Frequency and clinical features of patients with sensorineural hearing loss associated with the A3243G mutation of the mitochondrial DNA in otorhinolaryngic clinics

Abstract The A3243G mutation of the mitochondrial gene is a cause of maternally inherited diabetes and deafness. The aim of this study was to evaluate the frequency and clinical features of this mutation in patients with sensorineural hearing loss (SNHL) in otorhinolaryngic clinics. The frequency of the A3243G mutation in 230 patients with SNHL was 1.74% (4/230). Three of the four patients had diabetes mellitus (DM) and were already aware that they had the mutation. The other had cardiomyopathy but not DM, and proved to have the mutation in this study. The frequency of the mutation was 12.9% (4/31) in patients with a family history of possible maternal inheritance of SNHL, 10.3% (3/29) in patients with DM, and 50% (3/6) in patients with both. The age of onset of SNHL in these patients and their families was between their teens and their forties. The chance of diagnosing the A3243G mutation in patients with SNHL in otorhinolaryngic clinics is probably less than 1%. Association of DM, cardiomyopathy, a family history of possible maternal inheritance of SNHL, and an onset of SNHL between the teens and the forties are signs suggesting the mutation. These signs provide us with a reason for genetic testing for the mutation.

Key words Mitochondrial DNA · Point mutation · Genetic test · Sensorineural hearing loss · Diabetes mellitus · Cardiomyopathy

Introduction

Heteroplasmic mutations of the mitochondrial DNA (mtDNA) are important causes of maternally inherited diseases. Variability in the level of mutant mtDNA in tissues as well as in individuals is believed to explain the diversity of clinical manifestations associated with identical mtDNA mutations. One such mutation potentially associated with various clinical syndromes is the A to G point mutation at nucleotide position 3243 (A3243G) in the mitochondrial tRNALeu(UUR) gene. This mutation was first identified in patients with MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) syndrome (Goto et al. 1990). Subsequent work showed that the identical mutation is also causative for maternally inherited diabetes associated with deafness (Kadowaki et al. 1994; van den Ouweland et al. 1992, 1994), and the name MIDD (maternally inherited diabetes and deafness) was proposed for this syndrome (van den Ouweland et al. 1994, 1995). Furthermore, the A3243G mutation was shown to be a cause of an overlapping syndrome of MERRF (myoclonic epilepsy with ragged-red fibers) and PEO (progressive external ophthalmoplegia) (Verma et al. 1996), PEO (Koga et al. 2000; Pang et al. 1999), Kearns-Sayre syndrome (Pang et al. 1999), Leigh syndrome (Koga et al. 2000), progressive non-diabetic kidney disease (Cheong et al. 1999; Jansen et al. 1997), and cardiac diseases (Shiotani et al. 1998; Yamagata et al. 2000).

Majamaa et al. (1998) studied an adult population of 245,201 individuals, and screened for the A3243G mutation in 480 selected subjects who had one or more of the following diseases: diabetes mellitus (DM), sensorineural hearing impairment, epilepsy, occipital brain infarct, ophthalmoplegia, cerebral white-matter disease, basal-ganglia calcifications, hypertrophic cardiomyopathy, or ataxia. They found the mutation in 11 pedigrees, and clinical evaluation of the probands revealed that the most frequent syndrome consisted of hearing impairment, cognitive decline, and short stature. Damian et al. (1995) studied a large pedigree with the A3243G mutation and analyzed clinical manifestations in 26 cases positive for the mutation but unassociated with stroke-like episodes. They observed hearing impairment in 15 cases, diabetes in 6 cases, nephropathy in 7 cases, mild myopathy in 4 cases, cardiomyopathy in 2 cases, cerebellar diseases in 4 cases, mental retardation in 2 cases, and 8 cases were asymptomatic. Thus, these studies suggest that hear-
ing impairment is probably the most frequent symptom associated with the A3243G mutation.

