SEDEU JONAS LAURENT GUIEN

CARRIER FREQUENCY OF THE CONNEXIN 26 GENE IN HEALTHY POPULATIONS IN NORTH

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APPROVAL

We certify that we have read the thesis submitted by **SEDEU JONAS LAURENT GUIEN** titled **"CARRIER FREQUENCY OF THE CONNEXIN 26 GENE IN HEALTHY POPULATIONS IN NORTH CYPRUS"** and that in our combined opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Medical Biology & Genetics.

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DECLARATION

I hereby declare that all information, documents, analysis, and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of the Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

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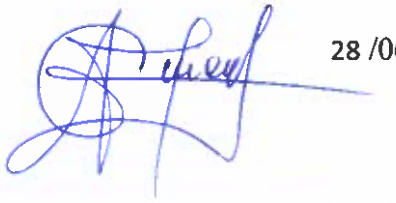

28 /06 /2024

DEDICATION

Father, mother, it is with great joy that I present this book to you; you who are at the origin of my fulfillment.

Thank you my dad GUIEN Gonzleu Jeannot,

Thank you my mom KPEA Makado Julienne.

To my fire Grandfather GUIEN Benoit and Grandmother GUIEN Jeannette.

To my ancestors.

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SEDEU JONAS LAURENT GUIEN

ABSTRACT

Carrier Frequency of The Connexin 26 Gene in Healthy Populations in North Cyprus

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The *GJB2* (connexin-26) gene mutations account for almost 50% of all instances of prelingual recessive hereditary non-syndromic deafness in Europe. The goal of this study is to determine the carrier frequency of congenital deafness causing *GJB2* variation such as these 10 mutations *35DelG, 167delT, M34T, L90P, R184P (G>C), V37I, IVS 1+1 G>A, W24X, 312Del* and *E47X* in Turkish Cypriot population. To develop public awareness for congenital deafness in the population. Hearing impairment is a common condition that impacts around 1 in 1000 children, with roughly 50% of instances being genetically related. Genetic deafness is categorized into syndromic (30%) and non-syndromic (70%) types. Genetic mutations in the *GJB2* gene, which codes for the connexin 26 protein, have been identified as a significant cause of early-onset, sensorineural, and non-syndromic deafness. An evolving natural population is likened to a large family tree, with genetic material passed down through generations according to Mendelian inheritance. Population genetic studies often use present-day genetic variation patterns or changes in allele frequencies to understand the impact of genetic drift, natural selection, and gene flow on evolutionary processes. Structural variation, such as copy number variants in single-copy loci, is increasingly recognized in adaptation and speciation processes. So, it very important for us to understand how mutations in the GJB2 gene affect the hearing function and what are the different variants or change that are normally observed during the disorder with the aid of the Hibrigen DNA isolation kit, genomic DNA was extracted from blood samples obtained from 100 Turkish Cypriot individuals. To find the concentration of deoxyribonucleic acid (DNA), a Nanodrop spectrophotometer is utilized. With the aid of the Connexin multiplex real-time PCR kit (Cat. No.: 100R-20-10) ten mutations on the Connexin 26 gene (*GJB2*) were found: *W24X, 312Del, M34T, 35DelG, R184P (G>C), V37I, IVS 1+1 G>A, L90P*, and *E47X*. Unique fluorescent probes included in the Connexin multiplex Real-Time PCR Kit were used to identify and quantify the amplification of the required DNA sequences. During every cycle of amplification, fluorescence signals were monitored in order to provide real-time PCR data. Data from real-time PCR were used to identify the genotypes of the *GJB2* polymorphisms. The precise *GJB2* allele combinations present in each sample were

identified via genotyping. Utilizing the chi-square test, the genotype distribution and allele frequencies were examined. We looked at deviations from expected genotype frequencies using the Hardy-Weinberg equilibrium and other computed factors. A sample of one hundred healthy individuals with unequal gender distribution participated in this investigation. Together, the average age of the participants is 35.14 years. The average age of the male participants is 36 years old, while the average age of the female participants is 64 years old, or 31.93 years. A statistically significant age gap exists between the genders. The allele frequency varies considerably according to the variant expression. The frequency of alleles equal to 1.000 for both deletions case *35DelG* and *167DelT* and for the others mutations *M34T, 312Del, R184P(G>C), IVS 1+1 (G>A),* and *W24X*. Based on Table 2, these mutations are statistically indistinguishable. This suggests that nearly all members of the population under investigation carry the G and T alleles and the M, T, I, D, R, P, G, A, W, and X alleles, respectively. The allele frequencies for V and I in V37I are 0.97 and 0.03, respectively, while we have the allele frequencies for L and P which are 0.99 and 0.01 in L90P respectively. There may be a considerable degree of genetic resemblance within this specific demographic group because the genotype distribution and allele frequencies of the Turkish Cypriot population closely match the expected genetic distribution. An analysis is conducted to assess and compare the distributions of genotypes and allele frequencies with the anticipated distribution. The observed and projected distributions are not significantly different. The frequency of the G and T allele and M, T, I, D, R, P, G, A, W, X all the allele are expressed respectively in *M34T, 312Del, R184P(G>C), IVS 1+1 (G>A),* and *W24X*, there is no mutation compared to V and I in V37I and L and P in L90P. The connexin 26 gene or *GJB2* in Turkish Cypriot individuals is normal or close to normal.

Keywords: *alleles frequencies, connexin 26 gene, GJB2 gene, genes variants, Hardy-Weinberg equilibrium.*

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List of Abbreviations

DNA……Deoxyribonucleic acid

CNVs……...Copy number variants

SVs……...Structural variations

SNPs……. Single Nucleotide Polymorphism

GWAS…….. Genome-wide Association Studies

dB…… ……decibel

HUGO……..…The Human Genome Organization

WHO……..World Health Organization

NICU…….……..Neonatal Intensive Care Unit

SNHL……. …Sensorineural Hearing Loss

CHL…….….. Congenital conductive hearing loss

UHNS…….…Universal hearing neonatal screening

IHC ……... Inner Hair Cells

OHC……Outer Hair Cells

NBHS ……..…….....Newborn Hearing Screening

AABR ……..… Automated Auditory Brainstem Response

OAEs …….……..…Otoacoustic Emissions

IPOG……International Pediatric Otolaryngology Group

Chapter I

Introduction

Human Genome Organization

The Human Genome Organization (HUGO), established in 1988, coordinates global genomic research and facilitates knowledge dissemination. It promotes collaboration among scientists and institutions, enabling data sharing and innovation in human genetics, contributing to genomic advancements (Liu, 2009). Despite our deep understanding of human gene function, the recent sequencing and assembly of the entire human genome has been more difficult than we first thought (Amaral et al., 2023).

The Human Genome Project, a global initiative, successfully sequenced the human genome and other organisms, using microscopy techniques to reveal subnuclear characteristics and chromosome arrangement (Boninsegna et al., 2022). Since 2001, human genome research has produced a reference sequence for transcription, evolution, genetics, and biological substances, with quality being crucial for medicinal applications due to reduced sequencing costs (Zerbino et al., 2020). Genomes' spatial arrangement and structure significantly influence gene transcription and nuclear processes, with abnormal chromatin folding linked to diseases like cancer and developmental abnormalities, often in noncoding regulatory areas (Mohanta et al., 2021).

The cell nucleus stores, transcribes, and replicates eukaryotic organisms' genetic material, requiring intricate topological structure and compression to fit within its limited space. The genome also ensures precise gene expression programs in specific tissues and cell types (Misteli, 2020). Gene editing technique modifies human DNA, raising concerns about heritable genome editing, which modifies fetal DNA, potentially causing passed-down changes to subsequent generations, a topic of global discussion since 2018 (Soni, 2024)

Human Variations and Populations

Previous human genome-sequencing studies have mainly focused on urban populations, leaving much to be learned about genetic variation, historical population divisions, interbreeding, adaptability, population size changes, and prehistoric gene introductions. More genomic sequences from diverse populations is needed (Bergström et al., 2020). Genome sequences from diverse human groups are crucial for understanding genetic

heterogeneity and historical aspects of different populations. This data can reveal how genetic changes have spread over time, providing insights into ancient human migration and genetic variation, ultimately enhancing our understanding of human evolution (Bergström et al., 2020).

The genetic makeup of individuals is more similar than diverse, with an average of 99.6% identical genomes and 0.4% different ones. Genomic variants, which include single-nucleotide and multiple nucleotide variations, influence an individual's distinctiveness. Factors like diet, environment, lifestyle, and social context also contribute to an individual's uniqueness. Most genomic variants do not affect an individual's genome's operation (Alberts et al., 2002). Genetic association studies primarily focus on Europeans, causing bias in illness risk prediction. This lack of ethnic diversity in human genome studies hinders understanding of disease genetics, amplifies health disparities, and risks translating genetic research into clinical practice or public health policy (Sirugo et al., 2019).

Mendelian disorders are caused by pathogenic mutations, causing disease development in different populations due to allelic heterogeneity, which can complicate diagnosis and treatment processes (Rahit & Tarailo-Graovac, 2020).

Importance of allele frequencies and genotype distribution among populations

The evolution of a natural population is similar to a family tree, with genetic material passed down through generations using Mendelian inheritance principles. Population genetic studies use present-day genetic variation patterns and allele frequencies to understand genetic drift, natural selection, and gene flow (N. Chen et al., 2019).

Scientific research highlights the importance of structural variation in adaptation and speciation processes, with copy number variants (CNVs) in single-copy loci altering phenotypes and preventing allele recombination, but less information exists on their prevalence and distribution in natural populations (Tigano, 2020). For example, over 170 million people are infected with the hepatitis C virus, causing chronic liver disease and potentially cirrhosis liver and hepatocarcinoma. Geographical location affects genotype

distribution, with three patterns identified: high genetic diversity, risk groups, and single subtypes.(Cantaloube et al., 2005)

The effect of human polymorphisms on diseases

Genomic surveys have identified over 80 million autosomal single nucleotide polymorphisms (SNPs), including 8 million common variants, indicating significant genetic variation. Genome-wide association studies (GWAS) have linked SNPs to disease risk, but most are in non-coding regions, limiting understanding of specific genes, cell types, and functional relationships. Epigenomic investigations show that disease risk mutations are more prevalent in cis-regulatory DNA regions (Schmiedel et al., 2018).

