

BASIC RESEARCH IN OTOLARYNGOLOGY

Mitochondrial DNA (mtDNA) haplotypes and dysfunctions in presbycusis

Correlazioni tra gli aplotipi del DNA mitocondriale (mtDNA) e la presbiacusia

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SUMMARY

The aim of this study was to investigate the presence of mitochondrial DNA (mtDNA) alterations and metabolic dysfunctions in patients with presbycusis, and to discover correlations between presbycusis and the degree of hearing loss and mitochondrial damage. Seventy patients with presbycusis were examined, including 40 Egyptian patients and 30 Italian patients. Forty eight normal subjects were included as control group, including 24 Egyptians and 24 Italians. There was no common point mutation, and A1555G, A3243G, A7445G not were detected in any patients or controls. Haplogroup U was significantly common in patients in comparison to controls. Mutation of antioxidant genes (GSTT1, GSTM1) were significantly present in only Italian patients compared to Italian controls.

KEY WORDS: Presbycusis • Mitochondria • Haplogroup • Antioxidant genes

RIASSUNTO

Lo scopo dello studio è stato quello di indagare la presenza di alterazioni del DNA mitocondriale (mtDNA) in pazienti affetti da presbiacusia e di evidenziare eventuali correlazioni tra la presbiacusia, il grado di deficit uditivo e le alterazioni mitocondriali. Sono stati esaminati settanta pazienti affetti da presbiacusia, tra cui quaranta pazienti egiziani e trenta italiani. Quarantotto soggetti normoacusici sono stati utilizzati come gruppo di controllo, tra cui ventiquattro soggetti egiziani e ventiquattro italiani. In nessun paziente, né nei controlli, abbiamo identificato le mutazioni puntiformi A1555G, A3243G, A7445G. L'aplogruppo U era significativamente più comune nel gruppo di pazienti in confronto al gruppo dei controlli. La mutazione dei geni Antioxidant (GSTT1, GSTM1) sono state riscontrate più comunemente nei pazienti italiani rispetto ai controlli italiani, in maniera significativa.

PAROLE CHIAVE: Presbiacusia • Mitochondria • Aplotipi • Geni antiossidanti

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Introduction

Presbycusis is a complex disorder, influenced by genetic, environmental/lifestyle and stochastic factors. Approximately, 13% of those over age 65 show advanced signs of presbycusis. By the middle of 21st century, the number of people with hearing impairment will have increased by 80%, partly due to an aging population, and partly to the increase of social, military and industrial noise¹.

According to Portmann and Portmann², presbycusis is a biologic phenomenon that no one can escape, starting at 20-30 years of age, and becomes socially bothersome when the person reaches 40-50 years. Early diagnosis and intervention in presbycusis are paramount to provide the elderly with a good quality of life.

Even though all individuals show a steady decline in hearing ability with ageing, there is a large variation in age of onset, severity of hearing loss and progression of disease,

which results in a wide spectrum of pure-tone threshold patterns and word discrimination scores. Presbycusis has always been considered to be an incurable and an unpreventable disorder, thought to be part of the natural process of ageing, but nowadays, it is recognized as a complex disorder, with both environmental and genetic factors contributing to its aetiology. This also means that it is not an inevitable disorder, and presbycusis should be considered as any other complex disease with a possible treatable and/or preventable nature. Scientific research should aim at the elucidation of the contributing factors³. Forward genetic examination is a promising approach. Isolating all the genes in the human genome, as well as identifying and cataloguing the functional variants within them in the human population, will allow assessment of the impact of genotype on phenotypic outcome of interest. Additionally, complementary strategies, based on

functional genomics technology involving microarrays and proteomics, can be used to develop predictors of disease susceptibility based on biological pathways physiologically relevant to presbycusis. Nevertheless, choices in study designs will still be a major factor in the probability of success. Determination of the genetic variants involved in presbycusis should provide new insights into the disorder mechanism, which may uncover new leads for pharmaceutical intervention and could result in the development of screening kits to identify individuals at increased risk⁴.

