BMJ Neurology Open

Neurological manifestations in adult patients with the m.3243A>G variant in mitochondrial DNA

Kari Majamaa ,[1,2] Mikko Kärppä,[1,2] Jukka S Moilanen[3,4]

To cite: Majamaa K, Kärppä M, Moilanen JS. Neurological manifestations in adult patients with the m.3243A>G variant in mitochondrial DNA. *BMJ Neurology Open* 2024;**6**:e000825. doi:10.1136/bmjno-2024-000825

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjno-2024-000825).

Received 09 July 2024
Accepted 09 September 2024

[1]Research Unit of Clinical Medicine, Neurology, University of Oulu, Oulu, Finland
[2]Medical Research Center Oulu and Neurocenter, Oulu University Hospital, Oulu, Finland
[3]Research Unit of Clinical Medicine, Clinical Genetics, University of Oulu, Oulu, Finland
[4]Medical Research Center Oulu, Oulu University Hospital, Oulu, Finland

Correspondence to
Professor Kari Majamaa; kari.majamaa@oulu.fi

ABSTRACT

Background The m.3243A>G variant in mitochondrial DNA (mtDNA) is the most common cause of the MELAS (Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes) syndrome usually commencing in childhood or adolescence. In adults, the variant presents with versatile and mostly neurological phenotypes, but MELAS may not be common.

Objective To examine the frequency of phenotypes in adults with m.3243A>G in a population-based cohort and in a meta-analysis of reported case series.

Methods We clinically examined 51 adult patients with m.3243A>G to determine the frequency of phenotypes and to analyse the contribution of variant heteroplasmy, age, sex and mtDNA haplogroup to the phenotypes. The frequencies of neurological features were also assessed in a meta-analysis on 25 published case series reporting 1314 patients.

Results Sensorineural hearing impairment (HI), cognitive impairment and myopathy were the most common manifestations, whereas stroke-like episodes were infrequent. Variant heteroplasmy and age were only modest predictors of the phenotypes, although heteroplasmy correlated significantly with disability and Kaplan-Meier analysis showed progression of phenotypes with age. Male sex predicted more severe disability, whereas haplogroup UK was associated with no significant disability. Meta-analysis revealed substantial heterogeneity of phenotype frequencies and preferential inclusion of the MELAS phenotype.

Discussion In adult patients with m.3243A>G sensorineural HI, cognitive impairment and myopathy are common manifestations with lifetime prevalences approaching unity. Stroke-like episodes are rare. Variant heteroplasmy, age, sex and mtDNA haplogroup contribute to the severity of the disease. Meta-analysis provided a solid estimate of the various neurological symptoms in adults with m.3243A>G.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The m.3243A>G variant in mitochondrial DNA (mtDNA) presents with versatile and mostly neurological phenotypes, but the reported frequencies of the phenotypes vary across studies.

WHAT THIS STUDY ADDS

⇒ Hearing loss, cognitive impairment and myopathy were the most common neurological phenotypes in our population-based cohort of m.3243A>G carriers, whereas stroke-like episodes were rare and a considerable proportion of patients was free of neurological manifestations. Meta-analysis identified hearing loss and myopathy as common phenotypes, but the differences between the frequencies of other phenotypes were not conspicuous. Male sex predicted more severe disability, whereas mtDNA haplogroup UK was associated with no significant disability.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The proportions of affected patients provide credible *a priori* probabilities for clinicians in the counselling of patients with m.3243A>G.

The m.3243A>G variant in the *MTTL1* gene is the most frequent cause of mitochondrial disease[1 2] and the most frequent cause of MELAS (mitochondrial encephalopathy, lactic acidosis and stroke-like episodes), one of the classical mitochondrial syndromes.[3 4] The syndrome was originally described as a childhood disease that was characterised by stroke-like episodes and seizures.[5] The age at onset of such severe syndrome has been estimated to be lower than 20 years in almost 70% of patients.[6] However, phenotypes other than MELAS are far more common in adults, who may present with phenotypes ranging from asymptomatic carriers to variable adult-onset multiorgan diseases.[4] The neurological manifestations may include epilepsy, myopathy, migraine-like headache, peripheral neuropathy, cognitive impairment, ophthalmoplegia, sensorineural hearing impairment (SNHI) and stroke-like attacks.[3]

Clinical features of patients with m.3243A>G have been described in many clinical series, but the studies have been very different in size, and the reported frequencies of phenotypes have been variable[7 8] and occasionally contaminated by preferential reporting of the MELAS phenotype.[9] The causes of this variability include differences

in patient selection, variation in the proportion of paediatric and adult patients and variable use of prospective or retrospective data. The common neurological manifestations of patients with m.3243A>G have been established, but the frequencies of neurological features among unselected adult patients with m.3243A>G are not completely defined. To obtain a more comprehensive view of the spectrum of phenotypes and to obtain a more realistic view of the frequencies of neurological symptoms, we set out to examine the spectrum of neurological involvement in a population-based cohort of adults with m.3243A>G[10] and in a meta-analysis based on reported case series.

