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Detection rates and phenotypic spectrum of m.3243A > G in the *MT-TL1* gene: A molecular diagnostic laboratory perspective

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ABSTRACT

The nucleotide change A to G at position m.3243 in the mitochondrial tRNA leucine (UUR) gene (*MT-TL1*) is the most common point mutation reported in association with the Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS) syndrome. Since the original description of this disorder, factors including random mitochondrial segregation and consequent variable tissue heteroplasmy are recognised to contribute to a much broader phenotypic spectrum associated with the *MT-TL1* m.3243A > G mutation, often rendering the process of making a diagnosis complex. Reliance on clinicians' referral patterns means that for most molecular diagnostic laboratories, their positive identification rates for the common pathogenic mitochondrial DNA (mtDNA) mutations, including *MT-TL1* m.3243A > G, is often relatively low compared to those reported in clinically targeted research studies. Herein, we report our results of consecutive prospective screening of 745 patients with a clinically suspected mitochondrial syndrome encompassing features associated with *MT-TL1* m.3243A > G mutation.

1. Introduction

The disorder characterised by Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS) is a well-recognised syndrome in the field of mitochondrial diseases. Goto and colleagues (Goto et al., 1990) were among the first to identify the most common point mutation associated with MELAS m.3243A > G, located in the tRNA leucine (UUR) gene (*MT-TL1*) of the mitochondrial genome. In their study, genetic testing on skeletal muscle biopsies of patients with MELAS revealed 80% of these patients harboured the point mutation (Goto et al., 1990, 1991). The consequent invariant criteria established for the clinical diagnosis of MELAS includes (1) stroke-like episodes before the age of 40 years; (2) encephalopathy characterized by seizures, dementia, or both; and (3) lactic acidosis, ragged-red fibres (RRF) or both. Diagnosis would be considered certain if there are also at least two of the following: normal early development, recurrent headache or recurrent vomiting (Ciafaloni et al., 1992; Hirano et al., 1992; Pavlakis et al., 1984).

Subsequent to the seminal reports, the clinical spectrum of the m.3243A > G mutation has been recognised to be much more diverse and very heterogeneous (Ciafaloni et al., 1992; Jean-Francois et al., 1994; Nesbitt et al., 2013) and there are many other distinct syndromes reported in association with *MT-TL1* m.3243A > G, such as Myoclonic Epilepsy and Ragged Red Fibres (MERRF) (Fabrizi et al., 1996), MERRF/MELAS overlap syndrome (Campos et al., 1996), Progressive External Ophthalmoplegia (PEO) (Moraes et al., 1993), Chronic Progressive External Ophthalmoplegia (CPEO) (Bosbach et al., 2003; Koga et al., 2000; Mariotti et al., 1995), MERRF/CPEO overlap syndrome (Verma et al., 1996), Maternally Inherited Diabetes and Deafness (MIDD) (Van den Ouweland et al., 1992) and Leigh's syndrome (Koga et al., 2000). Non-syndromic phenotypes associated with this point mutation include hypertrophic cardiomyopathy (Koga et al., 2000; Silvestri et al., 1997), cluster headache (Shimomura et al., 1994), pancreatitis (Kishnani et al., 1996) subacute dementia with myoclonus mimicking Creutzfeldt-Jakob Disease (Isozumi et al., 1994) and myoclonous with ragged red fibre phenotype (Nesbitt et al., 2013). According to the studies by Chinnery et al. (1999) and Frederiksen et al. (2006), there appears to be a uniform distribution of mutant mtDNA throughout the three germ layers in embryogenesis; however, there are significant differences between heteroplasmic levels of the individual tissue types, indicating tissue-specific segregation of *MT-TL1* m.3243A > G later in embryogenesis, which is believed to explain the diversity of clinical phenotypes.