Human health is influenced by factors like genetics, behavior, socioeconomic conditions, environment, and healthcare access. However, the exact impact on community health remains uncertain. Environmental and occupational elements, including dietary choices, smoking, alcohol, and drug use, can lead to chronic illnesses, accelerated aging, and reduced life expectancy (Chiarella et al., 2023). Genetic polymorphisms are being studied for their impact on environmental and occupational exposures, with a statistical approach proposed to predict ethnic-specific susceptibilities using public databases (Chiarella et al., 2019).

Single nucleotide polymorphisms (SNPs) are DNA sequence differences caused by changing a single nucleotide. They are crucial in studying the human genome, examining genetic variations' impact on disease susceptibility, treatment sensitivity, and healthcare. SNP databases aid in association studies (Sripichai & Fucharoen, 2007).

Another example of the impact of human polymorphism is, the impact of immune gene polymorphisms on viral infection outcomes, particularly in areas critical for viral clearance and immunopathogenesis, is a major concern (Hashemi et al., 2021).

Congenital Hearing Loss

Congenital hearing loss occurs from birth due to ear impairment in converting sound vibrations into electrical energy for nerve impulses. Classified based on location, it affects the outer or middle ear or inner ear (Capra et al., 2023). Hearing loss is a prevalent neurosensory impairment, affecting children's academic performance, social relationships, and speech and language development. Early detection and personalized treatments can mitigate negative impacts, while untreated conditions can cause delays and lower self-esteem. Prompt identification and treatment improve children's potential (Al-Qahtani et al., 2021).

About 1 in 500 babies experience severe hearing loss by 80 years old, with genetic factors accounting for 50% of cases. Pre-lingual causes account for even more deafness. Non-genetic causes include infections, developmental abnormalities, hypoxia, trauma, and drug use in neonates and early babies (Thorpe & Smith, 2020).

Hearing loss affects 34 million children and 432 million people globally, with one to three per thousand healthy infants and two to four per thousand in high-risk categories having bilateral moderate to severe hearing loss. Early identification and treatment can significantly impact a newborn's development and quality of life (Al-Ani, 2023).

Epidemiology

The WHO reports 34 million children worldwide suffer from hearing loss, with prevalence rates varying across nations and localities. This can be attributed to healthcare systems and universal newborn hearing screening programs. Causes include genetics, environmental influences, aging, inflammation, and a combination of factors. (Zhou et al., 2022).

Hearing impairment affects over 500 million people globally, with normal hearing having thresholds of ≤25 dB. Hearing loss, defined by the WHO, occurs when the average pure tone exceeds 25 dB in the better-hearing ear. Disabling hearing loss is over 40 dB in adults and 30 dB in children (Sheffield & Smith, 2019). Neonatal hearing screening programs

in North America, Europe, and developed countries show a gradual increase in permanent bilateral hearing loss prevalence, likely due to progressive, acquired, or late-onset hereditary factors (Korver et al., 2017a). The prevalence of hearing loss in countries lacking universal neonatal screening programs varies significantly, with high-income countries having higher rates due to different diagnostic methods and risk factors (Neumann et al., 2022).

Hearing impairment is a common sensory issue in Mexican babies, with over 50% of cases linked to genetics and 30% to syndromic entities. Research shows that pathogenic variations are most frequently associated with the *GJB2* gene, with additional mutations found in *GJB6, SLC26A4*, and *CHD23*. Initial screening of these genes could be a potential diagnostic approach (Torre-González et al., 2022).

Risk Factors

A study in China found that risk factors like craniofacial deformities, NICU admission, family history, and advanced maternal age may be linked to congenital hearing loss, sensorineural hearing loss (SNHL), and congenital conductive hearing loss (CHL), potentially contributing to its development (Zhou et al., 2022). Permanent childhood hearing problems are conditions where both ears experience over 40 dBHL on average in the frequency range of 0.5, 1, 2, and 4 kHz. Caused by genetic and environmental factors, they affect 7% of the global population.

Newborns admitted to the NICU are 10-20 times more likely to have permanent congenital hearing loss, affecting cognitive and language growth during the first 36 months (Alhazmi, 2023). Universal hearing neonatal screening (UHNS) effectively identifies and treats hearing loss in newborns. Risk factors include a family history of deafness (47%), syndromic deafness (41%), intrauterine growth retardation or preterm (19.7%), and prolonged NICU stay (18%). These factors account for 74% of cases, with probable hereditary deafness accounting for most congenital hearing loss cases (Paul et al., 2024).

The Joint Committee on Infant Hearing revised its position statement in 2007 to identify risk factors for delayed hearing loss in newborns, including hearing loss-associated syndrome, caregiver concerns, family history, neonatal intensive care, infections, craniofacial anomalies, neurodegenerative disorders, postnatal infections, head trauma, and chemotherapy (Appelbaum et al., 2018).

Pathophysiology

Congenital hearing loss can be inherited or acquired, with genetic variants often caused by gene mutations. Acquired hearing loss can occur later in life due to exposure to loud noise, infections, or ototoxic drugs. Understanding these types is crucial for effective therapy (Renauld & Basch, 2021).

Genetic alterations can affect the auditory pathway, with syndromic hearing loss often associated with a specific syndrome. Non-syndromic hearing loss is identified using prefixes like DFNA, DFNB, and DFNX. For instance, DFNB1 is associated with autosomal recessive non-syndromic hearing loss, while DFNB4 is associated with auditory impairment in Pendred syndrome. Understanding the genetic basis of hearing loss can aid in diagnosis and treatment (Korver et al., 2017). The inner ear, responsible for balance and hearing, has a complex structure for auditory processing. Hearing relies on peripheral and central pathways, and understanding the auditory system's structure is crucial for understanding hearing loss diseases (Capra et al., 2023).

The human cochlea, a hard shell, converts sound into electrical impulses. Pregnancy alters the immune system, making pregnant women more susceptible to infections. Viral infections can cause sensorineural hearing loss through direct cytotoxic effects or indirect tissue damage (Casale et al., 2024). The cochlea is a hearing organ that converts sound energy into electrical signals for the brain. It is a spiral-shaped tube filled with fluid, consisting of three chambers: vestibular ramp, tympanic ramp, and cochlear duct. The scala media houses the endolymph, a liquid with high K+ levels, and the perilymph, which has elevated Na+ levels (Rask-Andersen et al., 2012).

Hair cells, found in the Corti organ, are specialized mechano-sensors in the cochlea. There are two categories: inner hair cells (IHC) and outer hair cells (OHC). IHCs transmit signals through the auditory nerve, with variations in nerve terminals and synapses (Driver & Kelley, 2020). Synapses between nerve fibers and inner hair cells remain intact across species. Efferent terminals terminate at dendritic terminals, while outer hair cells (OHCs) act as mechanical amplifiers, increasing cochlea noise volume. Efferent inputs regulate these functions (Caprara & Peng, 2022).

The Organ of Corti converts mechanical vibrations into electrical signals, affecting the cochlear frequency. Its central pillar is the bone-like modiolus, with a spiral lamina protruding into the bony canal. The spiral ganglion, a group of nerve cell bodies, transmits information from the cochlea to the brain (White et al., 2024). The *GJB2* gene, encoding the connexin26 protein, is responsible for 50% of non-syndromic hearing loss. With 340 mutations, most result in recessive non-syndromic deafness, specifically DFNB1A. Mutations affect protein expression, channel features, and gap junction plaque formation (P. Chen et al., 2022).

Genetic congenital hearing loss

The genetic cause of congenital hearing loss is determined by determining if it is a syndromic presentation or an isolated characteristic. Examples include Usher, Pendred, Waardenburg, and Norrie syndromes. Most children's hearing loss is caused by hereditary factors, most of which are single gene defects. It is classed as either syndromic or non-syndromic, with autosomal recessive nonsyndromic accounting for 80% of instances and autosomal dominant nonsyndromic accounting for 20% (Korver et al., 2017).

Hearing loss is a manifestation of a pathological alteration in the auditory system, with genetic factors responsible for 80% of cases before language development. Identifying the genetic origin provides insights into prognosis, non-syndromic and syndromic hearing loss, habilitation options, therapies, and genetic counseling to predict recurrence (Sanjuán & Domingo-Calap, 2021). Hearing loss affects 0.1-0.2% of neonates and is primarily caused by genetic alterations in the *GJB2* gene. Over 100 harmful mutations have been

discovered, leading to hearing loss. The most prevalent mutation among Caucasians is c.35 delG, while other prevalent mutations, such as c.235delC in Japan and c.167delT in Ashkenazi Jews, are more widespread in other ethnic populations (Koohiyan, 2019).

Acquired congenital hearing loss

Acquired hearing loss is a common form of auditory impairment caused by damage to the inner ear, which converts sound waves into electrical signals. The cochlea contains hair cells that cannot regenerate, resulting in irreversible damage and the development of hearing impairment (Podury et al., 2023). Hearing loss in children is primarily caused by acquired factors like infections, postnatal delivery issues, ototoxic drugs, and autoimmune illnesses.

The World Health Organization attributes 31% of hearing loss to these, while preventable causes include trauma, infection, and ototoxic drugs. (Lieu et al., 2020). Identifying the cause of deafness can provide predictive insights, aid in rehabilitation, and improve patient prognosis. Hearing loss can be prelingual, occurring before speech development, or post lingual, occurring gradually after normal speech development.

Hearing loss is caused by infections, illnesses, trauma, and aging. Post lingual deafness may not impair communicative abilities (Božanić Urbančič et al., 2020).

