidosis and extensive neuroendocrine dysfunction [3]. The prevalence of A3243G mutation has been investigated in different countries with different study design. Eight studies have previously screened patients

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Figure 1. The map of the investigated area.

with sensoneural hearing loss, epilepsy, juvenile stroke syndromes, myopathy and ataxia for the A3243G mtDNA substitution and revealed this mutation in 0.07% to 6.5% of their patients [4-11]. The highest frequency has been observed in the Finnish population where the prevalence was calculated to be 16.3:100.000 in the adult population of the investigated area [5]. The aim of our study was to establish the frequency of A3243G mtDNA mutation in Hungarians with symptoms implying mitochondrial diseases.

2. Material and Methods

A total of 631 (361 female and 270 male) Hungarian patients were tested in this study from January 1999 till September 2008. The mean age of patients was 36.3 years (female: 38.1 years, male: 34.4 years). All of patients had in a clinical presentation highly suggestive of mitochondrial diseases with stroke, ataxia, maternal sensoneural hearing loss, myopathy, or hypotonia from North-East, South-West and Central Hungary (Counties Szabolcs Szatmár, Borsod- Abaúj-Zemplén, Hajdú-Bihar, Pest, Baranya and the city Budapest) (Figure 1). Informed consent was obtained from all patients involved in our study. The study was approved by the local institutional ethical committee. Molecular genetic analysis was performed for diagnostic purposes in all investigated patients. DNA was extracted from blood (n=615) and/or skeletal muscle tissue (n=53) by ABI Prism 6100 Nucleic acid isolation systems (Applied Biosystems, Foster City, California, USA) according to the manufacturer's instructions. The A3243G mutation in the $tRNA^{Leu(UUR)}$ gene of the mtDNA was screened according to the method described earlier using restriction fragment length polymorphism (RFLP) with HaeIII restriction endonuclease after PCR amplification [12]. The mutation frequency was calculated on the score of the ratio of patients with A3243G substitution/ total investigated patients during the period of analysis.

3. Results

The A3243G mutation of the mitochondrial $tRNA^{Leu(UUR)}$ gene was found in heteroplasmic form in 6 patiens (5 female and 1 male) from the investigated 631 patients. In one patient the mutation was detected only in the muscle tissue. The ratio of heteroplasmy varied between 22 and 80% (Table 1), in the course of familiar segregation analysis further 8 cases (7 female and 1 male) were detected. All of the investigated family members had clinical symptoms. The clinical symptoms of the patients and their family history are shown in Table 1. The distribution of the positive cases was 43% in Budapest (6 cases), 35% in Country Hajdú-Bihar (5 cases) and 22% Country Baranya (3 cases). The frequency of A3243G mutation was 2.22% in the investigated patients

Table 1. Clinical symptoms and results of familiar segregation of the patients with mtDNA A3243G substitution.

Family	Gender	Beginning of the symptoms	Ratio of heteroplasmy (blood)	Ratio of heteroplasmy (muscle)	Clinical features	Family history	Number of positive family member	Reference
1	Female	17 years	35%	45%	CPEO, diabetes mellitus, hypacusis, myopathy, myalgia, short stature	CPEO, diabetes mellitus, hypoacusis, myalgia, hypotonia	4(3 female, 1 male)	[18]
2	Female	35 years	35%	Not investigated	Stroke, depression, psychosis	Not investigated	Not investigated	
3	Male	13 years	40%	Not investigated	Sensoneural hearing loss, myalgia,	Hypoacusis, myocardial infarction	2(2 female)	[23]
4	Female	2 years	80%	Not investigated	Stroke, lactate acidosis, mental retardation	Hypoacusis	2(2 female)	[24]
5	Female	3 years	Not detected	22%	Diabetes mellitus, hypogonadisms, ataxia, mental retardation	Negative	0	
6	Female	30 years	30%	Not investigated	Sensoneural hearing loss, migraine	Not investigated	Not investigated	

CPEO – chronic progressive external ophthalmoplegia

4. Discussion

This is the first genetic epidemiology study of the A3243G mutation of the mtDNA in middle-east Europe in patients with neurological symptoms. The revelaed frequency of the A3243G mutation in 631 subjects with different neurological symptoms with an assumed mitochondrial etiology was 2.22% in North-East, South-West and Central Hungary. We found that the A3243G mutation frequency in Hungary does not differ significantly from other countries. Similar frequency was also found in Scandinavian, Portuguese and Japanese patients with diabetes mellitus as well [13-15]. In our study mitochondrial disease was assumed based on previous laboratory data and family anamnesis. Pang et al. confirmed the assumption that the A3243G mutation is one of the most frequent between mtDNA disorders [16]. In their study 32 of 177 patients with mitochondrial disorders harboured this mtDNA substitution, in 18 cases the G11778A mutation characterizing Leber hereditary optic neuropathy, in 17 patients the mtDNA common deletion, in 9 patients the A8344G mutation, and in 1 and 2 patients the T8993C and T8993G mutations of Leigh syndrome were found respectively. A pilot study in Croatia investigated the association between mtDNA A3243G mutation and type 2 diabetes mellitus in 22 patients [17]. The substitution was present in oral mucosal DNA samples of two patients, while the mutation analysis from their blood derived DNA has not detected this mtDNA substitution.

The mutation frequency varied in different countries, it may be due to the variety of population, further the choice of tissues for analysis may contribute to variable frequencies as different tissues of the same individual may express variable level of mutant mtDNA.