Therefore, a series of ancillary clinical and laboratory investigations including biochemical assays (lactic acid levels and respiratory chain complex activities), neuroimaging (computed tomographic (CT) or magnetic resonance imaging (MRI) for evidence of focal brain abnormalities) (Sparaco et al., 2003), tissue histochemistry (to detect the presence of ragged red fibres or cytochrome-oxidase deficient fibres),

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and molecular mitochondrial DNA analysis need to be considered in order to provide a definitive diagnosis for MELAS syndrome.

In this report, we describe the detection rate of the *MT-TL1* m.3243A > G mutation and the associated clinical features for 745 adult patients referred for mitochondrial genetic screening specifically directed at this mutation in the setting of a suspected mitochondrial disorder. A detailed summary of the associated clinical features is discussed, including evaluation of the percentage of patients fulfilling the invariant criteria for MELAS as compared to those who manifest only some of the symptoms or show other distinct clinical features associated with this point mutation.

2. Patients and methods

From 2002 to 2012, our laboratory received specimens from 1673 patients with a suspected mitochondrial disorder, of which 745 cases were screened for the *MT-TL1* m.3243A > G mutation. The majority of referrals were from Victoria with some from New South Wales and Tasmania. Requisition forms are routinely sent out to referring medical practitioners, with the majority being neurologists, to accompany the patient specimens in order to obtain informed genetic consent and to indicate the salient clinical features evident in their patients (Marotta et al., 2004). Diagnostic screening and further studies were carried out with the patient's consent. Seventy-one percent of the referred patient specimens were accompanied by a tick box requisition form indicating clinical signs and symptoms.

Genomic DNA from patient's muscle, blood, hair follicles and/or urine was extracted using the QIAmp Mini kit (Qiagen) or Puregene Blood Core kits (Qiagen) as appropriate according to the manufacturer's protocol. The mtDNA point mutation analysis was carried out using Restriction Fragment Length Polymorphism (RFLP). DNA was amplified using Polymerase Chain Reaction (PCR), end labelled with radioactive dATP alpha-^{32}P (Perkin Elmer) and digested with restriction enzyme *Hae III* (Promega) at 37 °C. The digested samples were then run on a 10% polyacrylamide gel, exposed on a phosphor screen and analysed using the Phosphor-imager SI (Molecular Dynamics). The approximate quantification of the heteroplasmy level to the total mtDNA was analysed using image analysis software, ImageQuant (Molecular Dynamics).

Point mutations identified by RFLP were confirmed by targeted Sanger sequencing. From 2010 onwards, sequencing of the entire *MT-TL1* gene was adopted as the routine testing method carried out for all patients referred for MELAS and if mutations were detected, RFLP was used as the second method for confirmation. Detection methods related to variants associated with MERRF, LHON and PEO/CPEO syndromes have been described previously (Marotta et al. 2004).

3. Results

3.1. Clinical features of patients with MT-TL1 m.3243A > G mutation

Over the period of 11 years, a total of 745 patients were systematically screened for *MT-TL1* m.3243A > G with 187 cases solely screened for this specific mutation. Sequencing of the entire *MT-TL1* gene was carried out in 160 patients to search for other uncommon sequence variants, including m.3252A > G, m.3256C > T and m.3291 T > C associated with MELAS. Of the 160 patients referred from 2010 to 2012 that had their entire *MT-TL1* gene screened, only the common m.3243A > G point mutation was detected in eight of these patients and none of the rarer point mutations spanning the *MT-TL1* gene were detected.

A total of 35 out of the 745 patients (4.7%) were found to harbour the *MT-TL1* m.3243A > G point mutation and the age of onset ranged from 7 to 67 years. Twenty-four of the 35 patients (69%) were female. Maternal inheritance was reported in 54.3% of cases and the clinical signs and symptoms indicated by the referring doctors of these 35 patients with *MT-TL1* m.3243A > G are summarised in Table 1. The most frequently reported clinical feature in these patients was hearing loss (62.8%) followed by diabetes mellitus (51.4%), proximal limb weakness (31.4%), headache/migraine (28.6%), short stature (22.9%), elevated blood lactate (22.9%), strokes (20%), seizures (20%) and nausea/vomiting (17.1%).