Diagnosis, screening, and prevention

Clinical phenotyping and family history are crucial for identifying potential genes in hearing loss illnesses. However, the correlation between genotype and phenotype is not uniform, and multiple genes can contribute to disorders. Gene panels are used to evaluate multiple genes or variations in a single analysis due to diverse physical and genetic characteristics (McDermott et al., 2019). The physiological newborn hearing screening (NBHS) method uses a portable automated auditory brainstem response (AABR) or otoacoustic emissions (OAEs) for initial screening.

If abnormal, a formal ABR test is conducted. The International Pediatric Otolaryngology Group recommends both tests for children with hearing loss risk factors. In 2017, over

98% of US babies had screening, and the median age for severe-to-profound hearing loss diagnosis decreased from 2 years to 2 months (Thorpe & Smith, 2020). The International Pediatric Otolaryngology Group (IPOG) released guidelines in 2016 for evaluating pediatric hearing loss, including sensorineural, conductive, mixed, and auditory neuropathy spectrum disorders. For sensorineural hearing loss, CMV testing is recommended, while genetic testing is recommended for both ears. Temporal bone imaging is recommended for one ear (Qian et al., 2021).

The Connexin 26 gene (*GJB2*)

The *GJB2* gene encodes connexin 26, also known as gap junction beta 2, which forms channels for nutrient, ion, and signaling movement between cells. The specific connexin proteins determine the channel dimensions and particles. (Zappalà et al., 2023). Connexin 26, a protein found in the inner ear, plays a crucial role in hearing by regulating the concentration of potassium ions within the cochlea. This protein is essential for the development of certain cells in the cochlea, which is a snail-shaped structure in the inner ear (Mammano, 2019). Hearing loss is a common and genetically diverse sensory impairment, affecting millions globally. In infants, it occurs 1 in 1000, with 50% caused by genetic abnormalities.

It can be categorized into syndromic and non-syndromic forms, with syndromic disorders affecting organ systems and non-syndromic disorders affecting the inner ear. Autosomal recessive non-syndromic hearing loss (ARNSHL) is the most prevalent type (Mishra et al., 2018). Over 200 genes, including Connexin 26, are linked to hearing loss in humans, with over 90 identified. *GJB2*, the gene responsible for Autosomal Recessive Non-Syndromic Hearing Loss, is responsible for up to 50% of pre-lingual hearing loss cases. Over 70 distinct mutations have been reported, with the c.35delG mutation being the most prevalent globally (Hegde et al., 2021).

Gap junctions (GJs) are crucial for the movement of ions, metabolites, and signaling molecules across cells' cytoplasm. Connexin 26, produced by the *GJB2* gene, plays a crucial role in GJ formation in epithelial organs like the inner ear and palmoplantar

epidermis. Its transmembrane structure consists of four domains (DeMille et al., 2018). Non-syndromic hearing loss (NSHL) is primarily autosomal recessive, affecting 75% to 85% of cases. Over 100 genes are associated with NSHL, with the gap junction protein connexin 26 (*GJB2*) having the highest mutation frequency. *GJB2* creates gap junctions near cells, affecting up to 50% of individuals from different populations (Al-janabi et al., 2021). Non-syndromic hearing loss (NSHL) is a genetic disorder affecting two-thirds of congenital hearing loss cases. It is caused by a single gene mutation and has a high degree of genetic diversity.

The *GJB2* gene has over 200 variants, with the 35delG mutation responsible for 60% to 70% of all mutations in Northern and Southern European and American Caucasian populations. The 35delG mutation is most prevalent in Mediterranean, European, and Turkish populations (Geden & Seneldir, 2023).

Structure

Human connexin 26, a protein with a molecular weight of 23 to 62 kDa, is one of 21 members with 226 amino acids. It is divided into five subgroups and shares structural features with other connexins. It forms intercellular channels and plasma membrane gap junctional plaques (Posukh et al., 2023). The *GJB2* gene, located on chromosome 13q12, encodes the Cx26 protein, which consists of four transmembrane domains, two extracellular loops, an amino-terminal domain, a cytoplasmic loop, and a carboxy-terminal domain.

The protein's cytoplasmic loop and N-terminus control voltage and ions flow, regulating channel selectivity. The gene's structure is straightforward, with exon 1 located 3179 base pairs away from exon 2 (Iossa et al., 2011). The *GJB2* gene, located on chromosome 13q12, produces Connexin 26 (Cx26), a protein with 226 amino acids. Cx26 has four transmembrane domains, two extracellular domains, and three intracellular domains. Connexons, also known as hemichannels, can be homomeric or heteromeric and are transported to the cell surface for connection (Mao et al., 2023). The human Cx26 gene, which is conserved and essential for maintaining hemostasis, is involved in cell

proliferation, differentiation, and development, as its elimination during embryonic development results in death in mice. (Mao et al., 2023). Hereditary hearing loss is primarily caused by mutations in the Gap Junction Beta 2 (*GJB2*) gene, which produces gap junction proteins like connexin 26, which maintain potassium ion balance in the inner ear. Non-syndromic deafness accounts for 75% of cases. Mutations 35delG and Cys169Tyr disrupt the protein structure, causing truncated Cx26 and disrupting the protein structure (Missoum, 2018).

Function

The *GJB2* gene, involved in the etiology of hearing impairment is responsible from connexin 26 coding. Mutations in this gap junction protein cause most deafness. The gene has two exons, but only exon 2 codes for Cx26. Sound waves from the outer ear contact the cochlea, were hair cells sense vibrations. Connexin 26 gap junctions allow potassium ions to enter hair cells, sending an electrical impulse to the auditory nerve (Maslova et al., 2021). Gap junctions are areas of the cell membrane with intercellular communication channels, facilitating the transfer of molecules like ions, metabolites, nucleotides, and peptides. These channels are created when connexons dock end-to-end, playing a crucial role in biological processes like development, differentiation, and immunological responses. Connexin mutations can cause disorders (Maeda et al., 2009).

Connexins, the building blocks of gap junctions, mediate potassium circulation in the cochlea, ensuring high potassium levels for auditory physiology. *GJB2* and *GJB6* genes produce most connexins in cochlear supporting cells, responsible for human connexin 26 and 30 proteins. Autosomal recessive changes in CX26 are found in NSHL populations (Su et al., 2020). Connexin, found in the stria vascularis and supporting cells of the organ of Corti, acts as undocked hemichannels and GJ channels for cell signal transmission. It produces endo-cochlear potential and endolymph, and its expression patterns suggest vast interconnected networks. Cx26 is expressed on supporting cells' surfaces in areas lacking cell-cell connections (Verselis, 2019). Connexins, the molecules that form gap junctions, consist of four transmembrane segments, unstructured C and N terminals, one cytoplasmic loop, and two extracellular loops. Humans have 21 connexins, with 19

shared. Their molecular weight determines their nomenclature, and they are expressed in almost every cell in the human body. Subtypes are expressed limitedly and determined by anatomical site (Yamasaki, 2018). Connexins act as gap junction channels, allowing ions and molecules to flow between cells in multicellular animals. They are expressed in tissue- or cell-type-specific patterns, with specific protein domain sequences affecting oligomerization (Posukh et al., 2023).

Pathogenic variations in the *GJB2* gene

Genetic deafness is often caused by harmful mutations in the *GJB2* gene, causing various clinical effects like type 1A, type 3A, and syndromic forms that combine hearing loss with skin disorders (Posukh et al., 2023). *GJB2* gene mutations cause severe to profound hereditary hearing loss in various populations. In several studies, GJB2 gene mutations have been linked to hearing loss (Bouzaher et al., 2020). Genetic variations in *GJB2* contribute to genetically inherited hearing loss in various regions, including Europe, the Middle East, East Asia, Southeast Asia, India, Ashkenazi Jews, and Ghana, but their exact extent remains unclear (Bouzaher et al., 2020). *GJB2* pathogenic variants cause various phenotypes, including autosomal recessive non-syndromic hearing loss (DFNB1A), a common form due to a mutant Cx26 form.

Gain-of-function variations often display hyperactive connexons, leaky hemi-channels, and gap junctions. Other variants may result in syndromic skin conditions or autosomal dominant diseases (Xiang et al., 2023). Biallelic missense mutations in the *GJB2* gene cause varying auditory thresholds in individuals. Despite ongoing controversy due to technical discrepancies, the pathogenicity of R143W variants has been established, leading to non-syndromic hearing impairment in Ghana. Hearing loss is linked to N206S and N206T variants (Namba et al., 2024).

Treatment strategies and *GJB2*

Gene therapy is a promising treatment for genetic abnormalities, particularly hereditary hearing loss (HHL), a prevalent form of deafness. Research is increasing to explore the potential of gene therapy in treating HHL, with clinical trials and CRISPR-Cas tools being explored (Jiang et al.,

2023). Advancements in understanding the genetic basis of deafness have identified around 150 genes. However, there is still a lack of clinical procedures to restore normal hearing. Genetic treatment aims to alter, replace, or restore genetic elements responsible for disorders. Improvements in technology have facilitated the rapid growth of gene therapy for hereditary hearing loss (Hahn & Avraham, 2023).

Chapter II

Methodology

Research Design

This research is a retrospective descriptive cohort study.

Data collection tool

This study is conducted to obtain comprehensive data on connexin 26 gene in the healthy population north Cyprus.

Study Group

This study includes 64 females and 36 males from the Healthy Population in Northern Cyprus, the study group consisted of 100 participants for the research.

Reagents and Instrument

SNP Biotechnology R&D Ltd., Ankara, Turkey; Hibrigen Biotechnology, Gebze/Kocaeli, Turkey); Warfarin Real-Time PCR Kit (Cat. No: 13r-10-03); Real-time PCR equipment (HiMedia, Mumbai, India).

Collection of Samples:

The approval for the study's protocol had been given by the Near East University Ethics Committee. Before any samples were taken, the participants' informed consent was acquired. Clear information on the protocol, purpose, and potential benefits and dangers of the study was received by participants. An opportunity was given to the attendees to clarify any concerns they might have and ask questions.

