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Research Article

An Overview of Pathways Network Analysis of Pendred Syndromic Genes

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Abstract: Pendred syndrome, mainly labeled as sensorineural hearing loss and goiter, is an autosomal recessive disorder that forms an association to worsen the disease severity. It is the most known and prevalent form of the audiological disease. Some studies showed that the disorder may account for almost 10% of inherited deafness. Like all other disorders, a set of genes are known to characterize the Pendred Syndrome, where each one of them is segregated with the disease according to the families they belong to. The proposed study focusing on exploring the extent of segregation of each gene that is playing a role in the disorder. SLC26, a protein family of ion transporters, is known to play a significant role in this accord. The mutations in the genes mainly SLC26A4 underlie the major anomalies and malfunctioning that characterize the Pendred Syndrome. The biological pathway analysis using different online tools and databases like Ensemble decision analysis, DAVID (Database for Annotation, Visualization, and Integrated Discovery), IPA (Ingenuity Pathway Analysis software) was performed to see Cellular Enrichment Components and Biological Enrichment Processes followed by gene network analysis, which determines the candidate gene interactions including the ion transporter family genes that are present on the plasma membrane, out of which two genes belonged to the focused protein family SLC26. The proposed study has highlighted the central role of the SLC26 protein family in hearing impairment and hearing loss.

Keywords: Pendred syndrome, Hearing impairment, PDS gene, SLC26A4, Gene network, Ingenuity Pathway Analysis software (IPA).

1. INTRODUCTION

The inner ear of vertebrates acts as a sensory organ with complex sensitivity along with the structure [1]. The recurring utilization of pathways of common signaling along with the roles for transmembrane proteins is one of the noteworthy mechanisms that is involved in the development and arrangement of the inner ear that arises from the otic placode [2-4]. Hearing loss (HL) is the most occurring and well-known sensorineural disorder and its pathogenesis involves dozens of genes. The approach of genetic diagnosis of Hearing Loss is of incredible significance for patients with hearing anomalies and plays a key role in evaluating the danger of recurring in families [5]. HL influences one in 500–1000 newborn babies [6] and around half of these cases have a hidden hereditary reason for their abnormality. More than 400 hereditary disorders are associated with HL, and practically 80% of disorders linked to familial Hearing Loss are Non-Syndromic Hearing Loss (NSHL) [7, 8].

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Despite huge advancements in the identification of deafness-related genes, the hereditary reasons for acquired NSHL regularly stay indistinct because of extraordinary ethnicity-explicit variety and constrained phenotypic variance [9, 10].

In the current era, the Next-Generation Sequencing (NGS) technologies have empowered the specialists to recognize the obscure damaging variations among the noteworthy count of Hearing Loss (HL) patients [11]. The merging of initial linkage analysis and NGS techniques for narrowing down the chromosomal regions related to variations has proved to be a new strategy for examining deafness-related genes [12]. Genome-wide association studies (GWAS) measuring a huge number of Single Nucleotide Polymorphisms (SNPs) from thousands of subjects have massively recognized several relationships of genetic variations underlying 80 or more diseases approximately [13].

Pendred Syndrome (PDS), the extensively recognized reason for syndromic deafness is a blend of cochlear formative abnormalities, sensorineural hearing misfortune, vestibular dysfunction, and goiter representing 10% of all reported genetic hearing loss [14]. Mutations in the SLC26 gene family root both DFNB4 and Pendred disorder, which are two autosomal recessive disorders, share hearing misfortune as an emblematic characteristic [15]. The PDS gene (SLC26A4) which encodes Pendrin protein is positioned on chromosomal region 7q31 and was recognized by Bioinformatics positional cloning in 1997 [16]. More than 50 autonomous gene variations have been considered for instigating PDS and non-syndromic deafness, which was re-affirmed by a discharge test featuring perchlorate. PDS mutations are dispersed throughout the coding region, being recognized in 18 of the 21 exons. Most transformations insertions, deletions, missense, splice site, and frameshift mutations [17, 18]. The infrequent permutation of goiter and deafness experienced in Pendred disorder brings up some intriguing issues that how flaws with regards to a similar protein (Pendrin) prime to the impacts that are tissue-explicit [17]. To pick up the answers to these intriguingly arising questions, the expression of PDS and localization of Pendrin at subcellular locations in several tissues were examined [19].

