Int J Mol Epidemiol Genet 2014;5(4):200-204
www.ijmeg.org /ISSN:1948-1756/IJMEG0005092

Original Article

Screening for the mitochondrial A1555G mutation among Egyptian patients with non-syndromic, sensorineural hearing loss

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Received December 21, 2014; Accepted December 30, 2014; Epub December 15, 2014; Published December 30, 2014

Abstract: Background & Aim: Hearing loss is the most frequent form of neurosensory deficit in humans. Although the majority of hereditary hearing loss is due to nuclear gene mutations, it has become clear the significant contribution of mitochondrial genes. The first mitochondrial mutation shown to cause non-syndromic hearing loss in humans was the A1555G mutation in the small ribosomal RNA gene (12S rRNA). It has been detected in hundreds of families of different ethnic backgrounds, making it one of the prevalent genetic causes of hearing loss currently identified. However, there are major differences between ethnic groups regarding the frequency of this mutation. Few studies have been made in Arab countries, especially in Egypt. Here we report the prevalence of the mitochondrial mutation A1555G among patients with non-syndromic hearing loss (NSHL) and in healthy individuals with normal hearing in the Egyptian population. Subjects & Methods: The study was conducted on 97 patients with SNHL and 300 unrelated healthy Egyptian individuals, with normal hearing, as normal control subjects. Polymerase chain reaction followed by restriction enzyme digestion was used to screen the DNA samples of all subjects for the A1555G mutation. Results: Participants included 97 cases with SNHL, 46 males and 51 females. Their ages ranged from 1 month to 65 years with the mean age 6.2 years (SD ± 8.2). Paternal consanguinity was reported in 46% (35/76) of the studied families. The A1555G mutation was found in one of the 97 patients (1.3%), while it has not been detected in the 300 control samples. Conclusion: Our findings indicate that, even in absence of exposure to aminoglycosides, the mitochondrial A1555G mutation is one of the potential causes of non-syndromic SNHL in the Egyptian population.

Keywords: 12S rRNA, A1555G, mitochondrial DNA, hearing loss, Egypt

Introduction

Hearing loss is the most frequent form of neurosensory deficit in humans [1]. Thus, it represents a major public health concern due to its high incidence globally. At birth, about 3 per 1000 in developed countries and more than 6 per 1000 in developing countries have hearing problems [2]. The etiology of hearing loss may be either genetic, non-genetic or a combination of both. It is important to note that the presence of an environmental cause does not necessarily exclude the existence of an underlying genetic predisposition. This is true both in early-onset and late-onset hearing losses, where the interaction between environmental and genetic factors is even more complex [3].

It is estimated that at least 60% of hearing impairment is due to a genetic cause [4]. Although the majority of hereditary hearing loss is due to nuclear gene mutations, it has become clear the significant contribution of mitochondrial genes [5]. Various mutations in mtDNA, causing both syndromic and non-syndromic hearing loss, have been identified [6]. The first mitochondrial mutation shown to cause non-syndromic hearing loss in humans was the A1555G mutation in the small ribosomal RNA gene (12S rRNA) [7]. The precise frequency of this mutation is unknown, but it has been detected in hundreds of families of different ethnic backgrounds, making it one of the prevalent genetic causes of hearing loss currently identified [8].

The A1555G mtDNA mutation has been implicated in both non-syndromic and aminoglycoside induced hearing loss. The phenotypic

expression of the A1555G mutation is extremely variable, including cases with normal hearing. Hearing loss, in affected cases, shows variable severity and age of onset; some individuals have congenital deafness, whereas others show a slow, progressive hearing loss of adult-onset [9].

The aim of this study was to report the prevalence of this mutation in patients with non-syndromic sensorineural hearing loss as well as amongst healthy individuals with normal hearing in the Egyptian population.

Materials and methods

Patients and methods

In the period from January 2012 to December 2013, we recruited 97 patients (58 sporadic and 39 familial) from 76 families with bilateral sensorineural hearing loss (SNHL) of varying severity. The patients were selected from those attending the Audiology Clinic of the main University Hospital in Alexandria, and the clinic of the Human Genetics Department, Medical research Institute, Alexandria, Egypt. In addition, three hundred Egyptian normal hearing individuals were included as controls.

The study was approved by the Ethics Committee of the Medical Research Institute, Alexandria University, Egypt. All of the members participating in this study had provided an informed consent form based on the international guidelines outlined by the Declaration of Helsinki.

