ORIGINAL ARTICLE

Prevalence of migraine in persons with the 3243A>G mutation in mitochondrial DNA

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Keywords:
headache, mDNA 3243A > G, migraine, mitochondrial dysfunction, mutation

Received 22 May 2015
Accepted 4 August 2015

European Journal of Neurology 2016, **23:** 175–181

doi:10.1111/ene.12832

Background and purpose: Over the last three decades mitochondrial dysfunction has been postulated to be a potential mechanism in migraine pathogenesis. The lifetime prevalence of migraine in persons carrying the 3243A>G mutation in mitochondrial DNA was investigated.

Methods: In this cross-sectional study, 57 mDNA 3243A>G mutation carriers between May 2012 and October 2014 were included. As a control group, a population-based cohort from our epidemiological studies on migraine in Danes was used. History of headache and migraine was obtained by telephone interview, based on a validated semi-structured questionnaire, performed by trained physicians.

Results: The prevalence of migraine is significantly higher in persons carrying the 3243A>G mutation than in controls (58% vs. 18%; $P < 0.001$). This applies for both subforms of migraine, migraine without aura (47% vs. 12%; $P < 0.001$) and migraine with aura (18% vs. 6%; $P < 0.001$), and in females (58% vs. 24%; $P < 0.001$) and males (58% vs. 12%; $P < 0.001$) for any migraine.

Conclusions: A high prevalence of migraine in persons with the mDNA 3243A>G mutation was found. This finding suggests a clinical association between a monogenetically inherited disorder of mitochondrial dysfunction and susceptibility to migraine. Mitochondrial DNA aberrations may contribute to the pathogenesis of migraine.

Introduction

Over the last three decades, mitochondrial dysfunction has been suggested to be a potential mechanism in migraine pathogenesis [1]. It has been postulated that abnormal oxidative metabolism in the brain due to mutations in mitochondrial DNA (mDNA) may increase the susceptibility to migraine [2]. A number of biochemical, physiological and morphological studies have shown elevated lactic acid, abnormal brain metabolism assessed by phosphorus magnetic resonance spectroscopy and ragged red fibers in muscle cells for at least some subtypes of migraine [1,3–7]. However, genetic studies have so far failed to demonstrate firm evidence supporting a mitochondrial dysfunction in migraine [8]. A single study found two common mDNA polymorphisms to be associated with pediatric cyclic vomiting syndrome and migraine [9].

The most common mitochondrial mutation in the northern European population is the mDNA 3243A>G mutation with a prevalence of 0.16% [10]. Since 80%–90% of patients with the rare mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome are caused by this mutation [11], it is often referred to as the MELAS mutation. This is so, even though other milder phenotypes associated with mDNA 3243A>G, such as

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EUROPEAN JOURNAL OF NEUROLOGY

diabetes, hearing impairment, exercise intolerance and ptosis, are much more common than MELAS. A few studies have shown that headache with migraine features is highly prevalent (70%–90%) in patients diagnosed with the clinical syndrome of MELAS [12–14]. However, none of these studies described migraine according to the diagnostic criteria of the International Classification of Headache Disorders [15], and no study has systematically investigated the prevalence of migraine by direct diagnostic interview in a substantial sample size of persons carrying the mDNA 3243A>G mutation. One study has investigated the prevalence of migraine in two maternal lineages with MELAS and chronic progressive external ophthalmoplegia in which the probands were carriers of the mDNA 3243A>G mutation [16].

Therefore, the clinical association between mDNA mutation and migraine was investigated by describing the prevalence and migraine characteristics of persons carrying the mDNA 3243A>G mutation. It is hypothesized that the lifetime prevalence of migraine without aura (MO) and migraine with aura (MA) is higher in persons with the mDNA 3243A>G mutation than in the general population. As a control group, population-based cohorts from our epidemiological studies on migraine in Danes were used [17].

