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Review Article

A systematic review and bioinformatics analysis on DNA methylation status in age-related hearing loss

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ABSTRACT

Many individuals experience age-related hearing loss (ARHL), yet it frequently goes unnoticed. Its prevalence increases two-fold with every ten years of age. Epigenetics is linked to heritable modifications in gene activity that are not caused by alterations in DNA sequence. In this review, our goal is to assess the variety of DNA methylation in individuals with ARHL. Methods: We conducted a systematic review of studies that report changes in DNA methylation among patients with presbycusis. The study used the PECO framework to establish the eligibility criteria for patients with ARHL. A search was performed on Embase, PubMed, Scopus, Google Scholar, and ScienceDirect. Furthermore, a bioinformatics analysis was performed. Results: A total of seven studies were reviewed. Eighteen genes including P2RX2, KCNQ5, SOCS3, ERBB3, TCF25, POLE, ESPN, TNFRSF25, FGFR1, CDH23, SLC26A4, MEF2D, LCK, ACP6, ALG10, DUSPK4, C21orf58 and GBX2 were differentially methylated. To contextualize these in ARHL pathways, we integrated them with 136 ARHL-associated genes for PPI network analysis, identifying nine hub genes. Then we performed the overlap analysis using R to identify intersections between 18 methylated genes and the hub genes. After conducting enrichment analysis for hub genes, differentially methylated genes, and intersected genes, it was revealed that ERBB3, P2RX2, and FGFR1 were involved in the calcium signaling pathway. Besides, the overlap of methylated genes with ARHL hubs identifies SLC26A4, ERBB3, and CDH23 as key candidates. Conclusion: This review demonstrated that DNA methylation could cause the development of ARHL. Changes in gene methylation can disrupt the calcium signaling pathway, potentially leading to presbycusis.

Keywords:
DNA Methylation
Age-Related Hearing Loss
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Bioinformatics Analysis

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Introduction

Hearing loss is a common condition, but it's often not diagnosed by patients and doctors, leading to lower estimates of its prevalence. The prevalence of hearing loss is believed to double with every decade of age (Nieman and Oh 2020). Presbycusis, also referred to as age-related hearing loss (ARHL), is a common condition characterized by the gradual decline in hearing sensitivity as individuals advance in age. The majority of older people are impacted by this natural aging process, which can greatly influence their quality of life (Wang and Puel 2020). The World Health Organization (WHO) states that around one out of every three individuals who are 65 years old and older experience hearing loss that limits their daily activities, demonstrating the commonality of this problem among the elderly (Organization 2018).

Epigenetics involves the study of alterations in gene expression caused by modifications to structure of DNA, rather than alterations to the genetic code itself (Niknazar et al., 2017a; Waddington 2012). It is crucial in cell differentiation and regulating gene expression in the developing organism (Bird 1986; Holliday 1990). Even though the genetic code stays the same throughout one's life, alterations in gene expression caused by epigenetic changes can vary and develop throughout a person's lifetime (Fraga et al., 2005; Niknazar et al., 2017b). One of the fundamental epigenetic processes studied in genetics is DNA methylation. This process involves adding a methyl group to the fifth carbon molecule of a cytosine base, resulting in the formation of 5-methylcytosin (Rakyan et al., 2011).

Both genetic variation and environmental exposure can impact epigenetic changes (Bell et al., 2011; Wong et al., 2010). Environmental factors can cause changes in DNA methylation, leading to changes in gene expression. This connection between our surroundings and gene expression may explain how a healthy individual can develop hearing disorders (Buschdorf and Strätling 2004; Donoso et al., 2003; Wilkin et al., 2000).

To explore how these methylated genes fit into broader ARHL networks, we integrated them with ARHL-associated genes from databases, hypothesizing that methylated hubs represent key epigenetic regulators. This approach links systematic review findings to bioinformatics, focusing on the role of methylation while using expanded networks for context.

Material and Methods

Object

The study aims to examine research papers reporting DNA methylation changes in patients with age-related hearing disorders, following the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021). The study protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) and was assigned the registration code CRD42024535891.

Eligibility criteria

The PECO framework was used to study patients with age-related hearing disorders and evaluate the effects of aging on hearing. The framework does not involve any comparator and aims to measure DNA methylation as the outcome.

- Inclusion criteria
- Studies on human
- Articles written in English
- Studies that assessed DNA methylation in patients with age-related hearing disorders
- Original articles comprising cohort, retrospective and prospective cross-sectional, randomized and non-randomized studies, and conference abstract with peer reviewed articles
- Exclusion criteria
- Studies not involving humans
- Studies without any report of DNA methylation
- Review articles, book chapters, conference abstracts without peer-reviewed articles, case reports, and case series.
- Search strategies
- Databases including PubMed/Medline, Scopus, Embase, ScienceDirect, and Google Scholar were searched until October 2023. The keywords “hearing loss”, “age-related hearing loss”, “hearing disorders”, “presbycusis”, and “DNA methylation” were used. No limitations were placed on the date and year of publication, and language was considered.
- Study selection and data extraction

The process of retrieving and analyzing data included the following steps: Initially, articles were gathered from all databases and recorded in an Excel spreadsheet. Duplicate entries were removed, and studies were narrowed down based on eligibility criteria after reviewing titles

and abstracts. Related articles were further reviewed for a complete text examination. The final articles were selected for data extraction, and the following subsequent details were documented: the first author's name, publication year, research type, number of patients, gender, mean age, and methylated gene. Two reviewers independently conducted the selection process and data extraction, and a third reviewer verified their work.