Venous blood samples were taken from 100 Turkish Cypriots between April and June using a sterile venipuncture procedure. Appropriate blood collection tubes containing anticoagulants like EDTA were used to avoid blood clotting. Each sample was labeled with

the initials of the participant. In order to prevent contamination, blood samples were carefully handled. Blood samples were kept at +4°C and -20°C.

Method

Isolation of DNA

Hibrigen DNA Isolation Kit was used to isolated the DNA from white blood cells.
This involved cell lysis with a lysis buffer, followed by protein precipitation and removal.

Wash buffers were used to eliminate contaminants after genomic DNA was bound with a binding buffer to a silica-based membrane on a spin column. After being stored at either -20°C or -80°C, the final pure genomic DNA was examined with a spectrophotometer to determine its concentration and purity.

Measuring DNA Concentration

Nanodrop spectrophotometer was used to measure DNA concentration. The absorbance levels were measured at 260 and 280 nm wavelengths.

Preparation of PCR Reaction Mixes

Connexin multiplex real time PCR kit (Cat. No: 100R-20-10) was used to detect ten mutations on the Connexin 26 gene (*GJB2); 35DelG, 167delT, M34T, L90P, R184P (G>C), V37I, IVS 1+1 G>A, W24X, 312Del* and *E47X*.

Wild type and mutant Real-Time Master mixes were used to test each extracted DNA sample. The system offers reagents in a master-mix format that is ready for use and has been specially designed for 5' nuclease PCR utilizing proprietary SNP analysis. Sequence-specific primers and probes are intended for use with the test system.
For mutation analysis, FAM was used as the fluorescence, and HEX/JOE dye was also used to tag the internal control present in every master mix. A different set of tubes was ready for each combination. The master mixtures were carefully pipetted together before the work was started.. Using micropipettes fitted with sterile filter tips, 10 µl of the master mix was meticulously dispensed into each optical white strip or tube in each sample. 2. 5

µl of DNA was then added to each tube, and the experiment was carried out according to the prescribed protocol.

PCR Amplification and Genotyping

Following the guidelines that came with the Connexin multiplex real-time PCR kit, the polymerase chain reaction mix was prepared. Primers were designed according to the desired region of the GJB2 gene. A real-time PCR device and the recommended cycling conditions from the manufacturer were used for the PCR amplification. The amplification of the *GJB2* gene region was observed on the screen while the PCR continued. Special fluorescent probes included in the Connexin multiplex Real-Time PCR Kit were used to identify and quantify the amplification of the required DNA sequences. Fluorescence signals were monitored throughout each amplification cycle in order to produce real-time PCR data. Real-time PCR data was used to establish the genotypes of the *GJB2* polymorphisms. The genotyping of each sample was noted and provides information on the specific GJB2 allele combinations.

Statistical Analysis

Utilizing the chi-square test, the genotype distribution and allele frequencies were examined. Deviations from expected genotype frequencies were looked at using the Hardy-Weinberg equilibrium and other computed factors. The genotype distribution and allele frequencies were compared to information from other databases and communities.

In the Turkish Cypriot population, the response to medication, the risk assessment for diseases and the conditions for individualized medication were investigated.

Chapter III

Results

In this study, 100 individuals from a healthy population of Turkish Cypriot in Northern Cyprus whose blood samples were collected at the Near East University Genetics Laboratory were evaluated retrospectively.

Table 1.

General Characteristic for the studied group

	N (%)	**Age**
Female	**64 (64)**	**31.93 ∓ 12.249**
Male	**36 (36)**	**40.84 ∓ 18.000**
Total	**100 (100)**	**35.14 ∓ 15.197**

GJB2 35DelG, 167DelT:

Particularly, *35DelG* and *167DelT* genetic mutations were investigated in the GJB2 gene.

35DelG Mutation

GG: The patient is homozygous to the G allele. G-: The patient is heterozygous for the G allele, with one copy of the G allele and one copy of the deleted G allele. -: The patient exhibits a homozygous deletion of the GG allele. The patient has a genetic variation where the G allele is absent at position 35. The genotype distribution of the *GJB2 35DelG* mutation in the Turkish Cypriot population is represented as GG in the chart. The number of individuals with the GG genotype is 100, as is the predicted number. The letter G

represents allele frequencies; the G variation has an allele frequency of 1.000. This means that the G allele is present in nearly 100% of the population under study.

There was no mutation, so there is no heterozygous genotype (refer to NaN in tab2).

GJB2 167DelT Mutation

TT: The patient is homozygous to the T allele. T-: The patient is heterozygous for the T allele and has a T allele deletion. -: The patient has a twofold deletion of the TT allele. The patient carries a deletion T allele at location 167. The genotype distribution of the *GJB2 167DelT* variant in the Turkish Cypriot population is represented by a chart. The observed of individuals with the TT genotype is 100, while the expected number is also 100. The representation of allele frequencies is denoted as T: The T variant has a frequency of 1.000 for the allele. This indicates that almost 100% of the population being examined possesses the T allele.

There was no mutation, so there is no heterozygous genotype (refer to NaN in tab2).

For both deletions case 35DelG and 167DelT, the Chi-squared value and p-value were not calculated (refer to NaN in tab2). The Chi-squared test is a statistical method that is employed to analyze the significant correlation between the observed and anticipated frequencies. Given the absence of mutations, it is not possible to compute the Chi-squared value and the p-value in this scenario.

GJB2 M34T, 312Del, exce R184P(G>C), IVS 1+1 (G>A), W24X Mutations

M34T, 312Del, R184P(G>C), IVS 1+1 (G>A), and *W24X*. Based on Table 2, these mutations are statistically identic.

GJB2 M34T Mutation

The *GJB2* gene involves *M34T* genetic variations.

MM: The patient is homozygous for the M allele. MT: The patient possesses one copy of the M allele and one copy of the T allele.

TT: The patient is homozygous for the T allele. The genotype distribution of the *GJB2 M34T* variant in the Turkish Cypriot population is depicted as MM in a chart. The actual count of individuals having the MM genotype is 100, whereas the projected number is also 100.

The MT genotype was not found in any individuals, which is consistent with the expected number of 0. The number of individuals observed with the TT genotype is zero, whereas the expected count was also zero.

Allele frequencies are denoted by the symbol M: The M variant has an allele frequency of 1.000. This indicates that almost 100% of the population being examined possesses the M allele. The T variation has a frequency of 0.000 for its allele. This indicates that approximately 0% of the population being examined possesses the T allele. The Chi-squared value and p-value were not calculated, because there was no mutation.

Table 2.

Allele Frequency and genotype Distribution of GJB2 variant in studied population

GJB2 Gene Variations	**Genotype Distribution**				**Allele Frequency**			
	Genotypes	GG	G-	--	G	-		
35delG	**Observed**	100	0	0	1.000	0.000	*P-value*	NaN
	Expected	100	0	0			X^2	NaN
		TT	T-	--	T	-		
167delT	**Observed**	100	0	0	1.000	0.000	*P-value*	NaN
	Expected	100	0	0			X^2	NaN

		MM	MT	TT	M	T		
M34T	**Observed**	100	0	0	1.000	0.000	*P-value*	NaN
	Expected	100	0	0			X^2	NaN
		LL	LP	PP	L	P		
L90P	**Observed**	98	2	0	0.99	0.01	*P-value*	0.102
	Expected	98	2	0			X^2	0.010
		RR	RP	PP	R	P		
***R184P* (G>C)**	**Observed**	100	0	0	1.000	0.000	*P-value*	NaN
	Expected	100	0	0			X^2	NaN
		VV	VI	II	V	I		
V37I	**Observed**	94	6	0	0.97	0.03	*P-value*	0.757
	Expected	94	5	1			X^2	0.095
		GG	GA	AA	C	T		
IVS 1+1 G>A	**Observed**	100	0	0	1.000	0.000	*P-value*	NaN
	Expected	100	0	0			X^2	NaN
		WW	WX	XX	W	W		

W24X	**Observed**	100	0	0	1.000	0.000	*P-value*	NaN
	Expected	100	0	0			X^2	NaN
		II	ID	DD	I	D		
312del	**Observed**	100	0	0	1.000	0.000	*P-value*	NaN
	Expected	100	0	0			X^2	NaN
		EE	EX	XX	E	X		
E47X	**Observed**	98	2	0	0.99	0.01	*P-value*	0.102
	Expected	98	2	0			X^2	0.010

The Chi-squared test is the statistical method that was employed to analyze the significant correlation between the observed and anticipated frequencies. Given the absence of mutations, it is not possible to compute the Chi-squared value and the p-value in this scenario.

GBJ2 (V37I, L90P, E47X) Mutations

The various allele combinations (also known as gene variants) that might arise at the *GJB2* gene's position "*V37I, L90P, E47X"* are shown in the column below. This column displayed each genotype's expected frequency in the population under investigation based on different theoretical. Known genetic distributions or presumptions about the population under study were usually the basis for these computations. The observed frequency of each genotype in the studied population is shown in table 2.

GJB2 V37I Mutation

This genotype had two copies of the "VV" allele at location 37. 94 was the measured frequency even though it was the predicted frequency. This genotype had one "V" and one "I" allele at position 37. Whereas the anticipated frequency was 5, the measured frequency was 6. This genotype had two copies of the "I" allele at location 37. The observed frequency was zero, even though the expected frequency was one. The frequency of the two distinct alleles ("C" and "T") at position 37 in the group under investigation were shown in these two rows.

In other words, around 97% of people will carry the "V" allele, according to the predicted frequency of 0.97. Around 3% of people are expected to carry the "I" allele, according to its predicted frequency of 0.03. A statistical metric called the chi-squared value is employed to ascertain if the difference between the observed and expected frequencies is statistically significant. This instance has an estimated chi-squared value of 0.095. When the discrepancy between the actual and predicted values is larger, the chi-squared value increases. Under these circumstances, the p-value was 0.757.