To date, genetic analysis of presbycusis has been limited. However, with recent major advances in hearing research, the human genome sequence completed and a comprehensive map of human genetic variation (Hap Map) available, together with a growing awareness of the importance of healthy ageing as global populations begin to age, a search for susceptibility alleles for presbycusis can be justifiably undertaken⁵.

Screening of cochlear tissue from blood of patients with presbycusis have identified mtDNA mutations, which could be related to age-related hearing loss. Many mitochondrial mutations cause defects in mitochondria from tissues such as lymphocytes and fibroblasts, yet the only phenotype associated with these mutations is hearing loss⁶. This tissue specificity may be the result of environmental factors. Tissue-specific patterns of gene expression are also likely to influence the effect of mtDNA mutations, with several examples of nuclear background affecting the defect caused by a mutation of the mtDNA⁷. The A3243G mutation, a variant located in the mitochondrial tRNA^{Leu} (UUR) gene and also known as MTTL1, is one of the most frequent heteroplasmic mitochondrial mutation⁸. Individuals carrying the A3243G mutation may manifest either as syndromic or non-syndromic mitochondrial disorders, and others may be asymptomatic. The most frequent syndromic manifestation of this mutation is mitochondrial encephalopathy, lactic acidosis and stroke-like episodes syndrome (MELAS)⁹. The original disorder in which the mutation was found is MELAS, but the majority of patients with the A3243G mutation show other clinical symptoms such as maternally inherited diabetes and deafness (MIDD), progressive external ophthalmoplegia (PEO) or other symptoms which include presbycusis, kidney disease, cardiomyopathy, neuropathy or endocrinopathies different from diabetes¹⁰.

The A7445G mutation, identified as T7445C in some earlier papers, was first described in a Scottish family and confirmed and established in two unrelated pedigrees from New Zealand and Japan¹¹. The penetrance of this mutation in the Scottish pedigree is quite low, while in the New Zealand and Japanese pedigrees is very high. Thus, mutation of A7445G itself may not be sufficient to cause hearing loss, but requires additional genetic or environmental factors, which seem to be rare in the Scottish

pedigree and common in the New Zealand and Japanese pedigrees¹².

The A1555G mutation in the mitochondrial 12S rRNA gene is one of the most common causes of sensorineural hearing loss and aminoglycoside-induced deafness. This mutation was first discovered in a large Arab-Israeli family, and subsequently found in various ethnic groups from Europe, Asia and Africa, with a variable prevalence. In the absence of aminoglycoside exposure, the phenotype observed is extremely variable in terms of the severity of hearing loss and age of onset. Moreover, a significant portion of individuals has normal hearing for their entire life. In many families with the A1555G mutation, a mild and progressive hearing loss occurs even in the absence of exposure to aminoglycosides. The hearing loss in affected cases shows a varied age of onset and severity, whereas others show a slow, progressive hearing loss beginning in their 40s, often preceded by tinnitus¹³.

Mitochondrial DNA (mtDNA) "polymorphisms" are maternally transmitted and typically reflect different ethnic backgrounds. Specific mtDNA polymorphisms have now been classified into a number of specific mitochondrial haplogroups. MtDNA haplogroups, determined by polymorphisms that occurred tens of thousands of years ago, are today high-prevalence population-specific substitutions. mtDNA has been used for a couple of decades as a molecular marker in population genetics¹².

Certain mtDNA haplogroups may increase the risk of presbycusis in some individuals, and mtDNA haplogroups are genetic markers for presbycusis. The mtDNA haplogroups U and K are independent genetic markers for moderate to severe presbycusis and may modify susceptibility associated with some known risk factors, but the precise mechanism underlying how mtDNA haplogroups increase genetic risk for presbycusis remains to be clarified. If these findings are confirmed, introduction of preventive strategies to minimize environmental causes could be implemented to reduce the overall risk of a genetically susceptible individual of developing presbycusis¹⁴.

