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ORIGINAL ARTICLE

CONNEXIN 26 MUTATION CORRELATIONS WITH NON-SYNDROMIC SENSORINEURAL HEARING LOSS IN A NIGERIAN POPULATION

Michael C. Ukaegbu[1], Clement Nwawolo[2], Tobechukwu E. Okeke[1], Patricia O. Iheme[3], Jamey P. Mairiga[4]

[1]Department of Biology, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State, [2]Department of Surgery, Lagos University Teaching Hospital, Idi-Araba, [3]Department of Cell Biology and Genetics, University of Lagos, [4]Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria.

ABSTRACT

Background: Connexin 26 mutation is known to cause non-syndromic sensorineural hearing loss (SNHL) in many populations however, mutation differs according to races. The objectives of the study are to determine the distribution of Non-syndromic SNHL by mode of inheritance, severity of hearing loss, frequency of hearing loss, age of onset and also determine the contribution of CX 26 and CX 30 genes mutation in pathogenesis of Non-syndromic SNHL in Lagos State Nigeria.

Materials & Methods: Patients who registered for hearing loss treatment at Ear Nose and Throat unit (ENT) of Lagos University Teaching Hospital (L.U.T.H), Idi-Araba Logos Nigeria from January 01, 2020 to December 31, 2021 were enrolled for the study**.** This study examined the mode of inheritance of non-syndromic SNHL using three generations pedigree. Data on the age, sex, region of the country where the patient comes from, age at onset of hearing loss, number of affected ears and family history of the patients were obtained through a structured questionnaire. Individuals whose hearing loss was as a result of environmental influence were excluded. The frequency and severity of hearing loss was obtained from the pure tone audiometry.

Results: A total of 148 patients (98.7 %) had autosomal recessive mode of inheritance while only 2 patients (1.3 %) had autosomal dominant mode of inheritance. None had neither maternal nor sex-linked mode of inheritance. Among the 102 patients that did audiological evaluations (48 did not undergo audiological evaluations), 43 patients (42.1 %) had moderately severe SNHL, 28 (27.4 %) had severe SNHL, 24 (23.5 %) had profound SNHL, 7 (6.8 %) had moderate SNHL while none had mild SNHL. Fifty four patients (53 %) had high frequency SNHL, 30 (29.4 %) had middle frequency SNHL while 18 (17.6 %) had low frequency SNHL.

Conclusion: The results of the study demonstrate that autosomal recessive mode of inheritance is the commonest mode of inheritance of non syndromic SNHL in the studied population. A novel mutation, Leu 56 His which caused non-syndromic SNHL, was discovered through sequencing.

KEY WORDS: Gap junction beta 2 gene; Connexin 26; Autosomal recessive non syndromic hearing loss; Deafness; Mutation.

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1. INTRODUCTION:

Hearing loss can be assessed by checking the person's hearing level deterioration as a result of some adverse influence. Hearing impairment is a sensory disability that affects millions of people all over the world. Though not life-threatening, it can become a major burden in social and professional life.[1] There are three basic types of hearing loss; conductive hearing loss, sensorineural hearing loss and mixed hearing loss. Sensorineural hearing loss occurs when there is damage to the inner ear (Cochlea), the nerve pathways from the inner ear to the brain and the auditory cortex of the brain.[2] Sensorineural hearing loss may be genetic, non genetic or of unknown causation.[3] About 50% of cases of sensorineural hearing loss

Corresponding Author:

Dr. Michael Chidi Ukaegbu
Department of Biology
Alex Ekwueme Federal University Ndufu-Alike,
Ebonyi State, Nigeria
E-mail: mikey2k71@yahoo.com

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are genetic, 25% non-genetic and 25% of unknown causation.[4] Genetic sensorineural hearing loss is classified as syndromic or non-syndromic. About 30% of genetic hearing loss is syndromic and the remaining 70% is non-syndromic. The inheritance of genetic hearing loss (both syndromic and non syndromic) may be autosomal dominant, autosomal recessive, sex linked or mitochondrial. Within the non syndromic hearing loss group, inheritance is 75-80% autosomal recessive, 20-24% autosomal dominant, 1% sex linked and less than 1% due to mitochondrial inheritance.[5]

