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Mitochondrial respiratory chain disorders in the Old Order Amish population

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ABSTRACT

The Old Order Amish populations in the US are one of the Plain People groups and are descendants of the Swiss Anabaptist immigrants who came to North America in the early eighteenth century. They live in numerous small endogamous demes that have resulted in reduced genetic diversity along with a high prevalence of specific genetic disorders, many of them autosomal recessive. Mitochondrial respiratory chain deficiencies arising from mitochondrial or nuclear DNA mutations have not previously been reported in the Plain populations. Here we present four different Amish families with mitochondrial respiratory chain disorders. Mutations in two mitochondrial encoded genes leading to mitochondrial respiratory chain disorder were identified in two patients. In the first case, MELAS syndrome caused by a mitochondrial DNA (mtDNA) mutation (m.3243A>G) was identified in an extended Amish pedigree following a presentation of metabolic strokes in the proband. Characterization of the extended family of the proband by a high resolution melting assay identified the same mutation in many previously undiagnosed family members with a wide range of clinical symptoms. A MELAS/Leigh syndrome phenotype caused by a mtDNA mutation [m.13513G>A; p.Asp393Asn] in the *ND5* gene encoding the ND5 subunit of respiratory chain complex I was identified in a patient in a second family. Mutations in two nuclear encoded genes leading to mitochondrial respiratory chain disorder were also identified in two patients. One patient presented with Leigh syndrome and had a homozygous deletion in the *NDUFAF2* gene, while the second patient had a homozygous mutation in the *POLG* gene, [c.1399G>A; p.Ala467Thr]. Our findings identify mitochondrial respiratory chain deficiency as a cause of disease in the Old Order Amish that must be considered in the context of otherwise unexplained systemic disease, especially if neuromuscular symptoms are present.

1. Introduction

The mitochondrial respiratory chain requires hundreds of proteins encoded by the nuclear and mitochondrial genomes for normal assembly and function [1]. Mutations in the genes for many of these proteins lead to a wide range of clinical phenotypes related to energy deficiency [2]. Mutations in the mitochondrial DNA (mtDNA) introduce an additional variable of clinical heterogeneity. Cells have many mitochondria, and each mitochondrion has multiple copies of mitochondrial DNA. Distribution of both is random in dividing cells and mitochondria. Thus a cell can contain two populations of mitochondria, normal and mutant, in varying ratios, a genetic situation known as heteroplasmy [1,2]. The extreme phenotypic heterogeneity of mitochondrial respiratory chain deficiency and variable degrees of heteroplasmy in different tissues for mtDNA mutations pose a practical challenge in recognizing these conditions. Estimates of the prevalence of mitochondrial disease vary among reports, and may be as high is 1:5000 individuals [3,4]. An m.3243A>G mutation in the *MT-TL1* gene encoding the mitochondrial $tRNA^{Leu(UUR)}$ is among the most commonly reported mitochondrial mutations, and is causal for MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) syndrome [5]. Clinical symptoms associated with this mutation are broad and include maternally inherited diabetes and deafness (MIDD), diabetes, stroke-like episodes, dementia, epilepsy, lactic acidemia, myopathy, cardiomyopathy, hearing loss, migraine headaches, Hypertrichosis, gastrointestinal dysmotility, failure to thrive, and short stature. Approximately 10% of individuals with the m.3243A>G mutation exhibit the most severe end of the clinical

Abbreviations: HRM, high-resolution melt profiling; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers; mtDNA, mitochondrial DNA.

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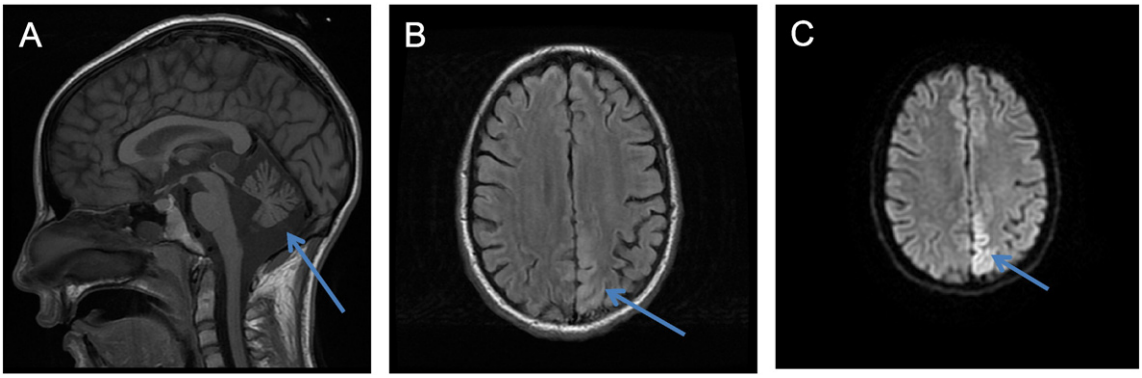

Fig. 1. MRI of the brain of Patient 1 with MELAS mutation m.3243A>G. (A) Sagittal T1 demonstrates cerebellar atrophy. (B,C) Axial T2/FLAIR and diffusion weighted imaging shows hyperintensity in the medial left occipital lobe associated with restricted diffusion consistent with a left occipital lobe infarct.

spectrum; another 10% of mutation carriers are asymptomatic, largely based on the level of heteroplasmy. Between these two extremes, intermediate phenotypes exist including single organ involvement or multiorgan involvement [5]. Other classic mitochondrial syndromes, including Leigh and MERRF (myoclonic epilepsy with ragged red fibers) syndromes, have also been associated with the m.3243A>G mutation [5–8].