Enzymes involved in glutathione metabolism (glutathione S-transferase, glutathione peroxidase, glutathione reductase) and enzymes involved in the breakdown of superoxide anions (catalase) have been suggested to be linked with presbycusis¹⁵. Glutathione S-transferase comprises several gene classes including GSTM and GSTT that show genetic variability in humans. Individuals who have null genotypes for GSTM1 and GSTT1 cannot conjugate metabolites specific for these enzymes. These individuals are thus thought to be more prone to damage caused by oxidative stress and possibly more susceptible to presbycusis¹⁶. In this study, the aim was to detect the presence of mtDNA alterations and mitochondrial metabolic dysfunctions in patients with presbycusis, and to discover any correlations between presbycusis and the degree of hearing loss and mitochondrial damage.

Materials and methods

Patients

Seventy patients with presbycusis were examined, including 40 Egyptian patients and 30 Italian patients. The Egyptian patients were examined in the clinic of Audiology Unit, ENT Department, Assiut university Hospital, Egypt. Italian patients were examined in the clinic of the Audiology Unit, ENT Department, Pisa University, Italy. Each patient had to fulfil the following criteria: Age above 40 years; bilateral more or less symmetrical sensorineural hearing loss, pure tone hearing threshold poorer to the normative values per age, determined by international committees; absence of any known cause of sensorineural hearing loss; absence of exposure to ototoxic drugs, otologic diseases, cranial trauma, acoustic trauma, chronic exposure to noise; and absence of any other pathology potentially correlated to sensorineural hearing loss¹⁷.

Forty eight normal individuals were included as control group, including 24 Egyptians and 24 Italians, with the following criteria: age above 40 years; pure tone hearing threshold within normal values per age as determined by international committees.

Informed consent was obtained from personal participants.

Audiological evaluation

All individuals in the study and control groups were subjected to:

- full medical history, including pedigree chart;
- ENT examination;
- pure tone audiometry with speech audiometry and tympanometry with measurement of the stapedial reflex. Of those volunteers who passed medical exclusion criteria, air conduction thresholds were measured at 250, 500, 1000, 2000, 3000, 4000 and 8000 Hz and bone conduction thresholds at 500, 1000, 2000 and

4000 Hz, according to clinical standards (ISO 8253-1, 1989).

All audiograms were categorized according to severity of hearing loss into five categories: mild, moderate, moderately severe, severe and profound hearing loss; according to the mean of thresholds at 500,1000,2000 and 4000 Hz¹⁷ (Table I).

Molecular evaluation

Peripheral blood was obtained from all participants. About 5 ml blood were obtained in suitable EDTA tubes. DNA was isolated from blood using a Nucleospin©Tissue kit (2006). mtDNA point mutations were determined by PCR-RFLP analysis. A set of primers, that amplifies all the tRNA coding regions for screening of A1555G, A3243G and A7445G point mutations were used. Reactions are performed in 25 µl of 10 mM Tris-HCl (pH 8.9) containing 0.4 µM each of the forward and reverse primers, 1.5 mM MgCl₂, 0.2 mM each of dATP, dGTP, and dTTP, 0.02 mM dCTP, 1 µCi of α-32[P] dCTP, and 1.25 U of Taq DNA polymerase (Roche, Indianapolis, IN). PCR conditions were: 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 30 sec, and a final extension step at 72°C for 7 min. Samples were denatured and separated on a 6% MDE polyacrylamide gel (BME, Rockland, ME) with 5% glycerol, according to the manufacturer's protocol. Confirmations of single-stranded DNA are visualized by autoradiography using BioMax film (Kodak, Rochester, NY). Samples with abnormal patterns were directly sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit and a 310 Automatic Sequencer (Applied Biosystems, Foster City, CA).