According to the Hereditary Hearing Loss Homepage (www.uia.ac.be/dnalab/hhh/), more than 50 genes have been mapped for non-syndromic hearing loss and those that have been characterized at the molecular level, code for proteins of diverse functions. The most common cause of autosomal recessive non-syndromic SNHL, accounting for around 50% of such cases, is mutation in connexin 26 genes. However mutations in this gene differ among races. The most common mutation in connexin 26 gene among people of European ancestry is a deletion of a single guanine from a string of six named 35 Del G. A deletion of thymine on codon 167 referred to as 167 Del T mutations is the most commonly identified mutation in the Ashkenazi Jewish Population. 235 Del C is the most prevalent in Asian populations. In Ghana, a study in a village revealed a homozygous mutation in which thymine was substituted for cytosine in the first codon 143 resulting in a non conservative amino acid exchange of a tryptophan for an arginine residue. This R143W mutation causes a recessive form of sensorineural hearing loss.[6]

Hereditary is a major contributor to neonatal sensorineural hearing loss which affects language and speech development. Genetic counseling brings the need to identify the kind of mutation and its pattern of inheritance in our environment. This study examined mutation and its pattern in a Nigerian environment in two genes, connexion 26 (GJB 2) and connexion 30 (GJB 6) known to be responsible for non-syndromic sensorineural hearing loss; using molecular techniques and sequencing. There is dearth of information on the kind of mutation responsible for Non-syndromic SNHL in Nigeria. The published study of this sort in West Africa was the work of Brobby et al., (6) in Ghana. The mutation discovered in Ghana is frequently ascribed as the commonest form of Non-syndromic sensorineural hearing loss in Africa south of the Sahara. The objective of this study was to determine the distribution of Non-syndromic SNHL by mode of inheritance, severity of hearing loss, frequency of hearing loss and age of onset, and also to determine the contribution of CX 26 and CX 30 genes mutation in Non-syndromic SNHL in Lagos State Nigeria.

2. MATERIALS AND METHOD.

2.1 Study Design/Duration and Data analysis

This study was done on patients who registered for hearing loss treatment at Ear Nose and Throat unit (ENT) of Lagos University Teaching Hospital (L.U.T.H), Idi-Araba Logos, Nigeria from January 01,2020 to December 31,2021 were enrolled for the study. Sampling was done every Tuesday and Thursday of the week; however, systematic random sampling was used for the study. Informed consent was obtained from all the participating subjects; in case of individuals under 18 years old, consent was obtained through their parents or guardians. Participation in the study was entirely voluntary. Questionnaire was administered on the participating members. The work was reviewed by the Institutional Review Board of the Nigerian Institute of Medical Research, Yaba Lagos. One hundred and fifty patients and twenty controls were used for the study.

The work was divided into 3 stages; data and sample collection, analysis of clinical parameters and molecular studies. Structured questionnaire was used to take detailed history for each patient. Details included: Obstetric history, perinatal history, job of parents, address, consanguinity, occupation, age at onset, course and duration of hearing loss, history of repeated ear discharge, starting time of use of hearing aids if present, history of chronic diseases like diabetes and thyroid malfunction, history of drug intake, noise exposure especially the kind and duration of noise, history of trauma, fever or ear operations. Blood samples were collected into ethylene diamine tetra acetic acid (EDTA) tubes. Collected blood samples were kept inside a refrigerator at a temperature of 4^0 C and DNA was extracted from the blood. Blood sample collection was designed to last for twelve months while molecular and data analysis lasted for six months.

Patients were sent to the laboratory for audiological evaluation. This includes: (a) Pure-tone audiometry (PTA), to determine the degree of hearing for both ears. (b) Auditory brain stem response (ABR), to confirm hearing threshold. (c) Otoacoustic emissions (OAEs) and CT scan done in some cases to exclude central auditory system affection. Data taken from audiological evaluations were analyzed using SPSS package version 17. PCR samples were sent to Inquaba Laboratory in South Africa for sequencing. Sequenced probands were aligned with control sequences using CLC Bio Main Workbench software.