This p-value demonstrated the probability of seeing the genotype distribution (VV, VI, II) due to the independence between the actual and expected numbers. The hypothesis regarding the substantial difference between the anticipated and observed distributions was not strongly supported (p-value = 0.757) according to the Chi-squared test. The results of the study show that no significant difference was found when the expected value for the genotype distribution of the *GJB2 V37I* variant and the observed value in the group were compared.

***GJB2 L90P Mutation*.**

This genotype had one "L" and one "P" allele at position 90. The LL genotype means that the patient carries the G allele in two copies. The LP genotype means that the patient carries one L allele copy and one P allele copy. PP genotype indicates, the patient carries the P allele in two copies. The genotype distribution of the *GJB2 L90P* variant in Turkish Cypriot population is represented in a chart as LL: The observed number of people with the LL genotype is 98, whereas the predicted number is 98. LP: The observed number of individuals genotype is 2. While the predicted number is also 2. PP: The observed number

of individuals genotype is 0, when the predicted number is 0. The alleles frequencies are represented as L: The L variation has an allele frequency of 0.99. This suggests that roughly 98% of the population under study carries the L allele. P: The P variant's allele frequency is 0.1. This shows that roughly 1% of the population under study carry the P allele. 0,102 is the Chi-squared value.

This p-value demonstrated the probability of seeing the genotype distribution (LL, LP) due to the independence between the observed and expected numbers. The hypothesis regarding the substantial difference between the anticipated and observed distributions was not strongly supported (p-value = 0.102) according to the Chi-squared test. The results of the study show that no significant difference was found when the expected value for the genotype distribution of the *GJB2 V37I* variant and the observed value in the group were compared.

The genotype PP is null (refer to tab2).

GJB2-E47X Mutation

This mutation has statistically the same characteristics as *L90P* above.

Chapter IV

Discussion, Conclusion and Recommendations

This study investigated the carrier frequency of the connexin 26 (*GJB2*) gene amongst healthy populations in North Cyprus retrospectively.

Discussion

The research observed that the carrier frequency of mutation in the connexin 26 (*GJB2*) gene among healthy individuals in North Cyprus to be zero. Mutations in *GJB2* are a leading cause of autosomal recessive non-syndromic hearing loss (ARNSHL) worldwide. However, no mutation was observed in any of the participants in the study. The majority of prelingual non-syndromic recessive deafness cases in Caucasians are attributed to alterations in the Connexin 26 gene (*GJB2*/Cx26). Various *GJB2* mutations were observed in Moroccan patients with autosomal recessive non-syndromic hearing loss, according to reports. Nevertheless, only a limited number of studies have investigated the prevalence of these mutations in healthy populations. This study aimed to determine the carrier frequencies of the *GJB2* mutations in the Moroccan population. A genetic analysis was conducted on 386 healthy, unrelated Moroccan adults without any documented hearing loss to examine the *35delG* mutation and other variants in the *GJB2* sequence (Abidi et al., 2008). Indeed, the *35delG* mutation is the primary cause of deafness in Caucasians. The prevalence of *GJB2*-associated deafness ranges from 61% in Switzerland to 93% in Slovakia (Minárik et al., 2012). For example, Connexin 26 (*GJB2*) gene mutations account for 19.4% of non-syndromic hearing loss (NSHL) cases in the Indian population. The *c.71G>A (W24X*) and *c.35delG* were the most common *GJB2* mutations, representing 72.2% (234 out of 324 total mutant alleles from 7 studies) and 15.4% (50 out of 324 total mutated alleles from 7 studies), respectively.(Mishra et al., 2018b). and the distribution of carrier frequency *c.35 delG* mutation in Russia exhibits distinct ethno-geographic distinctiveness. The prevalence of the *c.35 delG* mutation is commonly observed in Caucasian groups residing in the northwestern region of Russia, with rates reaching up to 7.5% in the Kaliningrad Oblast' and 5.9% in the Leningrad Oblast. The current population of the Kaliningrad Oblast is predominantly composed of Russians, accounting for approximately 80%, as a result of significant migration from European parts of the former USSR during the war. The indigenous Finno-

Ugric Vepsians residing in rural regions of the Leningrad Oblast' are expected to have a relatively high carrier frequency of *c.35delG*, which is consistent with the elevated rates observed in other Finno-Ugric populations. Specifically, Estonians have reported rates of 4.4-4.5%, while Mordvins have reported rates of 5.7-6.2%. The carrier frequencies of c. *c35 delG* in the Cauc*c.35 delG* or its absence were observed among various ethnic groups. The highest carrier frequencies were found among Abkhazians (3.8%) and Armenians (3.7%). This genetic variation was also present in Turkic-speaking peoples of the Volga-Ural region (Tatars, Bashkirs, Chuvashes), Siberia (Altaians, Tuvinians, Yakuts), Central Asia (Kazakhs, Uighurs, Uzbeks), and Mongolic-speaking Buryats (Siberia). The frequency of the *c.35 delG* carrier in Russians in Siberia ranges from 4.1% in Western Siberia (as reported in this study) to 2.5% in Yakutia (Eastern Siberia). The study examined chromosome 13 in individuals from Siberia who were homozygous for the *c.35delG* mutation and had hearing loss (Zytsar et al., 2018). By comparison, out of the 19 children with hearing impairment, only 3 (16%) had both copies of the *35 delG* and/or *167 delT* genetic mutations. The findings indicate that the characteristics of hearing loss differed depending on the genetic makeup, with certain genotypes having a higher likelihood of exhibiting a severe form of hearing loss compared to others. Additionally, the study revealed that the development of hearing loss was more prevalent than initially anticipated (Kenna et al., 2010). GG (homozygous) the G allele was present in two copies at location 35 in this genotype. G- (heterozygous) the G allele, with one copy of the G allele and one copy of the deleted allele. The genotype distribution of the *GJB2 35DelG* mutation in the Turkish Cypriot population is represented in the table2. The actual count of individuals with the GG genotype is 100, while the expected count is also 100.

The additional common genetic variations of *GJB2* identified in Iran include *R127H*, *delE120, W24X, R184P, 3170G>A (IVSI-1G>A)*, and *235delC (2931)*. In our present study there was no mutation for these specifics' variants *M34T*, *312Del, R184P (G<C), IVS 1+1 (G>A)* and *W24X*. The column then displayed the actual number of instances of each genotype found in the population under study using genetic analysis.

According to some studies conducted in Indian population. Several genetic investigations on HL have also demonstrated that the c.71G > A (W24*X)* mutation is the predominant

pathogenic alteration responsible with non-syndromic hearing loss (NSHL). In this study, 8 out of 35 probands exhibited the *c.71G > A (W24X)* mutation. This mutation is located on transmembrane domain 1 (TM1) of the connexin 26 protein. Consequently, the protein was shortened to only one-tenth of the sequence of the wild-type protein. The presence of a G > A transition at position c.71 causes a stop codon to form at position p.24 *(W24X*) of connexin 26 (Hegde et al., 2022)

In our study population, A significant prevalence of the W24X mutation was observed by us, while the absence of the c.35 delG, c.235 delC, and 167 delT mutations may be distinctive to this particular community. And 40% (6/15) of individuals with non-syndromic hearing loss (NSHL) were discovered to have *GJB2* mutations, with 33.3% (2/6) of these variants being monoallelic. Four out of six patients were found to have bi-allelic *GJB2* mutations. The most prevalent *GJB2* mutation discovered was *c.71G > A (p.W24X),* accounting for 30% of all *GJB2* mutant alleles. A total of six investigations, encompassing 1119 patients with non-syndromic hearing loss (NSHL), were examined. Among these studies, four reported that the most prevalent mutation was c.*71G > A (p.W24X)*, whereas two studies identified *c.35delG* as the most common mutation. *GJB2* mutations accounted for 10.9%–36% of cases of non-syndromic hearing loss (NSHL). In the present investigation, a total of sixteen mutations in the *GJB2* gene were found in Indian patients. Among these variants, six were different from the *c.71G > A (p.W24X)* mutation. These six mutations were specifically identified as *c.35delG, c.1A > G (p.M1V), c.127G > A (p.V43 M), c.204C > G (p.Y86X), c.231G > A (p.W77X),* and *c.439G > A (p.E147K*) (Mishra et al., 2018)

Based on the findings of another study, 24.57% of patients were found to have a causal mutation on both chromosomes, whereas an additional 9.9% had a cause mutation on only one chromosome. A total of seven polymorphisms were identified: *V27I, M34T, F83L, 354 C→T, R127H, V153I, 684 C→A*. Additionally, eleven causative mutations were detected: *IVS1+1 G→A, 35delG, W24X, V37I, E47X, 167delT, L90P* (Minárik et al., 2012). MM (homozygous) the M allele was present in two copies at location 34 in the *GJB2 M34T Mutation*. While 100 was the predicted frequency, the 100 frequencies were seen during the analysis. MT (heterozygote) at location, this genotype had both a “M” and an “T” allele. The observed frequency was 0, compared to the expected frequency of 0. TT (mutant

homozygote) The "T" allele was present in the *GJB2 M34T Mutation*. The observed frequency was 0, compared to the predicted frequency of 0. The table above provided information about the distribution of the *GJB2 "M34T"* genetic variant in the studied population, along with statistical values used to assess the significance of observed deviations from the expected distribution.

Various *GJB2* mutations were observed in Moroccan patients with autosomal recessive non-syndromic hearing loss, according to reports. Nevertheless, only a limited number of studies have investigated the prevalence of these mutations in healthy populations. This study aimed to determine the carrier frequencies of the *GJB2* mutations in the Moroccan population. A genetic analysis was conducted on 386 healthy, unrelated Moroccan adults without any documented hearing loss to examine the *35delG* mutation and other variants in the *GJB2* sequence (Abidi et al., 2008).