Bioinformatics analysis

Text data mining

The genes related to ARHL were obtained from GeneCard (https://www.genecards.org/), CTD (https://ctdbase.org/), and Core Mine (https://coremine.com/medical/). We used age-related hearing loss or presbycusis to find genes associated with ARHL. Then we used the union of all the genes obtained. As a result, 136 ARHL-associated genes were retrieved to provide context for the 18 methylated genes identified in the review, enabling the identification of methylated regulated interactions.

PPI Network construction and identification of MCODE clusters

A network of genes associated with ARHL was built using the online database STRING (https://string-db.org/) with a threshold of a confidence score greater than 0.4. The networks from both platforms were imported into Cytoscape (version 3.10) to analyze the data and determine the clusters (Shannon et al., 2003). All unconnected, isolated nodes in the networks were eliminated. The Molecular Complex Detection (MCODE) plugin in Cytoscape was utilized to detect the clusters (Degree cut-off >5, node, score cut-off >2, K-core >2, and max depth = 100) in the network. Finally, we determined the highest-scoring clusters in the PPI networks to identify potential genes related to ARHL.

Overlap analysis

To identify methylation-regulated hub genes, we computed the intersection between the 18 methylated genes and the nine hub genes using set operations in R (Venn. Diagram package). Overlaps were visualized in a Venn diagram and discussed for biological relevance.

Functional enrichment

Functional classification and annotation of the reported genes were conducted using the online database DAVID (Data base for Annotation, Visualization and Integrated Discovery) (http://david.abcc.ncifcrf.gov/) (Huang da et al., 2009), the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto 2000) and gene ontology (GO) were used to analyze the genes to identify pathways, biological processes, cellular component, and molecular functions. Enrichment was performed separately on: 1) the 18 methylated genes from the systematic review; and 2) the hub genes from PPI network

Results

Study characteristic

After searching several databases, a total of 833 articles were found (PubMed=79, Google Scholar=35, Embase =243, Scopus =349, Science Direct =127). Out of these, 153 duplicate articles were removed, leaving 677 articles for further screening. During the screening process based on the article titles and abstracts, 576 articles were excluded. Finally, out of a total of 32 articles that underwent full-text assessment, seven studies met the eligibility criteria and were used for data synthesis (Figure 1).

Quality assessment

All of the studies included in the systematic review were relevant to the topic. Most of the articles included in the review were case-control studies. The quality of the articles included in the review was assessed using the NOS scale, and the results are presented in Table 1. The main issues identified in the majority of the studies were related to the exposure section.

Quality synthesis

This study reviewed 936 individuals with age-related hearing issues to examine DNA methylation levels. Eighteen differentially methylated genes were identified across the studies. The information from the research is shown in Table 2.

ARHL

Seven studies evaluated the association between ARHL and DNA methylation of various genes compared to patients without ARHL. The following genes were differentially methylated: Purinergic receptor p2x2 (P2RX2), KQT-like subfamily Q member 5 (KCNQ5), Erb-b2 receptor tyrosine kinase (ERBB3), Suppressor

TABLE 1: Quality assessment of the observational studies (case-control) included in the meta-analysis (The NOS tool).

	Selection				Comparability	exposure			
Author, Year	Is the case definition adequate?	Representativeness of cases	Selection of controls	Definition of control	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-response rate	Total Quality Score
Bouzid et al., 2018	1	1	1	1	1	1	1	0	7
Wolber et al., 2014	1	1	1	0	1	1	1	0	6
Bouzid et al., 2018	1	1	1	1	1	1	1	0	7
Guo et al., 2023	1	1	1	1	1	1	1	0	7
Xu et al., 2017	1	1	1	1	1	1	1	0	7
Roche et al., 2022	1	1	1	1	1	1	1	0	7
Kuo et al., 2021`	1	0	1	1	1	0	1	1	0