Mitochondrial respiratory chain deficiency has not been reported in the Old Order Amish, a unique population in the US with specific health risks that stem from unbalanced population sampling of European founders followed by genetic drift in derivative generations [9–11]. They are socially isolated with little genetic inflow, living in communities often established by as few as ten families. Some groups keep extensive genealogical records that are maintained by local religious leaders, and have adequate family records to enable clear identification of their founder populations. They have experienced many genetic bottleneck events caused by successive migrations [12]. McKusick first recognized the importance and the potential of Amish populations for the study of human genetics, originally describing 26 genetic disorders identified in Amish families from different demes throughout Pennsylvania and the Midwest [13,14]. Subsequently, 37 Amish disorders were described in 2009 among demes from Pennsylvania, Ohio, and Maryland [9]. Most genetic disorders reported in Old Order Amish populations are autosomal recessive [9,13]. In 2011 a database (www.biochemgenetics.ca/plainpeople) was created focusing on single-gene Mendelian disorders and mutations specific to the Amish, Mennonite, and Hutterite groups (known collectively as Anabaptist sects and the Plain People) [12]. It was initially compiled based on searches of the scientific literature on Anabaptist populations, but now includes data from personal observation and communication with other genetic researchers. New disorders are added through periodic review of the literature. Ninety disorders are reported in the database in the Amish population, including seven of them with clear familial patterns but no causative gene identified [12]. Here, we report the first series of Amish patients with genetically verified disorders of the mitochondrial respiratory chain, including genetic studies on one large extended family with MELAS syndrome due to a m.3243A>G mutation.

2. Case reports

Patient 1 was an unimmunized 15-year-old Amish female with developmental delay, thin body habitus, Hypertrichosis, hearing loss, and consistently poor energy and appetite from Mercer County, PA, a center of the Northwestern Pennsylvania Amish community. Following a very long walk and slight dehydration, she presented at 15 years of age with an acute episode of vomiting followed by altered mental status, status epilepticus, visual impairment and lactic acidosis (lactic acid 9.7 mmol/L; normal 0.5–2.2). Her EEG was markedly abnormal showing status epilepticus with more than 35 electrographic seizures of right posterior quadrant onset and lack of normal background activity. An MRI of the brain showed a small, focal left occipital lobe infarct and cerebellar atrophy (Fig. 1). She was treated with fosphenytoin, levetiracetam, levocarnitine, phenobarbital, midazolam infusion and broad spectrum antibiotics, including Acyclovir, for suspected meningoencephalitis.

Past medical history revealed hearing loss, first noticed in early childhood and treated with hearing aids at 15 years of age. She subsequently had additional stroke-like episodes affecting the left occipital and temporal areas at 17 and 18 years of age. During these episodes she was treated aggressively with the intensive care unit status epilepticus protocol. This included the treatment given in first episode besides intravenous arginine since diagnosis was confirmed with m.3243A>G mutation. Valproic acid was specifically avoided due to the history of mitochondrial disease and its relative contraindication. Between the first and third episode the patient was started on low dose of oral arginine 45 mg/kg/day divided in 2 doses at age 17 but the dose was adjusted to 320 mg/kg/day divided in three doses (10.6 g/m^2 body surface area) after she had a stroke and she had no further strokes after the adjustment. She has been maintained since then on levetiracetam monotherapy for seizures. She was also placed on coenzyme Q_{10} (CoQ_{10}) 8.5 mg/kg/day and L-carnitine 30 mg/kg/day. Genetic evaluation identified the common MELAS mutation m.3243A>G with 74% heteroplasmy in saliva. Her clinical symptoms (detailed in Table 1, member AF013) have progressed slowly following the institution of therapy. She is 21 years old at the time of this writing. The family history on the maternal side was significant for multiple family members with variable combinations of hearing loss, diabetes mellitus, renal disease, migraine headaches, thin body habitus, and developmental delay. None received genetic evaluation. Further characterization of other family members who are carriers of m.3243A>G was performed as detailed in the Materials and Methods section.

Patient 2 was an Amish boy from Guernsey County, Ohio, with history of developmental delay who presented at 12 years of age with generalized convulsive epilepsy and a visual field loss. MRI revealed cerebral thrombosis and cerebral infarction. Ophthalmology evaluation revealed optic neuropathy and visual loss. He had a history of temporary vision loss and headache the year prior to his stroke. His lactate was mildly increased at (2.3 mmol/L), along with elevated alanine (820 μmol/L; normal 67–597). He subsequently had recurrent strokes, refractory seizures including epilepsy partialis continua, and developed basal ganglia and brainstem lesions consistent with Leigh syndrome (Fig. 2) with progressive spasticity, dysphagia, and weakness.

He also has optic atrophy with visual acuity to light perception only and bilateral hearing loss. Genetic evaluation revealed a mitochondrial