Mitochondrial hearing loss mutations among Finnish preterm and term-born infants

Heidi K. Soini,1-3 Minna K. Karjalainen,1-3 Reetta Hinttala,1-4 Arja Rautio,5 Mikko Hallman,1-3 Johanna Uusimaa1-4

1The Research Unit of Pediatrics, Pediatric Neurology, Pediatric Surgery, Child Psychiatry, Dermatology, Clinical Genetics and Obstetrics and Gynecology, Otorhinolaryngology and Ophthalmology (PEDEGO), University of Oulu; 2Medical Research Center, University of Oulu and Oulu University Hospital, Oulu; 3Department of Children and Adolescents, Oulu University Hospital, Oulu; 4Biocenter Oulu, Oulu University, Oulu; 5Faculty of Medicine, Arctic Health and Thule Institute, University of Oulu, Finland

Abstract

Mitochondrial ribosomal 12S subunit gene (MTRNR1) is a hot spot for hearing loss mutations. Mutations such as m.1555A>G, m.1494C>T and m.1095C>T cause sensitivity to aminoglycosides. Aminoglycoside treatment induces permanent hearing loss or deafness in the carriers and should therefore be avoided. The prevalence of these sensitivity mutations varies in different countries and populations. Over 90% of preterm children need aminoglycoside treatment during their first weeks of life. Infants who carry a mitochondrial sensitivity mutation can develop a lifelong sensorineural hearing impairment as a side-effect of aminoglycoside treatment.

Total of 813 Finnish preterm (born <36 gestational weeks, N=624) and term-born (born ≥37 gestational weeks, N=189) infants were genotyped for m.1555A>G, m.1494T>C and m.1095C>T mutations.

The population prevalence of m.1555A>G was determined to be 0.12% in Finland. M.1494C>T and m.1095C>T mutations were absent. Out of the 813 infants, a term-born infant was found to harbor m.1555A>G at 81% heteroplasmia, while his mother’s heteroplasmia was 68%. Both had normal hearing and had not received aminoglycosides. Mothers with a family history of hearing loss who are at risk of preterm labor would benefit from antenatal genotyping of m.1555A>G mutation. The prevalence of m.1555A>G in Finns was close to other European countries. M.1494C>T and m.1095C>T mutations either do not occur in the Finnish population or they are very rare. This study highlights the importance of population-specific genotyping of MTRNR1 aminoglycoside sensitivity mutations, especially in countries with liberal aminoglycoside use.

Introduction

Approximately 10% of all live births are premature, which is the leading cause of perinatal morbidity in the developed world.1 A very common disability among preterm infants is sensorineural type hearing loss or deafness, which is detected in 7% of preterm infants. Important risk factors include the use of aminoglycoside antibiotics in the treatment of infections, noise made by the neonatal intensive care unit (NICU) machines/life support and brain hypoxia.2 Gestational age contributes to hearing loss, as the auditory system remains underdeveloped if a child is born before term.3 Severe hyperbilirubinemia, which affects 80% of preterm infants additionally increases the risk of sensorineural hearing loss. Premature infants’ hearing loss can be progressive or delayed-onset; the child develops hearing loss later by three years of age.4

Mitochondrial 12S ribosomal subunit gene MTRNR1 is a known hotspot for non-syndromic sensorineural hearing loss mutations. In total, over 30 mutations have been reported to cause non-syndromic hearing loss in MTRNR1, such as m.1555A>G, m.1494C>T and m.1095T>C mutations.6,7 Mitochondrial hearing loss is often bilateral, progressive and sensorineural with multiple maternal generations of impaired hearing. Additional neurological symptoms are most of the time absent. Severity of the hearing defect varies from normal/mild hearing impairment to total deafness. The penetrance of m.1555A>G has been estimated to be...
between 28-75%, average being around 60%. Age of onset varies from early childhood to adulthood.9

The prevalence of mitochondrial hearing loss mutations seem to vary considerably from population to population. Frequency of m.1555A>G has been estimated to be higher in Asian countries compared to rest of the world,8,10-13 but it also varies among European countries. The frequency of m.1555A>G in Finnish non-syndromic hearing loss patients has been estimated to be around 2.6%, whereas in Japan it is estimated to be 5% of the hearing impaired and up to 23% among Spanish hearing loss patients. M.1494C>T and m.1095T>C mutation frequencies are similarly reported to be higher among Asian populations, and remain mostly unknown for other populations.14-16