A complete history was taken from each patient to document the age of onset of hearing loss, parental consanguinity, other cases in the family and the use of ototoxic drugs (aminoglycosides) and also to exclude other environmental causes such as maternal-fetal infections, neonatal jaundice, meningitis and head trauma. Patients were subjected to a thorough clinical evaluation and appropriate investigations were done to exclude syndromic forms of hearing impairment. Assessment of peripheral hearing sensitivity comprised pure tone audiometry or auditory brain stem response, according to the age of the child. The severity of hearing loss was classified as mild (25-40 dB), moderate (41-55 dB), moderately severe (56-70 dB), severe (71-90 dB), or profound (> 90 dB).

Molecular study

Genomic DNA was extracted from whole blood by standard salting out technique [10]. PCR amplification of the region of 12S rRNA gene that encloses the A1555G mutation was done in a Veriti Thermal Cycler (Applied Biosystems), in a total reaction volume of 25 ul. The sequence of the primers used was as follow: forward primer -5'-GCT CAG CCT ATA TAC CGC CAT CTT CAG CAA-3' and reverse primer -5'-TTT CCA GTA CAC TTA CCA TGT TAC GAC TGG-3'. Following amplification, the PCR products were digested by specific restriction enzyme; HaeIII (Fast Digest, Thermoscientific). Then, the digested restriction fragments were resolved on a 3% agarose gel and visualized under UV light [11]. The PCR-RFLP positive results were confirmed by direct sequencing.

Results

The age of the studied patients ranged from 1 month to 65 years with the mean age 6.2 years (SD ± 8.2). The cohort of patients included 46 males (47.4%) and fifty one females (52.6%), with a male to female ratio of about 1:1.1. Paternal consanguinity was reported in 46% (35/76) of the studied families. Prior exposure to aminoglycosides couldn't be determined in the patients' history due to lack of the data about drug intake especially in cases of hospital admission. Fifty eight families (76.3%) had isolated (sporadic cases) where the deaf proband was the only affected individual in the family. For the other eighteen families (23.7%), deafness formed a familial trait. According to the audiologic findings, 6 of the studied patients (6.2%) had moderately severe SNHL, 59 (60.8%) severe SNHL and 32 (33%) profound SNHL.

Only one of the participating patients was found to carry the mtDNA A1555G mutation. This result was confirmed by direct sequencing of the corresponding PCR product (**Figure 1**). The proband harboring A1555G mutation was the offspring of non-consanguineous healthy parents with no family history of SNHL. No history of prior exposure to aminoglycosides was reported. Hearing loss of the proband was prelingual profound bilateral and sensorineural. The family declined further molecular testing of its members. The A1555G mutation was not found in any of the tested 300 control samples.