The proposed study aimed to investigate the biological pathways that help in the analysis of the trend of PDS related genes in human deafness by looking into the behavior of candidate genes in disease states and the effects of drugs by constructing their network. Various bioinformatics tools and databases were used to investigate the interactions among candidate genes in human deafness and sensory perception of sound across multiple biological aspects including disease processes, molecular interactions, and cellular processes. The biological behavior of most common protein families that are involved in sensory perception of sound and highlighted several genes as potential targets of genetic hearing loss for diagnosis and treatment purposes was also explored.

2. MATERIALS AND METHODS

2.1 Gene Mining associated with Hearing Impairment

Gene mining is a supervised learning process for the identification of contributed genes in a specific biological pathway. Gene mining can also be employed to figure out the gene associated with any syndrome. To acquire the data of genes allied with Hearing Impairment (HI), an exhaustive literature survey was performed to find out the genes exclusively associated with Pendred Syndrome. Only (PDSO) using the analytical method based on Pearson`s correlation matrix by ensemble decision analysis of all 80 genes.

2.1.1 Cellular Enrichment Components and Biological Enrichment Process

The behavior of the candidate genes in biological pathways and the labeling of the associated genes were predicted by analyzing the gene ontology, cellular enrichment components, and biological enrichment process of the PDSO gene using DAVID [20], a Database for Annotation, Visualization and Integrated Discovery database (https://david.ncifcrf.gov/).

2.1.2 Gene Network

The output of gene ontology predicting the biological pathways is the broad range of information that is necessary to be narrowed down to find out the larger

gene families that are responsible for the hearing impairment, especially in Pendred syndrome. For this purpose, the confidence score of <=0.05 was used for the prediction of the interactions among associated genes for top-ranked protein family based on the well-characterized cell signaling and metabolic pathways including molecular and biochemical studies, a network analysis was performed using Ingenuity Pathway Analysis software (IPA; Ingenuity Systems, Redwood City, CA, USA) [21], software connected to world`s largest knowledge-based biological network system. The most interconnected molecules in a network were referred to as a central node. The network of each group was studied to estimate the likelihood that all genes of a group fit into the same network. Statistical analysis of network and pathways was performed in IPA using the right-tailed Fisher`s exact test to filter pathways by using the cut off value of ($p<^{10-27}$) to find the whole drug-able statistical background.

3. RESULTS

Based on an exhaustive literature review, we find out 80 genes and after applying the filtration

Table 1. Complete information about Pendred syndrome-associated genes and their phenotypic characteristics. The fifth column shows relevant literature.

Hearing Impairment in Pendred Syndrome				
Genes	**Chromosome**	**Syndromes**	**Phenotypical Effect**	**References**
GJB3	1	Vestibular aqueduct syndrome (EVAS), Pendred syndrome (PDS)	Hearing loss	[34]
PJVK	2	Nonsyndromic hearing loss, Pendred syndrome.	Hearing loss	[35]
GRXCR1	4	Usher syndrome, Pendred syndrome,	Otoacoustic loss (OAE loss), Hearing loss	[36]
MARVELD2	5	Alport and CHARGE syndromes, Waardenburg syndrome, Pendred syndrome, type 2 Baraitser-Winter syndrome	Hearing loss, Sensorineural hearing loss (SNHL) phenotype	[37]
DIAPH1		Pendred syndrome, Hearing Loss (HL) syndromes	Hearing Loss age of onset, severity, audiogram shape	[38]
FOXI1		Pendred syndrome	Pendred syndrome (PDS) phenotype, deafness phenotype	[39]
GRXCR2		Waardenburg syndrome, auditory-pigmentary syndrome, multiple neoplasia syndrome, Stickler syndrome, Usher syndrome, Pendred syndrome, Jervell and Lange-Nielsen syndrome	Hearing loss (HL), hearing and speech disorders	[40]
EYA4	6	Waardenburg syndrome, deafness syndrome (EVA Syndrome), Usher and Pendred/Enlarged vestibular aqueduct syndrome	congenital Hearing Loss (HL), pigmentation anomalies in eyes, skin, and hair	[41]
DFNA5	7	Muckle-Wells syndrome, Pendred syndrome, Usher syndrome, Waardenburg syndrome	audiometric phenotype, (unilateral Hearing Impairment)	[42]
SLC26A3		Bartter's syndrome, Pendred Syndrome (PDS)	Deafness	[43]