Aminoglycosides are a class of antibiotics designed to treat gram negative bacteria, anaerobic bacilli and mycobacteria by binding to the bacterial ribosome and thus inhibiting protein synthesis.17 Aminoglycosides can also bind to human mitochondrial ribosomes if mitochondrial DNA (mtDNA) mutations are present in the mitochondrial 12S ribosome subunit gene MTRNR1. It has been suggested, that these mutations transform the human mitochondrial ribosome into resembling a bacterial ribosome, enabling the drug molecules to bind to it with higher affinity compared to wild-type human mitochondrial ribosome. Adverse effects of aminoglycosides range from mild gastro-intestinal irritation, acute kidney damage to ototoxic effects on the hearing. Hair cells of the inner ear are abundant in mitochondria, making them very susceptible to the ototoxic effects of aminoglycosides. Aminoglycosides damage hair cells by triggering apoptosis and increasing ROS (reactive oxygen species) production, which in addition activates an oxidative stress reaction in the cell.18 Neurons in the spiral ganglion are also affected. Hearing loss is irreversible and it has been reported to occur in 2 to 25% of patients receiving aminoglycoside treatment.19 A single dose of aminoglycosides for a MTRNR1 mutation carrier can trigger hearing loss at any age,20 but preterm infants and small children are especially vulnerable. Infections and sepsis are common among premature infants, up to 90% of preterm infants will receive aminoglycoside treatment. Aminoglycosides have a low level of antibiotic resistance and are very effective, which is why they are often used to treat preterm infants. Also in countries with less generous aminoglycoside use such as Europe and North America. High noise level in the NICU filled with machines has also been reported to add to the ototoxic effects of aminoglycosides.21

Materials and Methods

Study population

A total of 813 infants born in the Oulu University hospital in Northern Finland were enrolled in the study (Table 1). 624 of the children were born prematurely and 189 were term-born infants. The children were born during 1973 to 2012. Buccal, blood or umbilical cord samples were collected. 93 of the preterm infant samples came from families, which had more than one preterm child. Only one preterm sibling per family was included in the study.

Research ethics

The ethical committee of Oulu University Hospital (PPSHP) approved the study protocol (EETMK: 123/2003) according to the Helsinki Declaration. Each child’s guardian signed an informed, written consent for the child’s participation in the study. Participants were only informed of the findings if it was imperative to their individual health and well being.

Molecular methods

Three MTRNR1 mutations associated with aminoglycoside-induced hearing loss were genotyped from Finnish preterm and term born infants; m.1555A>G, m.1494C>T and m.1095T>C. Genomic DNA was extracted using the UltraClean DNA Blood Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) or UltraClean DNA Blood Spin Isolation Kit (MO BIO) for whole blood samples. Umbilical cord tissue DNA was extracted with the Gentra Puregene Tissue Kit (Qiagen, Hilden, Germany). Chelex 100 (Bio-Rad, Hercules, CA, USA) was used for buccal cell samples. Buccal cell DNA was whole-genome amplified with the Illustra GenomePhip V2 DNA Amplification Kit (GE Healthcare Sciences, Cardiff, USA) following by purification with Illustra Microspin G-50 columns (GE Healthcare Sciences).

MtDNA fragments covering the MTRNR1 variants were amplified in two standard polymerase chain reactions (PCR) using Phire Hot Start II DNA polymerase (ThermoFisher Scientific, Waltham, MA, USA). Standard restriction fragment length polymorphism (RFLP) protocol was used for mutation detection. A 14061 (ThermoFisher Scientific) was used for m.1555A>G detection, Hph1 (ThermoFisher Scientific) for m.1494C>T detection and BspCNI (ThermoFisher Scientific) for m.1095T>C detection.

Mutation heteroplasm quantification was determined using 35S-dATP (Perkin-Elmer, Wellesley, MA, USA) labeled RFLP protocol. The PCR fragment was amplified in the presence of 35S-daATP, digested using Aho26I and electrophoresed through 6% polyacrylamide gel. The intensities of the fragments were then quantified using autoradiography (Quantity One; Bio-Rad, Hercules, CA, USA).

Results

A total of 813 Finnish newborns were genotyped for m.1555A>G, m.1494C>T and m.1095T>C hearing loss mutations in MTRNR1. We discovered a single heteroplasmic m.1555A>G mutation in a term-born infant. This child harbored the mutation at 81% heteroplasm level in his blood. The child’s mother carried m.1555A>G at 68% heteroplasm in her blood (Figure 1). The mother reported no hearing impairment in the child, herself or their immediate family. Unfortunately detailed hearing tests and sibling samples were unavailable. The child harbouring m.1555A>G had passed all the routine hearing test so far; newborn hearing test (otoacoustic emission/automated auditory brainstem response test) and audiometry tests at 5 y and 7 y of age. These were performed by the Finnish municipality child health clinic services. Based on our results, the population frequency of m.1555A>G was estimat-

| Table 1. Study population descriptives. |
|-----------------------------------------|
|                                         |
| Mean gestational age, week              |
| <36 week, n                            |
| 30.3±2.74 (22.7-35.9)                  |
| ≥37 week, n                            |
| 40.1±1.10 (37.9-42.3)                  |
| Birth weight, mean                     |
| Male/female ratio                      |
| 1481 g                                 |
| 103/86                                 |

