Am. J. Hum. Genet. 63:447–454, 1998

Epidemiology of A3243G, the Mutation for Mitochondrial Encephalomyopathy, Lactic Acidosis, and Strokelike Episodes: Prevalence of the Mutation in an Adult Population

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Summary

Mitochondrial diseases are characterized by considerable clinical variability and are most often caused by mutations in mtDNA. Because of the phenotypic variability, epidemiological studies of the frequency of these disorders have been difficult to perform. We studied the prevalence of the mtDNA mutation at nucleotide 3243 in an adult population of 245,201 individuals. This mutation is the most common molecular etiology of MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes), one of the clinical entities among the mitochondrial disorders. Patients with diabetes mellitus, sensorineural hearing impairment, epilepsy, occipital brain infarct, ophthalmoplegia, cerebral white-matter disease, basal-ganglia calcifications, hypertrophic cardiomyopathy, or ataxia were ascertained on the basis of defined clinical criteria and family-history data. A total of 615 patients were identified, and 480 samples were examined for the mutation. The mutation was found in 11 pedigrees, and its frequency was calculated to be ⩾16.3/100,000 in the adult population (95% confidence interval 11.3–21.4/100,000). The mutation had arisen in the population at least nine times, as determined by mtDNA haplotyping. Clinical evaluation of the probands revealed a syndrome that most frequently consisted of hearing impairment, cognitive decline, and short stature. The high prevalence of the common MELAS mutation in the adult population suggests that mitochondrial disorders constitute one of the largest diagnostic categories of neurogenetic diseases.

Received December 29, 1997; accepted for publication May 22, 1998; electronically published June 29, 1998.

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0002-9297/98/6302-0020$02.00

Introduction

The mitochondrial genome (mtDNA) is a double-stranded, circular molecule of 16,569 nucleotide pairs that is present in 2–10 copies in each mitochondrion (Wallace 1994*a;* Johns 1995; Shoffner 1996; Schon et al. 1997). This extranuclear genome is inherited maternally, and it is passed from the mother to all her offspring. mtDNA encodes 13 of >80 subunits in the mitochondrial respiratory-chain complexes, and it also contains genes for two rRNAs and 22 tRNAs. Mutations in mtDNA may therefore cause a respiratory-chain deficiency. Tissues with a high energy expenditure—for example, brain and muscle—suffer the most, although any organ may be affected, thus contributing to the clinical variability observed in mitochondrial diseases. Another factor involved in the phenotypic heterogeneity is the degree of mutant heteroplasmy. The normal genome and the mutant variant coexist in cells, and a high proportion of the mutant variant predicts a more severe clinical expression of the disease.

The acronym "MELAS" (mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes [MIM 540000]) denotes the classic clinical features of one of the mitochondrial syndromes (Pavlakis et al. 1984). Although the multiorgan involvement and the phenotypic variability of the disease make clinical diagnosis of MELAS syndrome difficult, the discovery of an A→G point mutation at nucleotide 3243 (A3243G) in the $tRNA^{Leu}$ gene of mtDNA (Goto et al. 1990; Mitomap) has enabled a definitive diagnosis to be established. Clinical evaluation of patients carrying this mutation has also revealed novel features, including phenotypes such as sensorineural hearing impairment, hypertrophic cardiomyopathy, ataxia, basal-ganglia calcifications, and ophthalmoplegia (Mariotti et al. 1995; Morgan-Hughes et al. 1995), as well as the quite commonly occurring diabetes mellitus (Kadowaki et al. 1994).

The frequency of pathogenic mtDNA mutations in a general population is unknown, but we assume that the prevalence of a given mutation may be estimated. Fin-

land provides good opportunities to perform epidemiological studies, since there is universal access to health care and the inhabitants are assigned to publicly funded health services on a regional basis. The registers available in the health-care system represent, to a large extent, the total morbidity in the population. We attempted to identify as many patients with the A3243G mutation as possible in the adult population of 245,201 in the province of Northern Ostrobothnia in northern Finland. Using various regional and municipal health-care registers, we first identified patients with clinical disorders that are commonly seen in association with the A3243G mutation. Subgroups of patients were then ascertained by reference to clinical criteria and family-history data and were analyzed for the presence of the mutation. In this way we estimated for the first time a prevalence for the common MELAS mutation A3243G in the adult population, determined its frequencies in several patient groups, and demonstrated that it has arisen independently on several occasions in a relatively small population.