METHODS

Subjects

We have previously ascertained 17 pedigrees with m.3243A>G in the province of North Ostrobothnia, Finland. Eleven probands were identified in a population-based epidemiological survey, the setting of which has been described elsewhere.[10] In addition, six other probands were encountered in the following years at the Neurology Outpatient Clinic of Oulu University Hospital that is the only provider of neurological services in the province and that has had a specific interest in mitochondrial diseases. The probands, their mothers and one complete sibship from each pedigree were intended to be included in the present study. There were 122 adult subjects in the matrilineal sibships. Non-participants numbered 71 subjects (35 men and 38 women) including 39 subjects who had died before the start of the study and 32 subjects who were excluded from the study (11 subjects not living in the province; 5 subjects not harbouring m.3243A>G; 16 subjects not volunteering to the study). Finally, the 17 probands and 34 adult maternal relatives (18 men and 33 women) aged 18–72 years (45±14 years) with the m.3243A>G variant were recruited prospectively to the study. The maternal relatives included both symptomatic and asymptomatic carriers.

Clinical evaluation

The subjects were thoroughly interviewed, and they underwent a complete neurological examination. The phenotype was ascertained by clinicians with expertise in mitochondrial disorders (KM and MK). Standard clinical evaluations were performed on all patients included in the study. The age of onset of symptoms was determined based on medical history or appropriate medical files. The date of the first abnormal finding in clinical examination was used to define the age of onset if the history was inconclusive. The diagnoses were established or confirmed on clinical grounds for for myopathy, ophthalmoplegia, retinopathy, ataxia, epilepsy, migraine-like headache and stroke. The diagnoses of peripheral neuropathy,[11] SNHI[12] and hypertrophic cardiomyopathy[13] were based on ancillary examinations as described previously. Trail making test was used to assess cognitive impairment that was diagnosed if the time to complete the test exceeded the mean of age-stratified normative data by two SDs.[14] Short stature was defined as a height below two SDs of the mean height of the Northern Finnish population (men <165 cm, women <152 cm).[15] Diabetes mellitus was defined according to WHO recommendations for the diagnostic criteria for diabetes and intermediate hyperglycaemia.[16] Severity of disease was estimated using modified Rankin Scale (mRS, 0–5)[17] and Clinical Global Impression of Severity of Illness Rating (CGISIR grade 1–7).[18]

Molecular methods

The m.3243A>G heteroplasmy was determined in the buccal epithelium of 41 subjects, in skeletal muscle of 37 subjects, and in blood of two subjects by using an Apa I restriction digestion of a 390 bp mitochondrial DNA (mtDNA) fragment (spanning between positions m.3150 and m.3550) amplified by PCR in the presence of ^{35}S-ATP.[19] The intensities of the fragments were determined by using autoradiography. Heteroplasmy was determined both in the muscle and buccal epithelium of 31 subjects, and it was found to be correlated, so that muscle heteroplasmy=22.3+0.86×buccal epithelium heteroplasmy (R^2=0.709, p<0.0001). This equation was used to estimate muscle heteroplasmy given the buccal heteroplasmy in the 12 cases, where only buccal sample was available. The estimated values were then used to impute the 12 missing values and together with the 37 values of muscle heteroplasmy they composed the variable of imputed muscle heteroplasmy that was used in subsequent statistical analyses.

mtDNA haplogroups were determined by restriction fragment analysis of informative polymorphisms.[20] Twenty-eight patients belonged to mtDNA haplogroup UK, 12 to haplogroup H or V, six to haplogroup T, four to haplogroup I and one patient to haplogroup Z. The D loop was amplified in a fragment spanning nucleotides 15 975–725, and the sequence between nucleotides 16024 and 400 was determined by use of forward primers with their 5' nucleotides at positions 15975 and 16449, respectively.[20]

Meta-analysis

The identification and review of publications for the meta-analysis were carried out according to the guidelines in the PRISMA 2020 checklist (https://www.prisma-statement.org/prisma-2020-checklist). We searched articles until 31 January 2023 in PubMed database (figure 1). The following text words were used to search: (1) "A3243G"; (2) "m.3243A>G"; (3) "(3243 clinical) OR (3243 phenotype) AND mitochondrial NOT (case report) NOT m.3243A>G" and (4) "MELAS AND clinical". In addition, a search was performed using the terms: (5) "MELAS-(title) NOT (case report)(text word)". In searches (1)–(3) the date limit of 1 January 1990 was applied, and in searches (4) and (5), the limit was set at 1 January 1984. Two authors (KM and MK) were blinded to each other's