DNA extraction was carried out on blood samples using DNA Blood Mini Kit (Zymo Research Tissue and Insect DNA microprep U.S.A), according to manufacturer's instruction.One milliliter of each DNA sample was put into a NanoDrop spectrophotometer (ND1000) and read. Readings were obtained at absorbance of 260 nm and 280 nm wavelengths of UV light. The ratio of the absorbance at 260 nm to 280 nm with values between 1.6 and 1.9 were noted as those with good quality DNA.

3. RESULTS

Mode of inheritance, severity of hearing loss, frequency of hearing loss and age of onset are expressed in figures 1,2,3 and 4 respectively.

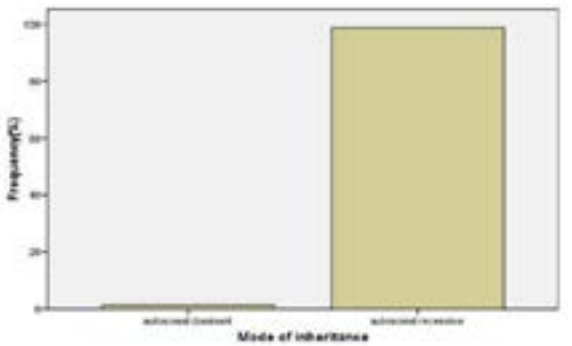

Fig. 1

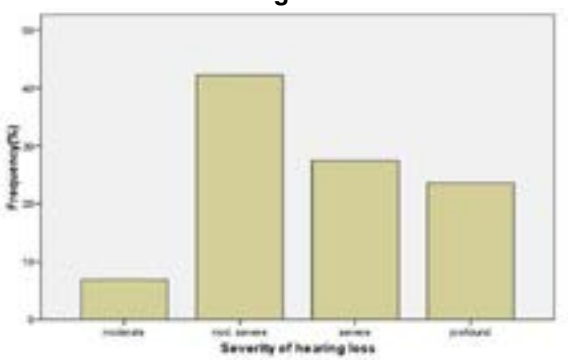

Fig. 2

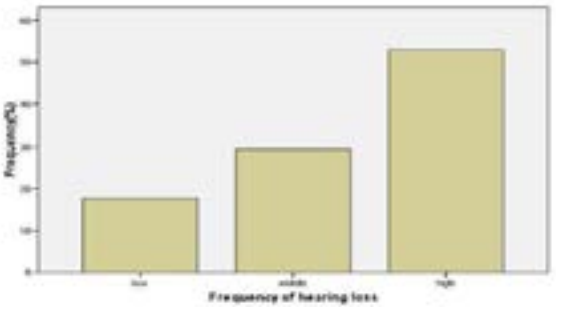

Fig. 3

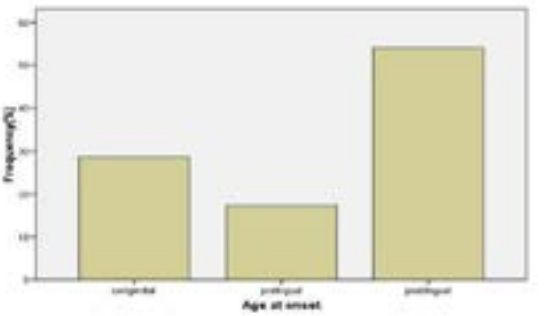

Fig. 4

Figures 1-4. Audiological parameters of probands.