Methods

Setting

The prevalence area considered here is Northern Ostrobothnia, a province that extends across the northern part of Finland, from the Gulf of Bothnia in the west to the Russian border in the east. The area of the province is 29,965 km^2, and, administratively, it is divided into 42 local government districts, with populations of 720–106,419 (median 4,793). The total population of the province was 353,895, and people $\geqslant$20 years of age numbered 245,201 on December 31, 1994 (hereafter termed the "prevalence date").

Primary health care in Finland is provided by the local authorities, and specialized medical care is provided at the provincial level. In Northern Ostrobothnia, it is Oulu University Hospital (OUH) that provides specialized medical care. The inhabitants are entitled to services at this institution, and, in fact, they may seek publicly funded medical care at institutions in other parts of the country only if they obtain the permission of their own local health authority. Private hospitals account for 8.4% of total hospital-care expenditure in the country, and private practitioners account for 6.8% of all outpatient visits in Northern Ostrobothnia (NAWH National Research and Development Centre for Welfare and Health 1994). This means that the files of the local health-care units and the institutions providing specialized medical care provide a fairly good representation of the prevalence of diseases in the population.

The Departments of Neurology and Cardiology at OUH are the only centers for their disciplines in Northern Ostrobothnia, as is the Department of Otorhinolaryngology in the provision of audiological services. Computerized discharge files are available from 1976 onward, for in-patients, and from 1984 onward, for outpatient visits. The findings of each radiological examination are coded, and the codes have been filed electronically since 1980.

Identification of Patient Cohorts

Adult patients with diagnoses that are commonly associated with the A3243G mutation (Johns 1995; Shoffner 1996) were defined as being at risk for a mitochondrial disorder. We therefore screened for patients >20 years of age who had disorders such as diabetes mellitus, sensorineural hearing impairment, cardiomyopathy, and brain infarct; patients >20 years of age with symptoms such as epilepsy, ataxia, and subjective visual disturbance (including field defects and double vision); and patients >20 years of age with radiological findings (in either a brain computed-tomography scan or magnetic-resonance imaging) such as an occipital lesion, basal-ganglia calcifications, and cerebral white-matter lucencies (table 1).

In the entire population of Northern Ostrobothnia, the total number of patients with diabetes mellitus, sensorineural hearing impairment requiring a hearing aid, or epilepsy was 18,642. Population-based cohorts of patients with defined clinical characteristics were then identified in the appropriate registers (table 1). To minimize screening, we assessed the family history, with respect to any combination of diabetes mellitus, hearing loss, and epilepsy, in first- and second-degree relatives, by means of a questionnaire (table 1); the mean response rate was 83%. Patients with any one or any combination of the three disorders in maternal relatives were eligible for inclusion within the series to be examined.

Using the discharge files of OUH, we identified 2,580 patients in the remaining six diagnostic groups. Their medical charts were reviewed, and, by reference to the defined criteria, they were deemed to be eligible for examination (table 1). Family history was not used as a selection criterion for these patients.

A blood sample was requested from each patient fulfilling the selection criteria (table 2). After the A3243G mutation had been found, a muscle-biopsy sample or buccal-epithelial-cell sample also was taken, and, in addition, the adult members of the families concerned were requested to visit the outpatient clinic, where similar samples were obtained from those who had volunteered.