When reviewing all the clinical features reported in the 35 patients with *MT-TL1* m.3243A > G (Table 2), only two patients (5.7%) could be categorised as satisfying the invariant criteria sufficient to diagnose the "classic MELAS syndrome" as described by Ciafaloni et al. (1992), Hirano et al. (1992) and Pavlakis et al. (1984). One patient (2.9%) manifested the invariant criteria but with stroke after 40 years, with onset at 67. Nine patients (25.7%) were oligo-symptomatic, whereby there was evidence of one or more "invariant criteria" with or without stroke but not manifesting the "complete syndrome." Interestingly, one patient's mother who harboured this point mutation was clinically diagnosed with Kearns-Sayre Syndrome and the patient's clinical phenotype featured ptosis, progressive ophthalmoplegia and ataxia. One patient (2.9%) manifested headache/migraine, nausea/vomiting coupled with exercised intolerance with evidence of maternal inheritance. Three patients (8.6%) could be categorised as Maternally Inherited Diabetes and Deafness (MIDD) syndrome whilst five patients had diabetes and deafness with maternal inheritance and additional clinical features including but not limited to CPEO, ataxia, proximal limb weakness, intellectual disability (refer to Table 2). Three patients (8.6%) either had deafness or diabetes but not both and maternal inheritance with additional features such as short stature and myopathy. One patient (2.9%) manifested ophthalmoplegia, respiratory insufficiency with, exercise intolerance and proximal limb weakness. Four patients (12%) were mono-symptomatic: two patients manifested hearing loss only; one presented only with non-insulin-dependent diabetes mellitus and a 25-year-old female displayed multi-focal myoclonus as the only reported symptom. There were two asymptomatic patients where family members harboured the *MT-TL1* m.3243A > G.

In some cases, the mutation was not detected at the initial screening but was detected several years later. One patient (case 23, Table 2) manifested seizures after a hypoglycaemic episode, bilateral deafness, ptosis, external ophthalmoplegia, dysarthria, basal ganglia calcification on a CT scan and elevated creatine kinase and blood lactate. Testing mtDNA from hair follicles failed to show the presence of the m.3243A > G, so further tests were carried out for the MERRF *MT-TK* m.8344A > G mutation and two of the primary point mutations associated with Leber's Hereditary Optic Neuropathy (LHON). Thirteen years later the patient additionally developed optic atrophy and cardiomyopathy. Repeat mtDNA analysis of a muscle biopsy, hair follicles and blood, detected the *MT-TL1* m.3243A > G mutation in all tissues, with the greatest heteroplasmic level in hair follicles (56%) followed by muscle (31%) and blood (17%). For another patient (case 26, Table 2), the doctor initially requested the three most common LHON mutations screen; however, re-examination 6 years later, after more clinical details were provided, the *MT-TL1* m.3243A > G was detected with the complete clinical features including maculopathy with cone dystrophy, headache/migraine, diabetes mellitus, deafness, cerebellar ataxia, hypothyroidism and myelopathy.

3.2. Clinical phenotype of patients where MT-TL1 m.3243A > G mutation was not detected

The clinical features and number of patients in which *MT-TL1* m.3243A > G was not detected despite referral specifically for "MELAS" or clinician providing clinical details and symptoms known to be associated with the *MT-TL1* m.3243A > G are summarised and presented in Table 3. From the clinical details provided by requesting clinicians, 4 out of the 710 (0.6%) "negative" patients manifested the MELAS syndrome fulfilling the invariant criteria with stroke before the age of 40 years. Two additional patients (0.3%) fulfilled the "invariant" criteria but with stroke after the age of 40 years. The number of patients with an incomplete syndrome with at least one or more invariant criteria with stroke