Materials and methods

Study population

The study was conducted in Denmark between May 2012 and October 2014. Ethnic Danes carrying the mDNA 3243A>G mutation, as confirmed by genetic testing at the Department of Clinical Genetics at the National University Hospital, Rigshospitalet, were recruited. The study group comprised 57 mDNA 3243A>G carriers including 23 probands and 34 mutation-positive maternal relatives. Although participants within certain families were obviously related, the families were not interrelated as indicated by a wide range of mDNA haplotypes. Probands were initially diagnosed at different centers in Denmark including Copenhagen Neuromuscular Center, Department of Neurology, Rigshospitalet, and Departments of Clinical Genetics at Odense University Hospital, Odense, Rigshospitalet, Copenhagen, Aalborg University Hospital, Aalborg, and at the Kennedy Center, Glostrup. The initial indication for testing of probands was mostly to investigate a clinical syndrome.

When probands were diagnosed as mDNA 3243A>G carriers, both probands and maternal relatives were invited to clinical follow-up, genetic counseling and predictive testing. After this clinical consultation, probands and all known mutation-positive maternal relatives were invited to participate in the present study. Possible candidates were recruited exclusively because they were mutation positive. Except for one participant, who was 17 years old and got parental consent to participate in the study, all subjects were older than 18 years.

The present study was approved by the Regional Committees on Health Research Ethics for Southern Denmark (no. S-20100112). The subjects received written information about the study before telephone contact, and written informed consent was obtained from all subjects before the interview. People with dementia or other diseases that would prevent informed consent were excluded.

Controls

Our control population was sampled from the Danish population and has previously been described [17]. In short, the study included 3471 randomly selected 40-year-old Danes (2576 males and 895 females) from the Danish Central Population Registry. Using national statistics data, the control population was regarded as representative of the total Danish population in age, sex and marital status.

Tissue sampling and isolation of DNA

Genetic analysis for mDNA 3243A>G performed on blood has a high sensitivity but a low specificity due to heteroplasmic conditions. Some carriers were probably lost but the true positive rate is high.

DNA from blood was isolated using a routine salting out procedure. Samples were stored at 5°C until DNA isolation. DNA extractions were carried out using the QIAampH DNA Mini Kit (Qiagen GmbH, Hilden, Germany), using the manufacturer's protocol.

An allele-specific polymerase chain reaction (PCR) assay was used for the detection of the 3243A>G mutation by a direct PCR. Two sets of primers were used in one reaction tube: 3014L (forward), GTG CAG CCG CTA TTA AAG GT; and 3262HM17 (reverse, with two mismatches), TTT TAT GCG ATT ACCGCG CC. The PCR product was 249 base pairs. A control product was co-amplified using the primers 5289–5309 (forward) and 5903–5884 (reverse). The PCR product was 614 base pairs.

Migraine diagnosis and description of the mDNA 3243A>G phenotype

Headache and migraine histories in our cases and controls were obtained by a telephone interview, based

on a validated semi-structured questionnaire, performed by trained physicians [18,19]. The age of onset, frequency, features and severity of migraine were assessed. For associated symptoms of migraine, questions were asked about aura, nausea, vomiting and sensitivity to light or sound. The questionnaire assessed treatment status including frequency of medication per month. Headaches were classified into the following diagnoses according to the third edition of the International Classification of Headache Disorders [15]: MO, MA, probable MO, tension-type headache (TTH) and other primary headaches. In addition, the questionnaire also included information on mDNA 3243A>G-associated symptoms and comorbidities as well as onset of these symptoms.

Statistical analysis

Calculation of sample size was based on the difference in migraine prevalence between persons with mDNA 3243A>G mutation and our control group of 3471 healthy subjects [17], at 5% significance with 80% power. It was hypothesized that migraine prevalence in persons with mDNA 3243A>G mutation was at least 40%. Based on these assumptions, it was estimated that the inclusion of a minimum of 24 subjects would be sufficient (http://www.statisticalsolutions.net/pss_calc.php based on [20].