Molecular Methods

DNA from the blood samples and buccal-epithelial-cell samples was purified by use of the QIAamp Blood Kit (QIAGEN), and that from the muscle-biopsy samples was purified by a standard extraction method. All the blood samples were screened for the A3243G mutation,

Table 1

Criteria Used in Screening of Patient Groups

Patient Group	Selection Criterion 1	No. of Patients Identified	Selection Criterion 2	No. of Patients Identified	No. (%) of Samples Received
Diabetes[a]	Insulin treatment started at age 20–45 years	479	Family history	169[b]	143 (85)
Hearing loss[c]	Sensorineural hearing impairment, hearing aid obtained at age ⩽45 years, current age ⩾20 years	242	Family history	108[b]	82 (76)
Epilepsy[d]	Outpatient visit to OUH, age ⩾20 years at visit, response to family-history questionnaire	945	Family history	223[b]	165 (74)
Brain infarct	Brain infarct, occipital lesion in computed-tomography scan or visual-field defect, age ⩽45 years at diagnosis	823	Occipital infarct, age 20–45 years at onset	35	29 (83)
Ophthalmoplegia	Double vision or ptosis, any age	799	Definite ophthalmoplegia or symmetric ptosis, age ⩾20 years at examination	15	15 (100)
Basal-ganglia calcification	Intracranial calcification, any age	635	Symmetric basal-ganglia calcification, significant size, age ⩾20 years at examination	10	8 (80)
White-matter disease	White-matter disease not related to vascular or demyelinating disorders or CNS radiotherapy, age ⩽55 years at examination	155	Significant extent, age ⩾20 years at examination	5	5 (100)
Hypertrophic or dilated cardiomyopathy	Any cardiomyopathy, unknown etiology	89	Hypertrophic cardiomyopathy, age 20–45 years at diagnosis	11	7 (64)
Ataxia	Any ataxia, unknown etiology	79	Idiopathic cerebellar ataxia, age ⩾20 years at visit	39	26 (67)
Total		4,246		615	480 (78)

[a] Patients with insulin-dependent diabetes mellitus obtain needles, syringes, insulin pens, and glucose sticks free of charge from the public-health-care units, and the supplies used are recorded. These patients were identified from the records of 40 of 42 of the local-authority health-care units. The discharge diagnoses from one of the two regional hospitals in the area, as well as the diabetes register of the other one, also were reviewed.

[b] Patients with any combination of diabetes, sensorineural hearing impairment, or epilepsy in first- or second-degree maternal relatives were included.

[c] The cost of hearing aids is completely refunded by the public health service, and they are supplied only by the Department of Otorhinolaryngology at OUH. The register of hearing aids supplied was reviewed, on the basis of the following clinical criteria: symmetric sensorineural hearing impairment with undefined etiology; hearing impairment >30 dB (pure-tone average of frequencies 0.5, 1, 2, and 4 kHz); a difference, between the ears, <10 dB; and use of a hearing aid at age <45 years.

[d] The majority of adult patients with epilepsy make regular follow-up visits at least once a year to the Outpatient Clinic of the Department of Neurology at OUH. During a 1-year period, a physician involved in the study checked the charts of the patients visiting the clinic every day. The diagnosis of epilepsy was confirmed on this occasion, and patients receiving regular antiepileptic medication were included. No distinction was made between the types or etiologies of epilepsy.

Table 2

Frequency of A3243G Mutation in Patient Groups

Patient Group	Family History[a]	No. of Patients Identified	No. of Samples Evaluated	No. (%) of Samples with A3243G Mutation
Diabetes	Diabetes	150	126	2 (1.6)
	Epilepsy or hearing loss	19	17	0
Hearing loss	Hearing loss	89	68	5 (7.4)
	Diabetes or epilepsy	19	14	0
Epilepsy	Epilepsy	114	87	1 (1.1)
	Diabetes or hearing loss	109	78	0
Occipital stroke	…	35	29	2 (6.9)
Ophthalmoplegia	…	15	15	2 (13)
Intracranial calcification	…	10	8	1 (13)
White-matter disease	…	5	5	0
Hypertrophic cardiomyopathy	…	11	7	1 (14)
Ataxia	…	39	26	0
Total		615	480	14

[a] Denotes presence of the disorder in first- or second-degree maternal relatives of the proband. An ellipsis (…) denotes that family history was not used as a selection criterion in the group.