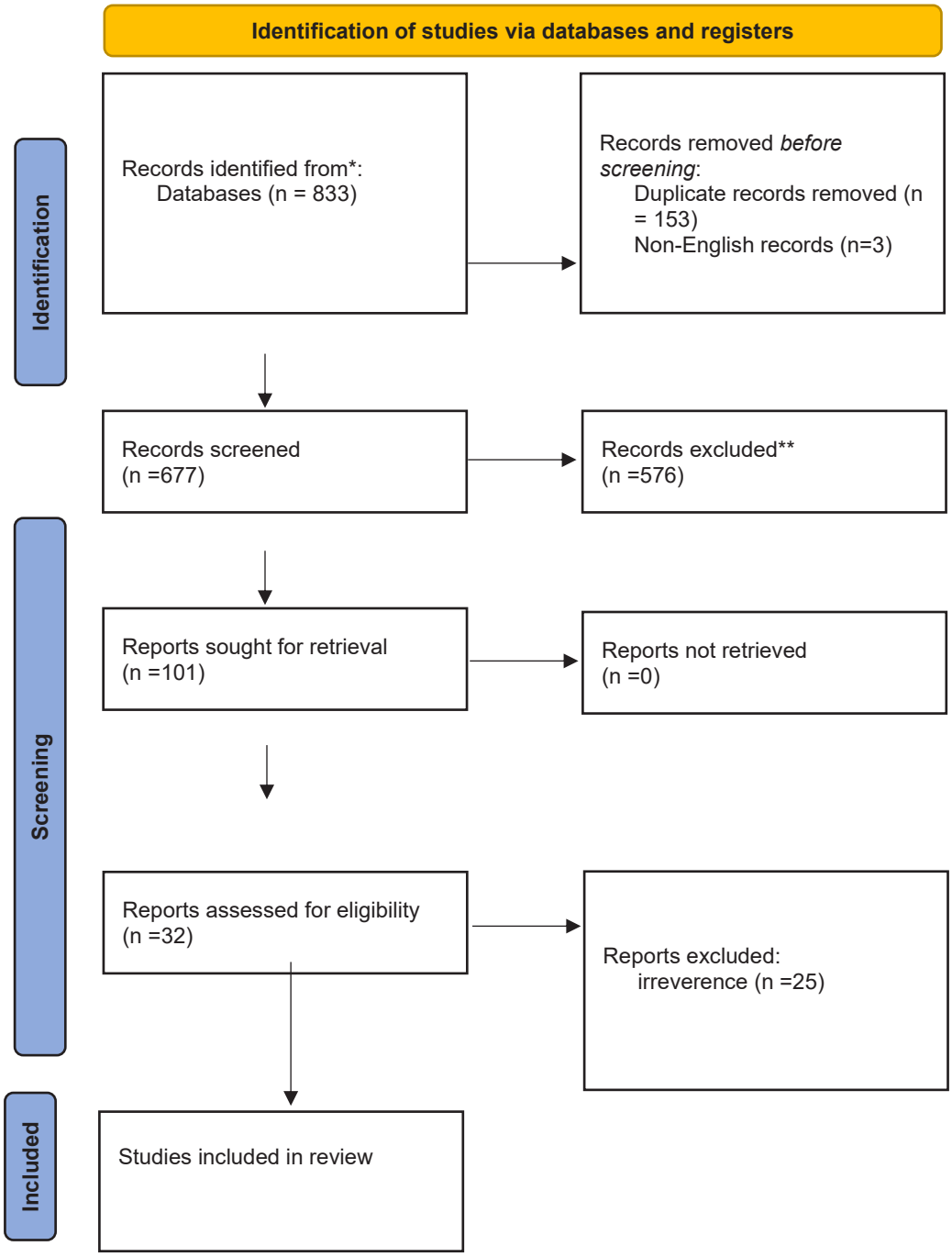

FIGURE 1. PRISMA Diagram resembling Electronic Database Search and Inclusion/Exclusion process of the review.Legend: the date of the search 10/20/2023

of cytokine signaling 3 (SOCS3), transcription factor 25 (TCF25), DNA Polymerase (POLE), Epsilon Catalytic Subunit (ESPN), tumor necrosis factor super family 25 (TNFRSF25), fibroblast growth factor receptor 1 (FGFR1), Cadherin related 23 (CDH23), dual specificity protein phosphatase 4 (DUSP4), Chromosome 21 Open Reading Frame 58 (C21orf58), Solute carrier family 26 member 4 (SLC26A4), Myocyte enhancer factor 2D (MEF2D), acid phosphatase 6 (ACP6), alpha-1, 2-glucosyl transferase (ALG10), C3, Gastrulation brain homeobox 2 (GBX2), and lymphocyte cell specific protein tyrosine kinase (LCK) (Bouzid et al., 2018a; Bouzid et

TABLE 3: shows the results of enrichment with DAVID.

Category	Term	Genes	Count	P-value
GOTERM_BP_DIRECT	Sensory perception of sound	CDH23, ESPN, FGFR1, P2RX2, SLC26A4	5	0.00001
GOTERM_BP_DIRECT	Transmembrane receptor protein tyrosine kinase signaling pathway	LCK, ERBB3, FGFR1	3	0.0042
GOTERM_BP_DIRECT	Animal organ development	FGFR1, MEF2D	2	0.026
GOTERM_BP_DIRECT	Negative regulation of signal transduction	ERBB3, SOCS3	2	0.043
GOTERM_BP_DIRECT	Peptidyl-tyrosine phosphorylation	LCK, FGFR1	2	0.043
GOTERM_BP_DIRECT	Positive regulation of kinase activity	ERBB3, FGFR1	2	0.047
GOTERM_BP_DIRECT	Inner ear morphogenesis	FGFR1, GBX2	2	0.048
GOTERM_CC_DIRECT	Receptor complex	ERBB3, FGFR1, P2RX2	3	0.013
GOTERM_CC_DIRECT	Stereocilia	CDH23, ESPN	2	0.028
GOTERM_CC_DIRECT	Plasma membrane	SLC26A4, POLE, LCK, TNFRSF25, CDH23, ERBB3, KCNQ5, P2RX2, FGFR1, C3	10	0.017
GOTERM_CC_DIRECT	Stereocilium	CDH23, ESPN	2	0.028
GOTERM_CC_DIRECT	Apical plasma membrane	ERBB3, P2RX2, SLC26A4	3	0.043
GOTERM_MF_DIRECT	Protein tyrosine kinase activity	LCK, ERBB3, FGFR1	3	0.0046
GOTERM_MF_DIRECT	SH2 domain binding	LCK, FGFR1	2	0.037
GOTERM_MF_DIRECT	Phosphotyrosine binding	LCK, SOCS3	2	0.043
KEGG_PATHWAY	Calcium signaling pathway	ERBB3, FGFR1, P2RX2	3	0.041

al., 2018b; Guo et al., 2023b; Roche et al., 2022; Wolber et al., 2014; Xu et al., 2017). The results are demonstrated in Table 2.

Most of the genes are related to the development and regulation of the cochlea. Genes like P2RX2, SLC26A4, and CDH23 are involved in sensory neurons and sensing sounds. EPSN and CDH23 play an essential role in the construction of stereocilia. Additionally, other genes like ERBB3, FGFR1, and SOCS3 are involved in signal transduction. The research demonstrated that other genes are involved in immune responses, regulation of cell growth and death, and ion channel activity (13-18).