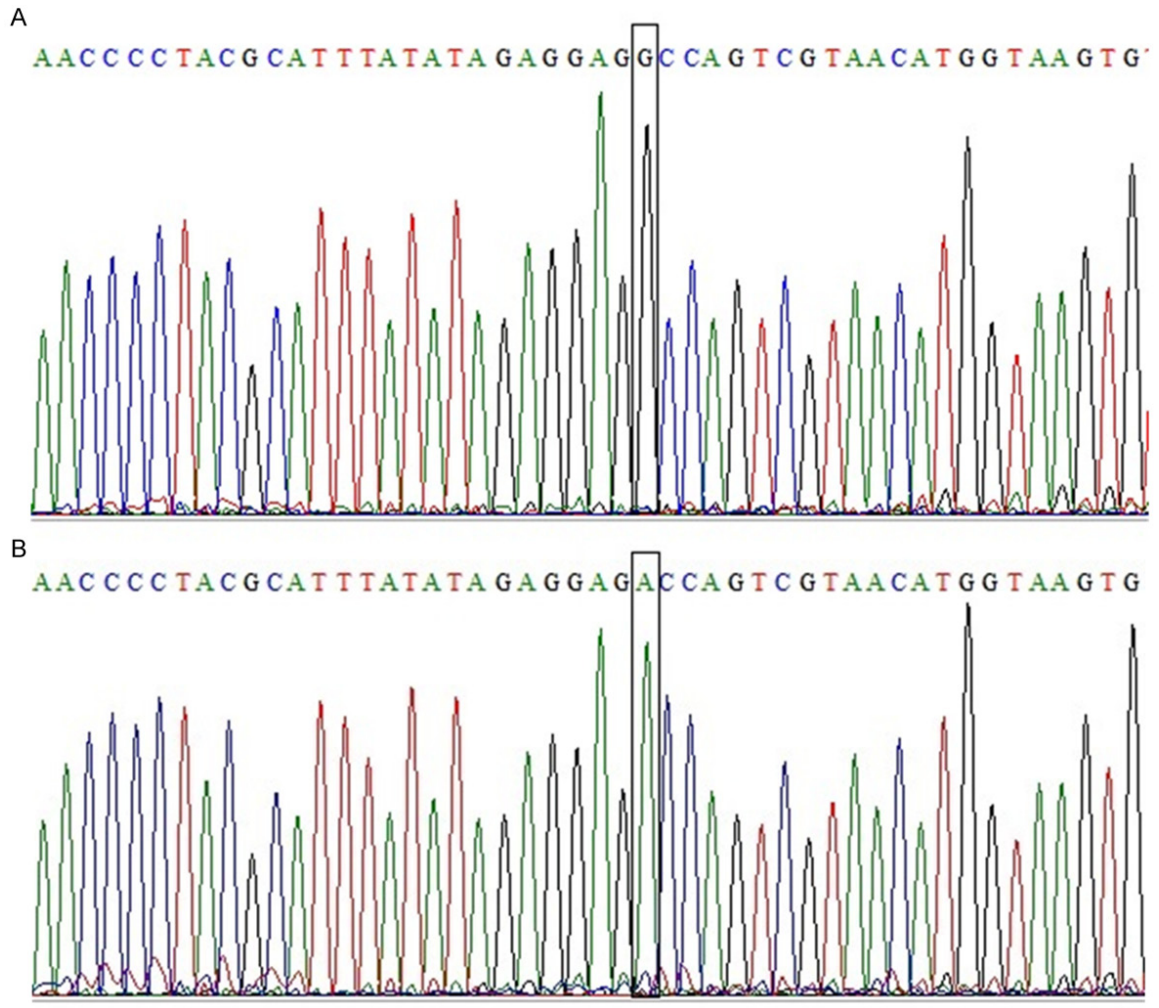

Figure 1. Partial sequence chromatograms of the mitochondrial 12S rRNA gene for a deaf patient carrying the A1555G mutation (A), and a normal hearing individual having a normal allele (B).

Discussion

Deafness is a clinically and genetically heterogeneous disorder with various genetic and environmental causes [12]. The A1555G mutation, which was the first mtDNA mutation identified as a cause of maternally inherited non-syndromic HL, has been associated with several phenotypes from completely normal hearing to profound deafness and has also been implicated in aminoglycoside associated HL [13, 14].

The mtDNA A1555G mutation was first described in a large Arab-Israeli family [7]. Subsequently, it was found in various populations with major differences of its prevalence; ranging from 0 to 27% [14-18]. As revealed by recent studies, the A1555G mutation has been a frequent cause of non-syndromic hearing loss in some Asian populations [15-17]. It is, however, rare or even absent in most of the European and American populations [19-22]. Thus, it seems that there is an ethnic-related variation in the prevalence of this mutation, presumably influenced by the overall mitochondrial genetic background.

In the present study, the A1555G mutation has been detected in only one of the studied patients (1.3%) and in none of the 300 controls, indicating that it might be rather uncommon among Egyptians. To our knowledge, there are no available data regarding the prevalence of the A1555G mutation among the Egyptian population. Therefore, we tried to discuss our results in view of the available reports from different Middle East countries. A quite similar finding was reported from Iran [15] where the

A1555G mutation was detected in 1.3% of the Iranian probands with NSHL, while it was not found in none of the 548 normal hearing controls. Furthermore, Mkaouar-Rebai et al. [23], identified the A1555G mutation in one out of 100 Tunisian families affected with NSHL (1%) but did not detect it in 100 control subject. Tekin et al. [18] reported a slightly higher frequency (1.8%) among children with prelingual SNHL in Turkey, while a recent study reported the occurrence of the A1555G mutation in 3.6% of hearing impaired Moroccan patients [24]. On the other hand, none of the 126 screened Qatari patients with NSHL carried the mutation [25].

Overall, the prevalence of the A1555G mutation in Middle East populations seems to be intermediate between the relatively high prevalence reported from Asian countries and the very low, and even null, frequencies in some Western populations. In fact, this observation, which reflects the halfway geographic location of the Middle East, suggests a gene flow effect from East to West.