by a restriction-fragment analysis. Specifically, PCR was used to amplify a 399-bp fragment of mtDNA in 30 cycles in the presence of [^{35}S]-dATP, a forward primer corresponding to nucleotide positions 3153–3172, and a reverse primer corresponding to nucleotide positions 3551–3531. The A3243G point mutation was detected by cleavage with the restriction enzyme *Apa*I (Kobayashi et al. 1990). The digested samples were electrophoresed through a 6% acrylamide gel, which was dried and autoradiographed at −72°C overnight, by means of Kodak XAR-5 film with an intensifying screen. The film was analyzed with a Bioimage scanner and image processing apparatus (Millipore), with a setting that enabled the detection of a band that differed from the background by 6%. On average, a minimum mutant heteroplasmy of 2% has been detectable in the restriction-fragment analysis (Smith et al. 1997; Wong and Lam 1997). The reproducibility of the method was controlled by including, in each electrophoresis run, a sample with the A3243G mutation.

PCR using allele-specific primers (Liu et al. 1997) was used to amplify templates in situations in which a minimal degree of mutant heteroplasmy was assumed. The method has a high sensitivity, and a detectable amount of the mutant genome may be amplified in the presence of a 10,000-fold excess of normal molecules (Liu et al. 1997). This method was used whenever the restriction-fragment analysis using *Apa*I did not find the A3243G mutation in the sample from a relative of the proband. The amount of total DNA used as template was 1 pg, in the case of blood cells, and 0.5 pg, in the case of muscle or other tissue. In the presence of the mutant genome, a PCR product was detected in a 1.5% agarose gel electrophoresis.

A solid-phase minisequencing method (Suomalainen et al. 1993; Tully et al. 1996) was used to determine the degree of mutant heteroplasmy. Both a 5′-biotinylated forward primer corresponding to nucleotide positions 3213–3232 and the reverse primer described above were used to synthesize the primary PCR product, which was quantified with a fluorometer (DyNA Quant; Hoefer Scientific Instruments). An aliquot of 500 pg of the product was immobilized on Dynabeads M-280 streptavidin paramagnetic particles (Dynal) and was subjected to minisequencing in the presence of a reverse primer corresponding to nucleotide positions 3263–3244 and either [^{3}H]-dCTP, to detect the mutant genome, or [^{3}H]-methyl TTP (Amersham), to detect the wild-type genome. Mutation heteroplasmy was calculated as the ratio of radioactivity in the two reactions after correction for the different specific activities of the nucleotides. A good correlation was observed between the degree of heteroplasmy obtained with this method and that with the quantification of the *Apa*I-generated restriction fragments and subsequent correction for heteroduplex formation (Pearson correlation coefficient .930, $P = .01$).

Genealogy and Haplogroup Analysis

The pedigrees of the probands with the A3243G mutation were assessed, including all the first- and second-degree maternal relatives and a maternal ancestor born during the 19th century. The pedigrees were compiled from the official population records, which contain information on births, deaths, marriages, and relocations of the individuals.

A set of 10 primer pairs were used for PCR amplification, to obtain 10 overlapping fragments covering the mtDNA (Passarino et al. 1993). mtDNA haplogroups were determined by analysis of RFLPs obtained with 14

restriction enzymes (Torroni et al. 1996). The 22 tRNA genes were sequenced in a dideoxy termination reaction (AmpliTaq; Perkin-Elmer) using forward primers corresponding to nucleotide positions 534–553, 3153–3172, 3951–3970, 5467–5488, 7392–7410, 8100–8121, 9911–9930, 10356–10380, 12095–12117, 14560–14585, and 15812–15838 and reverse primers for nucleotide positions 1696–1677, 4508–4489, and 5917–5898. Approximately 21% of the total mtDNA could be sequenced by use of these primers. Furthermore, the D-loop was sequenced in an automatic sequencer (ABI Prism; Perkin Elmer) using the forward primers corresponding to nucleotide positions 15975–15994, 16449–16468, and 328–350.

Ethical Considerations

The protocol was approved by the Ethics Committee of the Medical Faculty, University of Oulu. Permission for the chart review was obtained from the Finnish Ministry of Social Affairs and Health. All the persons who were initially contacted were patients of either OUH or the local-authority health-care units, and they were contacted with the consent of the attending physician. The samples were studied after informed consent was obtained from the patients.