Bioinformatics analysis

Identification of genes associated with ARHL

We conducted text mining on three datasets, Coremine, Gene cards, and CTd, to extract gene information related to ARHL. A total of 136 genes associated with ARHL were identified. The expanded network provides context without assuming methylation in all genes.

Nodes and edges analyzed by Cytoscape

A total of 114 nodes and 413 edges were selected for the plot PPI (Figure 2). Four important modules were identified. Based on degree, the most important genes were SRC, CDH23, ERBB3, HIF1A, MYO7A, GJB2, SLC26A4, MYO6, KCNQ4, with degrees of 37,23,22,20,19,19,18,17,17, respectively.

Cluster identification

After analyzing with MCODE, four clusters were identified, of which only one had the genes that were gathered from the literature (Figure 3).

Overlap analysis

Of the nine hub genes, three overlapped with the methylated gene set: SLC26A4 (degree 18), ERBB3 (degree 23), and CDH23 (degree 23). The resulting Venn diagram is displayed in Figure 4.

Enrichment analysis

Based on the degree, nine genes were selected for en-

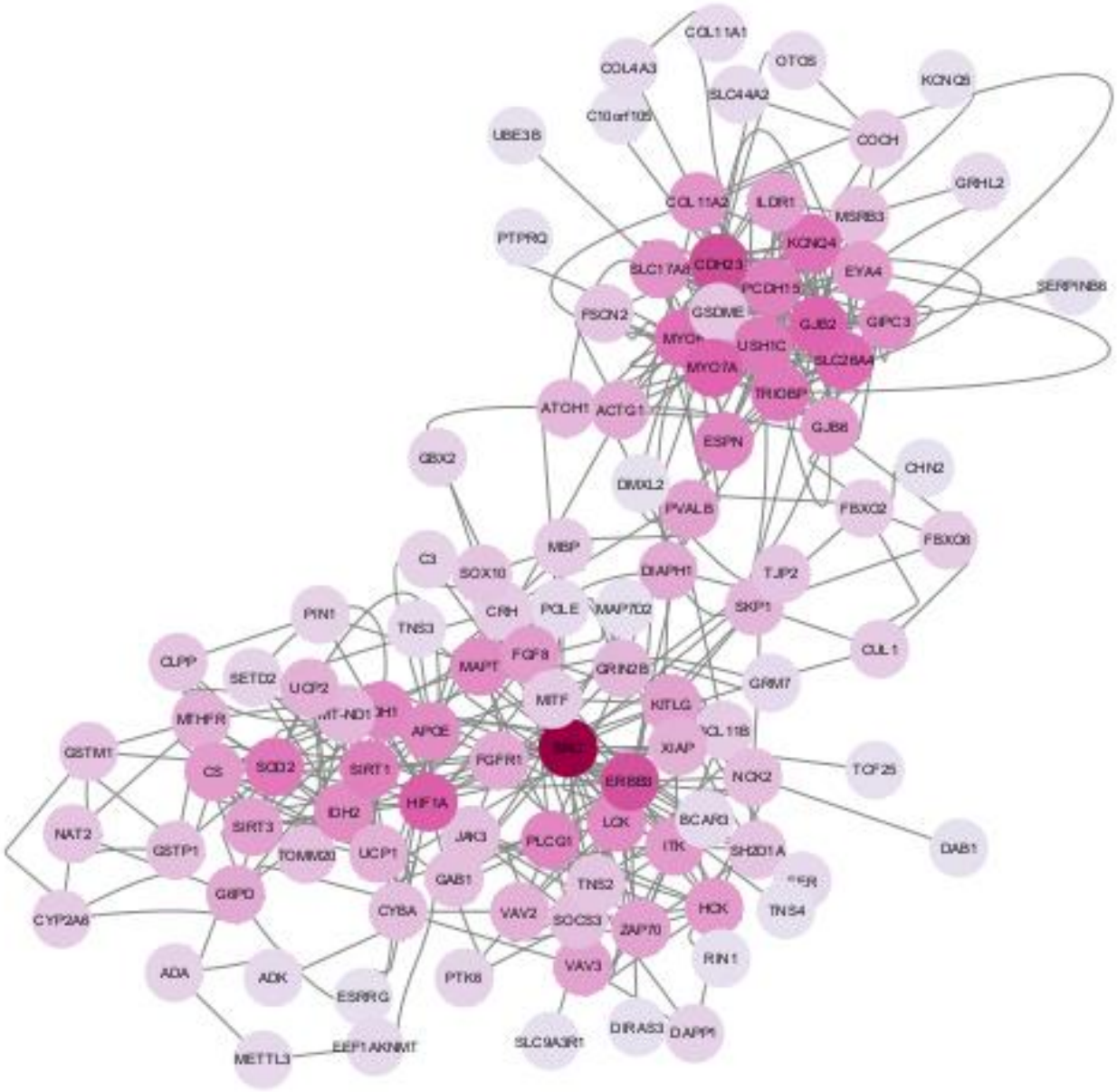

FIGURE 2. Shows the protein-protein interaction plot of genes. The color of the nodes relates to the degree of that node. Figure 2 shows the protein-protein interaction plot of genes. The color of the nodes relates to the degree of that node.

richment. GO and KEGG analysis were conducted for the genes extracted from databases for ARHL, the nine hub genes, and the intersected three genes. Enrichment of 18 methylated genes revealed involvement in sensory perception of sound, equilibrioperception, auditory receptor cell stereocillium organization, positive regulation of glycolytic process, positive regulation of epithelial cell migration, Inner ear morphogenesis, and neuron opoptotic proces in biological processes. Besides, all the included genes were related to the plasma membrane, actin filament based movement, apical plasma membrane, stereocillium, myosin complex, basal plasma membrane, and microvillus in chemical components. The results of the enrichment of 18 methylated genes are demonstrated in Table 3.