Eleven mtDNA haplogroups (H, T, J, U, K, V, I, W, X, O, L) were categorized by the presence or absence of the well-defined restriction enzyme recognition sites as determined by PCR and RFLP analysis, in all patients and controls¹⁸, (Revised Cambridge Reference Sequence "rCRS").

Table I. Categories of the audiogram according to configuration.

Category	Definition
Flat	Audiogram where the difference between the mean of 250/500 Hz thresholds, the mean of 1/2 kHz thresholds and the mean of 4/8 kHz thresholds, is less than 15 dB
High frequency gently sloping (HFGS)	Audiogram where the difference between the mean of 500 Hz/1 kHz thresholds and the mean of 4 kHz/8 kHz thresholds is greater than 15 dB and less than 29 dB
High frequency steeply sloping (HFSS)	Audiogram where the difference between the mean of 500 Hz/1 kHz thresholds and the mean of 4 kHz/8 kHz thresholds is greater than 30 dB
Low frequency ascending (LFA)	Audiogram where the difference between the poorer low frequency thresholds and better high frequency ones is greater than 15 dB
Mid frequency U-shape (MFU)	Audiogram where the difference between the poorest thresholds in the mid-frequencies and those at higher and lower frequencies is greater than 15 dB
Mid frequency reverse U-shape (MFRU)	Audiogram where the difference between the best thresholds in the mid-frequencies and those at higher and lower frequencies is greater than 15 dB

The genetic polymorphism analysis for the GSTM1 and GSTT1 genes was determined using the multiplex PCR procedure of Abdel-Rahman¹⁹. Isolated DNA (40 ng) was amplified in a 25 µl reaction mixture containing 25 pmol of each of the following: GSTM1 primers of 5'-GAA CTC CCT GAA AAG CTA AAG C-3', 5'-GTT GGG CTC AAA TAT ACG GTG G-3' and GSTT1 primers of 5'-TTC CTT ACT GGT CCT CAC ATC TC-3', 5'-TCA CCG GAT CAT GGC CAG CA-3'. As an internal control exon 7 of the CYP1A1, genes were co-amplified using the primers 5'-GAA CTG CCA CTT CAG CTG TCT-3' and 5'-CAG CTG CAT TTG GAA GTG CTC-3' in the presence of 200 µmol dNTP (deoxynucleotide triphosphate), 25 µl 10 × PCR buffer, 1.5 mM MgCl₂, and 1 U Taq polymerase. The PCR conditions consisted of an initial melting temperature of 94°C (5 min) followed by 35 cycles of melting (94°C, 2 min) and annealing (59°C, 1 min), and an extension step (72°C) for 10 min terminated the process. The PCR products were then analyzed electrophoretically on an ethidium bromide stained 1.5% agarose gel.

Data were analyzed by using the SPSS-11 statistics programme to detect haplogroup distribution and differences between patient and control groups for point mutations and antioxidant defects.

Results

The study included 70 patients with presbycusis. Forty patients were Egyptian and 30 were Italian. The study included also 48 subjects as a control group; 24 were Egyptian and 24 were Italian.

Thirty-six patients were male, and 34 were female. Twenty-four patients had positive history of consanguinity between their parents, and they were all Egyptian patients. Twenty-nine patients had familial history of presbycusis; 25 were Egyptian, and only four were Italian. Fig. 1 shows distribution of the patient group according to age. Mean (± SD) age for Egyptian patients was 61.2 ± 9.0 years, while the mean age for Italian patients was 69.1 ± 7.5 years. Mean (± SD) age for Egyptian controls was 54 ± 6.8 years, and the mean age for Italian controls was 60.1 ± 7.5 years. There was no statistically significant difference between the mean age in Egyptian and Italian patients.