The proband carrying the mtDNA A1555G mutation, detected in the current study, was suffering from prelingual profound SNHL with no family history of hearing impairment and was not previously exposed to aminoglycosides. In fact, carriers of A1555G mutation exhibit a wide variety of individual hearing levels, from normal to severe hearing loss [26]. Although the A1555G mutation increases sensitivity to aminoglycoside ototoxicity, it has been reported also in deaf individuals who have not been exposed to these antibiotics [27]. Different hypotheses have been proposed to explain the role of the A1555G mutation as a deafness predisposing mutation [28, 29]. Mitochondrial haplotypes may explain some of the differences between families and ethnic groups, but the variable severity of A1555G-related deafness could be the result of a combined action of other susceptibility and modifier genes with environmental factors [30, 31].

In conclusion, our findings indicate that even in absence of exposure to aminoglycosides, the mitochondrial A1555G mutation is one of the potential causes of non-syndromic SNHL. Despite its low prevalence among the Egyptian population, it should be a part of any genetic screening of hearing loss especially in cases where maternal mode of transmission is apparent.

Disclosure of conflict of interest

None to disclose.

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References

[1] Morton NE. Genetic epidemiology of hearing impairment. Ann N Y Acad Sci 1991; 630: 16-31.

[2] Feldman HM, Salinas MA, Tang BG. Sensory Disorders, in: Elzouki AY, Harfi HA, Nazer H, Oh W, Stapleton FB, Whitley RJ (Eds.). Textbook of Clinical Pediatrics: Springer Berlin; 2012. pp. 602.

[3] Smith RJ, Bale JF Jr, White KR. Sensorineural hearing loss in children. Lancet 2005; 365: 879-890.

[4] Abreu Alves FR, Quintanilha Ribeiro Fde A. Diagnosis routine and approach in genetic sensorineural hearing loss. Braz J Otorhinolaryngol 2007; 73: 412-417.

[5] Finsterer J, Fellinger J. Nuclear and mitochondrial genes mutated in nonsyndromic impaired hearing. Int J Pediatr Otorhinolaryngol 2005; 69: 621-647.

[6] Jacobs HT. Mitochondrial deafness. Ann Med 1997; 29: 483-491.

[7] Prezant TR, Agapian JV, Bohlman MC, Bu X, Oztas S, Qiu WQ, Arnos KS, Cortopassi GA, Jaber L, Rotter JI, et al. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nat Genet 1993; 4: 289-294.

[8] Vivero RJ, Ouyang X, Yan D, Du L, Liu W, Angeli SI, Liu XZ. Mitochondrial DNA mutation screening in an ethnically diverse nonsyndromic deafness cohort. Genet Test Mol Biomarkers 2012; 16: 1146-1148.

[9] Matsunaga T, Kumanomido H, Shiroma M, Ohtsuka A, Asamura K, Usami S. Deafness due to A1555G mitochondrial mutation without use of aminoglycoside. Laryngoscope 2004; 114: 1085-1091.

[10] Salazar LA, Hirata MH, Cavalli SA, Machado MO, Hirata RD. Optimized procedure for DNA isolation from fresh and cryopreserved clotted human blood useful in clinical molecular testing. Clin Chem 1998; 44: 1748-1750.

[11] Estivill X, Govea N, Barcelo E, Badenas C, Romero E, Moral L, Scozzri R, D'Urbano L, Zeviani M, Torroni A. Familial progressive sensorineural deafness is mainly due to the mtDNA A1555G mutation and is enhanced by treatment of aminoglycosides. Am J Hum Genet 1998; 62: 27-35.

[12] Yao GD, Li SX, Chen DL, Feng HQ, Zhao SB, Liu YJ, Guo LL, Yang ZM, Zhang XF, Sun CX, Wang ZH, Zhang WY. Combination of hearing screening and genetic screening for deafness-susceptibility genes in newborns. Exp Ther Med 2014; 7: 218-222.

[13] Guan MX. Mitochondrial 12S rRNA mutations associated with aminoglycoside ototoxicity. Mitochondrion 2011; 11: 237-245.

[14] Casano RA, Bykhovskaya Y, Johnson DF, Hamon M, Torricelli F, Bigozzi M, Fischel-Ghodsian N. Hearing loss due to the mitochondrial A1555G mutation in Italian families. Am J Med Genet 1998; 79: 388-391.

[15] Montazer Zohour M, Tabatabaiefar MA, Dehkordi FA, Farrokhi E, Akbari MT, Chaleshtori MH. Large-scale screening of mitochondrial DNA mutations among Iranian patients with prelingual nonsyndromic hearing impairment. Genet Test Mol Biomarkers 2012; 16: 271-278.