Most of the genetic epidemiological studies are performed from blood DNA. In one of our cases the mutation was detected only in postmitotic muscle tissue and was absent in the blood. This result may raise the possibility, that in some cases the low ratio of heteroplasmy in the blood hamper the detection of the A3243G mutation. In such cases the molecular genetic investigation of the buccal mucosal cells or muscle biopsy material have to be considered. Though buccal mucosal cells are not postmitotical cells, their turnover is lower than that of the cellular elements of peripheral blood, which inhibit the wash-out of mutation.

Table 2. The mtDNA A3243G mutation frequencies among the patients with diabetes mellitus in different countries (* Pilot study with oral mucosal DNA samples).

Mutation frequency	Number of investigated patients	Number of patients with A3243G mutation	Selection criteria	Country	Reference
9.09 *	22	2	DM2	Croatia	[17]
3.33	90	3	DM	China	[25]
3.01	133	4	DM2	China	[26]
2.92	240	7	DM, gestational diabetes	Japan	[13]
2.61	115	3	Early oneset DM	Sweden and Finnland	[14]
2.03	148	3	DM1, DM2	Portugal	[15]
0.72	138	1	DM1	Catalonia, Spain	[27]
0.47	428	2	DM2	China	[28]
0.41	244	1	DM2	China	[29]
0.41	733	3	DM1, DM2, MIDD, hearing loss	Portugal	[7]
0.07	1460	1	DM, hearing loss	Germany	[4]
0.00	129	0	DM2	Poland	[30]
0.00	184	0	DM2	China	[31]

DM – diabetes mellitus, CPEO – chronic progressive external ophthalmoplegia

Table 3. The mtDNA A3243G mutation frequencies among the patients with mitochondrial diseases in different countries (* Patients were selected on the basis of results of muscle biopsy; ** Patients were selected on the basis of the result of Mitochip microarray).

Mutation frequency	Number of investigated patients	Number of patients with A3243G mutation	Selection criteria	Country	Reference
44.33 *	97	43	Mitochondrial encephalomyopathy	China	[20]
22.35 *	85	19	Mitochondrial enceplalomyopathy, psychomotory regression, cardiomyopathy, reccurent strole-like episodes, sensoneural hearing loss, DM, renal diseases	Korea	[22]
18	177	32	Mitochondrial encephalomyopathy	Taiwan	[16]
12.50 *	8	1	Mitochondrial encephalomyopathy, CPEO	Cuba	[21]
6.5	615	40	DM, sensoneural hearing loss, epilepsy, occipital stroke, CPEO, Intracranial calcification, white mater disease, ataxia, hypertrophic cardiomyopathy	Finnland	[5]
6.17	1184	73	KSS, CPEO	Australia	[34]
3.45 **	29	1	Sensoneural hearing loss	France	[8]
1.74	230	4	Sensoneural hearing loss	Japan	[10]
1.2	166	2	Mitochondrial encephalomyopathy	French	[9]
0.81	124	1	Leigh- disease, Leigh-like syndrome	China	[35]
0.00	128	0	Mitochondrial disease - MIDD, MELAS, MERRF, PEO, hypertrophic cardiomyopathy, Leigh-syndrome	Tunesia	[19]
0.00	52	0	Idiopathic cardiomyopathy	UK	[32]
0.00	265	0	Adult-oneset ataxia, SCA	Taiwan	[11]

DM – diabetes mellitus, CPEO – chronic progressive external ophthalmoplegia, KSS - Kearns-Sayre syndrome, MELAS - Mitochondrial Encephalomyopathy, Lactic Acidosis, And Stroke-Like Episodes, MERRF - Myoclonus Epilepsy Associated With Ragged-Red Fibers, MIDD - Maternally Inherited Diabetes And Deafness, SCA - Spinocerebellar Ataxia

The mtDNA A3243G mutation mainly associates with juvenile stroke syndrome, while other symptoms indicating mitochondrial disease like ataxia, hypoacusis, short-stature, endocrine dysfunctions and diabetes mellitus with maternal inheritance are not strictly associated with these mutation [18]. Majamaa et. al investigated the A3243G mtDNA alteration in patients with stroke in the occipital lobe [6]. Two of the 38 investigated patients (5.26%) harboured the A3243G mtDNA substitution [6]. The mutation frequency of the A3243G mutation varies greatly depending on patient selection criteria (Table 2 and Table 3). In many studies the investigation focused on patients with DM (Table 2). The mutation frequency varied in the different countries.

Six studies screened patients with a large variety of mitochondrial phenotype for the A3243G mtDNA substitution and identified this mutation in 0% to 44.33% in the investigated cohorts [5,9,19-22]. From these, a higher number of A3242G mutations were detected in the three studies where patients were selected on the basis of pathological muscle biopsy e.g. which showed mitochondrial alterations. In these studies the frequency was between 12.5% and 44.33% (Table 3) [20-22]. By using similar selection criteria, a study in Finland found a much higher mutation frequency, while in France and Taiwan the frequencies were similar to those found in Hungary.

We conclude: that due to the broad clinical phenotype associated with the A3243G mutation, and the different mutation load in different tissues the study design has a huge impact on the result of the genetic epidemiological investigations concerning A3243G mutation frequency. The mutation frequency of the A3243G mutation in Hungary does not differ significantly from other countries.

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