Audiological results

Patients were classified according to the degree of hearing loss into five categories: mild, moderate, moderately severe, severe and profound hearing loss (Fig. 2). The mean (± SD) hearing threshold for Egyptian patients was 62.7 ± 4.7 dB HL, while the mean hearing threshold for Italian patients was 67.1 ± 8.2 dB HL. There was no statistical significant difference between the mean hearing threshold in Egyptian and Italian patients.

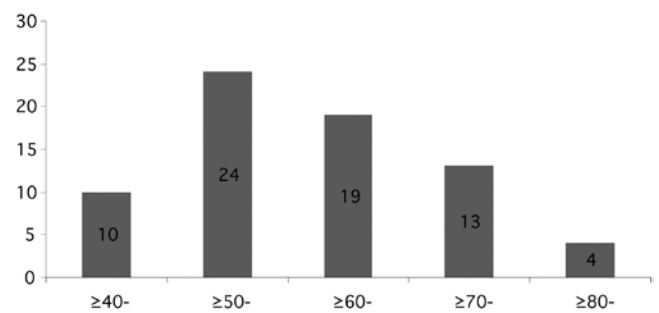


Fig. 1. Distribution of patients according to age.

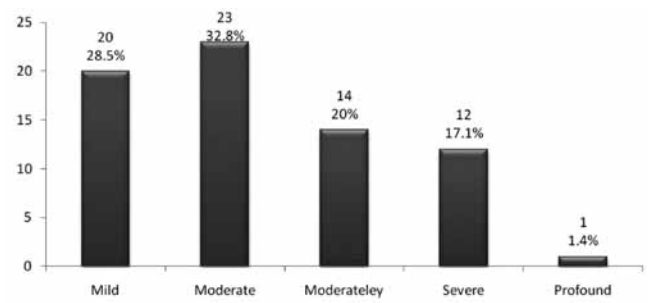


Fig. 2. Classification of patients according to degree of hearing loss.

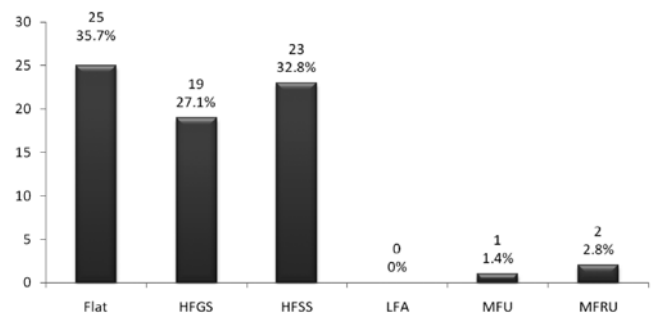


Fig. 3. Classification of patients according to shape of audiogram.

The control groups including Egyptians and Italian subjects had normal peripheral hearing.

Patients were also classified according to the shape of audiograms into five categories: flat, HFGS, HFSS, LFA, MFU and MFRU (Fig. 3).

Molecular results

Point mutations in A1555G, A3243G, and A7445G were not detected in any subject in either the study or control groups.

After identification of haplogroup distribution, the haplogroup U was the most common haplogroup (32.5%) among Egyptian patients, followed by haplogroup H (27.5%), haplogroup I (20%) and haplogroup T (7.5%). In Italian patients, haplogroup H was the most common haplogroup (30%), followed by haplogroup U (23.3%) hap-

Table II. Distribution of haplogroups in the study and control groups.

