Hearing Impairment in Mucopolysaccharidosis: A Systems Biology Approach

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Abstract

Mucopolysaccharidoses (MPS) are lysosomal diseases caused by deficiencies in lysosomal enzymes involved in the degradation of glycosaminoglycans (GAGs). Sensorineural hearing impairment is a common feature in MPS patients, but there is no consensus on its etiology. For this reason, we aimed to identify genes and pathways related to hearing loss and to correlate them with gene expression data in MPS. We used HPO and Disgenet to identify candidate genes. We constructed the network with string and Cytoscape, and hub genes were identified in Cytohubba. Expression data were obtained from the MPSBase website. We found the *NDUFA* gene family as the major hub genes and 114 enriched pathways related to hearing loss. These genes and biological pathways may serve as potential candidates for clinical studies to better understand hearing impairment mechanisms in lysosomal storage diseases like mucopolysaccharidosis.

Keywords

Ear development, hearing impairment, network analysis, lysosomal storage diseases.

Introduction

Mucopolysaccharidoses (MPS) are inborn errors of metabolism characterized by deficiencies in lysosomal enzymes involved in the degradation of glycosaminoglycans (GAGs) which are constituents of the extracellular matrix. These multi-systemic diseases are classified into types based on the differences between the enzyme deficiency and the accumulated GAGs. The ear-nose-throat (ENT) manifestations are common in MPS patients with recurring ear infections and frequently present hearing loss [1]. Hearing impairment significantly impacts patients' quality of life, affecting speech and language development [2].

Hearing loss is common in almost all types of MPS, except in MPS IVB and MPS IX, and can be classified as conductive, sensorineural, or mixed. Each MPS type has a specific type of hearing loss [3]. The conductive occurs when sound conduction is impeded through the ear [4] due to ossicular chain deformities or disruption, or seromucous otitis [1]. Sensorineural occurs due to problems in the cochlea or the neural pathway to the auditory cortex problems [4]. Sensorineural hearing impairment is a common feature in MPS patients, but there is no consensus on its etiology [3]. The mixed type is the concomitant conductive and sensorineural loss.

Although hearing impairment is a common feature in MPS patients, the available treatments do not significantly improve hearing loss [1,3]. The mechanisms of hearing loss in MPS remain to be elucidated. Studying and identifying pathways related to hearing can provide helpful information that can be used to develop new therapies.

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Methods

Gene Candidate Analysis

We searched for the terms "hearing loss" in the Human Phenotype Ontology - HPO [5] and in the Disgenet databases [6]. The HPO database provides a standardized vocabulary of phenotypic abnormalities encountered in human diseases, and their related genes. The Disgenet database has a similar approach, but the database integrates information of human gene-disease association and variant-disease associations from various external databases with information about Mendelian, complex and environmental diseases.

The related terms found in the HPO and Disgenet are shown in Table 1. Then, we used the unique genes between the two databases in the following analysis steps. The flowchart of the methodology is shown in Figure 1.

Network Analysis

A gene network was constructed with the selected candidate genes in the STRING database v.11.0 [7]. We used high confidence network scores (0.07) to obtain only experimental data, and text mining interactions were excluded from the analysis. Only the query proteins were considered, without first and second shell interactors. The analyses were performed in Cytoscape v.3.8, with curated plugins [8]. To identify the hub genes, we used Cytohubba v.0.1 with the local based method Maximal Clique Centrality, MCC [9].

Gene Set Enrichment and Expression Analysis

The functional enrichment was quantitatively assessed (p-value) using a hypergeometric distribution. Multiple test correction was also implemented by applying the FDR at a significance

Table 1. Related terms and phenotypes used in the network analysis.

Phenotype	Code	Related genes
	HPO	
Hearing abnormality	HP:0000364	1392
Hearing impairment	HP:0000365	1369
Sensorineural hearing impairment	HP:0000407	2182
Conductive hearing impairment	HP:0000405	367
Mild hearing impairment	HP:0012712	33
Childhood onset sensorineural hearing impairment	HP:0011474	16
Moderate hearing impairment	HP:0012713	29
Progressive hearing impairment	HP:0001730	58
Profound hearing impairment	HP:0012715	13
	Disgenet	
Sensorineural Hearing Loss (disorder)	C0018784	783
Conductive hearing loss	C0018777	291
Sensorineural hearing loss, bilateral	C0452138	117
Sudden sensorineural hearing loss	C4275242	72
Congenital sensorineural hearing loss	C1865866	68
Hearing Loss, High-Frequency	C0018780	39
Noise-induced hearing loss	C0018781	36
Hearing Loss, Mixed Conductive-Sensorineural	C0155552	32
Profound sensorineural hearing loss	C1848641	28
Hearing Loss, Extreme	C0086395	20
Hearing Loss	C3887873	18
Complete Hearing Loss	C0581883	20

level of p<0.05. We used the Biological Network Gene Ontology (BiNGO) plugin v.3.0.4 [10] to identify the biological processes (BP), molecular function (MF), and cellular component pathways. To determine the KEGG [11] pathways, we used the pathfindR package [12] in the R environment [13]. To evaluate the expression of the hub genes, we searched for datasets in the MPSBase [14]. We also selected the most frequent genes which appear in the pathways to evaluate their expression in the available transcriptomic MPS datasets, which were obtained from human IPS and Hela cells.

Results

In HPO, we found 1393 genes, and in Disgenet 1078 (Figure 1). In total, 1679 unique genes were present in either bank. After removing genes without any connections by String, 1617 remained. The Cytoscape network was composed of 827 nodes (genes), and 3777 edges (number of interactions between the genes).