Results

Prevalence of the A3243G Mutation in Adults

Prior to the present study, we were aware of five pedigrees with the A3243G mutation in the population of Northern Ostrobothnia. The screening revealed a total of 14 patients, including members of these five pedigrees and members of five newly identified pedigrees. Eight of the pedigrees were identified through a single proband, and two pedigrees were identified through more than one member. The frequency of the A3243G mutation in the various patient groups is shown in table 2. We also identified one additional pedigree with sensorineural hearing impairment; but none of the affected members fulfilled the selection criteria, and the pedigree was not found in the screening.

There were 36 members of the sibships of the probands and 87 members of the sibships of their mothers, constituting a cohort of 123 persons in all. If the probands are excluded, 68 of the 123 individuals were >20 years of age, and 33 of these volunteered to participate in the study. We found the A3243G mutation in 27 blood samples from these sibships and also in archival tissue samples from four members. The total number of verified mutation carriers >20 years of age who were living in the province of Northern Ostrobothnia was 25 on the prevalence date. Thus, the minimum estimate for the prevalence of the A3243G mutation in the adult population of 245,201 in Northern Ostrobothnia is 10.2/100,000 (95% confidence interval 6.2–14.2/100,000 [Gardner and Altman 1986]).

The first-degree maternal relatives of the verified mutation carriers were then defined as obligatory carriers. There were 36 obligatory carriers living in the province of Northern Ostrobothnia, and 15 of them were >20 years of age on the prevalence date. Thus, the number of verified and obligatory mutation carriers in the adult population in Northern Ostrobothnia was taken to be 40, suggesting that the prevalence of the common MELAS mutation is 16.3/100,000 (95% confidence interval 11.3–21.4/100,000). Analysis of the pedigrees, indeed, suggested a very high frequency of the mutation among the first-degree maternal relatives of a verified mutation carrier, since the mutation could be found in $\geqslant 14$ of the 17 adult siblings and in all seven mothers of the probands who were examined. Furthermore, a definite absence of the mutation was found only in one person, since a maternal uncle of the proband in family 2 did not have the mutation in blood, muscle, or a post-mortem cerebral cortical sample, even though at least three of his six siblings were mutation carriers.

Clinical Evaluation

Clinical evaluation of the 11 probands revealed syndromic features in 9 of them and isolated ophthalmoplegia in 2 of them (table 3). Sensorineural hearing impairment was the most common feature of the syndrome, being found in each of the nine syndromic probands. Only one of the probands had had symptoms since childhood: he had been examined for hearing impairment and retarded growth at 13 years of age. The median value for mutant heteroplasmy among the probands was 23% in blood and 67% in muscle.

The presence of symptoms of mitochondrial disease was evaluated in the 44 persons who gave a blood sample and who belonged to the sibship of either a proband or a proband's mother. The sensitivity of the radioactive restriction-fragment analysis in the detection of the mutation in blood DNA from clinically affected persons was .93. Furthermore, the sibships included 32 persons with the mutation in a tissue sample from whom a blood DNA was available. In 30 cases the mutation was also found in the blood DNA.

Haplogroup and Genealogy Analysis of the Pedigrees

RFLPs revealed that six probands belonged to mtDNA haplogroup U, two to haplogroup H, two to haplogroup T, and one to haplogroup I. Sequencing revealed differences in at least seven nucleotide sites within the 22 tRNA genes and the D-loop of the haplogroup H and T mtDNAs. Interestingly, among the six probands belonging to haplogroup U, we found only four dissimilar

Table 3

Clinical Features, Molecular Diagnostics, and Family History of Probands with A3243G mtDNA Mutation, Identified in the Population of Northern Ostrobothnia