Two mutations del(*GJB6*- D13S1830) and del(GJB6-D13S1854) known to be responsible for non-syndromic SNHL were investigated using multiplex PCR. Homozygous mutation for deletion *GJB6*- D13S1830 would give a band size of 460

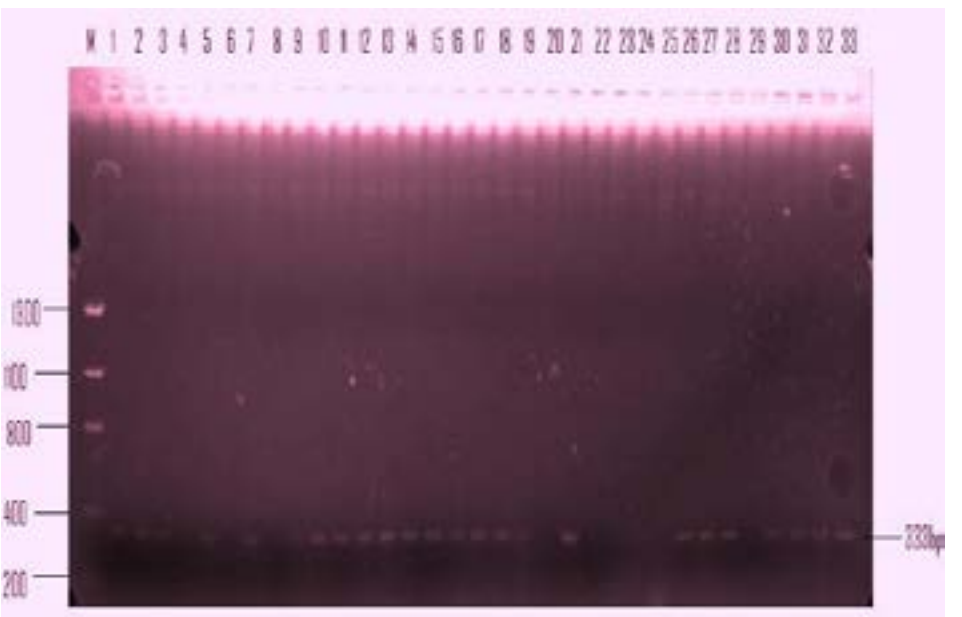

Figure 5. CX 30 multiplex PCR showing wild-type banding pattern. A heterozygous mutation would have shown two banding patterns, one at position 333 bp and the other at either 460 bp or 564 bp. Presence of only 333bp indicates no mutation.

Table 1: Summary of Connexin 26 mutations found in the study.

Proband	Mutation	Nucleotide change	Age at onset	Degree of hearing loss
17	L56H	T to A at 174	Congenital	Profound
31	L56H	T to A at 174	Congenital	Profound
54	L56H	T to A at 174	Congenital	Severe
59	L56H	T to A at 174	Congenital	Profound

base pairs, deletion GJB 6-D13S1854 would give 564 base pairs. Where there is no mutation it would give a band size of 333 base pairs. Heterozygous mutation involving either of the two mutations would give two bands, one at 333 base pairs and the other at either 460 base pairs or 564 base pairs.

During the course of this study amplification was only found at 333 base pairs showing that there was no Connexin 30 gene mutation in the population studied.

4. DISCUSSION

Patients affected with non-syndromic SNHL in Lagos State were evaluated with a view to ascertaining the kind of mutation that could be responsible for their condition. Thus, molecular techniques and sequencing were used to study the effect of mutation on Connexin 26 and Connexin 30 genes and its pattern in our environment. Pedigree analysis shows that 148 patients (98.7 %) had autosomal recessive mode of inheritance while only 2 patients (1.3 %) had autosomal dominant mode of inheritance. None had maternal or sex-linked mode of inheritance. Within the autosomal dominant mode of inheritance, Proband 19 had autosomal dominant late-onset progressive non-syndromic SNHL. Progression of hearing loss was with increasing age. All the affected members at the onset of hearing loss had high-frequency tinnitus. Hearing loss was bilateral. The late uncle who first had this impairment was profoundly hearing impaired before he died. The Proband that are currently 43 years started noticing hearing loss at the age of 31 years while the younger sister had hers manifest at the age of 23 years. Unfortunately we could not sample the sister because she is not resident in Lagos State. In family 2, the father had mild non-syndromic sensorineural hearing loss; however, 2 of his 3 children (male and female) had congenital profound sensorineural hearing loss. Hearing loss was bilateral. Differences in the degree of hearing loss could be due to differences in the degree of penetrance. Maternal inheritance was ruled out because both sexes were affected.