The clinical parameters were binary in character and were extracted from the semi-structured questionnaire. As the control population for migraine prevalence, data from a large population-based epidemiological study previously conducted at the Danish Headache Center were used [17]. The P values for comparisons of the migraine prevalence in our cases versus controls were calculated using the χ^2 test. To account for relatedness in our sample, and to calculate a corrected prevalence for migraine, a beta-binomial model based on the assumption that prevalence of migraine in each family follows a beta-distribution (on the interval from 0 to 1) was used. This model accounts for accumulation of migraine in specific families of our sample, as well as the size of the families. Statistical analysis was carried out using statistical software SAS version 9.3 (Copyright (c) 2002−2008 by SAS Institute Inc., Cary, NC, USA) for Microsoft Windows 10.

Results

In total, 57 subjects carrying the mDNA 3243A>G mutation were included and participated in the telephone interview. Thirty-four (60%) were women, and the mean age ± SD was 46 ± 16 years (range 17–85 years).

Clinical characteristics of the participants

Of the carriers of the mDNA 3243A>G mutation, only one subject was diagnosed with MELAS, which progressed rapidly and ended lethally during the course of the study (Table 1). The most frequent mDNA 3243A>G-associated symptoms were hearing impairment or deafness (60%) and migraine (MO or/and MA combined; 58%). Three subjects had migraine as the only symptom without any further mDNA 3243A>G-associated symptoms. The majority of the participants had at least one mDNA 3243A>G-associated symptom (84%). The mean age ± SD of migraine onset was 25 ± 9 years, which is much earlier than the mean age of onset for other mDNA 3243A>G-associated symptoms (35 ± 15 years). Only one subject developed migraine after age 40.

Characteristics of migraine

Thirty-three subjects [58%; confidence interval (CI) 44%–71%) had migraine: 23 (40%; CI 28%–54%) had MO only, six (11%; CI 4%–22%) had MA only and four subjects (7%; CI 2%–17%) had both MO and MA (Fig. 1). All subjects with MA had migraine with typical aura (MTA). Compared to the lifetime prevalence of migraine in the Danish population (18%) [17], the prevalence of migraine and its subforms was more than threefold higher in persons car-

Table 1 Description of mDNA 3243A>G-associated symptoms amongst the 57 subjects

	All subjects n = 57
Gender	
Male	23 (40%)
Female	34 (60%)
mDNA 3243A>G-associated symptoms	
Hearing impairment or deafness	34 (60%)
Migraine (MA or/and MO)	33 (58%)
Myopathy	30 (53%)
Diabetes	29 (51%)
Loss of vision	13 (23%)
Cardiovascular disorders	11 (19%)
Nephropathy	5 (9%)
Epilepsy	3 (5%)
Apoplexia	2 (4%)
Paresis	2 (4%)
Metabolic disorder (hypo/hyperthyroidism)	2 (4%)
No symptoms	9 (16%)
≥1 mDNA 3243A>G-associated symptom	48 (84%)
MELAS	
Subjects with MELAS (having a stroke-like episode)	1 (2%)
Subjects with first-degree relatives with MELAS	9 (16%)
Subjects with no relatives with MELAS	47 (82%)

Data are shown as numbers of subjects (n) and percentage of total subjects (%).