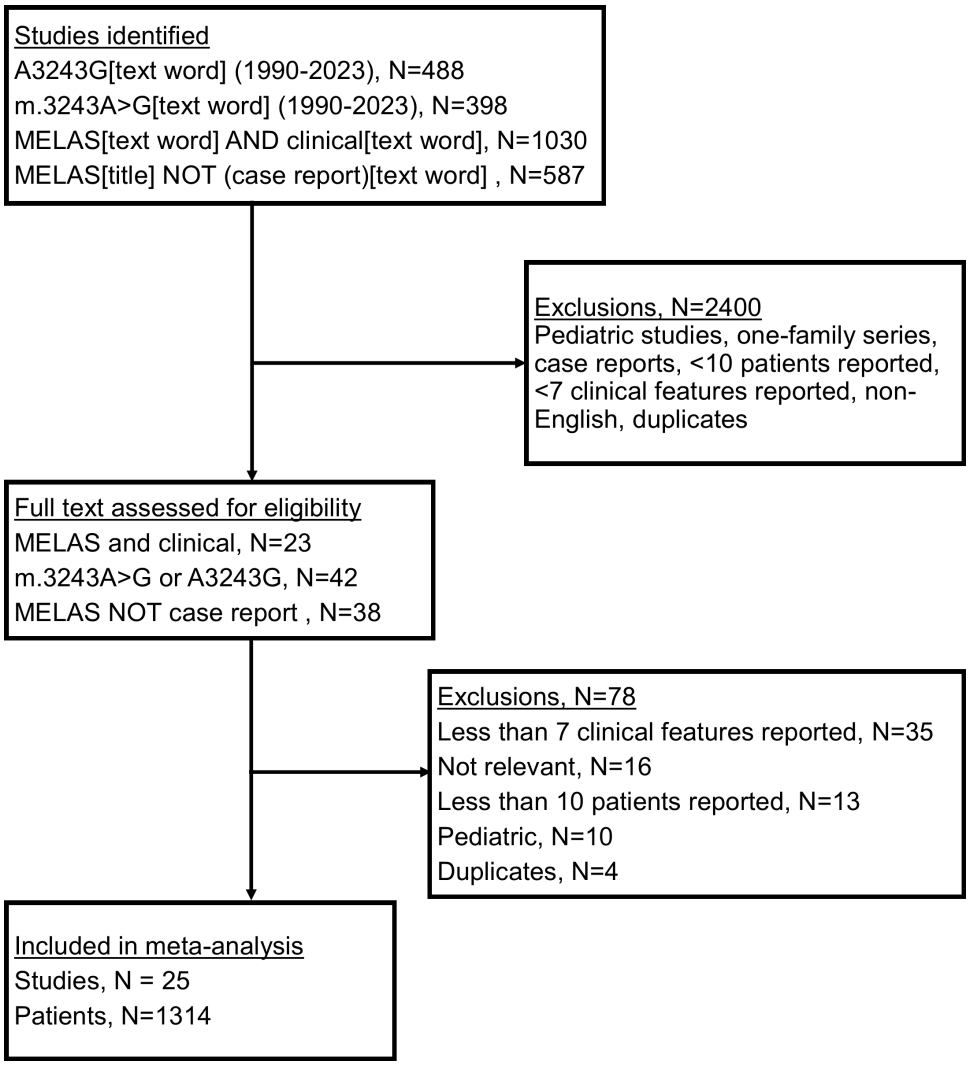

Figure 1 Screening of studies for the meta-analysis. MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes.

work and independently assessed the titles and abstracts of the retrieved articles for eligibility.

We included studies that reported case series with more than 10 individuals who were 18 years or older. We set the limit at 10 patients in order to avoid inclusion of case reports and small case series that may bring descriptions of more extreme phenotypes into the dataset. Studies were excluded if the cases were paediatric, or if cases had been ascertained based on the MELAS phenotype. Studies reporting phenotypes from samples combining children and adults were included only if the mean age of the cases was more than 18 years or if adult cases could be selected from the data. In addition, it was required that the study reported at least 6 out of the 13 phenotypes listed above. The lower limit was set at six phenotypes in order to avoid inclusion of publications that have examined selected phenotypes, such as diabetes and deafness, or stroke-like episodes. Focusing on a single phenotype carries the risk of ignoring other phenotypes. No restrictions based on sex or ethnicity were applied. Twenty-five studies (Dataset-25) were identified (table 1). Stroke-like episodes and epilepsy are frequent manifestations in the MELAS syndrome[5] and, therefore, we created a second dataset, where studies were excluded if the frequency of either phenotype was ≥50%. This exclusion was aimed to avoid preferential inclusion of the severe MELAS phenotype. Seventeen studies were included in Dataset-17.

Publication bias across individual studies was assessed using funnel plot inspection and the Begg's rank test.[21] Heterogeneity between included studies was assessed with I^2 statistics.[22] The random effects model was employed in the meta-analysis.

Statistical analyses

Demographic and clinical data were summarised using descriptive statistics, such as Mann-Whitney U test and Spearman's rank correlation test. χ^2 test was used to analyse associations between categorical variables. Binary logistic regression analysis was used to model the relationship between an outcome variable and independent variables using the enter method. Kaplan-Meier estimator was used to analyse the probability

Table 1 Studies included in Dataset-25 in the meta-analysis

Study author	Year	Patients with m.3243A>G (N)
Moraes CT *et al*	1993	21
Morgan-Hughes JA *et al*	1995	12
Hammans SR *et al*	1995	36
Mariotti C *et al*.*	1995	18
Damian MS *et al*	1995	29
Chinnery PF *et al*.*	1997	100†
McEntagart M *et al*	1997	12
Sue CM *et al*	1998	16
Deschauer, M *et al*	2001	15
Torroni A *et al*.*	2003	24
Tesarova M *et al*	2004	30
Kaufmann P *et al*	2009	123
Ma Y *et al*	2010	61
Yatsuga S *et al*.*	2011	38
de Laat P *et al*	2012	71
Liu CH *et al*.*	2012	22
Nesbitt V *et al*	2013	129
Chin J *et al*	2014	35
Mancuso M *et al*	2014	126
Dvorakova V *et al*	2016	33
Luigetti M *et al*.*	2016	12
Pickett SJ *et al*	2018	238
Brambilla A *et al*	2019	21
Chen H *et al*.*	2020	17
Wang W, *et al*.*	2023	87

*Studies reporting a frequency≥50% for stroke-like episodes or epilepsy were not included in Dataset-17.
†Phenotypes were reported as percentages. Number of patients taken as 100.

