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Three families with 'de novo' m.3243A > G mutation

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ABSTRACT

The m.3243A > G mutation is the most prevalent, disease-causing mitochondrial DNA (mtDNA) mutation. In a national cohort study of 48 families harbouring the m.3243A > G mutation, we identified three families in which the mutation appeared to occur sporadically within these families. In this report we describe these three families. Based on detailed mtDNA analysis of three different tissues using two different quantitative pyrosequencing assays with sensitivity to a level of 1% mutated mtDNA, we conclude that the m.3243A > G mutation has arisen *de novo* in each of these families. The symptomatic carriers presented with a variety of symptoms frequently observed in patients harbouring the m.3243A > G mutation. A more severe phenotype is seen in the *de novo* families compared to recent cohort studies, which might be due to reporting bias.
The observation that *de novo* m.3243A > G mutations exist is of relevance for both diagnostic investigations and genetic counselling. Firstly, even where there is no significant (maternal) family history in patients with stroke-like episodes, diabetes and deafness or other unexplained organ dysfunction, the m.3243A > G mutation should be screened as a possible cause of the disease. Second, analysis of maternally-related family members is highly recommended to provide reliable counselling for these families, given that the m.3243A > G mutation may have arisen *de novo*.

1. Introduction

In recent years, a large body of data concerning the inheritance of mitochondrial disorders has been published [1–3]. Knowledge relating to the mode of inheritance has great importance for both the diagnosis and counselling of patients. Due to the involvement of the nuclear and mitochondrial genomes, mitochondrial diseases can be transmitted in a Mendelian manner, in the case of a nuclear aetiology, be maternally transmitted in the case of primary mitochondrial DNA (mtDNA) defects or they may occur sporadically [4].

In the case of single, large-scale mtDNA deletions such as those observed in patients with the Kearns-Sayre Syndrome, Chronic Progressive External Ophthalmoplegia or Pearson's Syndrome, occurrence is usually sporadic and recurrence in the offspring of female carriers is limited to 4% of the patients [5]. Our study focuses on the m.3243A > G *MTTL1* gene mutation, first described by Goto, Nonaka and Horai [6] as a cause of the mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome but which also causes maternally-inherited diabetes and deafness (MIDD) [7]. Other clinical phenotypes include cardiac, ocular, gastrointestinal and renal involvement. The m.3243A > G mutation is the most prevalent, multi-system disease-causing mitochondrial DNA mutation, with a reported mutation prevalence of between 7.59 and 236 per 100,000 [8–10].

A considerable number of case series and small cohorts have been published describing the phenotypic expression of the m.3243A > G mutation [11–17]. In contrast to what is seen for other mtDNA mutations, the sporadic occurrence of the m.3243A > G mutation, with an expressed phenotype, is rare with only four *de novo* cases published to date [18–21] (see Table 1).

In our own cohort of Dutch m.3243A > G mutation carriers, we identified three families where the m.3243A > G mutation appears to have arisen *de novo*. The purpose of this paper is to document these families, describing the probands' presentation in detail

Abbreviations: mtDNA, mitochondrial DNA; MELAS, mitochondrial myopathy, encephalopathy, lactate acidosis and stroke-like episodes; MIDD, maternally inherited diabetes and deafness; MERRF, myoclonic epilepsy with ragged-red fibres.

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Table 1
An overview of all *de novo* reports of the m.3243A > G mutation.

Report: author (year)	Gender and age at onset	Probands clinical sign & symptoms	Heteroplasmy levels	Number of tested maternal family members and tested tissues	Special characteristics
Yamamoto (1995)	m, 21 years	MELAS syndrome; SLE, mild deafness, weakness,	Muscle: 89% Blood: 36%	3; all studied in muscle.	Mother was the *de novo* patient experiencing MIDD. (muscle 79%, blood 10%)
Campos et al. (1996)	m, 2 years	MELAS/MERRF overlap syndrome; epilepsy, SLE, weakness, psychomotor delay	Muscle: 70% Blood: 30%	4; all studied in blood, 1 studied in muscle, 1 studied in hair	
Ko et al. (2001)	m, 5 years	MELAS syndrome; epilepsy, SLE, ataxia, blurred speech, paralytic ileus,	Muscle: 54% Blood: 56% Hair: 70% Buccal saliva: 64%	6; all studied in blood, hair and buccal saliva	Mother was the *de novo* patient experiencing mild deafness, (blood 11%, hair 27%, buccal saliva 32%); the younger brother was a dormant carrier. (blood 65%, hair 79%, buccal saliva 70%)
Maassen et al. (2002)	f, 8 years	MIDD; bilateral deafness, diabetes, hypertension, proteinuria	Blood: 18% Buccal saliva: 55%	4; all studied in blood and buccal saliva	
Patient 1 (this study)	m, 34 years	MELAS syndrome; aphasia, encephalopathy, deafness, SLE, epilepsy, myopathy.	Muscle: 82% Blood: 23% UEC: 63% Buccal saliva: 40	16; all studied in blood, UEC and buccal saliva	
Patient 2 (this study)	m, 1 year	Transient hypotonia, ataxia, ptosis and ophthalmoplegia, motor retardation. Improvement to normal at age 8.	Muscle: 23% UEC: 38% Buccal saliva: 27%	6; all studied in blood, UEC and buccal saliva	Mother was the *de novo* patient, without clinical symptoms. (UEC 6%, Muscle 5%, undetectable in blood and buccal saliva)
Patient 3 (this study	m, 1 day	Foetal distress, transient tachypneu of the neonate, transient left ventricular hypertrophy.	Muscle: 12% Blood: 16% UEC: 20% Buccal saliva:16%	3; all studied in blood, UEC and buccal saliva	A sibling died post vaccination at age 4 months. No mutation load was found in muscle.