While most research on *GJB2* mutations has focused on individuals with hearing impairment, there is limited information available regarding the prevalence of these variants in the general population. A study aimed to assess the frequency of *GJB2* mutations that result in hereditary deafness in the whole Korean population was conducted wherein 2,072 blood samples were collected from babies who had normal hearing. After performing direct DNA sequencing, the dried blood samples were subjected to PCR to amplify the whole coding region of the *GJB2* gene. A total of 24 distinct sequence variants were found in the coding region of GJB2. These include eight pathogenic mutations (*p.V37I, p.G45E, p.R143W, c.176_191del16, c.235delC, c.292_298dup7, c.299_300delAT*, and *c.605ins46),* four polymorphisms (*p.V27I, p.E114G, p.G160S, and p.I203T)*, and six new variants (*p.W3T, p.I20L, p.K41E, c.147C > T, c.186C > T, and c.576A > G)* (Han et al., 2008).

Conclusion

In conclusion, the carrier frequency of connexin 26 gene in healthy populations in north Cyprus variation in this study provides insight into the genetic makeup of the Turkish Cypriot society in relation to warfarin metabolism. The alleles frequencies of *GJB2 variants* were statistically identic, there was no mutation. According to this study, the prevalence to have the *GJB2 (35DelG, 167delT, M34T, L90P, R184P (G>C), V37I, IVS 1+1 G>A, W24X, 312Del* and *E47X*) *Mutations* responsible from hearing loss in the Northern Cypriots population it is lower.

Recommendations

This study recommends the following:

1. Ethical considerations in genetic testing and carrier screening programs.
2. The importance of genetic counseling and providing support to individuals identified as carriers.
3. The ongoing development of gene therapies and potential future treatments for GJB2-associated hearing loss.

Future Directions:

Building on this study, future research could:

- Investigate the carrier frequency within specific ethnic subgroups within North Cyprus.
- Analyze genotype-phenotype correlations to understand the association between specific GJB2 mutations and the severity of hearing loss.
- Explore the potential benefits of implementing a GJB2 mutation screening program in North Cyprus.

References

Abidi, O., Boulouiz, R., Nahili, H., Bakhouch, K., Wakrim, L., Rouba, H., Chafik, A., Hassar, M., & Barakat, A. (2008). Carrier frequencies of mutations/polymorphisms in the connexin 26 gene (GJB2) in the Moroccan population. *Genetic Testing*, *12*(4), 569–574. https://doi.org/10.1089/gte.2008.0063

Al-Ani, R. M. (2023). Various aspects of hearing loss in newborns: A narrative review. *World Journal of Clinical Pediatrics*, *12*(3), 86–96. https://doi.org/10.5409/wjcp.v12.i3.86

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). How Genomes Evolve. In *Molecular Biology of the Cell. 4th edition*. Garland Science. https://www.ncbi.nlm.nih.gov/books/NBK26836/

Alhazmi, W. (n.d.). Risk Factors Associated With Hearing Impairment in Infants and Children: A Systematic Review. *Cureus*, *15*(6), e40464. https://doi.org/10.7759/cureus.40464

Al-janabi, A. M., Ahmmed, H. S., & Al-Khafaji, S. M. (2021). Connexin 26 (GJB2) gene mutations linked with autosomal recessive non-syndromic sensor neural hearing loss in the Iraqi population. *Journal of Medicine and Life*, *14*(6), 841–846. https://doi.org/10.25122/jml-2021-0152

Al-Qahtani, A., Altamimi, Z., & Chandran, R. K. (2021). Congenital Hearing Loss. In *Textbook of Clinical Otolaryngology* (pp. 105–110). Springer, Cham. https://doi.org/10.1007/978-3-030-54088-3_9

Amaral, P., Carbonell-Sala, S., De La Vega, F. M., Faial, T., Frankish, A., Gingeras, T., Guigo, R., Harrow, J. L., Hatzigeorgiou, A. G., Johnson, R., Murphy, T. D., Pertea, M., Pruitt, K. D., Pujar, S., Takahashi, H., Ulitsky, I., Varabyou, A., Wells, C. A., Yandell, M., … Salzberg, S. L.

(2023). The status of the human gene catalogue. *Nature*, *622*(7981), 41–47. https://doi.org/10.1038/s41586-023-06490-x

Appelbaum, E. N., Howell, J. B., Chapman, D., Pandya, A., & Dodson, K. M. (2018). Analysis of risk factors associated with unilateral hearing loss in children who initially passed newborn hearing screening. *International Journal of Pediatric Otorhinolaryngology*, *106*, 100–104. https://doi.org/10.1016/j.ijporl.2018.01.024

Bergström, A., McCarthy, S. A., Hui, R., Almarri, M. A., Ayub, Q., Danecek, P., Chen, Y., Felkel, S., Hallast, P., Kamm, J., Blanché, H., Deleuze, J.-F., Cann, H., Mallick, S., Reich, D., Sandhu, M. S., Skoglund, P., Scally, A., Xue, Y., … Tyler-Smith, C. (2020). Insights into human genetic variation and population history from 929 diverse genomes. *Science*, *367*(6484), eaay5012. https://doi.org/10.1126/science.aay5012

Boninsegna, L., Yildirim, A., Zhan, Y., & Alber, F. (2022). Integrative approaches in genome structure analysis. *Structure*, *30*(1), 24–36. https://doi.org/10.1016/j.str.2021.12.003

Bouzaher, M. H., Worden, C. P., & Jeyakumar, A. (2020). Systematic Review of Pathogenic GJB2 Variants in the Latino Population. *Otology & Neurotology*, *41*(2), e182. https://doi.org/10.1097/MAO.0000000000002505

Božanić Urbančič, N., Battelino, S., Tesovnik, T., & Trebušak Podkrajšek, K. (2020). The Importance of Early Genetic Diagnostics of Hearing Loss in Children. *Medicina*, *56*(9), 471. https://doi.org/10.3390/medicina56090471

Cantaloube, J.-F., Gallian, P., Attoui, H., Biagini, P., De Micco, P., & de Lamballerie, X. (2005). Genotype Distribution and Molecular Epidemiology of Hepatitis C Virus in Blood Donors from Southeast France. *Journal of Clinical Microbiology*, *43*(8), 3624–3629. https://doi.org/10.1128/JCM.43.8.3624-3629.2005

Capra, D., DosSantos, M. F., Sanz, C. K., Acosta Filha, L. G., Nunes, P., Heringer, M., Ximenes-da-Silva, A., Pessoa, L., de Mattos Coelho-Aguiar, J., da Fonseca, A. C. C., Mendes, C. B., da Rocha, L. S., Devalle, S., Niemeyer Soares Filho, P., & Moura-Neto, V. (2023). Pathophysiology and mechanisms of hearing impairment related to neonatal infection diseases. *Frontiers in Microbiology*, *14*, 1162554. https://doi.org/10.3389/fmicb.2023.1162554

Caprara, G. A., & Peng, A. W. (2022). Mechanotransduction in mammalian sensory hair cells. *Molecular and Cellular Neuroscience*, *120*, 103706. https://doi.org/10.1016/j.mcn.2022.103706

Casale, J., Kandle, P. F., Murray, I. V., & Murr, N. (2024). Physiology, Cochlear Function. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK531483/

Chen, N., Juric, I., Cosgrove, E. J., Bowman, R., Fitzpatrick, J. W., Schoech, S. J., Clark, A. G., & Coop, G. (2019). Allele frequency dynamics in a pedigreed natural population. *Proceedings of the National Academy of Sciences*, *116*(6), 2158–2164. https://doi.org/10.1073/pnas.1813852116

Chen, P., Wu, W., Zhang, J., Chen, J., Li, Y., Sun, L., Hou, S., & Yang, J. (2022). Pathological mechanisms of connexin26-related hearing loss: Potassium recycling, ATP-calcium signaling, or energy supply? *Frontiers in Molecular Neuroscience*, *15*, 976388. https://doi.org/10.3389/fnmol.2022.976388

Chiarella, P., Capone, P., & Sisto, R. (2023). Contribution of Genetic Polymorphisms in Human Health. *International Journal of Environmental Research and Public Health*, *20*(2), 912. https://doi.org/10.3390/ijerph20020912

Chiarella, P., Capone, P., Sisto, R., Chiarella, P., Capone, P., & Sisto, R. (2019). The Role of Genetic Polymorphisms in the Occupational Exposure. In *The Recent Topics in Genetic Polymorphisms*. IntechOpen. https://doi.org/10.5772/intechopen.86975

DeMille, D., Carlston, C. M., Tam, O. H., Palumbos, J. C., Stalker, H. J., Mao, R., Zori, R. T., Viskochil, D. H., Park, A. H., & Carey, J. C. (2018). Three novel GJB2 (connexin 26) variants associated with autosomal dominant syndromic and nonsyndromic hearing loss. *American Journal of Medical Genetics Part A*, *176*(4), 945–950. https://doi.org/10.1002/ajmg.a.38648

Driver, E. C., & Kelley, M. W. (2020). Development of the cochlea. *Development (Cambridge, England)*, *147*(12), dev162263. https://doi.org/10.1242/dev.162263

Geden, H., & Seneldir, L. (2023). Connexin 26 (GJB2) Mutations Associated with Congenital Hearing Loss in a Country of Different Migration Routes: Turkey. *Indian Journal of Otolaryngology and Head & Neck Surgery*, *75*(1), 628–634. https://doi.org/10.1007/s12070-023-03672-x

Hahn, R., & Avraham, K. (2023). Gene Therapy for Inherited Hearing Loss: Updates and Remaining Challenges. *Audiology Research*, *13*, 952–966. https://doi.org/10.3390/audiolres13060083

Han, S.-H., Park, H.-J., Kang, E.-J., Ryu, J.-S., Lee, A., Yang, Y.-H., & Lee, K.-R. (2008). Carrier frequency of GJB2 (connexin-26) mutations causing inherited deafness in the Korean population. *Journal of Human Genetics*, *53*(11–12), 1022–1028. https://doi.org/10.1007/s10038-008-0342-7