Group	H	T	J	U	K	V	I	W	X	O	L
Eg patients	11 (27.5%)	3 (7.5%)	1 (2.5%)	13 (32.5)	0 (0%)	0 (0%)	8 (20%)	0 (0%)	1 (2.5%)	2 (5%)	1 (2.5%)
It patients	9 (30%)	4 (13.3%)	1 (3.3%)	7 (23.3%)	0 (0%)	0 (0%)	4 (13.3%)	0 (0%)	1 (3.3%)	4 (13.3%)	0 (0%)
Eg controls	5 (20.8%)	5 (20.8%)	0 (0%)	3 (12.5%)	0 (0%)	0 (0%)	1 (4.1%)	1 (4.1%)	0 (0%)	1 (4.1%)	8 (33.3%)
It controls	14 (58.3%)	2 (8.3%)	0 (0%)	1 (4.1%)	0 (0%)	1 (4.1%)	2 (8.3%)	2 (8.3%)	0 (0%)	2 (8.3%)	0 (0%)

logroups T, I and O (13.3% each). In Egyptian controls, haplogroup L was the most common haplogroup (33.3%), followed by haplogroups H and T (20.8% each) and haplogroup U (12.5%). In Italian controls, haplogroup H was the most common (58.3%), followed by haplogroups T, I, W and O (8.3% each) (Table II).

Genetic polymorphism analysis for the GSTM1 and GSTT1 genes was performed in all patients and control subjects. The polymorphism analyzed in the GSTM1 and GSTT1 genes was the null genotype. For GSTM1, this mutation was identified in 25 patients (35%) (10 Egyptians, 15 Italians). Only four subjects in the control group (8.3%) had this mutation, and they were all Egyptians. There was a statistically significant difference ($p < 0.001$) between the patient and control groups, and between the Italian patient and control groups, but no statistically significant difference was detected between Egyptian patients and control groups (Table III, Fig. 4).

For GSTT1 gene, this mutation was identified in 21 patients (30%) (10 Egyptian patients, 11 Italian patients).

Table III. GSTM1 gene mutation in study groups.

Group	Positive GSTM1 in patients	Positive GSTM1 in controls	p value
Total	25/70 (35%)	4/48 (8.3%)	< 0.001
Egyptian	10/40 (25%)	4/24 (16.6%)	Not significant
Italians	15/30 (50%)	0/24 (0%)	< 0.001

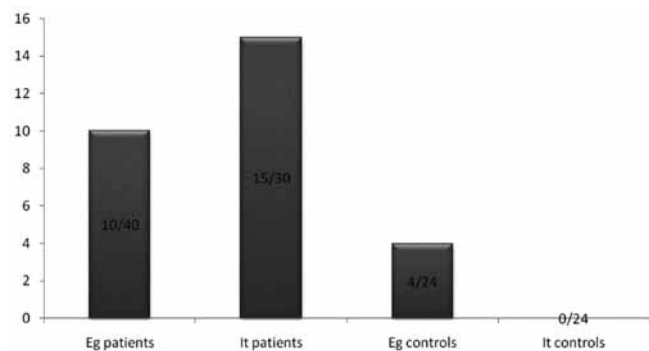


Fig. 4. Genetic polymorphism analysis for GSTM1.

Only nine control individuals (18.75%) had this mutation (six Egyptians and three Italians). There was statistically significant difference between both patient and control groups in total, and between the Italian patient and control groups, but no significant difference was detected between the Egyptian patients and control groups (Table IV, Fig. 5).

Table IV. GSTT1 gene mutation in study groups.

Group	Positive GSTT1 in patients	Positive GSTT1 in controls	p value
Total	21/70 (30%)	9/48 (18.75%)	< 0.001
Egyptians	10/40 (25%)	6/24 (25%)	Not significant
Italians	11/30 (36.6%)	3/24 (12.5%)	< 0.001

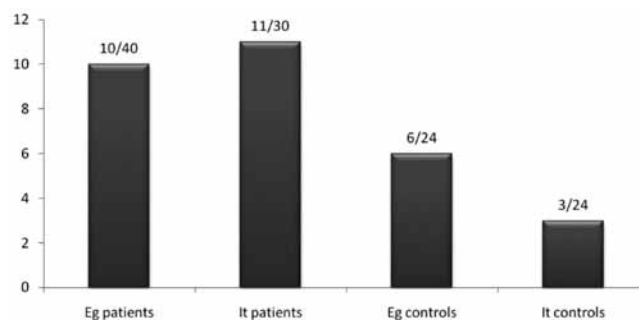


Fig. 5. Genetic polymorphism analysis for GSTT1.