m = male, f = female, SLE = stroke-like episodes, UEC = urinary epithelial cells.

and highlighting the family members in whom the m.3243A > G mutation was not detected. Maternal inheritance in a pedigree is a trigger for clinicians to consider the presence of an mtDNA mutation but even if there is no significant family history in the maternal line, the m.3243A > G mutation should be considered in patients with symptoms consistent with the MIDD or MELAS syndromes.

2. Methods

All patients participated in the m.3243A > G mutation cohort study at the Radboud Center for Mitochondrial Medicine [22]. 135 patients from 48 families were included in this study. The study was approved by the ethics committee of the Nijmegen–Arnhem region. Written informed consent according to the Helsinki agreement was obtained from all parents and patients ≥ 12 years.

Heteroplasmy levels were determined in urinary epithelial cells, blood and buccal cells of all participants using Pyrosequencing™ technology (Pyrosequencing, Uppsala, Sweden) as described before by Lowik, Hol, Steenbergen, Wetzels and van den Heuvel [23]. The pyrosequencing assay of the m.3243A > G mutation in the mtDNA (Genbank accession# NC_012920.1) has a precision of 1.5%, the precision relates to the correlation between independent measurements of the same sample. A sensitivity of 4.5% has been quoted for this technique, as determined by serial dilution of an m.3243A > G positive sample in a sample containing wild type mtDNA, and by determining the background signal + 3 × SD in a panel of 43 control samples.

The tissue samples of patient II-3 of family 3 were collected within 2 h post-mortem. Samples were snap frozen using liquid nitrogen and stored at −80 °C. DNA was extracted using a Genomic DNA Purification Kit (Gentra, Mineapolis, USA), following the manufacturer's procedures.

In the families in which a *de novo* m.3243A > G mutation was suspected, heteroplasmy levels in urinary epithelial cells from first degree family members were measured using a second pyrosequencing assay with a sensitivity calculated at 1% as previously described by Alston et al, [24].

The data of this study were submitted to the MITOMAP database (www.mitomap.org).

2.1. Family reports

2.1.1. Family 1

The proband (III-2, Fig. 1a) presented with aphasia and encephalopathy at the age of 34 years having experienced difficulty in speaking for a few months with a history of hearing impairment since age 5. During this episode of encephalopathy he spoke in short sentences and was unable to respond to complex commands. There were no abnormalities in the evaluation of cranial nerves, reflexes, motor skills or sensation. MRI imaging showed abnormal signal intensities in both cortical and subcortical areas of the left parieto-temporal lobe. MRI-spectroscopy demonstrated elevated levels of lactic acid in the affected areas. A muscle biopsy was performed showing diminished ATP production and decreased complex I activity (see Table 2). Unfortunately no histopathological data are available. m.3243A > G mutation screening was performed, revealing a high level of m.3243A > G mutation load (82%) in muscle, confirming the diagnosis of m.3243A > G related mitochondrial disease. Assessment of m.3243A > G mutation levels in leucocytes, urinary epithelial cells and buccal cells were 23%, 63% and 40%, respectively. During follow-up, the patient developed epilepsy at the age of 35 years and diastolic dysfunction on echocardiography. He was myopathic and unable to perform normal work. Lactate levels in blood were elevated to an average level of 4.0 mmol/L (reference <2.2 mmol/L). An extensive family study was performed. Family members with symptoms that could be consistent with the m.3243A > G mutation include a cousin (III-6) and her baby child (IV-6) who experienced severe congenital hearing impairment, and the proband's mother (II-3) who reported non-insulin dependent diabetes and mild hearing impairment at the age of 71 years. A total of 16 family members were tested for the presence of the m.3243A > G mutation, but this was not detected in any other family member apart from the proband (Fig. 1a). The underlying cause of the congenital hearing loss reported by (III-6) and (IV-6) was investigated by whole exome sequencing, and a nuclear-encoded mutation was identified which is likely to be pathogenic.

2.1.2. Family 2

The proband (IV-1, Fig. 1b) presented at the age of almost 2 years, with neurological deterioration following a viral infection resulting in