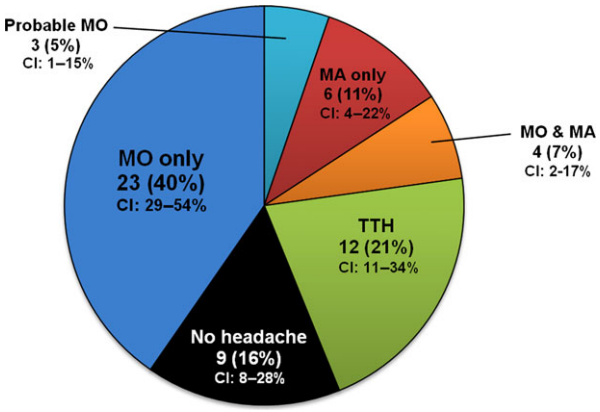

Figure 1 Headache classification of the 57 subjects carrying the mDNA 3243A>G mutation. Each subject occurs only once as subjects with both MO and MA trump other diagnoses; only MO or only MA trumps probable migraine. Furthermore all definite migraine and probable migraine trump TTH. Data are shown as *n* (%) with 95% confidence interval (CI). MO, migraine without aura; MA, migraine with aura; TTH, tension-type headache.

rying the mDNA 3243A>G mutation ($P < 0.001$) (Table 2). This was true for the prevalence of all migraine in females and males separately as well ($P < 0.001$). In addition, three subjects could not recall or did not fulfill one of the symptoms associated with migraine and were therefore classified as having probable MO. Otherwise there were no missing responses.

In total, 48 of 57 (84%; CI 72%–93%) suffered from a primary headache and the distribution of their headaches is shown in Fig. 1. Twelve subjects suffered from TTH (episodic $n = 11$ and chronic $n = 1$). Five of the six subjects who had MA were males. Eleven (33%) subjects had over 12 attacks in the last year and 19 (58%) had over 50 migraine attacks in their lifetime.

Headache of moderate to severe intensity was the most common symptom occurring in subjects with migraine (97%) followed by nausea (85%), aggravation with physical activity (82%), photophobia (82%) and phonophobia (79%) (Table 3).

At the time of the interview, 26 subjects (46%) used acute pharmacological treatment for headache. Eight used a triptan, 22 used simple analgesics and one used ergotamine.

Relatedness

Our 57 participants came from 23 families, but migraine prevalence was high in almost all families, and amongst subjects with no family members the prevalence was 78% (Table 4).

Migraine prevalence corrected for relatedness was 56.7% (CI 41.4%–72.0%), which is very similar to the crude prevalence of 58.0% (CI 44.1%–70.9%). The evidence of variation between family-specific prevalences for the families in our sample is quite small, meaning that relatedness only influences our result insignificantly and therefore cannot explain the high migraine prevalence reported. The lack of relatedness amongst families was also verified by the high haplotype variability in the families.

Discussion

The most important result of the present study is that migraine prevalence in persons carrying the mDNA 3243A>G mutation is significantly higher than in the general Danish population (Table 2). This was true for MO and MA as well as for the prevalence of all migraine in females and males separately. Relatedness was also accounted for and it was shown that this does not influence our finding.

Our own population-based epidemiological study of a representative sample from the Danish population was used as the control group, because the data from

Table 2 Sex-specific and overall prevalence of migraine in persons carrying the mDNA 3243A>G mutation compared to 40-year-old representative inhabitants in Denmark [17]

	mDNA 3243A>G mutation ($n = 57$)			Controls ($n = 3471$)			*P* value[b]		
	Males ($n = 24$)	Females ($n = 33$)	Overall[a]	Males ($n = 2576$)	Females ($n = 895$)	Overall[a]	Males	Females	Overall[a]
Migraine without aura	45.8% (25.6–67.2)	48.5% (32.5–64.8)	47.2% (34.0–61.0)	7.6% (6.6–8.8)	15.9% (13.5–18.3)	11.8% (10.5–13.1)	**< 0.001**	**< 0.001**	**< 0.001**
Migraine with aura	20.8% (7.1–42.2)	15.2% (6.2–31.4)	18.0% (8.8–30.0)	3.6% (2.9–4.3)	7.5% (5.8–9.1)	5.5% (4.6–6.4)	**< 0.001**	0.199	**< 0.001**
All migraine	58.3% (36.6–77.9)	57.6% (39.2–74.5)	58.0% (44.1–70.9)	11.7% (10.4–12.9)	23.7% (20.9–26.5)	17.7% (16.2–19.2)	**< 0.001**	**< 0.001**	**< 0.001**

The sum of MO and MA prevalence is higher than all migraine prevalence because migraine diagnoses are not mutually exclusive – a person with MO may also have MA. Values are presented as percentages (95% CI).
[a]Overall prevalence is adjusted for sex; [b]*P* value, $\chi 2$ test.
Bold type indicates statistically significant values.