Hearing Impairment in Pendred Syndrome

Genes	Chromosome	Syndromes	Phenotypical Effect	References
SLC26A4		Pendred syndrome (PDS),	Hereditary deafness	[44]
SLC26A5		Pendred syndrome (PDS), nonsyndromic recessive hearing impairment	hearing loss	[45]
LRTOMT	11	Pendred syndrome, Clouston syndrome, usher syndrome.	Age at onset, Penetrance, Expressivity key features, Audiological features.	[46]
RDX		clinically distinct syndromes, Usher syndrome, Pendred syndrome Clouston syndrome	Age at onset, Penetrance, Expressivity key features, Audiological features,	[47]
TECTA		Usher syndrome, Pendred syndrome and Jervell and Lange-Nielsen syndrome, Wolfram syndrome, Keratitis-Ichthyosis-Deafness (KID) syndrome	deafness, hearing loss phenotype	[7]
P2RX2	12	Waardenburg syndrome, auditory-pigmentary syndrome, multiple neoplasia syndrome, STL syndrome, Usher syndrome, Pendred syndrome (PDS)	unquestionable phenotypic manifestations, deafness, Hearing Loss, hearing impairment	[47]
STRC	15	auditory-pigmentary syndrome, multiple neoplasia syndrome, Stickler syndrome, Pendred syndrome (PDS), Jervell and Lange-Nielsen syndrome, Alport syndrome, Mohr-Tranebjaerg syndrome	hearing loss, malformations of the external ear progressive deafness	[48]
CRYM	6	Pendred syndrome (PDS), nonsyndromic hearing loss, Clouston syndrome	Age at onset, Penetrance, Expressivity key features, Audiological features, Syndromic features, hearing loss. Deafness	[49]
TBC1D24		Usher syndrome, Pendred syndrome (PDS)	prelingual profound hearing loss	[50]
LOXHD1	18	usher syndrome type 1B (USH1B) and USH3, Usher syndrome 1D (USH1D), Oto-Spondylo-Mega-Epiphyseal Dysplasia (OSMED) syndrome, syndrome type If (USH1F), Pendred syndrome (PDS), Smith-Magenis syndrome	retinal phenotypes, Deafness, hearing loss	[51]
GIPC3	19	Usher syndrome, Pendred syndrome (PDS)	prelingual profound hearing loss	[52]

through ensemble decision analysis the number reduced to 21, all having the association with Pendred Syndrome with hearing loss [22]. Table 1 depicts the overall chromosome wise distribution of the 21 genes associated with the syndrome and the phenotypic effect caused by a mutation in each gene. The results showed that Chr. 1,2,4,6,12,15,18,19 have only one PDS associated gene, Chr.16 and 11 have 2 and 3 PDS associated genes respectively and chromosomes 5 and 7 have 4 genes. It was also observed that some genes were responsible not only for hearing loss but also for the nonfunctioning of the audiological features. These genes were DIAPH1, LRTOMT, RDX, P2RX2, STRC, and CRYM. Some genes like GRXCR2 and EYA4 showed different behavior, for example, it was noted that due to the malfunctioning of the GRXCR2 gene hearing and speech disorders came into account while a mutation in EYA4 may cause pigmentation anomalies in the eye, skin, and hair.