Table 2 Demographic characteristics and neurological manifestations of 51 patients with m.3243A>G

Variable	Men	Women
Patients, N	18	33
Age, years	47.7±11.9	42.8±13.3
Heteroplasmy, muscle %	73.7±11.2	64.1±15.4
Heteroplasmy, epithelium %	54.5±8.6	49.4±14.8
Ataxia, N (%)	2 (11)	2 (6)
Cognitive impairment, N (%)	11 (79) *	10 (38)
Diabetes, N (%)	13 (72)	14 (42)
Epilepsy, N (%)	5 (28)	4 (12)
Hypertrophic cardiomyopathy, N (%)	11 (61)	14 (42)
Migraine-like episodes, N (%)	4 (22)	15 (45)
Myopathy, N (%)	10 (56)	15 (45)
Ophthalmoplegia, N (%)	3 (17)	6 (18)
Peripheral neuropathy, N (%)	5 (28)	3 (9)
Retinopathy, N (%)	4 (22)	9 (27)
Sensorineural hearing impairment, N (%)	15 (83)	20 (61)
Short stature, N (%)	13 (72)	15 (45)
Stroke-like episodes, N (%)	5 (28) *	1 (3)
Rankin score	2.3±0.9 **	1.2±0.9
CGISIR score	3.7±0.7 **	2.6±1.2

Values are means±SD. A clinical diagnosis of myopathy was made for 25 patients including 13 patients (25 %) with myopathy with moderate proximal limb weakness, 8 patients (16 %) with mild proximal limb weakness, and 4 patients (8 %) with ptosis or ophthalmoplegia. Any histological abnormality referring to myopathy was found in the muscle of 26 out of 36 patients.
*p<0.05, **p<0.001.
CGISIR, Clinical Global Impression of Severity of Illness.

of phenotypes over time, where onset of the phenotype was the outcome, and subjects were censored at the age of death or at present age. Statistical analyses were performed with the SPSS Statistics V.29 software package for Windows, and the package *meta* in R (V.4.3.1) was used for meta-analysis.[23] Difference was considered significant if p<0.05.

RESULTS

Neurological manifestations

Fifty-one patients with m.3243A>G (18 men) were examined neurologically (table 2). Forty-two patients had at least one neurological manifestation, while nine patients (18 %, mean age 39 years, range 28–56 years) were free of neurological manifestations. The mean age of onset of the first neurological manifestation was 31 years (range, 0–70 years). The most common neurological phenotypes were SNHI, cognitive impairment and myopathy, whereas only six patients (12 %) had suffered from stroke-like episodes (table 2). None of the patients was diagnosed with dementia, but 53% of the patients were diagnosed with cognitive impairment. In addition, cardiomyopathy, short stature and diabetes were common. Diabetes and SNHI were associated (p=0.0002) and other significant associations were between cognitive impairment and SNHI (p=0.003), between stroke-like episodes and epilepsy (p=0.006) and between cognitive impairment and retinopathy (p=0.009).

Functional capacity

Twenty-three patients with m.3243A>G had no significant disability (mRS 0 or 1), 18 patients had mild disability (mRS 2) and 10 patients had moderate disability (mRS 3). The clinical impression of severity of illness gave grades 1 or 2 (normal or borderline ill) for 17 patients, grade 3 (mildly ill) for 15 patients and grades 4–5 (moderately to markedly ill) for 19 patients. There was a good correlation between the two assessments (p<0.001). Two patient

groups with mRS 0–1 or mRS 2–3 were formed for binary logistic regression analysis, where the variables HI, myopathy and stroke-like episodes were selected to represent the correlating phenotypes. The analysis yielded a model with R^2 0.477 and classification 72.5%.

Factors contributing to the clinical severity of disease

Imputed muscle heteroplasmy, age, sex and mtDNA haplogroup were found to contribute to the clinical severity of disease. Heteroplasmy correlated with Rankin scores (p<0.001) and CGISIR grading (p<0.001) and patients with mild or moderate disability had higher heteroplasmy than those with no significant disability (p=0.009). The two groups did not differ in age (p=0.52).

Imputed muscle heteroplasmy was higher in patients with encephalopathic phenotypes compared with those without the phenotype (table 1 in online supplemental file 1). Differences between the groups in age were not conspicuous, but the penetrance of m.3243A>G increased with age. Late-onset phenotypes included myopathy, cognitive impairment and peripheral neuropathy, while SNHI manifested earlier in life. Kaplan-Meier analysis showed that the estimated proportion of myopathy and SNHI at age 70 years was 100 %, while that of epilepsy was 22% and that of stroke-like episodes were 11% (figure 2).

Men were more severely affected than women as estimated by means of Rankin score (p=0.003, χ^2 test) and CGISIR grade (p=0.001, χ^2 test). All the 18 men had at least one neurological manifestation, while nine women (27%) did not have any manifestations. Among the phenotypes, however, only the frequency of cognitive impairment (p=0.022, χ^2 test) and stroke-like episodes (p=0.017, χ^2 test) were higher among men. mtDNA haplogroup UK was associated with no significant disability (p=0.004, χ^2 test).