However, studies on the frequency of patients with the A3243G mutation in otolaryngology clinics are limited (Usami et al. 2000), whereas there have been many studies on the frequency of the mutation in diabetes clinics (Elbein and Hoffman 1996; Fukui et al. 1997; Fukunaga et al. 1997; Holmes-Walker and Boyages 1999; Kadowaki et al. 1994; Katagiri et al. 1994; Newkirk et al. 1997; Odawara et al. 1995; Otabe et al. 1994; Rigoli et al. 1997; Shigemoto et al. 1998; Vionnet et al. 1993). Thus, the significance of this mutation in clinical practice in an otolaryngology clinic appears to have not been determined. The aim of this study is to evaluate the frequency of the A3243G mutation in otolaryngology clinics.

Subjects and methods

Subjects

Two hundred and thirty subjects with bilateral sensorineuronal hearing loss (SNHL) were enrolled for this study. The SNHL in the patients was of unknown etiology, or probably associated with presbycusis in the most senile patients. Patients with other known etiology such as sudden deafness, drug-induced hearing impairment, acoustic trauma, Meniere’s disease, etc., were excluded from this study. All of the subjects were unrelated Japanese residents of the Tokyo area, primarily Chiba Prefecture, adjacent to Tokyo. They were outpatients of otolaryngology clinics of Chiba University Hospital or affiliated hospitals. All patients were subjected to measurement of hearing levels by pure-tone audiometry. The age at onset of hearing impairment was defined as the time when a patient was conscious of hearing loss or the time when it was diagnosed by audiometry. The judgment for association with DM was made on the basis of a history of diagnosis and treatment of DM, or the patients subjected to measurement of hearing levels by pure-tone audiometry. The age at onset of hearing impairment was estimated. This study was approved by the ethics committee of the Graduate School of Medicine, Chiba University.

Analysis of the A3243G mutation of mtDNA

A restriction endonuclease fragment polymorphism of a polymerase chain reaction product (PCR-RFLP) was used for analysis of the A3243G mutation. Genomic DNA extracted from peripheral blood was amplified by polymerase chain reaction (PCR) with primers 5'-ACGAAAGGACAAAGAAATAAGGCC-3' [3125-3149]; all nucleotide positions in the text are according to Anderson et al. (1981)] and 5'-CCACGTGGGGCTTGTGGTAG-3' (3424-3403) to incorporate the A3243G site into the PCR product. PCR was performed with reagents composed of 200nmol/l primers, 150µmol/l dNTPs, 20mmol/l Tris HCl at pH 8.4, 50mmol/l KCl, 1.5mmol/l MgCl2, and 1.5 units Taq DNA polymerase in a 100-µl reaction solution. The program was 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min for 35 cycles. The PCR product was precipitated with 20µg of glycogen (Roche Diagnostic, Mannheim, Germany), dissolved in 35µl of distilled water, and 10µl of the resulting solution was digested with 10U of Apa I (TAKARA, Kyoto, Japan) at 37°C overnight. Digested fragments were separated on a 6% polyacrylamide gel. The size of the PCR product was 300bp, and digested fragments cleaved at the A3243G site were 180bp and 120bp long. PCR products from the wild-type DNA were not cut with Apa I (Fig. 1). The results of the analysis were confirmed by direct sequencing of PCR products positive and negative for the A3243G mutation. In addition, the intensity of bands was assessed with a densitometer (Molecular Dynamics, Sunnyvale, CA, USA), and the percentage of mutant DNA was calculated.

Results

The method used in this study clearly and constantly detected the A3243G mutation when the percentage of mutant DNA was more than 10% in the total mtDNA in peripheral blood leukocytes (data not shown). The frequency of the A3243G mutation of the mtDNA in the 230 subjects with SNHL in our otolaryngology clinics was 1.74% (4/230) (Table 1); the percentage of mutant DNA was 41.1%, 36.1%, 19.0%, and 20.0%, respectively. Lane C is a negative control for the mutation. The size marker is molecular weight marker VIII (Roche Diagnostic, Mannheim, Germany).