3.1 Cellular Enrichment Components and Biological Enrichment Process

David database was used to assess the signal transmission in the cell. The results highlighted the cellular enrichment components including the pathway description of each cluster of genes. The Pendrin SLC264A gene was found in the largest group of 9 genes with Pathways ID GO.0042995 involved in the cell projection, a type of cellular protrusion. For the enrichment analysis shown in Table 2, the whole genome statistical background was assumed. A group of a maximum of 15 genes with Pathways ID GO.0007605 was observed to be involved in the sensory perception of sound with an FDR (False Discovery Rate) value of $2.59x10^{-25}$. It was also predicted that the SLC26 family genes were a member of each pathway involved in the overall biological enrichment processes in Gene Ontology (Table 3). IPA database supported the same results as predicted from the DAVID Database, shown in Fig 1.

3.2. Gene Network

Network analysis of genes including audiological features such as hearing and speech disorder, malformation of the external ear, and pigmentation anomalies in eye, skin, and hair was studied which revealed 3 networks. The network with the maximum number of 35 molecules with 10 focused

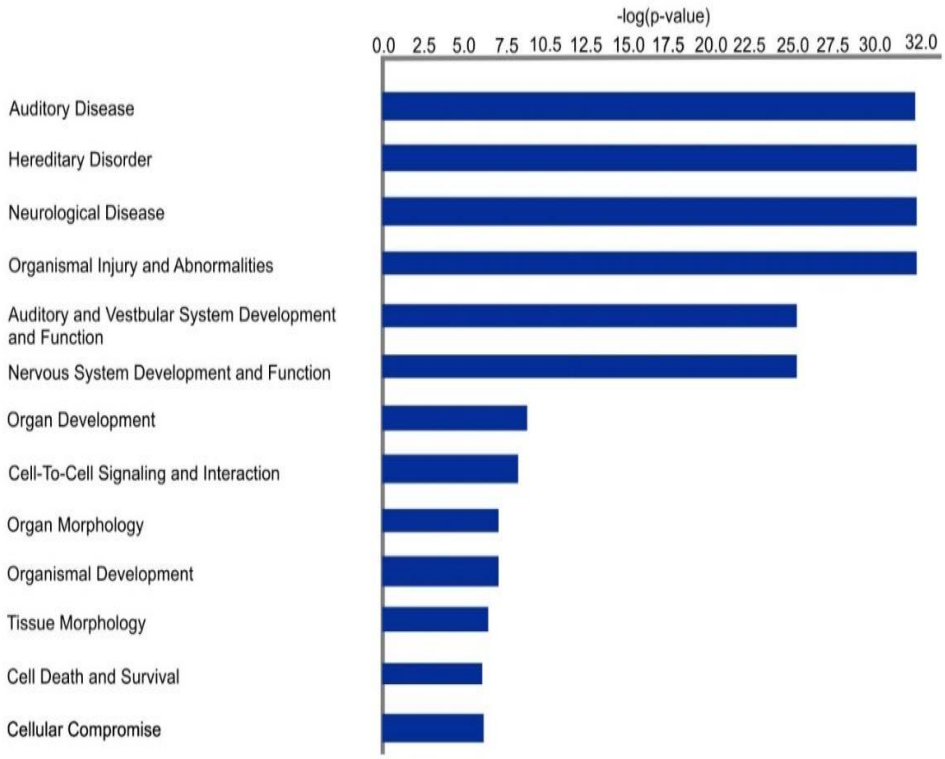

Fig 1. Gene Ontology behavior: disease-associated biological expressional analysis keeping the whole genome as a statistical background for the identification of Pendred syndrome: Hearing Loss (HL).