Neurological phenotype as a dependent variable and imputed muscle heteroplasmy, age, sex and mtDNA haplogroup as covariates were imported in binary logistic regression analysis. The ORs suggested that higher heteroplasmy more than higher age was a modest explanatory covariate for all phenotypes (table 2 in online supplemental file 1), whereas male sex was a significant covariate for stroke-like episodes and mild or moderate

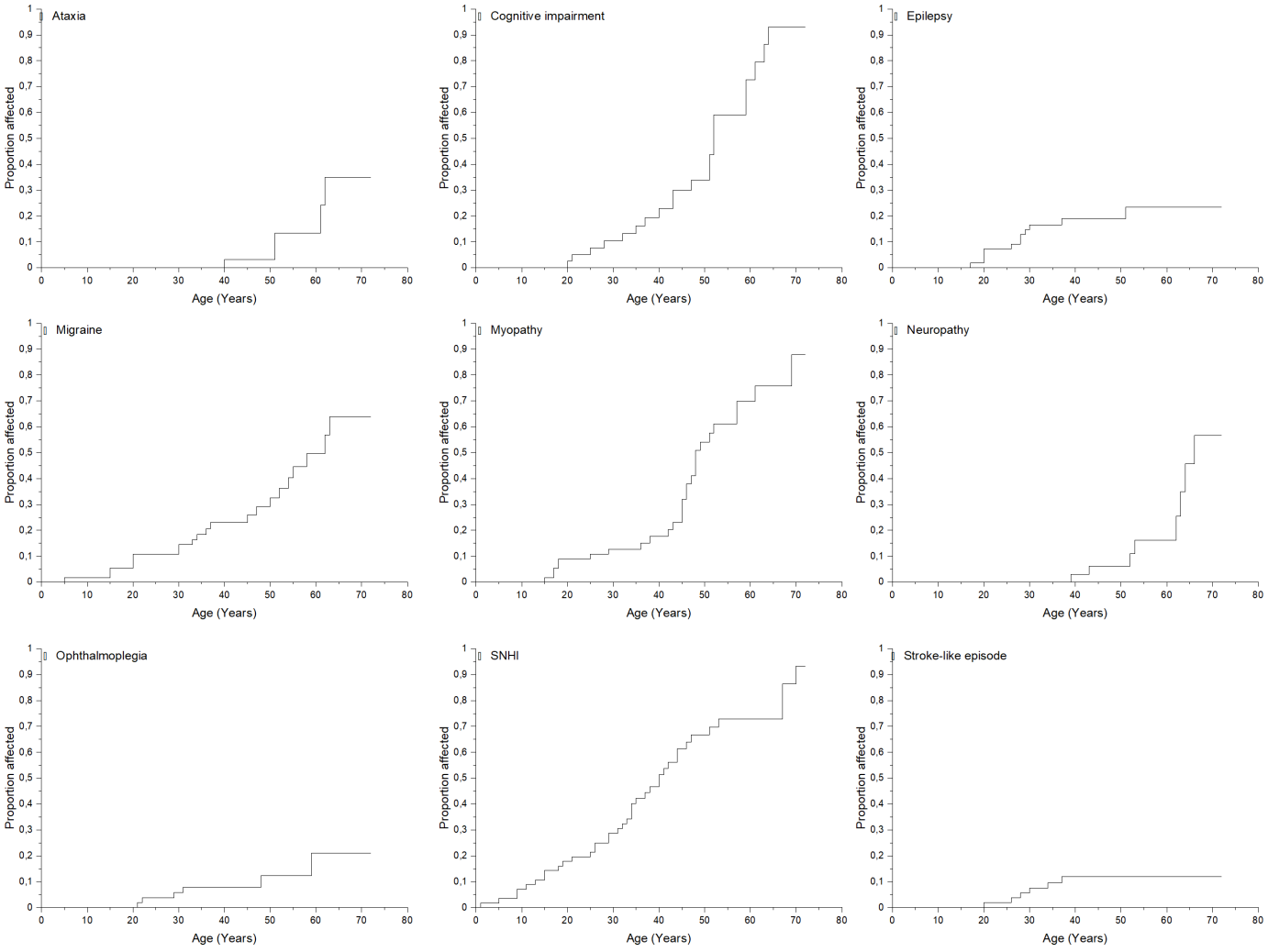

Figure 2 Kaplan-Meier plot for probability of phenotype in patients harbouring m.3243A>G. The event of interest was onset of the phenotype and patients were censored at the age of death or at present age. One minus survival is plotted.

disability and female sex for migraine. Haplogroup UK was an explanatory variable for the low frequency of most neurological phenotypes, significant only for epilepsy, and the phenotype of no significant disability. Regression analyses using muscle heteroplasmy and buccal epithelium heteroplasmy separately as covariates decreased the analytical power and did not provide additional information.

Meta-analysis

Twenty-five studies including 1314 patients with m.3243A>G (table 1) were identified for meta-analysis. The I^2 values were $0.5 \leq I^2 \leq 0.9$ suggesting substantial heterogeneity between the studies in all phenotypes and inspection of the phenotype frequencies revealed that stroke-like episodes or epilepsy were reported at a frequency of ≥50% in eight studies. Such high proportions suggested preferential inclusion of patients with the MELAS phenotype and, hence, the eight studies were removed creating a second dataset of 17 studies with 996 patients for the meta-analysis. Inspection of funnel plots and subsequent Begg's rank test revealed that possible bias remained in stroke-like episodes (p=0.048) and in epilepsy (p=0.182), and substantial heterogeneity prevailed between the 17 studies according to the I^2 values.

Meta-analysis was performed separately on Dataset-25 and Dataset-17. Peripheral neuropathy and retinopathy were rare phenotypes with estimated frequencies lower than 20%, and HI and myopathy belonged to the common phenotypes with frequencies higher than 35% (table 3; Forest plots are shown in online supplemental file 2). Except for ataxia, the estimated frequencies of the phenotypes were higher in Dataset-25 than in Dataset-17. The difference was less than 10% in most phenotypes, but the frequency of epilepsy was 45% and that of stroke-like episodes was 49% higher in Dataset-25.