Hashemi, S. M. A., Thijssen, M., Hosseini, S. Y., Tabarraei, A., Pourkarim, M. R., & Sarvari, J. (2021). Human gene polymorphisms and their possible impact on the clinical outcome of SARS-CoV-2 infection. *Archives of Virology*, *166*(8), 2089–2108. https://doi.org/10.1007/s00705-021-05070-6

Hegde, S., Hegde, R., Kulkarni, S. S., Das, K. K., Gai, P. B., & Bulagouda, R. S. (2022). Analysis of Genetic Variations in Connexin 26 (GJB2) Gene among Nonsyndromic Hearing

Impairment: Familial Study. *Global Medical Genetics*, *9*(2), 152–158. https://doi.org/10.1055/s-0042-1743257

Hegde, S., Hegde, R., Kulkarni, S. S., Das, K. K., Gai, P. B., & Bulgouda, R. (2021). Molecular alteration in the Gap Junction Beta 2 (GJB2) gene associated with non-syndromic sensorineural hearing impairment. *Intractable & Rare Diseases Research*, *10*(1), 31–36. https://doi.org/10.5582/irdr.2020.03157

Iossa, S., Marciano, E., & Franzé, A. (2011). GJB2 Gene Mutations in Syndromic Skin Diseases with Sensorineural Hearing Loss. *Current Genomics*, *12*(7), 475–785. https://doi.org/10.2174/138920211797904098

Jiang, L., Wang, D., He, Y., & Shu, Y. (2023). Advances in gene therapy hold promise for treating hereditary hearing loss. *Molecular Therapy*, *31*(4), 934–950. https://doi.org/10.1016/j.ymthe.2023.02.001

Kenna, M. A., Feldman, H. A., Neault, M. W., Frangulov, A., Wu, B.-L., Fligor, B., & Rehm, H. L. (2010). Audiologic Phenotype and Progression in GJB2 (Connexin 26) Hearing Loss. *Archives of Otolaryngology--Head & Neck Surgery*, *136*(1), 81–87. https://doi.org/10.1001/archoto.2009.202

Koohiyan, M. (2019). Genetics of Hereditary Hearing Loss in the Middle East: A Systematic Review of the Carrier Frequency of the GJB2 Mutation (35delG). *Audiology and Neurotology*, *24*, 1–5. https://doi.org/10.1159/000502201

Korver, A. M. H., Smith, R. J. H., Van Camp, G., Schleiss, M. R., Bitner-Glindzicz, M. A. K., Lustig, L. R., Usami, S., & Boudewyns, A. N. (2017a). Congenital hearing loss. *Nature Reviews. Disease Primers*, *3*, 16094. https://doi.org/10.1038/nrdp.2016.94

Korver, A. M. H., Smith, R. J. H., Van Camp, G., Schleiss, M. R., Bitner-Glindzicz, M. A. K., Lustig, L. R., Usami, S., & Boudewyns, A. N. (2017b). Congenital hearing loss. *Nature Reviews. Disease Primers*, *3*, 16094. https://doi.org/10.1038/nrdp.2016.94

Lieu, J. E. C., Kenna, M., Anne, S., & Davidson, L. (2020). Hearing Loss in Children: A Review. *JAMA*, *324*(21), 2195. https://doi.org/10.1001/jama.2020.17647

Liu, E. T. (2009). The Human Genome Organisation (HUGO). *The HUGO Journal*, *3*(1–4), 3–4. https://doi.org/10.1007/s11568-010-9139-9

Maeda, S., Nakagawa, S., Suga, M., Yamashita, E., Oshima, A., Fujiyoshi, Y., & Tsukihara, T. (2009). Structure of the connexin 26 gap junction channel at 3.5 Å resolution. *Nature*, *458*(7238), 597–602. https://doi.org/10.1038/nature07869

Mammano, F. (2019). Inner Ear Connexin Channels: Roles in Development and Maintenance of Cochlear Function. *Cold Spring Harbor Perspectives in Medicine*, *9*(7), a033233. https://doi.org/10.1101/cshperspect.a033233

Mao, L., Wang, Y., An, L., Zeng, B., Wang, Y., Frishman, D., Liu, M., Chen, Y., Tang, W., & Xu, H. (2023). Molecular Mechanisms and Clinical Phenotypes of GJB2 Missense Variants. *Biology*, *12*(4), Article 4. https://doi.org/10.3390/biology12040505

Maslova, E. A., Orishchenko, K. E., & Posukh, O. L. (2021). Functional Evaluation of a Rare Variant c.516G>C (p.Trp172Cys) in the GJB2 (Connexin 26) Gene Associated with Nonsyndromic Hearing Loss. *Biomolecules*, *11*(1), Article 1. https://doi.org/10.3390/biom11010061

McDermott, J. H., Molina-Ramírez, L. P., Bruce, I. A., Mahaveer, A., Turner, M., Miele, G., Body, R., Mahood, R., Ulph, F., MacLeod, R., Harvey, K., Booth, N., Demain, L. A. M., Wilson, P., Black, G. C., Morton, C. C., & Newman, W. G. (2019). Diagnosing and Preventing Hearing Loss in the Genomic Age. *Trends in Hearing*, *23*, 2331216519878983. https://doi.org/10.1177/2331216519878983

Minárik, G., Tretinárová, D., Szemes, T., & Kádasi, L. (2012). Prevalence of DFNB1 mutations in Slovak patients with non-syndromic hearing loss. *International Journal of Pediatric Otorhinolaryngology*, *76*(3), 400–403. https://doi.org/10.1016/j.ijporl.2011.12.020

Mishra, S., Pandey, H., Srivastava, P., Mandal, K., & Phadke, S. R. (2018a). Connexin 26 (GJB2) Mutations Associated with Non-Syndromic Hearing Loss (NSHL). *The Indian Journal of Pediatrics*, *85*(12), 1061–1066. https://doi.org/10.1007/s12098-018-2654-8

Mishra, S., Pandey, H., Srivastava, P., Mandal, K., & Phadke, S. R. (2018b). Connexin 26 (GJB2) Mutations Associated with Non-Syndromic Hearing Loss (NSHL). *Indian Journal of Pediatrics*, *85*(12), 1061–1066. https://doi.org/10.1007/s12098-018-2654-8

Missoum, A. (2018). The role of gene GJB2 and connexin 26 in hearing impairment. *The Ukrainian Biochemical Journal*, *90*(6), 5–11. https://doi.org/10.15407/ubj90.06.005

Misteli, T. (2020). The Self-Organizing Genome: Principles of Genome Architecture and Function. *Cell*, *183*(1), 28–45. https://doi.org/10.1016/j.cell.2020.09.014

Mohanta, T. K., Mishra, A. K., & Al-Harrasi, A. (2021). The 3D Genome: From Structure to Function. *International Journal of Molecular Sciences*, *22*(21), 11585. https://doi.org/10.3390/ijms222111585

Neumann, K., Mathmann, P., Chadha, S., Euler, H. A., & White, K. R. (2022). Newborn Hearing Screening Benefits Children, But Global Disparities Persist. *Journal of Clinical Medicine*, *11*(1), 271. https://doi.org/10.3390/jcm11010271

Paul, A., Bense, F., Boithias Guerot, C., De La Rubia, S., Lebeaux, C., & Papon, J.-F. (2024). Leading Risk Factors for Congenital Deafness in the Context of Universal Neonatal Screening: Our Observations in a Four-Year Retrospective Study. *International Journal of Neonatal Screening*, *10*(1), Article 1. https://doi.org/10.3390/ijns10010011

Podury, A., Jiam, N. T., Kim, M., Donnenfield, J. I., & Dhand, A. (2023). Hearing and sociality: The implications of hearing loss on social life. *Frontiers in Neuroscience*, *17*, 1245434. https://doi.org/10.3389/fnins.2023.1245434

Posukh, O. L., Maslova, E. A., Danilchenko, V. Y., Zytsar, M. V., & Orishchenko, K. E. (2023). Functional Consequences of Pathogenic Variants of the GJB2 Gene (Cx26) Localized in Different Cx26 Domains. *Biomolecules*, *13*(10), Article 10. https://doi.org/10.3390/biom13101521

Qian, Z. J., Chang, K. W., Ahmad, I. N., Tribble, M. S., & Cheng, A. G. (2021). Use of Diagnostic Testing and Intervention for Sensorineural Hearing Loss in US Children From 2008 to 2018. *JAMA Otolaryngology–Head & Neck Surgery*, *147*(3), 253–260. https://doi.org/10.1001/jamaoto.2020.5030

Rahit, K. M. T. H., & Tarailo-Graovac, M. (2020). Genetic Modifiers and Rare Mendelian Disease. *Genes*, *11*(3), 239. https://doi.org/10.3390/genes11030239

Rask-Andersen, H., Liu, W., Erixon, E., Kinnefors, A., Pfaller, K., Schrott-Fischer, A., & Glueckert, R. (2012). Human Cochlea: Anatomical Characteristics and their Relevance for Cochlear Implantation. *Anatomical Record (Hoboken, N.J. : 2007)*, *295*, 1791–1811. https://doi.org/10.1002/ar.22599

Renauld, J. M., & Basch, M. L. (2021). Congenital Deafness and Recent Advances Towards Restoring Hearing Loss. *Current Protocols*, *1*(3), e76. https://doi.org/10.1002/cpz1.76

Sanjuán, R., & Domingo-Calap, P. (2021). Genetic Diversity and Evolution of Viral Populations. *Encyclopedia of Virology*, 53–61. https://doi.org/10.1016/B978-0-12-809633-8.20958-8

Schmiedel, B. J., Singh, D., Madrigal, A., Valdovino-Gonzalez, A. G., White, B. M., Zapardiel-Gonzalo, J., Ha, B., Altay, G., Greenbaum, J. A., McVicker, G., Seumois, G., Rao, A., Kronenberg, M., Peters, B., & Vijayanand, P. (2018). Impact of Genetic Polymorphisms on

Human Immune Cell Gene Expression. *Cell*, *175*(6), 1701-1715.e16. https://doi.org/10.1016/j.cell.2018.10.022

Sheffield, A. M., & Smith, R. J. H. (2019). The Epidemiology of Deafness. *Cold Spring Harbor Perspectives in Medicine*, *9*(9). https://doi.org/10.1101/cshperspect.a033258

Sirugo, G., Williams, S. M., & Tishkoff, S. A. (2019). The Missing Diversity in Human Genetic Studies. *Cell*, *177*(1), 26–31. https://doi.org/10.1016/j.cell.2019.02.048

Soni, S. (2024). Recent Developments in the Regulation of Heritable Human Genome Editing. *Journal of Bioethical Inquiry*, *21*(1), 15–18. https://doi.org/10.1007/s11673-023-10332-w

Sripichai, O., & Fucharoen, S. (2007). Genetic polymorphisms and implications for human diseases. *Journal of the Medical Association of Thailand = Chotmaihet Thangphaet*, *90*(2), 394–398.