Moreover, the nine hub genes were enriched separately. The results of enriched genes are shown in Figure 5.

We further explored the intersected genes: ERBB3, SLC26A4, and CDH23. The results showed that these genes are involved in the biological process of sensory perception of sound. Additionally, ERBB3, SLC26A4, and CDH23 are associated with the apical plasma mem-

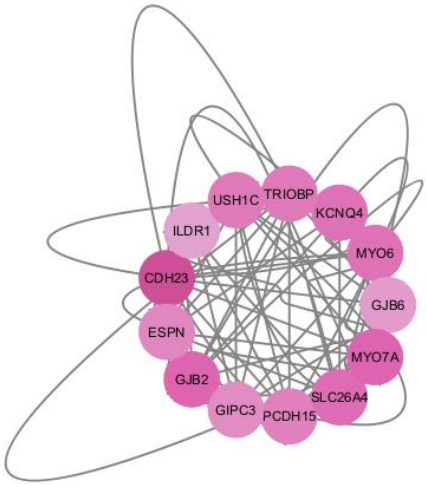

FIGURE 3. Shows the cluster that contained the genes that gathered from literature. The color of the nodes relates to the degree of that node.

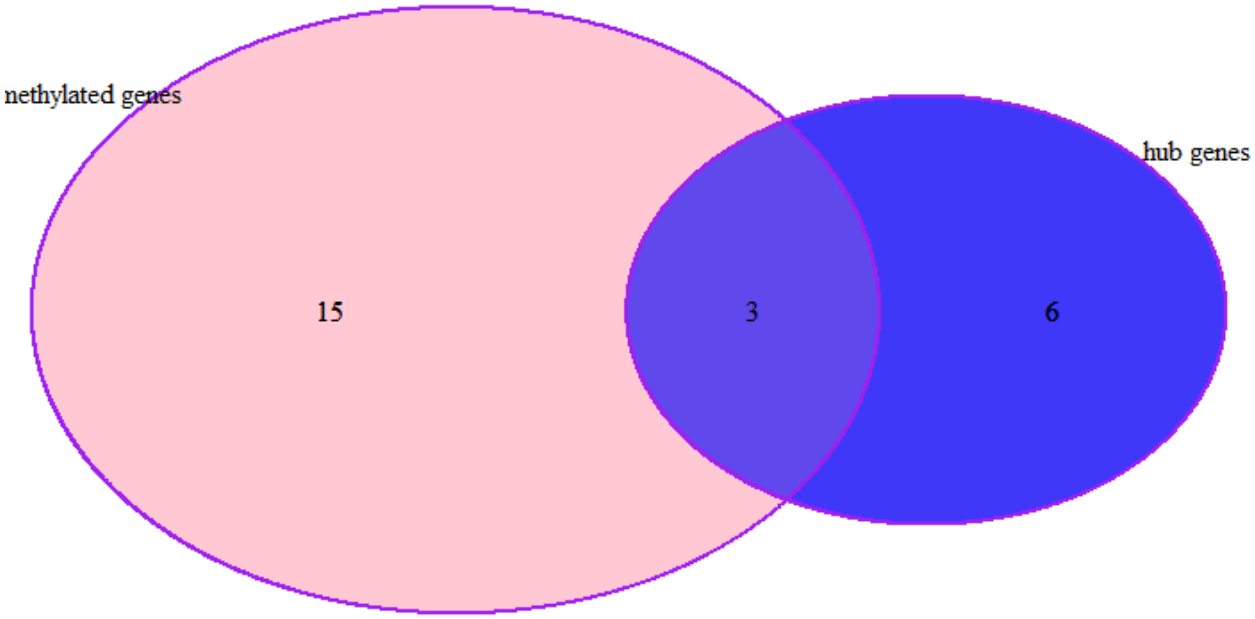

FIGURE 4. Shows the Venn diagram of intersected genes between hub genes and methylated genes.

brane and the plasma membrane in terms of their chemical composition.

Based on KEGG analysis, ERBB3, FGFR1, and P2RX2 are involved in the calcium signaling pathway. Additionally, enrichment analysis of the network obtained through data mining revealed that PCDH15 and USH1C participate in calcium ion binding, while MYO6 and MYO7A are involved in ADP binding in terms of molecular function. Collectively, these findings may highlight the critical role of calcium channels in the pathophysiology of ARHL.

Discussion

An estimated 30 million adults in the United States, representing almost 15% of the total population, are affected by varying degrees of hearing impairments (Mahboubi et al., 2018). This condition is most frequent in older individuals, with nearly 50% of adults in their 70s and 80% of those aged 85 or older being affected (Lin et al., 2011; Mahboubi et al., 2018). ARHL is a compli-