*Missing gender data for 5 infants.
mtDNA mutation. It has been estimated that up to 1:200 carries a deleterious mutation in the European population has turned out to be much higher than expected. A carrier of m.1555A>G mutation with normal-hearing can suddenly present with sensorineural hearing loss or even total deafness after a single dose of aminoglycosides. It has been proposed that the mutation changes the structure of the mitochondrial ribosome to be more bacterial-like. As a consequence, antibiotic molecules are strongly bound to both bacterial and human mitochondrial ribosomes. This triggers apoptosis and ultimately permanent cell damage in the mitochondria-abundant hair cells of the cochlea. If these MTRNR1 mutations are very prevalent in the population, a number of preterm infants will develop an irreversible disability due to genetic predisposition to aminoglycoside side-effects.

The frequency of pathogenic mtDNA mutations in the European population has turned out to be much higher than expected. It has been estimated that up to 1:200 carries a deleterious mtDNA mutation. The MTRNR1 gene has been noted to drastically increase the susceptibility to aminoglycoside ototoxicity. A carrier of m.1555A>G with normal-hearing can suddenly present with sensorineural hearing loss or even total deafness after a single dose of aminoglycosides. It has been proposed that the mutation changes the structure of the mitochondrial ribosome to be more bacterial-like. As a consequence, antibiotic molecules are strongly bound to both bacterial and human mitochondrial ribosomes. This triggers apoptosis and ultimately permanent cell damage in the mitochondria-abundant hair cells of the cochlea. If these MTRNR1 mutations are very prevalent in the population, a number of preterm infants will develop an irreversible disability due to genetic predisposition to aminoglycoside side-effects.

The estimated 0.12% population frequency of m.1555A>G mutations remained absent in this cohort of Finnish newborns.

### Table 2. Prevalence of m.1555A>G mutation among sensorineural hearing loss patients and populations around the world. Hearing loss patients (H), general population (P).

| Population           | m.1555A>G (P) | m.1095T>C (P) | m.1494C>T (P) |
|----------------------|--------------|---------------|---------------|
| Finland              | 0.12% (P)    | 0% (P)        | 0% (P)        |
|                      | 0.004% (H)   | -             | -             |
| Denmark              | 2.4% (H)     | -             | -             |
| Spain                | 15-20% (H)   | -             | -             |
| Germany              | <0.6% (P)    | -             | -             |
| Hungary              | <0.44% (P)   | -             | -             |
| Poland               | <1.1% (P), 3.6% (H) | -          | -             |
| Greece               | 0.4% (H)     | -             | -             |
| Italy                | 5.4% (H)     | -             | -             |
| United Kingdom       | 0.33% (P)    | -             | -             |
| China                | 0.27% (P), 0.6% (P), 0.024 (P) | -          | -             |
| Japan                | 3.5% (H)     | -             | -             |
| Australia*           | 0.2% (H)     | -             | -             |
| USA                  | 0.23-1.8% (P), 0.9% (H) | -          | -             |
| Brazil               | 0% (P, N=100) | -             | -             |
| Argentina            | 0% (P)       | -             | -             |
| Morocco              | 3.6% (H)     | -             | -             |
| South Africa°        | 0.9% (P)     | 0% (P)        | 0% (P)        |
| GenBank frequency*   | 0.15% (N=57) | 0.12% (N=45) | 0.01% (N=4)   |

P, population; H, hearing impaired. *Australian of European ancestry; °South African black population; #Reported in a single family; August 2015.

**Figure 1.** A) Sequencing electropherogram of m.1555A>G mutation. B) Heteroplasmy analysis of m.1555A>G patient and mother was conducted by 35S-dATP labeled RFLP using Alw26I. The fragment of m.1555A>G mtDNA remains uncut by Alw26I and produces a 319 bp band. The smaller 189 bp digested band is the wild type mtDNA part. 1. Patient 2. Mother.
Mitochondrial mutations (m.1555A>G, m.1494C>T and m.1095T>C) causing aminoglycoside sensitivity were screened among Finnish preterm and term-born infants. This study highlights the importance of population specific screening of hearing loss causing mtDNA mutations to assess the safety of aminoglycoside use.

Conclusions
Mitochondrial mutations (m.1555A>G, m.1494C>T and m.1095T>C) causing aminoglycoside sensitivity were screened among Finnish preterm and term-born infants. This study highlights the importance of population specific screening of hearing loss causing mtDNA mutations to assess the safety of aminoglycoside use.

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