Table 2. Gene Ontology behavior: cellular enrichment analysis keeping the whole genome as a statistical background (DAVID Database)

Cellular Enrichment Components (GO)				
Pathway ID	**Pathway description**	**Observed gene count**	**False discovery rate**	**Matching proteins clusters (labels)**
GO.0032420	stereo cilium	5	9.95E-08	GRXCR1, GRXCR2, LOXHD1, RDX, STRC
GO.0032421	stereo cilium bundle	5	2.74E-07	GRXCR1, GRXCR2, LOXHD1, RDX, STRC
GO.0005902	microvillus	5	6.93E-06	GRXCR1, GRXCR2, LOXHD1, RDX, STRC
GO.0042995	cell projection	9	0.00238	DIAPH1, GRXCR1, GRXCR2, LOXHD1, P2RX2, RDX, SLC26A4, STRC, TBC1D24
GO.0060091	Kino cilium	2	0.00445	GRXCR1, STRC

Table 3. Gene Ontology behavior: biological enrichment analysis keeping the whole genome as a statistical background for the identification of Pendred syndrome: Hearing Loss (DAVID Database)

Biological Enrichment Process (GO)				
Pathway ID	**Pathway description**	**Observed gene count**	**False discovery rate**	**Matching proteins in your network (labels)**
GO.0007605	sensory perception of sound	15	2.59E-25	CRYM, DFNA5, DFNB59, DIAPH1, EYA4, GJB3, GRXCR1, LOXHD1, LRTOMT, MARVELD2, P2RX2, SLC26A4, SLC26A5, STRC, TECTA
GO.0007600	sensory perception	14	6.62E-12	CRYM, DFNA5, DFNB59, DIAPH1, GJB3, GRXCR1, LOXHD1, LRTOMT, MARVELD2, P2RX2, SLC26A4, SLC26A5, STRC, TECTA
GO.0050877	neurological system process	13	6.62E-09	CRYM, DFNA5, DFNB59, DIAPH1, GJB3, GRXCR1, LOXHD1, LRTOMT, MARVELD2, SLC26A4, SLC26A5, STRC, TECTA
GO.0048839	inner ear development	5	0.00135	DFNA5, FOXI1, LRTOMT, SLC26A5, STRC
GO.0043583	ear development	5	0.00203	DFNA5, FOXI1, LRTOMT, SLC26A5, STRC
GO.0042491	auditory receptor cell differentiation	3	0.00608	GRXCR1, LRTOMT, STRC
GO.0060119	inner ear receptor cell development	3	0.00856	GRXCR1, LRTOMT, STRC
GO.0060113	inner ear receptor cell differentiation	3	0.0236	DFNA5, LRTOMT, STRC
GO.0002064	epithelial cell development	4	0.031	LRTOMT, MARVELD2, RDX, STRC

genes was selected with a high significance score of 28 ($p = 10^{-27}$) (Fig 2). The rest of the 2 networks were rejected because of their fewer molecules (one was with 3 molecules and one was having 2 molecules) with one focused gene in each network. All three networks were involved in auditory disease on top, hereditary disorder on the second number, and neurological disease on number three. As our focus was to determine only Pendred syndromic genes network so the study found a very small network with the middle nodule Ca^{2+} with 16 edges while 14 and 12 edges were connected to ELAVL1 and cyclic AMP respectively. Detailed information revealed that DIAPH1, EJB3, LRTOMT, MARVELD2, P2RX2, SLC26A4, SLC2645 were located on the plasma membrane while EYA4, DFNA5 were located on cytoplasm and GRXCR1, LOXHD1, STRC, AND TECTA were located in extracellular space as shown in Fig. 3. IPA databases also revealed that among the genes located on the plasma membrane, GJB3, SLC26A4, SLC26A5, and RX2 were ion transporters. Also, found that none of our candidate genes formed any cluster in the network which proved that these genes are indirectly interacting with each other in Pendred Syndrome. This network indicates many other genes playing direct and indirect roles in Pendred syndrome which may also be considered as candidate genes for auditory disease treatments. Aiming to determine the role of ion transporters in underlying causes of Pendrin protein found that the SLC26A4 and SLC26A5 protein playing a vital role in different transport mechanisms such as the iodide transport mechanism where the thyrocyte uptakes NA^{+}/I- cotransporter on the basolateral membrane where Pendrin (SLC26A4) permits iodide ions within the thyrocyte to pass through the apical membrane and into the colloid space where it quickly binds to Tg by a catalyzed reaction regulated by thyroid peroxidases (TPO) [12].