According to the audiometric results in this study, hearing loss was profound in 23% of patients, severe in 27.4%, moderately severe in 42.1% and moderate in 6.8%, with a predominance of high frequencies (4000-8000Hz). In patients who had mutations in GJB2 gene, the profound degrees of hearing loss were predominant (75%) while 25% had severe degree of hearing loss. Similar to this study, previous reports have shown that Connexin 26 gene mutations have a consistent picture of hearing loss: prelingual, bilaterally symmetrical and usually being severe or profound with a wide variability in the extent of hearing loss.[7,8,9] Fifty four percent of the patients had postlingual hearing loss while 46% had prelingual hearing loss. Analogous to other studies patients who had postlingual hearing loss were more than those with prelingual hearing loss. These could be found in figures one to eleven. This suggests a possible contribution of environmental factors to the hearing loss. Hearing loss is typically described as 50% genetic and 50% environmental in nature, involving wide range of both genetic and environmental factors.[10, 11] The incidence of congenital rubella has greatly decreased in more developed countries by the introduction of rubella vaccines in the late 1960s. However congenital rubella syndrome continues to rank as the most important cause of acquired SNHL in countries without a rubella vaccination programme.[10] Also congenital cytomegalovirus infection is generally recognized as one of the most frequent cause of acquired SNHL.[12] Most congenitally infected patients have no apparent signs of cytomegalovirus infection at birth but experience progressive postnatal deterioration in their hearing. It could therefore be adduced that some of the SNHL patients in this study probably had acquired mild congenital progressive hearing loss that does not become severe enough to be detected until early childhood.

For Connexin 26 gene analysis, seventy eight (78) samples were sent out for sequencing. PCR failed in some samples. This could be attributed to the long storage of the samples before they were used for the study. Sequencing was done in both directions. Alignments and analysis were performed using CLC Main Workbench version 6.7.1. Sequence analysis demonstrated that 4 probands had mutations. This gave a prevalence of 5.1 % (4 out of 78). The contribution of Connexin 26 gene mutation in this study is much lower than western populations; 40 % in U.S.A [13], 49 % in Italy[14], 54 % in Russia[5], 36.6 % in Iran[15], 22 % in Germany[7], 17.7 % in India[16]. The high frequency

of mutation in Connexin 26 gene in white population possibly is the result of a founder effect rather than a mutational hot spot. However, Cordeiro-Silva et al.,[17] reported a prevalence of 7.8 % in a Brazilian study and Chalestori et al.[18] had 7.8 % in an Iranian study. The low prevalence in this study could be attributed to the inability to sequence all the samples. It could also be that some of the hearing-impaired Proband had non genetic origin. Surprisingly the Arg 143 Trp mutation reported in most literature to be the most frequent among black Africans but was only identified in Ghana was not found in this study.

To fully assess the relationship between Connexin 26 gene variants and age of onset, genotype data is needed on individuals with congenital, non-congenital prelingual, postlingual and late-onset non-syndromic SNHL. In the absence of new born hearing screening, hearing loss is usually not diagnosed until late infancy or early childhood. Thus in most published studies it is difficult to distinguish between congenital and non-congenital prelingual SNHL. Only four studies have addressed the possibility of an association between Connexin 26 variants and postlingual hearing loss. Three of these studies did not detect any Connexin 26 variants among individuals with post lingual hearing loss. The fourth study which took place in Austria found 4 carriers of Connexin 26 variants among 16 individuals with postlingual hearing loss.[19] This present study detected one Connexin 26 variant in four patients with congenital non-syndromic SNHL.

5. CONCLUSION

The results of the study demonstrate that mutations in the connexin26 gene caused non-syndromic SNHL in the studied population. This highlights the importance of molecular tests for genetic counseling. Although, molecular analysis of hearing loss is not frequent in developing countries, it is essential to investigate connexin mutations for public health and genetic counseling purposes.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	MCU, CN
Acquisition, Analysis or Interpretation of Data:	MCU, CN, TEO, POI, JPM
Manuscript Writing & Approval:	MCU, CN, TEO, POI, JPM

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.