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

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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 life-long 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% heteroplasmacy, while his mother’s heteroplasmacy 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. 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. Gestational age contributes to hearing loss, as the auditory system remains underdeveloped if a child is born before term. 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.

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. 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 (EETTMK: 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).

MiDNA 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. Alw26I (ThermoFisher Scientific) was used for m.1555A>G detection, Hphl (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-dATP, digested using Alw26I 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% heteroplasmacy level in his blood. The child’s mother carried m.1555A>G at 68% heteroplasmacy 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-
ed to be 1:23:1000 in the population (0.5% of term-born infants, 0.12% of whole study population). M.1494C>T and m.1095T>C mutations remained absent in this cohort of Finnish newborns.

Discussion

Despite the well-known ototoxic properties of aminoglycosides, they are regularly used in treating infections in premature infants. Most of the infants born before term in Finland receive aminoglycoside treatment. Mutations in the mitochondrial MTRNR1 gene have 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 human 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 aminoglycoside sensitivity mutations have been reported to occur in varying frequencies among different populations across the globe. The Finns are a genetic outlier population among other European countries, especially Northern Finland with the indigenous Saami people’s genetic influence. Therefore, it is difficult to estimate mtDNA mutation frequencies in the Finnish population based on mtDNA frequencies from other European populations. About half of Finnish hearing-impaired children have a genetic etiology for the hearing disability and in addition, 13% have three or more perinatal risk factors (such as prematurity).

The estimated 0.12% population frequency of m.1555A>G based on the results of this study concurs with the previous estimates among Finnish hearing impaired children and the low mutation frequencies reported in other European populations, such as 0.19% in British children. The absence of m.1095T>C and m.1494C>T in our study can be interpreted that either these mutations are very rare and occur in extremely low numbers or that they do not exist in our population altogether. Similar results have been

### 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).

<table>
<thead>
<tr>
<th>Population</th>
<th>m.1555A&gt;G</th>
<th>m.1095T&gt;C</th>
<th>m.1494C&gt;T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finland</td>
<td>0.12% (P)</td>
<td>0% (P)</td>
<td>0% (P)</td>
</tr>
<tr>
<td>Denmark</td>
<td>2.4% (H)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spain</td>
<td>0.20% (H)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Germany</td>
<td>&lt;0.6% (P)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hungary</td>
<td>&lt;0.4% (P)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Poland</td>
<td>&lt;1.1% (P), 3.6% (H)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Greece</td>
<td>0.4% (H)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Italy</td>
<td>5.4% (H)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>0.33% (P)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>China</td>
<td>0.27% (P)</td>
<td>0.6% (P)</td>
<td>0.024 (P)</td>
</tr>
<tr>
<td>Japan</td>
<td>3.5% (H)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Australia</td>
<td>0.2% (H)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>USA</td>
<td>0.23-1.8% (P), 0.9% (H)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Brazil</td>
<td>0% (P, N=100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Argentina</td>
<td>0% (P)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Morocco</td>
<td>3.6% (H)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>South Africa°</td>
<td>0.9% (P)</td>
<td>0% (P)</td>
<td>0% (P)</td>
</tr>
<tr>
<td>GenBank frequency°</td>
<td>0.15% (N=57)</td>
<td>0.12% (N=45)</td>
<td>0.01% (N=4)</td>
</tr>
</tbody>
</table>

P, population; H, hearing impaired. *Australians 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.
reported in e.g. South Africa.\textsuperscript{16} M.1095T>C and m.1494C>T have mostly been genotyped in the Chinese population, where they occur in low frequencies ranging from 0.024 to 0.6\%\textsuperscript{14,15} (Table 2). Also m.1555A>G has been predominantly reported in Asian countries, such as in China and Japan.\textsuperscript{12,15} Many of the Asian countries have a more liberal aminoglycoside use policy compared to for example Europe and North America. Some aminoglycoside sensitivity mutations have turned out to be surprisingly common in some European populations, such as m.1555A>G among the Spanish hearing impaired (Table 1).\textsuperscript{29}

The m.1555A>G mutation found in this study turned out to be heteroplasmic at 81\% heteroplasm level. Heteroplasmic m.1555A>G families are reported less often, as homoplasy is more commonly observed for this mutation. The severity of hearing loss is interestingly not linked to heteroplasm levels such as in classical mitochondrial diseases, even though the hearing defect phenotype can vary greatly among mutation carriers in the same family. Sensorineural hearing impairment caused by m.1555A>G is very variable and can manifest symptomless or manifest in childhood, adolescence or adulthood. The mean age of onset is 14 years. At the moment, the heteroplasmic family in our study reported no hearing loss. The child is now 10 years old, so either he will continue to have normal hearing throughout his life or the defect will manifest later in life. His mother also reported to be normal-hearing. No audiological testing was done to confirm this by wishes of the family. Nuclear gene variants such as in \textit{TRMU} (rRNA 5-methylaminomethyl-2-thiouriylate methyltransferase) have been suggested to modulate the phenotype, but the results are still inconclusive.\textsuperscript{30}

In this study we genotyped 813 Finnish newborns for three \textit{MTRNR1} hearing loss mutations; m.1555A>G, m.1494C>T and m.1095T>C. We did not find m.1494C>T or m.1095T>C in this cohort, whereas m.1555A>G occurred in 0.12\% of the newborns in Northern Finland, in a term-born child. The risk of irreversible, permanent sensorineural hearing loss among fragile preterm infants is very real. Maternal history of non-syndromic hearing loss should be taken into account before administering aminoglycosides to preterm infants, as treatment and rehabilitation price for a life-long disability far outweighs the price of a genetic test. Mitochondrial DNA hearing loss mutations are present in the Finnish population in small frequencies. It is important to screen these mtDNA mutations globally in different countries, as their frequencies vary greatly among populations: \textit{MTRNR1} hearing loss mutations seem to be more prevalent among Asian ethnicities but also range considerably inside Europe (Table 2). Screening \textit{MTRNR1} and other mtDNA mutations among specific populations can unveil surprisingly common pathogenic and harmful mtDNA mutations, which can then be put into perspective to the rate of aminoglycoside use of the country. Population frequencies of aminoglycoside-induced deafness mutations are especially important to rate in countries with liberal aminoglycoside use. In these countries the mutation carriers are also more likely to be detected among sensorineural hearing loss patients.

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.

References

ototoxicity and targets of hair cell protection. Int J Otolaryngol 2011;937861.