IPA network analysis tool also provided information about upstream regulators involved in biological pathways interacting with our candidate genes. Table 4 shows Pendrin protein (SLC26A4) as a vital protein having interactions with most

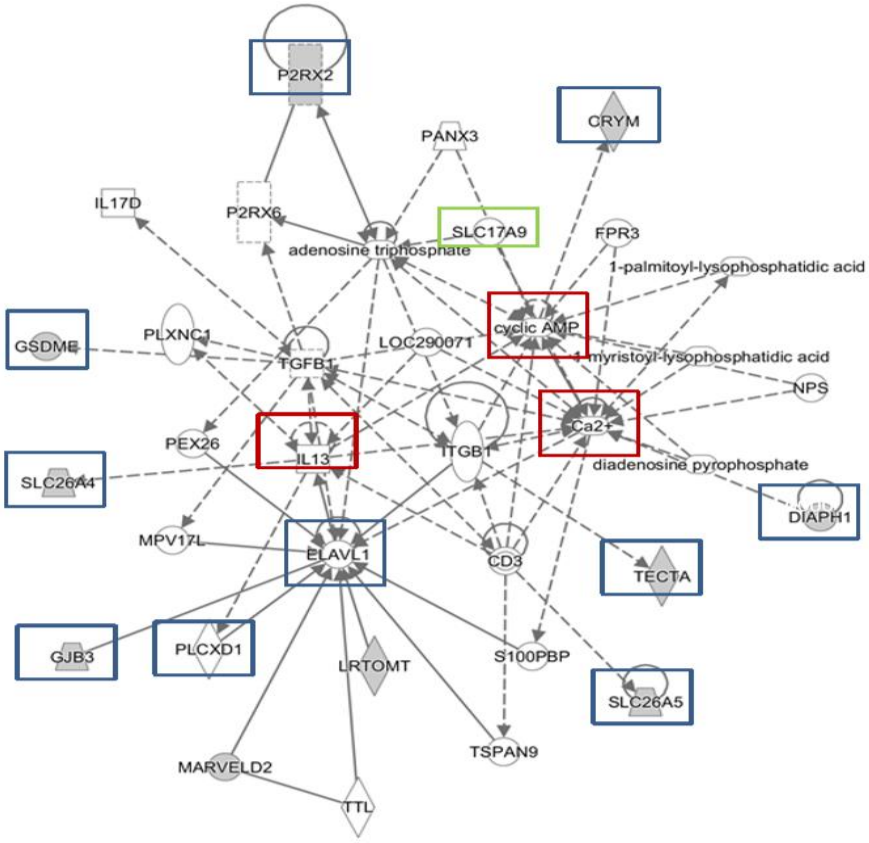

Fig 2. The most significant gene network produced by IPA network analysis: Three types of nodes are indicated here: central nodes with maroon color, candidate genes with dark blue color, and SLC17A9 gene is highlighted with green color as it belongs to the SLC family and it is not from the candidate gene list.