Table 3 Migraine characteristics of the 33 subjects with migraine

Migraine characteristics	
Number of migraine attacks within last year, average ≥12 attacks/year	11 (33%)
Number of migraine attacks in lifetime ≥ 50	19 (58%)
Duration of migraine headache > 24 h	4 (12%)
Pain characteristics	
Unilateral	20 (61%)
Pulsating	16 (48%)
Moderate/severe intensity	32 (97%)
Aggravation with physical activity	27 (82%)
Accompanying symptoms	
Nausea	28 (85%)
Vomiting	18 (55%)
Photophobia	27 (82%)
Phonophobia	26 (79%)
Osmophobia	7 (21%)

Data are shown as number of subjects (*n*) and percentage of total subjects with migraine (%).

Table 4 Family relatedness of the 57 subjects and the distribution of migraine within each family

Groups of family	Number of subjects	Subjects with migraine	Percentage of family with migraine
No related family	9	7	78
Family 1	12	9	75
Family 2	4	2	50
Family 3	4	1	25
Family 4	3	3	100
Family 5	3	3	100
Family 6	3	2	67
Family 7	3	2	67
Family 8	3	0	0
Family 9	3	0	0
Family 10	2	2	100
Family 11	2	1	50
Family 12	2	1	50
Family 13	2	0	0
Family 14	2	0	0
Total	57	33	

that study were obtained using the same validated semi-structured interview as used in the present study. In addition, the large sample size of 3471 Danish participants makes the data very robust. The prevalence of the mDNA 3243A>G mutation in the northern European population is only 0.16% [10]. The prevalence of this mutation is assumed to be the same in our control group. It cannot therefore influence our result significantly. It could also be argued that comparing our participants, who were on average 46 ± 16 years old, with 40-year-old controls could skew the migraine prevalence. However, the control data used are considered to be valid for comparison, because a smaller population-based epidemiological study with 740 Danish participants [21], with an age range as in the present study, found a similar migraine prevalence (16%) as in the controls used for comparison. In addition, prevalence of migraine tends to peak around 40 years of age [22], so if anything the threefold difference in migraine found in our study is underestimated.

The mDNA 3243A>G mutation is the most common mitochondrial pathogenic mutation [10] and some individuals may carry this mutation without experiencing any symptoms. The precise mechanism connecting the mutation with the clinical phenotype is still not fully understood but is certainly related to the mutation load in different tissues. Symptoms from the brain are probably elicited by increased demands for brain metabolism. Some studies have shown that the mutation reduces tRNALeu(UUR) aminoacylation, which results in a defective mitochondrial protein synthesis and reduced activities of respiratory chain complexes [23,24].

Mitochondrial disorders caused by the mDNA 3243A>G mutation affect various types of tissues and give rise to a wide range of phenotypes, but the involvement of the brain causing neurological symptoms is a prominent feature [25,26]. Migraine is a brain disorder and the predilection of maternal inheritance in migraine makes a mitochondrial etiology especially intriguing. Most of the subjects in the present study, interestingly, suffered from MO (40%). This is in contrast to previous assumptions of a more likely association between mitochondrial mutations and MA subtypes such as MTA, migraine with prolonged aura and hemiplegic migraine [27–31] based on the fact that the genetic relationship is stronger for MA than for MO [32]. In addition, several functional imaging studies have reported that MA patients may have a greater deficit in brain energy metabolism compared to MO patients [5,7,33–35]. However, one study has shown reduced energy metabolism in the occipital lobe of MO patients similar to what has been observed in MA [36].