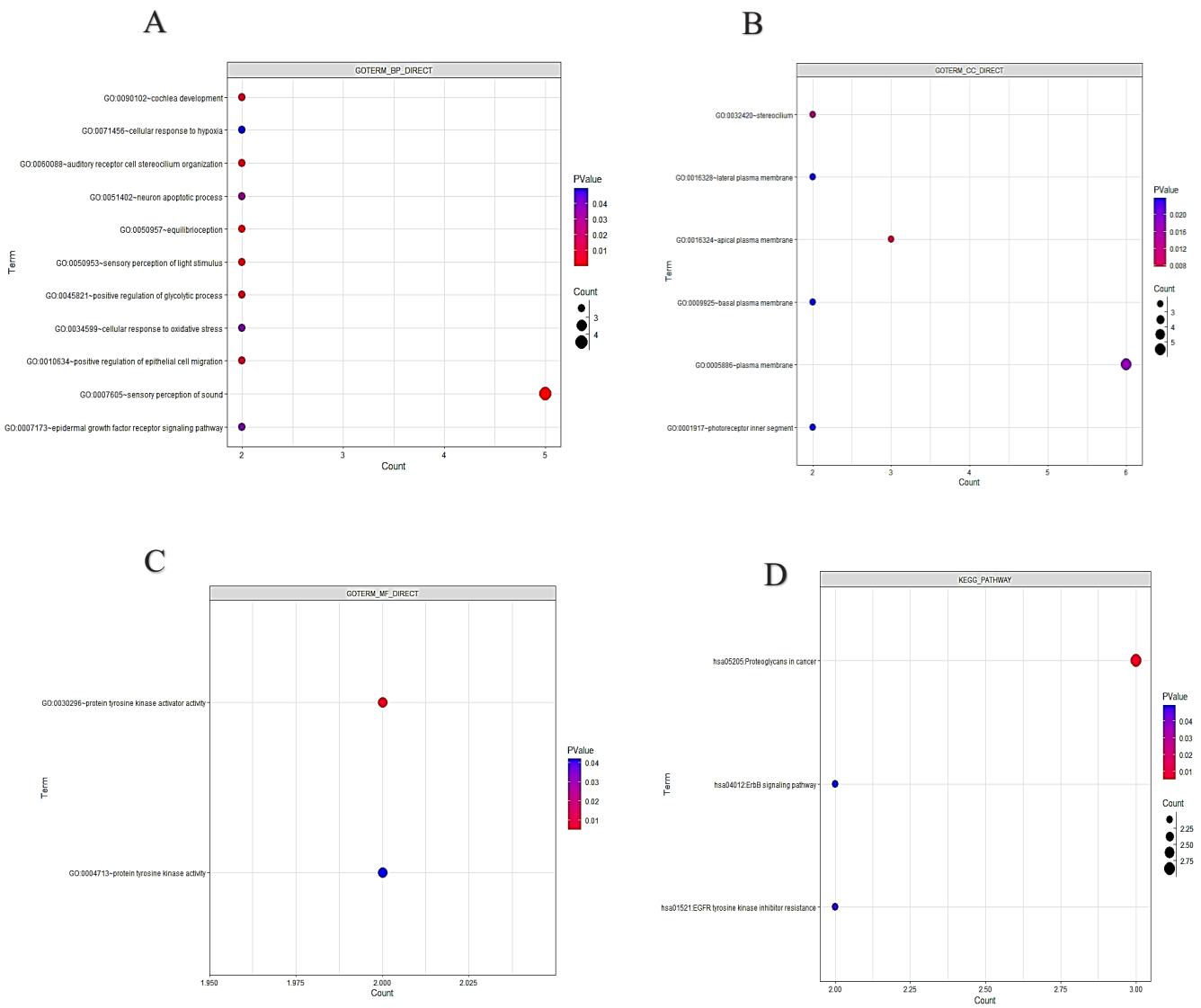

FIGURE 5. Shows the dot plot of enrichment of hub genes. A: biological process, B: chemical component, C: molecular function, and D: KEGG pathway.

cated condition that stems from the cumulative effects of aging on the auditory system. It is characterized by a gradual, symmetric decline in the ability to perceive sounds, particularly at higher frequencies (Agrawal et al., 2008).

Epigenetics is the study of changes in gene activity that can be passed down to offspring without changing the DNA sequence (Moore et al., 2013). It is now widely acknowledged that DNA methylation is a significant epigenetic factor that influences gene activity (Moore et al., 2013).

Recent studies have concluded that DNA methylation is crucial in various forms of hearing loss. This systematic review and bioinformatics analysis investigated DNA methylation in patients with ARHL, identifying 18 methylated genes across seven studies comprising 936 individuals. To conceptualize these findings, we integrate 18 methylated genes with 136 ARHL-associated genes from established data bases (CTD, GeneCards, and Coremine) for PPI network analysis, hypothesizing that the overlapping methylated hubs represent key epigenetic regulators in ARHL pathogenesis. Functional enrichment analysis, was performed initially on the 18 methylated genes using DAVID tools, and subsequently on PPI-derived hub genes. The results of enrichment revealed involvement of ERBB3, P2RX2, and FGFR1 in the calcium signaling pathway. Critically, an overlap analysis conducted by intersecting 18 methylated

genes with nine hub genes from PPI network identified SLC26A4, ERBB3, and CDH23 as the most relevant methylation regulated candidates, representing a 33% overlap and warranting focused interpretation.