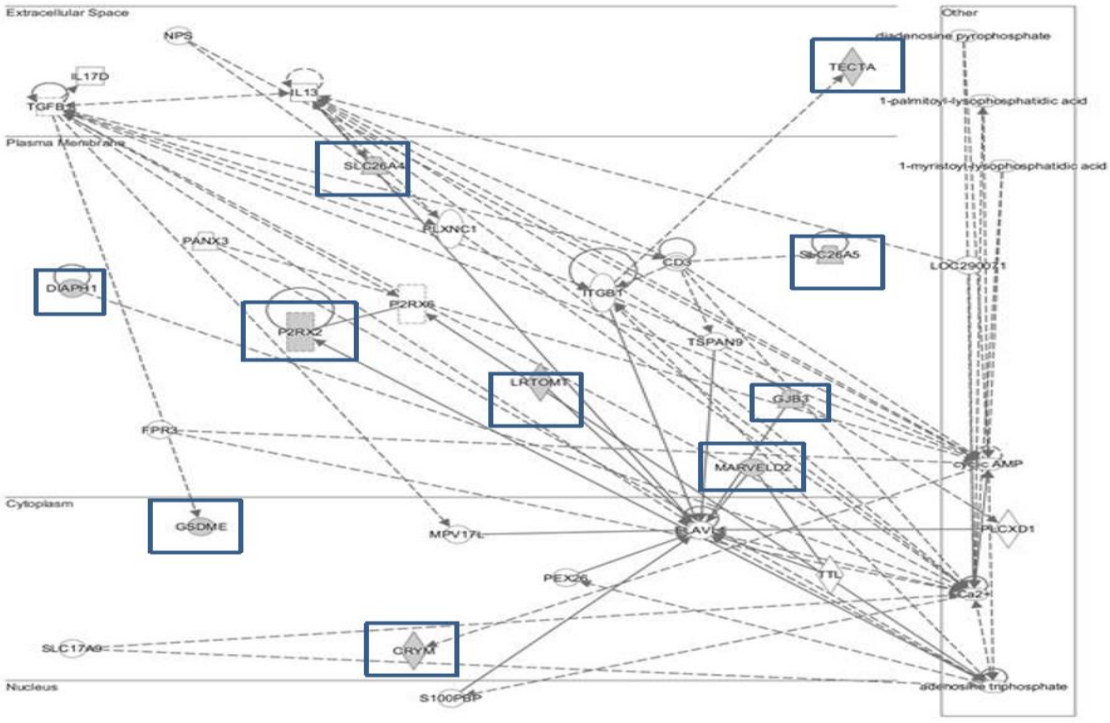

Fig. 3. The candidate genes are categorized based upon their working in cellular pathways including ion transportation, catalytic activities, and their locations in extracellular space, plasma membrane, cytoplasm, and nucleus.

Table 4. The most common Pendred syndromic upstream regulator interacting with candidate genes.

Upstream Regulator	Molecule Type	p-value of overlap	Target molecules in the dataset
GUCA2B	Other	0.000692	SLC26A4
Chloride	chemical - endogenous mammalian	0.00207	SLC26A4
Mineralocorticoid	chemical drug	0.00207	SLC26A4
FOXI1	transcription regulator	0.00346	SLC26A4
Iodide	chemical - endogenous mammalian	0.00415	SLC26A4
Acetazolamide	chemical drug	0.00415	SLC26A4
LASP1	Transporter	0.00552	MARVELD2
Hormone	chemical drug	0.00896	SLC26A4
miR-9-5p (and other miRNAs w/seed CUUUGGU)	mature microRNA	0.0124	DIAPH1
NOX1	Enzyme	0.0124	DIAPH1
ADCY	Group	0.0151	CRYM
Sucrose	chemical - endogenous mammalian	0.0158	CRYM
deoxycorticosterone acetate	chemical drug	0.0165	SLC26A4
BTG2	transcription regulator	0.0172	DIAPH1
TG	Other	0.0212	SLC26A4
miR-200b-3p (and other miRNAs w/seed AAUACUG)	mature microRNA	0.0287	GJB3
N-formyl-Met-Leu-Phe	chemical reagent	0.03	DIAPH1
Methimazole	chemical drug	0.0347	SLC26A5
ESR2	ligand-dependent nuclear receptor	0.0349	EYA4, GJB3
miR-141-3p (and other miRNAs w/seed AACACUG)	mature microRNA	0.048	GJB3
PAX7	transcription regulator	0.0494	EYA4

of the chemical drugs, transcription regulators, and endogenous mammalians. By having a cut-off value ($p = 4.15*10^{-3}$) found that only Pendred protein was interacting with GUCA2B, chloride, mineralocorticoid, FOXII, iodide, and acetazolamide. GUCA2B and FOXI1 are the genes, mineralocorticoid and acetazolamide are chemicals and chlorides are iodides are ions.