We then estimated confident ranges for the reported proportions of affected patients in Dataset-25 by defining limits that were set at mean proportion±1 SD. Fifty-two of the 186 proportions (28 %) were outliers, and deviation was most common in the phenotypes of migraine, stroke-like episodes, ataxia and epilepsy. In our study, we found deviation above the upper limit in the proportion of cognitive impairment and deviation below the lower limit in that of stroke-like episodes and ataxia.

DISCUSSION

We found that among adult patients with m.3243A>G, the three most common neurological manifestations were SNHI, cognitive impairment and myopathy, each with a lifetime prevalence approaching unity. Classical MELAS syndrome was rare, as only six patients met the diagnostic criteria of the syndrome giving a frequency of 12%. The frequency is markedly lower than that in most of the previous studies but is consistent with a large recent study.[24] Furthermore, 18% of the patients were free of neurological manifestations. The population prevalence of m.3243A>G is some 10-fold higher[25 26] than the prevalence obtained from the screening of clinically affected subjects.[10] This discrepancy suggests that the proportion of unaffected subjects or monosymptomatic subjects may be even higher.

Table 3 Proportions of affected patients with various phenotypes based on a meta-analysis on Dataset-17 and Dataset-25 separately

	Dataset-17			Dataset-25		
Phenotype	Studies (N)	Events (N)	Proportion (%)	Studies (N)	Events (N)	Proportion (%)
Ataxia	12	218	27.4	19	266	23.6
Cardiomyopathy	13	206	22.3	17	238	22.8
Cognitive impairment	14	225	27.4	18	275	28.8
Diabetes	15	323	33.8	21	404	32.4
Epilepsy	15	198	23.2	23	382	33.6
SNHI	17	516	53.7	24	673	54.1
Migraine	12	261	27.9	16	350	32.5
Myopathy	16	383	38.9	22	522	42.2
Neuropathy	7	74	16.1	10	99	19.4
Ophthalmoplegia	12	156	23.0	18	219	24.5
Retinopathy	7	42	17.9	12	75	19.8
SLE	16	198	21.0	24	360	31.3

Comparison of the proportions shows that an excess of studies reporting patients affected with epilepsy or stroke-like episodes are included in Dataset-25. The meta-analysis employed random effect model.
SLE, Stroke-like episodes; SNHI, sensorineural hearing impairment.

We estimated the lifetime frequencies of the various manifestations by using Kaplan-Meier analysis. The age of onset of the manifestations was intended to be as accurate as possible, but inevitable inaccuracies were introduced, because some of the ages were obtained retrospectively and because age at examination rather than age at onset was used for many phenotypes. Therefore, phenotypes with exact time of onset and phenotypes with time at diagnosis were examined separately. An early analysis of patients with the MELAS syndrome, most of whom had been diagnosed before the discovery of the m.3243A>G variant, suggested that the median age at onset is 6–10 years.[27] The frequency of the MELAS syndrome was low among our patients, which explains that the mean age at onset of any neurological symptom was 32 years. The mean age of onset of diabetes has been reported to be 38 years in two studies on patients with m.3243A>G and the age of onset of SNHI to be 41 years. Furthermore, the mean age at onset has been reported to be 24.4±20.5 years in an analysis of a nationwide database of 1100 patients with any mitochondrial disease with or without a genetic diagnosis.[28] Although the medical history of our 51 patients covered their childhood, no children from the 17 pedigrees were included, which may affect the estimates on lifetime frequency. The Kaplan-Meier plots showed the contribution of age to the phenotypes and revealed two groups of manifestations including cognitive impairment, neuropathy and myopathy with late onset, and epilepsy and stroke-like episodes with early onset.

A different view on the frequency of phenotypes was obtained from a meta-analysis. We omitted studies that had employed disparate selection criteria, such as the full-blown MELAS phenotype, leaving 25 studies reporting 1314 patients with m.3243A>G (table 1). However, avoiding ascertainment bias is a difficult task. Even if all the patients in the included studies were adults and harboured m.3243A>G, all patients in the population had not been included, the phenotypes and their diagnostic criteria had not been consistent, and studies were selected for inclusion based on the number of patients and the number of phenotypes in the study. Especially, over-reporting of severe phenotypes and under-reporting of mild phenotypes may skew the estimates. However, the meta-analysis gave a good impression on the frequency of various phenotypes (table 3). By far, SNHI was the most common manifestation, followed by cognitive impairment and myopathy. There was substantial heterogeneity with I^2 values higher than 0.5 between studies in the proportions of affected patients. Several causes of heterogeneity can be identified including the small number of studies, differences in study designs, differences in case ascertainment and differences in the age of the patients. Overall, the phenotypes with clear-cut diagnostic criteria such as SNHI and diabetes proved to be consistent across studies. Meta-analysis on the two datasets showed that the frequency estimates were rather similar except for the frequency of epilepsy and stroke-like episodes, suggesting that preferential inclusion of patients with MELAS does not affect the other phenotypes. Our study was not included in the meta-analysis making a comparison possible. The ratio of our frequency to that of the meta-analysis varied from 1.68 for cognitive impairment to 0.29 for ataxia, the median being 1.13.