After statistical analysis of the results with a One Way Anova test, no statistical significance was detected between groups (one way Anova > 0.05)

After analysis there was no correlation between separate groups, and no significant correlations were identified between any audiological or molecular variables including age, degree of hearing loss, shape of audiogram, haplogroup distribution, GSTM1 and GSTT1 mutations among Egyptian, Italian and all patients (Table V).

Table V. Correlation between audiological and molecular variables in the study group.

Variable	Age	Degree of hearing loss	Shape of audiogram	Haplogroup distribution	GSTM1 mutation	GSTT1 mutation
Age		NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)
Degree of hearing loss	NS ($p > 0.05$)		NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)
Audiogram shape	NS ($p > 0.05$)	NS ($p > 0.05$)		NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)
Haplogroup distribution	NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)		NS ($p > 0.05$)	NS ($p > 0.05$)
GSTM1 mutation	NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)		NS ($p > 0.05$)
GSTT1 mutations	NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)	NS ($p > 0.05$)	

Discussion

The aim of epidemiological and genetic studies is to identify risk factors for presbycusis, such as environmental factors or genetic factors. Presbycusis is defined as a disorder, often quantified by a certain degree of hearing loss. In genetic terms, any study uses only 'one' phenotype to define the presence/absence of hearing impairment due to ageing, and to determine possible risk factors or causes²⁰. Most patients with presbycusis have mild (28.5%) or moderate (32.8%) SNHL, and the 'flat-configuration' was most dominantly represented (35.7%), followed by HFSS (32.8%), while the 'LFA', 'MFU' and 'MFRU' configurations were very rare (together less than 1%). These data agree with previous studies evaluating audiometric data on presbycusis²¹⁻²³. As animal studies have shown that aging alone does not cause outer hair cell loss, some Authors have emphasized that in humans, typically associated with a HFSS audiometric configuration, this has little to do with age.

It has been well documented that mutations in the 12S rRNA gene are hot spots that are antibiotic-induced and/or associated with presbycusis, and several deafness-associated mtDNA mutations have been identified in this gene. These mutations in a highly conserved decoding site of 12S rRNA could associate with both aminoglycoside treatment and presbycusis hearing loss in families with different ethnic backgrounds. The most common mutations in 12S rRNA causing presbycusis are A1555G, A3243G and A7445G. These gene mutations have also been involved in aminoglycoside-induced hearing impairment¹⁵. The progressive breakdown of mitochondrial function with age might result in presbycusis. Fischel-Ghodsian²⁴ reported mutations in the mitochondrially encoded cytochrome oxidase II gene in the auditory system of five patients with presbycusis with large individual variability in both quantity and cellular location of these mutations.

However in our study, these mutations were not detected in any patient. Recent studies indicate that the phenotypic expression of mtDNA mutations is highly variable, which indicates that these mutations may not be present, and some differences in either the nuclear gene content or activity appears to contribute significantly to the biochemical defect²⁵. Because of heteroplasmy, the proportion of

mutant mtDNA varies in cells/tissues of an individual, and affected organs can be variable in patients with the 12S rRNA gene mutation²⁶.

We found a significantly higher prevalence of presbycusis in subjects with mtDNA haplogroups U and I in Egyptian patients in comparison with Egyptian controls, while haplogroup H was not statistically significant between Egyptian patients and controls. The Egyptian controls had significantly high prevalence of haplogroups L and T compared to Egyptian patients.

In Italian patients, haplogroup H is not statistically greater, while haplogroup U was significantly higher in comparison to Italian controls who had a significantly high prevalence of haplogroup H.