A genetic search for the mDNA 3243A>G mutation in cohorts of migraine patients has been negative [8]. However, the largest studies in MO [37,38] and MTA [28,29,31,37,38] have included only 25 individuals, which is insufficient to exclude the mDNA 3243A>G mutation as a contributor to the pathogenesis in migraine. Also, the detection of the mutation may have been missed, because genetic analyses were performed on blood-derived cells in which the mutation may decline with time due to selection against the mutation with aging [39]. In any case, the mDNA 3243A>G mutation does not appear to be a main contributor to migraine pathogenesis in the general population, but our results suggest a possible explanation for migraine pathogenesis related to perturbed mitochondrial metabolism.

Mitochondrial dysfunction might perhaps cause increased neuronal excitability leading to a decreased migraine threshold [8]. The hypothesis is based on the assumption that migraine is a threshold disorder [40], induced when a certain metabolic threshold is reached in the brain, e.g. by triggering factors causing increased energy demand [36]. Epilepsy studies have shown that disturbed brain metabolism is able to increase neuronal excitability [41,42]. Reduced mitochondrial energy reserve may thus be one of the many factors determining the migraine threshold. A recent proof-of-concept study showed that ketogenic diet, which is effective for epilepsy, could also improve migraine [43,44]. The authors proposed that the underlying mechanisms of ketogenic diet efficacy might be related to its ability to enhance mitochondrial energy metabolism and counteract neural inflammation. This hypothesis is supported by the observation that cofactors that facilitate respiratory chain function, such as riboflavin [45] and coenzyme Q10 [46], may be effective as a preventative treatment for migraine. In addition, a study showed that riboflavin response was related to a specific mitochondrial haplotype [47].

Although our sample size is relatively small and a risk for ascertainment bias cannot be excluded, the strength of the present study is that trained physicians conducted all interviews and diagnosed primary headaches according to the criteria of the International Classification of Headache Disorders [15]. The limitation in our study that physicians who established the diagnosis of migraine knew that the persons they interviewed were carriers of the mDNA 3243A>G mutation is acknowledged. In contrast, investigators in the control group conducted interviews without any prior knowledge of the subjects.

In conclusion, the present study is the first to demonstrate an association between mitochondrial dysfunction and the susceptibility to migraine in a cohort of persons carrying the mDNA 3243A>G mutation, in whom prevalence of migraine was threefold higher than in a representative control population. Since the mutation is rare, it cannot explain the occurrence of migraine in the general population, but our findings contribute to an understanding of pathogenic mechanisms underlying migraine. It is unknown whether other pathogenic mitochondrial mutations have the same association with migraine which merits further investigation in future studies.

Acknowledgements

The authors thank all the subjects who participated in this study. Further thanks for funding from Novo Nordisk Foundation (NNF11OC1014333) and Independent Research-Medical Sciences (FSS) (DFF-1331-00210A), Lundbeck Foundation (R155-2014-171) and the FP7-EUROHEADPAIN (602633). Special thanks to Lene Theil Skovgaard from the Biostatistical Department at Copenhagen University for statistical support regarding relatedness.

Disclosure of conflicts of interest

Song Guo has received a travel grant from Autonomic Technologies Inc. Jes Olesen has received grants/research support from and/or has been a consultant/scientific adviser for, and/or has been in the speakers' bureau of Allergan Inc, AstraZeneca Pharmaceuticals LP, Boehringer Ingelheim, Eli Lilly, GlaxoSmithKline, Janssen Pharmaceutical Products, Lundbeck, Merck, Amgen, Alder and Pfizer. Messoud Ashina has received consulting fees, speaking/teaching fees and/or honoraria – Alder, Allergan, Amgen, ATI and Eli Lilly. John Vissing has received travel grants from Genzyme/Sanofi, Novartis and Reata Pharmaceuticals, and is scientific adviser for Genzyme Corporation and NOVO Industries.

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