The biological significance of these overlapping genes underscores DNA methylation's potential role in disrupting auditory pathway. SLC26A4, encoding the pendrin protein involved in anion transport and inner ear fluid hemeostasis, exhibits elevated promoter CpG sites, which correlates with an increased risk in men and sensorineural hearing loss phenotypes such as enlarged vestibular aqueduct(Xu et al., 2017). Hypermethylation of SLC26A4 may silence its expression, impairing bicarbonate and iodide transport in cochlea, thereby contributing to age related ionic imbalances and hair cell degeneration(Ito et al., 2011). Similarly, ERBB3, a receptor tyrosine kinase in ErbB family, shows down-regulation via DNA hypermethylation in elderly women with ARHL, potentially disrupting neurotrophic signaling essential for spiral ganglion neuron survival(Bouzid et al., 2018b; Guo et al., 2023a). This aligns with ERBB3's role in modulating inflammatory response and oxidative stress in auditory system, where methylation-induced repression could exacerbate presbycusis progression(Peng et al., 2022). CDH23, encoding cadherin-23 critical for stereocillia bundling in hair cells, is hypermethylated in ARHL patients, linking epigenetic changes to structural defects in the cochlea(Guo et al., 2023a). Genetic variants in CDH23 are already implicated in age-related and noise-induced hearing loss, and methylation may compound this by reducing cell adhesion and mechanotransduction efficacy(Jiao et al., 2024). These genes' methylation status thus positions them as epigenetic bridges between environmental exposures (e.g., age related oxidative stress) and ARHL onset.

Based on literature review ESPN and TNFRSF25 became more methylated as the hearing threshold deteriorated (Roche et al., 2022). Espins are multifunctional proteins that regulate the actin cytoskeleton. They affect the dynamics, dimensions, and signaling capabilities of microvilli, which mediate sensory transduction in various cells (Sekerková et al., 2004). Lack of espin proteins found in stereocilia can cause hearing loss in mice (Donaudy et al., 2006). Sekerkova et al. showed that the homozygous jerker mouse lacks espin proteins, resulting in abnormally short, thin, and unstable stereocilia (Sekerková et al., 2011). In GO analysis we also showed that ESPN participate in sensory perception of sound and stereocilium. Methylation of Espins may lead to ARHL by contributing to hair cell degeneration and progressive hearing loss(Ahmed et al., 2018; Donaudy et al., 2006).

The TNFRSF25 can bind necrosis factors and plays a role in apoptosis via its cysteine-rich extracellular domain (Schreiber et al., 2011). TNFRSF25 has not been detected in hair bundle preparation and has no known effect on hair cells (Krey et al., 2017). It may influence inflammation-related cochlear damage, reducing auditory function over time (Roche et al., 2025).

Bouzid et al. compared nine women suffering from ARHL with nine healthy women with normal hearing threshold. They showed that hypermethylation of KCNQ, P2RX2, SOCS3, and ERBB3 are associated with ARHL (Bouzid et al., 2018b). KCNQ5 is a potassium channel type that is present in the inner ear of adult zebrafish, mouse models, guinea pig, and rat cochlea (Liang et al., 2006; Spitzmaul et al., 2013; Wu et al., 2014). These potassium channels regulate neural excitability. Down-regulation of KCNQ is observed in ARHL, potentially accelerating ARHL by affecting cochlear potassium hemeostasis and vestibular function(Manville et al., 2019).

P2RX2 is an essential molecule involved in multiple cellular reactions, specifically in the excitatory postsynaptic responses of sensory neurons. It is a crucial signaling molecule that plays a role in maintaining normal cochlear homeostasis and sensitivity to sounds (Thorne et al., 2002). A decrease in P2RX2 receptor-mediated regulation of endocochlear potential in aged mouse cochlea results in decreased hearing sensitivity (Telang et al., 2010). Mutations in P2RX lead to severe age-related and noise-induced hearing impairment by disrupting channel function, making it a key factor predisposing to ARHL(George et al., 2019; Liu et al., 2020; Yan et al., 2013). Down-regulation of SOCS3 is associated with blocking Akt3, a protein kinase that boosts hair cell protection against ototoxic medications and is crucial for healthy hearing (Ghosh and Pahan 2012). Additionally, SOCS3 plays a negative role in controlling cytokines that trigger the JAK-STAT3 pathway, crucial for the regeneration of hair cells in zebrafish by activating stem cells, promoting cell division, and inducing differentiation (Liang et al., 2012). SOCS3 may contribute to

the inflammatory response in the cochlea, exacerbating ARHL. Supporting cells in the organ of Corti also express ERBB (Stankovic et al., 2004). As illustrated by previous research, all three genes play an active role in the inner ear. Furthermore, according to the enrichment analysis, P2RX2 plays a critical role in the sensory perception of sound and hearing. Additionally, SOCS3 and ERBB3 control the inhibition of signal transduction and nervous system development, respectively.

Wolber et al. performed a case-control study on participants with ARHL to explore methylated genes. They determined that the methylation of FGFR1, TCF25, and POLE was linked to ARHL (Wolber et al., 2014). It is thought that the FGFR1 gene is essential for maintaining the health of glial cells in the spiral ganglion and cochlear neurons (Wang et al., 2009). FGFR1 may contribute to ARHL potentially due to disrupted signaling in glial cells(Wang et al., 2009). TCF25 belongs to the basic helix-loop-helix transcription factor family and is extensively present in various organs, such as the mouse embryonic dorsal root ganglia (Olsson et al., 2002). Changes may affect the gene regulation in auditory pathways contributing to ARHL, though the direct mechanism is not clear (Patil et al., 2024). Besides, the elongation of the leading strand during cell division requires a DNA polymerase known as POLE. Additionally, POLE regulates the cell cycle, which in effect regulates several other biological processes (Olsson et al., 2002). Methylation alteration in ARHL may link to genomic instability in cochlear cells, potentially accelerating age-related damage and hearing impairment (Patil et al., 2024; Wang et al., 2009).