Several factors contributing to the phenotype of adult patients with m.3243A>G were identified. Imputed muscle heteroplasmy was significantly higher in patients with SNHI, epilepsy, stroke-like episodes, retinopathy, migraine and cognitive impairment than in patients without the phenotype. On the other hand, age differed only in epilepsy and neuropathy. Regression analysis including imputed muscle heteroplasmy, age, sex and mtDNA haplogroup as covariates, however, revealed only a modest contribution of heteroplasmy and age to the phenotypes confirming previous findings.[29] Kaplan-Meier analyses showed that the penetrance of phenotypes was age dependent in line with the progressive nature of mitochondrial diseases. In addition to age, we found that sex was associated with the phenotype. The severity of disease as estimated by the Rankin score was higher in men, but among the phenotypes, only the frequency of stroke-like episodes differed between the sexes, as five of the six patients with the MELAS phenotype were men. Male predominance is consistent with previous similar findings,[29 30] although the number of patients is small, and the sex distribution of participants was different from that of non-participants. Interestingly, variant heteroplasmy in urine epithelium has been reported to be 19% higher in men than in women,[31] but we did not find such a difference in the heteroplasmy of muscle or buccal epithelium.

It is well established that the risk of visual failure in Leber hereditary optic neuropathy is greater when m.11778G>A or m.14484T>C variants are present in haplogroup J mtDNA.[32] In addition, an analysis of 142 patients with m.3243A>G has revealed that the frequency of haplogroup J was lower than that in the general population,[33] whereas other studies have not found differences between patients and the general population.[24 34] We found that patients with m.3243A>G who belonged to mtDNA haplogroup UK were less likely to present with a phenotype than patients belonging to other haplogroups, most notably haplogroup HV. Nine maternal pedigrees belonged to haplogroup UK and their D loop sequences indicated that the m.3243A>G variant has arisen at least eight times in the population. It, therefore, seems unlikely that the association of haplogroup UK with a milder phenotype is contributed by a single family. Interestingly, haplogroup UK has previously been associated with longevity[35] and reduced risk for Parkinson disease compared with haplogroup HV,[36] and haplogroup U has been associated with a reduced risk of cognitive disease progression in Parkinson's disease.[37] mtDNA haplogroups seem to influence the range of disease phenotype.

We have previously ascertained a population-based cohort of patients with m.3243A>G. Here, we determined the spectrum of neurological symptoms among 51 adult patients and carried out a meta-analysis on

data in 25 previous publications. The proportions of affected patients provide credible *a priori* probabilities for clinicians in the counselling of patients with m.3243A>G. The probabilities were further elaborated by Kaplan-Meier estimates. SNHI, cognitive impairment and myopathy were the most common neurological features, and their estimated life-time frequency approached unity. Variant heteroplasmy and age were rather modest predictors of the phenotypes, whereas male sex was associated with more severe disability and mtDNA haplogroup UK with milder disability. Meta-analysis not only revealed substantial heterogeneity between studies but also gave a more solid estimate on the frequency of various manifestation in patients with m.3243A>G. However, multicentre studies will be needed to describe an unbiased phenotype and to analyse covariates that contribute to the phenotype of m.3243A>G.

Acknowledgements The expert technical assistance of Anja Heikkinen and Pirjo Keränen is acknowledged. This work was supported in part by grants from the Sigrid Juselius Foundation.

Contributors KM, guarantor: conceptualisation; investigation; writing—original draft; analysis and interpretation of data; methodology; validation; visualisation; data curation; project administration; supervision; funding acquisition. MK: conceptualisation; investigation, writing—review and editing; data curation; interpretation of data. JSM: conceptualisation; investigation; writing—review and editing.

Funding This work was supported in part by grants from the Sigrid Juselius Foundation.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The study was approved by the Ethics Committee of the Medical Faculty, University of Oulu (25.9.1995) and it has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer-reviewed.

Data availability statement Data are available upon reasonable request.