[16] Li Q, Yuan YY, Huang DL, Han DY, Dai P. Rapid screening for the mitochondrial DNA C1494T mutation in a deaf population in China using real-time quantitative PCR. Acta Otolaryngol 2012; 132: 814-818.

[17] Usami S, Abe S, Akita J, Namba A, Shinkawa H, Ishii M, Iwasaki S, Hoshino T, Ito J, Doi K, Kubo T, Nakagawa T, Komiyama S, Tono T, Komune S. Prevalence of mitochondrial gene mutations among hearing impaired patients. J Med Genet 2000; 37: 38-40.

[18] Tekin M, Duman T, Bogoclu G, Incesulu A, Comak E, Fitoz S, Yilmaz E, Ilhan I, Akar N. Frequency of mtDNA A1555G and A7445G mutations among children with prelingual deafness in Turkey. Eur J Pediatr 2003; 162: 154-158.

[19] Dent KM, Kenneson A, Palumbos JC, Maxwell S, Eichwald J, White K, Mao R, Bale JF Jr, Carey JC. Methodology of a multistate study of congenital hearing loss: preliminary data from Utah newborn screening. Am J Med Genet 2004; 125C: 28-34.

[20] Gravina LP, Foncuberta ME, Estrada RC, Barreiro C, Chertkoff L. Carrier frequency of the 35delG and A1555G deafness mutations in the Argentinean population. Impact on the newborn hearing screening. Int J Pediatr Otorhinolaryngol 2007; 71: 639-643.

[21] Maniglia LP, Moreira BC, Silva MA, Piatto VB, Maniglia JV. Screening of the mitochondrial A1555G mutation in patients with sensorineural hearing loss. Braz J Otorhinolaryngol 2008; 74: 731-736.

[22] Hutchin TP, Thompson KR, Parker M, Newton V, Bitner-Glindzicz M, Mueller RF. Prevalence of mitochondrial DNA mutations in childhood/congenital onset non-syndromal sensorineural hearing impairment. J Med Genet 2001; 38: 229-231.

[23] Mkaouar-Rebai E, Tlili A, Masmoudi S, Louhichi N, Charfeddine I, Ben Amor M, Lahmar I, Driss N, Drira M, Ayadi H, Fakhfakh F. Mutational analysis of the mitochondrial 12S rRNA and tRNASer(UCN) genes in Tunisian patients with nonsyndromic hearing loss. Biochem Biophys Res Commun 2006; 340: 1251-1258.

[24] Nahili H, Charif M, Boulouiz R, Bounaceur S, Benrahma H, Abidi O, Chafik A, Rouba H, Kandil M, Barakat A. Prevalence of the mitochondrial A 1555G mutation in Moroccan patients with non-syndromic hearing loss. Int J Pediatr Otorhinolaryngol 2010; 74: 1071-1074.

[25] Khalifa Alkowari M, Girotto G, Abdulhadi K, Dipresa S, Siam R, Najjar N, Badii R, Gasparini P. GJB2 and GJB6 genes and the A1555G mitochondrial mutation are only minor causes of nonsyndromic hearing loss in the Qatari population. Int J Audiol 2012; 51: 81-185.

[26] Usami S, Abe S, Kasai M, Shinkawa H, Moeller B, Kenyon JB, Kimberling WJ. Genetic and clinical features of sensorineural hearing loss associated with the 1555 mitochondrial mutation. Laryngoscope 1997; 107: 483-490.

[27] Fischel-Ghodsian N. Mitochondrial deafness mutations reviewed. Hum Mutat 1999; 13: 261-270.

[28] Bindu LH, Reddy PP. Genetics of aminoglycoside-induced and prelingual non-syndromic mitochondrial hearing impairment: a review. Int J Audiol 2008; 47: 702-707.

[29] Guaran V, Astolfi L, Castiglione A, Simoni E, Olivetto E, Galasso M, Trevisi P, Busi M, Volinia S, Martini A. Association between idiopathic hearing loss and mitochondrial DNA mutations: a study on 169 hearing-impaired subjects. Int J Mol Med 2013; 32: 785-794.

[30] Bravo O, Ballana E, Estivill X. Cochlear alterations in deaf and unaffected subjects carrying the deafness-associated A1555G mutation in the mitochondrial 12S rRNA gene. Biochem Biophys Res Commun 2006; 344: 511-516.

[31] Yang AF, Zheng J, Lv JX, Guan MX. Modifier factors influencing the phenotypic manifestation of the deafness associated mitochondrial DNA mutations. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2011; 28: 165-171.