4. DISCUSSION

In this study, expression of Pendred syndrome and subcellular localization of Pendrin in different tissues and their biological pathways were analyzed. The networks and pathways helped us in understanding the role of candidate genes in human deafness and the effect of drugs upon them [23]. After Gene mining and Ensemble decision analysis, 21 out of 80 genes were selected and gene enrichment analysis was performed through DAVID [20]. IPA software was used to predict interactions among the associated genes. Among the shortlisted 21 genes, the SLC26 gene family was most significant having more than 1 gene associated with PDS. Genes namely DIAPH1, LRTOMT, RDX, P2RX2, STRC, and CRYM not only cause loss of hearing but also non-functioning of audiological features [24].

Gene enrichment and pathway analysis by DAVID showed a somewhat bigger picture of genes interacting with each other. In cellular enrichment analysis, a total of five pathways were identified. The largest pathway was 'cell projection' which consisted of 9 genes including the SLC26A4 gene with 0.00238 FDR value. Other pathways were stereo cilium, stereo cilium bundle, microvillus, and Kino cilium. Biological enrichment analysis showed 15 candidate genes involved in sensory perception of the sound pathway with an FDR value of $2.59x10^{-25}$. Other pathways identified during biological enrichment analysis were neurological system process, inner ear development, ear development, auditory receptor cell differentiation, inner ear receptor cell development, inner ear receptor cell differentiation, and epithelial cell development [25].

In IPA analysis 1 out of 3 gene networks with 35 molecules and 10 focused genes were selected. ELAVL1, cycAMP, and CA^{2+} being central nodes of the selected network were connected to 12, 14, and 16 nodes respectively. Plasma membrane and cytoplasm genes were identified, and it was reported that some genes present on the plasma membrane were ion transporters. These genes are GJB3, SLC26A4, SLC26A5, and RX2. SLC26A4 is the most vital gene in PDS involved in several important ion transport mechanisms[26] like the iodide transport mechanism producing thyroid peroxidases [27]. SLC26A4 also interacts with several molecules and genes including GUCA2B, chloride, mineralocorticoid, FOXII, iodide, and acetazolamide that directly initiate PDS [28]. FOXI1 gene is a transcriptional activator that is responsible for the sense of balance in the development of normal hearing [29]. It plays a vital role in the expression of SLC26A4, JAG1, and COCH genes which contribute to the development of the endolymphatic system in the inner ear and also help in the differentiation of intercalated cells [30] in the distal renal tubule`s epithelium by supporting SLC4A1, SLC4A9, and ATP6V1B1 genes [31]. GUCA2B gene encodes proteolytic preprotein [32] for producing multiple proteins such as uroguanylin and guanylate cyclase-C receptor`s [33] endogenous ligands. This gene being the transcriptional regulator stimulates SLC26A4 (PDS) indirectly.

The proposed study has highlighted the central role of the SLC26 protein family in hearing impairment and hearing loss. The future benefit associate with the proposed system is the identification of genes, pathways, or networks that will be responsible for hearing impairment and hearing loss. It allows the pharmaceutical scientist and drug designers to target the respective genes or pathways to develop a candidate drug-using docking mechanism.

5. CONCLUSIONS

This study focused on the cellular enrichment, network and pathway analysis of human Pandered syndromic genes. By studying the ontology behavior of genes, we constructed a network to understand the role of these genes and their interactions with each other. Our analyses provided a platform to understand the role of the Pandered syndrome in different cellular pathways and a cutting-edge manifesto to treat and design new drugs not only for deafness but also for the inner

ear receptor cell development, epithelial cell development, and neurological system process. Our study used knowledge-based databases that are being evolved day by day and provide updated integrated information.

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