ORCID iD

Kari Majamaa http://orcid.org/0000-0002-9070-3791

REFERENCES

1 Gorman GS, Schaefer AM, Ng Y, *et al*. Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. *Ann Neurol* 2015;77:753–9.
2 Martikainen MH, Majamaa K. Incidence and prevalence of mtDNA-related adult mitochondrial disease in Southwest Finland, 2009-2022: an observational, population-based study. *BMJ Neurol Open* 2024;6:e000546.
3 Montano V, Gruosso F, Simoncini C, *et al*. Clinical features of mtDNA-related syndromes in adulthood. *Arch Biochem Biophys* 2021;697:108689.
4 Boggan RM, Lim A, Taylor RW, *et al*. Resolving complexity in mitochondrial disease: Towards precision medicine. *Mol Genet Metab* 2019;128:19–29.
5 El-Hattab AW, Adesina AM, Jones J, *et al*. MELAS syndrome: Clinical manifestations, pathogenesis, and treatment options. *Mol Genet Metab* 2015;116:4–12.
6 Sproule DM, Kaufmann P. Mitochondrial encephalopathy, lactic acidosis, and strokelike episodes: basic concepts, clinical phenotype, and therapeutic management of MELAS syndrome. *Ann N Y Acad Sci* 2008;1142:133–58.
7 Moraes CT, Ciacci F, Silvestri G, *et al*. Atypical clinical presentations associated with the MELAS mutation at position 3243 of human mitochondrial DNA. *Neuromuscul Disord* 1993;3:43–50.
8 Luigetti M, Sauchelli D, Primiano G, *et al*. Peripheral neuropathy is a common manifestation of mitochondrial diseases: a single-centre experience. *Eur J Neurol* 2016;23:1020–7.
9 Lu Y, Deng J, Zhao Y, *et al*. Patients with MELAS with negative myopathology for characteristic ragged-red fibers. *J Neurol Sci* 2020;408:116499.
10 Majamaa K, Moilanen JS, Uimonen S, *et al*. Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: prevalence of the mutation in an adult population. *Am J Hum Genet* 1998;63:447–54.
11 Kärppä M, Syrjälä P, Tolonen U, *et al*. Peripheral neuropathy in patients with the 3243A>G mutation in mitochondrial DNA. *J Neurol* 2003;250:216–21.
12 Uimonen S, Moilanen JS, Sorri M, *et al*. Hearing impairment in patients with 3243A-->G mtDNA mutation: phenotype and rate of progression. *Hum Genet* 2001;108:284–9.
13 Majamaa-Voltti K, Peuhkurinen K, Kortelainen ML, *et al*. Cardiac abnormalities in patients with mitochondrial DNA mutation 3243A>G. *BMC Cardiovasc Disord* 2002;2:12.
14 Karstens AJ, Christianson TJ, Lundt ES, *et al*. Mayo normative studies: regression-based normative data for ages 30-91 years with a focus on the Boston Naming Test, Trail Making Test and Category Fluency. *J Int Neuropsychol Soc* 2024;30:389–401.
15 Sovio U, Bennett AJ, Millwood IY, *et al*. Genetic determinants of height growth assessed longitudinally from infancy to adulthood in the northern Finland birth cohort 1966. *PLoS Genet* 2009;5:e1000409.
16 WHO Document Production Services. *Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation*. Geneva, Switzerland, 2006.
17 RANKIN J. Cerebral vascular accidents in patients over the age of 60. II. Prognosis. *Scott Med J* 1957;2:200–15.
18 Guy E. *Clinical global impressions. Early Clinical Drug Evaluation (ECDEU) assessment manual for psychopharmacology*. Rockville: National Institute of Mental Health, 1976:218–22.
19 Kobayashi Y, Momoi MY, Tominaga K, *et al*. A point mutation in the mitochondrial tRNA(Leu)(UUR) gene in MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes). *Biochem Biophys Res Commun* 1990;173:816–22.
20 Finnilä S, Lehtonen MS, Majamaa K. Phylogenetic network for European mtDNA. *Am J Hum Genet* 2001;68:1475–84.
21 Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994;50:1088–101.
22 Higgins JPT, Thompson SG, Deeks JJ, *et al*. Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557–60.
23 Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial. *Evid Based Ment Health* 2019;22:153–60.
24 Nesbitt V, Pitceathly RDS, Turnbull DM, *et al*. The UK MRC Mitochondrial Disease Patient Cohort Study: clinical phenotypes associated with the m.3243A>G mutation--implications for diagnosis and management. *J Neurol Neurosurg Psychiatry* 2013;84:936–8.
25 Manwaring N, Jones MM, Wang JJ, *et al*. Population prevalence of the MELAS A3243G mutation. *Mitochondrion* 2007;7:230–3.
26 Elliott HR, Samuels DC, Eden JA, *et al*. Pathogenic mitochondrial DNA mutations are common in the general population. *Am J Hum Genet* 2008;83:254–60.
27 Hirano M, Pavlakis SG. Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS): current concepts. *J Child Neurol* 1994;9:4–13.

28 Orsucci D, Angelini C, Bertini E, *et al*. Revisiting mitochondrial ocular myopathies: a study from the Italian Network. *J Neurol* 2017;264:1777–84.
29 Pickett SJ, Grady JP, Ng YS, *et al*. Phenotypic heterogeneity in m.3243A>G mitochondrial disease: The role of nuclear factors. *Ann Clin Transl Neurol* 2018;5:333–45.
30 Mancuso M, Orsucci D, Angelini C, *et al*. The m.3243A>G mitochondrial DNA mutation and related phenotypes. A matter of gender? *J Neurol* 2014;261:504–10.
31 Grady JP, Pickett SJ, Ng YS, *et al*. mtDNA heteroplasmy level and copy number indicate disease burden in m.3243A>G mitochondrial disease. *EMBO Mol Med* 2018;10:e8262.
32 Hudson G, Carelli V, Spruijt L, *et al*. Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background. *Am J Hum Genet* 2007;81:228–33.
33 Pierron D, Rocher C, Amati-Bonneau P, *et al*. New evidence of a mitochondrial genetic background paradox: impact of the J haplogroup on the A3243G mutation. *BMC Med Genet* 2008;9:41.
34 Torroni A, Campos Y, Rengo C, *et al*. Mitochondrial DNA haplogroups do not play a role in the variable phenotypic presentation of the A3243G mutation. *Am J Hum Genet* 2003;72:1005–12.
35 Niemi AK, Hervonen A, Hurme M, *et al*. Mitochondrial DNA polymorphisms associated with longevity in a Finnish population. *Hum Genet* 2003;112:29–33.
36 Autere J, Moilanen JS, Finnilä S, *et al*. Mitochondrial DNA polymorphisms as risk factors for Parkinson's disease and Parkinson's disease dementia. *Hum Genet* 2004;115:29–35.
37 Liu G, Ni C, Zhan J, *et al*. Mitochondrial haplogroups and cognitive progression in Parkinson's disease. *Brain (Bacau)* 2023;146:42–9.