Different mtDNA haplogroups may cause mild deleterious bioenergetic abnormalities. Impairment in mitochondrial function due to mutations in the mitochondrial genome is associated with an insidious decline in physiologic and biochemical performance that contributes to the aging process and to the ultimate death of the organ. There is a growing body of evidence to suggest that presbycusis may be associated with a reduction in mitochondrial function. In keeping with this, mutations in mtDNA and reduced mitochondrial function have been reported in human models of presbycusis²⁷.

Our findings suggest that haplogroup U may be used as a genetic marker for presbycusis susceptibility. Our results further support the concept that certain mtDNA haplogroups may cause mild deleterious bioenergetic abnormalities rather than merely representing the "neutral" polymorphisms reflecting different ethnic backgrounds. It is possible that genetic variants in specific mtDNA haplogroups may impair respiratory chain function within the cochlea to increase the risk of developing presbycusis.

A number of studies have suggested associations between various mtDNA haplogroups and a variety of medical conditions, including Parkinson's disease, Alzheimer's disease, occipital stroke in migraine, Leber hereditary optic neuropathy and multiple sclerosis. Of particular interest, haplogroup U has previously been associated with occipital stroke, azoospermia and Alzheimer's disease²⁸.

In many population studies, the prevalence of mtDNA haplogroup U, reported from studies of European and North American populations^{29,30}, was similar to our data,

suggesting that our results would likely be applicable to other populations.

Several antioxidant enzymes have been demonstrated to be active in the adult cochlea, for instance, enzymes involved in glutathione metabolism and enzymes involved in the breakdown of superoxide anions. The GSTM1 and GSTT1 genes show genetic variability in humans. Genotypes can be classified as null genotypes, heterozygotes, or wild type on the basis of whether they are homozygotes or heterozygotes. Approximately half of the white and black population are homozygotes for the deletion (null genotype). However, the percentage of the population carrying the null genotype varies in different geographic regions and ethnicities³¹. Individuals who are null genotypes for GSTM1 and GSTT1 cannot conjugate metabolites specific for these enzymes. These individuals are thus thought to be more prone to damage caused by oxidative stress and possibly more susceptible to presbycusis³².

The present study highlights the genotypic variability of antioxidant enzymes among different ethnic populations. This study emphasizes ethnic discrepancies and how they relate to presbycusis. These ethnic variabilities may play a role in genotype-phenotype associations, antioxidant enzymes and presbycusis. This study demonstrated an increased risk of presbycusis among Italian subjects carrying the GSTM1 and GSTT1 null genotype, but no increased risk was demonstrated in Egyptian patients. Of clinical importance, recent studies in animals have shown the potential importance of antioxidant enzyme supplementation in the prevention of presbycusis. Bared³³ showed an increased risk of presbycusis among white subjects, and presbycusis was more prevalent in white Hispanics than in white non-Hispanics.

These results are in contrast with previously published data. In a study of 68 white subjects of Turkish descent, Ates¹⁶ did not find a statistically significant correlation between individuals with a GSTM1 or GSTT1 null genotypes and presbycusis. Similarly, Unal³⁴ found no association between the GSTT1 and the GSTM1 null genotypes and presbycusis in white subjects of Turkish descent. However, In contrast to these studies, Bared³³, showed a clinically significant correlation for the development of presbycusis among subjects with the GSTT1 mutant allele and the GSTM1 mutant allele.

In conclusion, our findings suggest that mtDNA haplogroups U are independent genetic markers for presbycusis and may modify susceptibility associated with some known risk factors. Mitochondrial DNA may play a role in the pathogenesis of presbycusis, but our findings need to be corroborated by future studies to confirm the prevalence of mtDNA haplogroups in other populations of individuals affected with presbycusis. The precise mechanism underlying how mtDNA haplogroups increase genetic risk for presbycusis remains to be clarified. If these findings are confirmed, the introduction of preven-

tive strategies to minimize environmental causes could be implemented to reduce the overall risk in a genetically-susceptible individual of developing presbycusis.

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