Guo et al. conducted a study in China on 57 monozygotic twin pairs. They showed that the methylation of DUSP4 gene is associated with high-frequency speech, C21orf5858 and ALG10 with high-frequency, C3 and LCK with speech and low-frequency, and GBX with low-frequency ARHL (Guo et al., 2023a). ALG10 is homologous to mouse Alg10b, which codes for a membrane-associated protein and is probably essential in maintaining the outer hair cells' function (Probst et al., 2013). ALG10 is involved in glycosylation, and methylation changes may disrupt protein maturation in auditory cells, contributing to ARHL progression(Guo et al., 2023a). A strong association has been discovered between tumor-related deafness and Perilymph C3. Furthermore, a relation has been identified between hearing deficiencies and the C3-S complement (Edvardsson Rasmussen et al., 2018; Lassaletta et al., 2019; Parving et al., 1993). Evidence showed that C3 was increased in the cochlea of older mice, suggesting a possible role of the complement system in either acoustic trauma or aging (Su et al., 2020). Gbx2 in mice is orthologous to GBX2 (Choo et al., 2006; Sánchez-Calderón et al., 2002b). The inner ear's development in mice depends on this particular gene. As demonstrated, GBX2 plays a role in the development of the nervous system. Methylation of GBX may alter developmental gene expression, leading to increased vulnerability to ARHL(Guo et al., 2023a; Sánchez-Calderón et al., 2002a). The LCK gene is a part of the Src family of protein tyrosine kinases and produces a protein that is unique to lymphocytes (Guo et al., 2023b). The LCK gene may play a role in immune-related cochlear aging, contributing to ARHL susceptibility.

Patil et al. conducted a systematic review on the role of DNA methylation in different types of hearing impairments. The majority of the included studies focused on ARHL. They concluded that while there is evidence supporting the role of DNA methylation in ARHL, inconsistencies in study designs make it difficult to definitively assess the exact impacts of each gene on ARHL(Patil et al., 2024). In contrast to Patil et al.'s study, we specifically examined presbycusis to determine the influence of DNA methylation on this condition and to obtain more definitive findings. However, due to differences in study methodologies, genes analyzed, and a limited number of participant groups, the results were inconclusive. Consequently, we performed a bioinformatic analysis to propose a hypothesis regarding the potential pathway through which these methylated genes may contribute to ARHL.

In this study, we applied a data mining approach to identify key gene-disease associations involved in age-related hearing loss (ARHL), using multiple established biological databases. Among the genes identified, those with the highest degree centrality were considered potentially important hubs in the ARHL-related gene interaction network. Interestingly, four out of the top ten high-degree genes were already mentioned in our literature review based on previous experimental or clinical studies. This overlap not only reinforces the biological significance of these genes but also highlights the robustness of our integrative approach, which combines

computational analysis with existing knowledge from the literature. Such consistency suggests that data mining can serve as a powerful tool to prioritize candidate genes for further experimental validation, especially when used in conjunction with comprehensive literature-based insights.

After analysis, we found that FGFR1, ERBB3, and P2RX2, are hypermethylated in individuals with ARHL and play a role in the calcium-signaling pathway. Besides other important genes with highest degree were shown to participate in calcium ion and ADP binding. Calcium is essential for the functioning of hair cells and serves as an important signal transducer. It plays several roles in hearing, entering the hair cell through mechanotransduction channels at the top of the cell and voltage-gated calcium channels at the base. Calcium is buffered by calcium-binding proteins in the cytoplasm and organelles such as mitochondria and the endoplasmic reticulum. Furthermore, it is eliminated by the Ca-ATPase pump distributed across the plasma membrane. Calcium also controls the transmission of signals in the central nervous system (Richard et al., 2023). Age-dependent declines in calcium-related activity within the central auditory pathway, including reduced expression of voltage-gated channels like Cav1.3 and Cav3.1, promote oxidative stress, mitochondrial dysfunction, and neural apoptosis-hallmarks of ARHL (Bao and Ohlemiller 2010; Gröschel et al., 2014; Perez and Bao 2011). Our findings suggest that methylation-induced interference in this pathway could explain why healthy individuals develop hearing disorders, supporting prior evidence that calcium disturbances contribute to hair cell death and auditory nerve degeneration (Gröschel et al., 2014; Pan et al., 2021). These results align with emerging literature on epigenetics in hearing loss, where DNA methylation is linked to both monogenic and polygenic forms, though evidence remains limited to a few genes in complex disorders like ARHL (Patil et al., 2024).

Limitation

The systematic review has some limitations. First, only five databases were searched. Due to the different genes and methodologies used to determine DNA methylation, a meta-analysis could not be performed. Additionally, the number of included studies was low, and most of them were case-control studies, which may have impacted their quality. While the expanded 136-gene PPI provides ARHL context, its genes lack confirmed methylation status, limiting direct epigenetic interferences. Future epigenome-wide studies could validate methylation in additional hubs like GJB2.

Conclusion

In conclusion, numerous studies have demonstrated that people with ARHL possess various differentially methylated genes. Methylation is believed to play a crucial role in the development of ARHL. This study highlights DNA methylation's role in ARHL via 18 genes, with overlaps identifying priority candidates for therapeutic targeting. The main genes identified in multiple studies were associated with the calcium signaling pathway. The findings of these studies suggest that targeting DNA methylation related to the calcium signaling pathway could be a promising approach for developing new treatments for ARHL.

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Conflict of Interests

There are no conflicts of interest to declare.

Author contribution

SN designed the research, SK and NB collected data, NB, SN, SR and HM analyzed data, NB and SR wrote the draft. SN, SK, and EA revised the draft . All authors reviewed the manuscript.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Informed consent

Not applicable.

Ethical Statement

The present study was approved by the research ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1402.752).

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