Su, H.-A., Lai, T.-W., Li, S.-Y., Su, T.-R., Yang, J.-J., & Su, C.-C. (2020). The Functional Role of CONNEXIN 26 Mutation in Nonsyndromic Hearing Loss, Demonstrated by Zebrafish Connexin 30.3 Homologue Model. *Cells*, *9*(5), 1291. https://doi.org/10.3390/cells9051291

Thorpe, R. K., & Smith, R. J. H. (2020). Future directions for screening and treatment in congenital hearing loss. *Precision Clinical Medicine*, *3*(3), 175–186. https://doi.org/10.1093/pcmedi/pbaa025

Torre-González, C. de la, Villanueva-García, D., García-Delgado, C., Castillo-Castillo, S., Huante-Guido, M., Chichitz-Madrigal, J., Juárez-Torres, M. E., Sánchez-Sandoval, A. L., Barrón-Palma, E. V., & Morán-Barroso, V. F. (2022). Congenital hearing loss: A literature review of the genetic etiology in a Mexican population. *Boletin Medico Del Hospital Infantil De Mexico*, *79*(4), 206–214. https://doi.org/10.24875/BMHIM.21000186

Verselis, V. K. (2019). Connexin hemichannels and cochlear function. *Neuroscience Letters*, *695*, 40–45. https://doi.org/10.1016/j.neulet.2017.09.020

White, H. J., Helwany, M., Biknevicius, A. R., & Peterson, D. C. (2024). Anatomy, Head and Neck, Ear Organ of Corti. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK538335/

Yamasaki, R. (2018). Connexins in health and disease. *Clinical and Experimental Neuroimmunology*, *9*(S1), 30–36. https://doi.org/10.1111/cen3.12433

Zappalà, A., Romano, I. R., D'Angeli, F., Musumeci, G., Lo Furno, D., Giuffrida, R., & Mannino, G. (2023). Functional Roles of Connexins and Gap Junctions in Osteo-Chondral Cellular Components. *International Journal of Molecular Sciences*, *24*(4), Article 4. https://doi.org/10.3390/ijms24044156

Zerbino, D. R., Frankish, A., & Flicek, P. (2020). Progress, Challenges, and Surprises in Annotating the Human Genome. *Annual Review of Genomics and Human Genetics*, *21*, 55–79. https://doi.org/10.1146/annurev-genom-121119-083418

Zhou, X., Wang, L., Jin, F., Guo, Y., Zhou, Y., Zhang, X., Zhang, Y., Ni, X., Li, W., & Liu, H. (2022). The prevalence and risk factors for congenital hearing loss in neonates: A birth cohort study based on CHALLENGE study. *International Journal of Pediatric Otorhinolaryngology*, *162*, 111308. https://doi.org/10.1016/j.ijporl.2022.111308

Zytsar, M., Barashkov, N. A., Bady-Khoo, M. S., Shubina-Olejnik, O. A., Danilenko, N. G., Bondar, A. A., Morozov, I. V., Solovyev, A. V., Danilchenko, V. Y., Maximov, V. N., & Posukh, O. L. (2018). Updated carrier rates for c.35delG (GJB2) associated with hearing loss in Russia and common c.35delG haplotypes in Siberia. *BMC Medical Genetics*, *19*(1), 138. https://doi.org/10.1186/s12881-018-0650-5

Appendix A

NEAR EAST UNIVERSITY
SCIENTIFIC RESEARCH ETHICS COMMITTEE

RESEARCH PROJECT EVALUATION *REPORT*

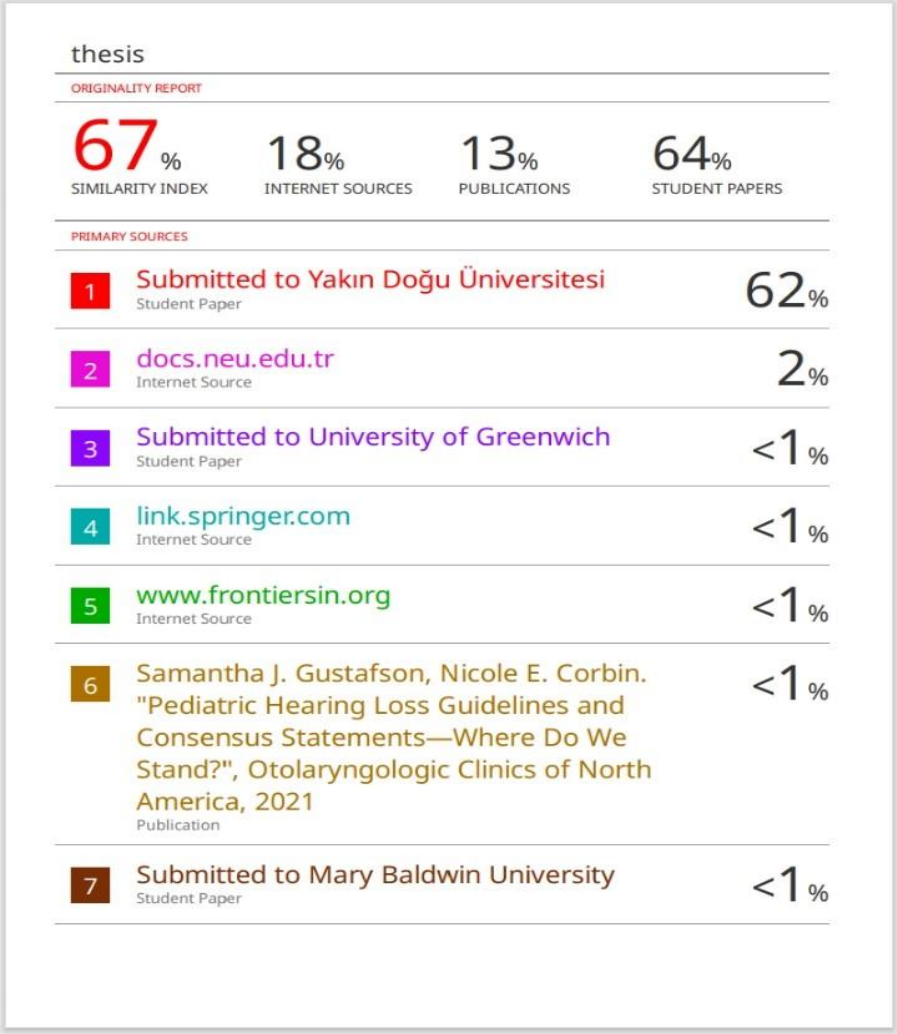
Meeting date :30.05.2024
Meeting Number :2024/124
Project number :1834

The project entitled **"The Carrier Frequency of The Connexin 26 Gene Variants in Healthy Populations"** (Project no: NEU/2024/124-1834), which will be conducted by Assoc. Prof. Dr. Mahmut Çerkez Ergören has been reviewed and approved by the Near East University Scientific Research Ethical Committee.

Prof. Dr. Şanda Çalı
Near East University
Head of Scientific Research Ethics Committee

Committee Member	Role	Meeting Attendance *Attended(✓)/Not attended(X)*	Decision *Approved(✓)/Rejected(X)*
1. Prof. Dr. Şanda Çalı	Head	✓	✓
2. Assoc. Prof. Dr. Gulifeiya Abuduxike	Rapporteur	✓	✓
3. Prof. Dr. Tamer Yılmaz	Member	✓	✓
4. Prof. Dr. Şahan Saygı	Member	✓	✓
5. Prof. Dr. İlker Etikan	Member	✓	✓
6. Assoc. Prof. Dr. Dilek Sarpkaya Güder	Member	✓	✓
7. Prof. Dr. Burçin Şanlıdağ	Member	✓	✓

Appendix B



thesis

ORIGINALITY REPORT

67%	18%	13%	64%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Yakın Doğu Üniversitesi Student Paper	62%
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3	Submitted to University of Greenwich Student Paper	<1%
4	link.springer.com Internet Source	<1%
5	www.frontiersin.org Internet Source	<1%
6	Samantha J. Gustafson, Nicole E. Corbin. "Pediatric Hearing Loss Guidelines and Consensus Statements—Where Do We Stand?", Otolaryngologic Clinics of North America, 2021 Publication	<1%
7	Submitted to Mary Baldwin University Student Paper	<1%

Appendix C

CURRICULUM VITAE

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ACADEMIC BACKGROUND

Year	University	Field
2022-2024	Near East University	Master in Medical Genetics
2020-2022	University of Jean Lorougnon Guede of Daloa	Master in Herbal Medicine of Natural Substances
2019-2020	University of Jean Lorougnon Guede of Daloa	Bachelor in Life and Earth Science
2017-2019	University of Jean Lorougnon Guede of Daloa	Agroforestry and environment

EXPERIENCE

June 2024	Genetics Laboratory Near East Hospital
2022-2024	Mechanics Work
2017-2024	Driver
2017-2021	Lecturer Private College in Daloa
2021-2022	Laboratory university of Cocody in Abidjan