Family	Sex	Age at First Examination[a] (years)	Age at Diagnosis (years)	Mutant Heteroplasmy (%)		Clinical Features	Family History
				Blood	Muscle		
1	F	28	33	23	67	Epilepsy,[b] cognitive decline, hearing impairment, basal-ganglia calcifications, short stature	Hearing impairment, diabetes, cognitive decline, epilepsy, short stature
2	M	28	28	42	83	Epilepsy, hearing impairment, basal-ganglia calcifications, occipital stroke,[b] hypertrophic cardiomyopathy	Hearing impairment, diabetes
3	F	21	40	26	60	Hearing impairment, ataxia, ophthalmoplegia, diabetes, cardiomyopathy[b]	Diabetes
4	F	18	30	51	89	Hearing impairment,[b] diabetes, cognitive decline, epilepsy, short stature	Hearing impairment, diabetes, short stature
5	M	48	58	14	58	Cognitive decline, short stature, polyneuropathy	Diabetes, basal-ganglia calcifications[b]
6	F	32	31	0	38	Ophthalmoplegia[b]	Ophthalmoplegia
7	M	13	20	39	89	Hearing impairment,[b] short stature, cognitive decline	Hearing impairment, diabetes, short stature
8	F	26	46	40	72	Hearing impairment[b]	Hearing impairment
9	F	43	46	18	77	Hearing impairment[b]	Hearing impairment
10	F	24	26	6	10	Ophthalmoplegia[b]	Ophthalmoplegia
11[c]	F	37	37	9	31	Hearing impairment,[b] short stature	Hearing impairment, short stature

[a] Performed because of a symptom that may be considered to be of mitochondrial origin.
[b] Symptom or disorder that led to the identification of the proband. Family 5 had been identified earlier, on the basis of the proband identified, and another family member was ascertained to be in the group of patients with basal-ganglia calcifications.
[c] Identified during the present study but not in the initial screening.

sequences. Different mtDNA genotypes in at least nine of the pedigrees suggest that the mutation has arisen in the population several times.

A maternal ancestor was identified in each pedigree (two to four generations from the proband), the median year of birth being 1873 (range 1808–94). Three of the 11 maternal ancestors had been born in Northern Ostrobothnia, and, interestingly, 6 of them had been born in a much smaller population in northeastern Finland including the province of Kainuu; of these 6 ancestors, 4, including the 3 with identical mtDNA genotypes, belonged to haplogroup U. Indeed, two of these pedigrees were found to have a common ancestor born in 1864. Genealogical analysis extending to the years 1808 and 1837 did not, however, reveal any ancestors common to both this pedigree and the third pedigree sharing the mtDNA genotype.

Discussion

This is the first assessment of the epidemiology of mitochondrial diseases. We found a prevalence of $\geqslant$10.2/100,000 for the A3243G mutation in the adult population—or, if it is assumed that all the first-degree maternal relatives of a verified mutation carrier also harbored the mutation, a prevalence of $\geqslant$16.3/100,000. The analysis of the pedigrees suggested that this was a reasonable approach, since the frequency of the mutation was found to be very high among the first-degree maternal relatives of the probands and only one examined relative was definitely without the mutation. The high prevalence suggests that mitochondrial disorders may constitute one of the largest diagnostic categories of neurogenetic diseases among adults.

The observed prevalence represents a minimum estimate for the frequency of the A3243G mutation, for several reasons. Second-degree maternal relatives were not considered obligatory mutation carriers even in the presence of a family history suggesting mitochondrial disease such as diabetes mellitus or sensorineural hearing impairment. Cases were lost during the process of patient identification, because of nonresponse to inquiries concerning family history, nonrecovery of patient charts for review, and nonparticipation in blood-sample collection. Furthermore, the mutation was detected in the blood samples of the affected persons, with a sensitivity of .93. The patient groups studied represent the clinical phenotypes known to be associated with the A3243G mutation, and only adult patients were identified. For

example, pigmentary retinopathy was considered a noncontributory phenotype when the study was initiated. However, a recent analysis of four pedigrees suggests that the frequency of this phenotype may be high among patients with the A3243G mutation (Sue et al. 1997). Children were not ascertained in this study, and, therefore, we may have lost pedigrees with exclusively pediatric cases. The effect of these biases is parallel, and accounting for them would increase the prevalence.

The clinical phenotype of the ascertained probands suggested a multiorgan disorder in nine cases, most frequently including sensorineural hearing loss, cognitive decline, or short stature. We observed isolated ophthalmoplegia in two probands, and ocular symptoms alone were reported in other members of both families. Interestingly, no cases with childhood-onset classic MELAS syndrome (Pavlakis et al. 1984) could be found either among the probands themselves or among the 59 children of either the female probands or the sisters of the probands. These results suggest either that the mutation may cause lower morbidity in children than in adults or that childhood cases and adult cases do not occur concurrently in families.

A frequency of 0.9% for the A3243G mutation has been observed in unselected patients with diabetes (Maassen and Kadowaki 1996). The corresponding rate in the present study was 0.5%, whereas the frequency among the patients with matrilinear diabetes was 1.6%. Similar ratios, in the range of 2.0%–5.5%, have been reported among patients with familial diabetes (Gerbitz et al. 1995; Maassen and Kadowaki 1996). The minimum frequency of the mutation among unselected adult patients with hearing loss requiring a hearing aid was 0.07%, whereas that in the selected cohort of patients with matrilinear sensorineural hearing loss was 7%, suggesting that the mutation is more frequent among these patients than among patients with matrilinear diabetes mellitus (Gerbitz et al. 1995; Maassen and Kadowaki 1996). We found mutation frequencies on the order of 10% among patients with ophthalmoplegia or hypertrophic cardiomyopathy. Previously, no etiology had been identified in the patients selected for the mutation analysis, in either group. These data suggest that a search for an mtDNA mutation is worthwhile in the diagnosis of young patients with these disorders. The frequency of the mutation was also fairly high among young patients with an occipital brain infarct (Majamaa et al. 1997).

RFLPs and the tRNA and D-loop sequences of the 11 probands revealed nine distinct mtDNA genotypes, suggesting that the mutation had arisen in the population several times. A similar finding, based on the variability of nucleotides in the D-loop, has been reported for the A3243G mutation (Morten et al. 1995) and for the A1555G mutation (Hutchin and Cortopassi 1997). We found an apparent clustering in haplogroup U, since six probands belonged to this haplogroup. Three of these six had an identical mtDNA genotype, however, and two of them were found to be distant maternal relatives.

The maternal ancestors, whose median year of birth was 1873, were mainly of northeastern Finnish descent. Of the 10 families, 5 originated from the province of Kainuu and the easternmost district of Northern Ostrobothnia, an area with a total population of 55,593 in the year 1900, whereas 3 families originated from the remaining part of Northern Ostrobothnia, an area with a population of 170,424 in 1900. These figures suggest that the frequency of families with the A3243G mutation may have been $\geqslant$5.1-fold higher in Kainuu than in Northern Ostrobothnia. This is interesting in view of the fact that the province of Kainuu did not belong to the area from which the initial population for this survey was taken.

Finland is known for >30 heritable disorders that are more prevalent there than in other countries (Norio et al. 1973; de la Chapelle 1993; Peltonen et al. 1995), and the concept of a Finnish disease heritage has been used to describe this phenomenon (Norio et al. 1973). The clustering of diseases is due partly to the founder effect and partly to the Finnish population's isolation because of geographical, linguistic, and cultural reasons. We found that the A3243G mutation in mtDNA may be one of the most common single mutations among neurogenetic diseases in the adult Finnish population. That we encountered a cluster of the A3243G mutation within the country is unlikely, since our preliminary results suggest that the average frequency of the mutation is similar in the provinces of Central Ostrobothnia and Kainuu (authors' unpublished observations). In the absence of other studies of the epidemiology of mitochondrial disorders, we are not able to assess whether the mutation is part of the Finnish disease heritage or whether its frequency is similar to that in other populations. The former alternative may be true if nuclear mutations are required to provoke mtDNA mutations; the latter may be true if only a constant rate of new mutations in mtDNA is involved.

Acknowledgments

The expert technical assistance of Ms. Anja Heikkinen is acknowledged. This work was supported in part by grants from the Medical Research Council of the Academy of Finland and from the Sigrid Juselius Foundation.

Electronic-Database Information

URLs for data in this article are as follows:

Mitomap, http://www.gen.emory.edu/mitomap.html

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for MELAS [MIM 540000])

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