The top hub genes and the related neighbors are shown in Figure 2. Most of them are part of the NADH Ubiquinone Oxidoreductase family, like *NDUFB7*, *NDUFS7*, *NDUFB8*, *NDUFA13*, *NDUFS2*, *NDUFV1*, *NDUFV2*, *NDUFS3*, *NDUFA9*, *NDUFA2*, *NDUFB5*, *NDUFA6*, *NDUFB9*, *NDUFB10*, *NDUFS1*, and *NDUFA7*. In addition, we also identified as hub genes the Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase genes, like *MTND6*, *MTND2*, and *MTND3*. Another hub gene identified in our analysis is the Ubiquinol-Cytochrome C Reductase, Rieske Iron-Sulfur Polypeptide - *UQCRFS1* gene.

Regarding the pathway analysis, the most frequent genes were *MAPK1*, *PIK3CA*, *PIK3R1*, *AKT1*, *KRAS*, *MAP2K1*, *NRAS*, *PRKCB*, *RAF1*, and *NFKB1*. There were 114 enriched pathways related to the hearing loss gene list (Table 2). The top KEGG-related pathways are shown in Figure 3. We also constructed the KEGG maps to understand how the gene hub list affects the enriched pathways (Figure 4, Supplementar File 1).

Gene expression analysis showed *NDUF* genes (the top hub genes) to be up-regulated in MPS IIIB, while *NDFUS7* is down-regulated in MPS I. *NDUFV2* and *NDUFS3* are not identified as differentially expressed. The same pattern is seen in the gene pathways list (Table 3).

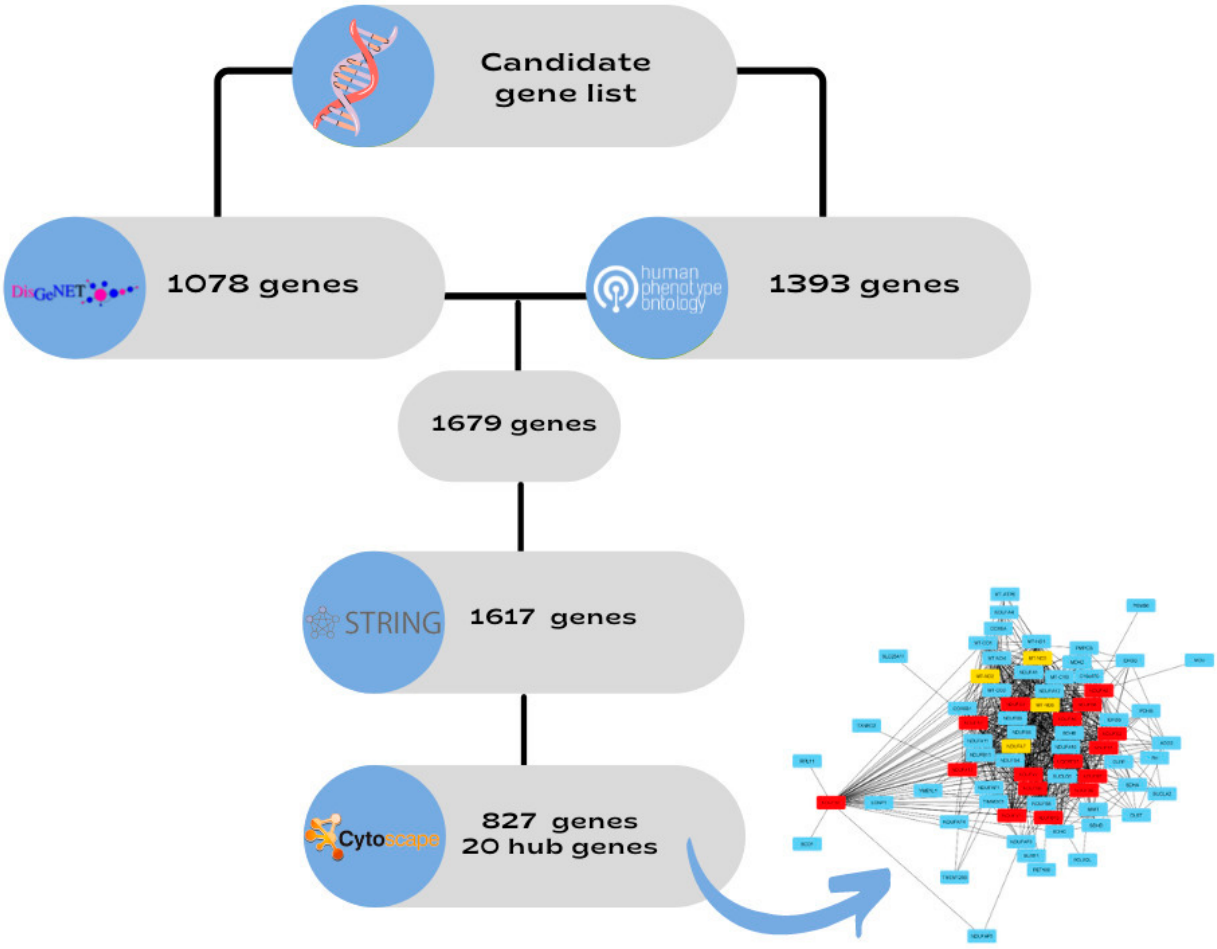

Figure 1. Bioinformatics pipeline.

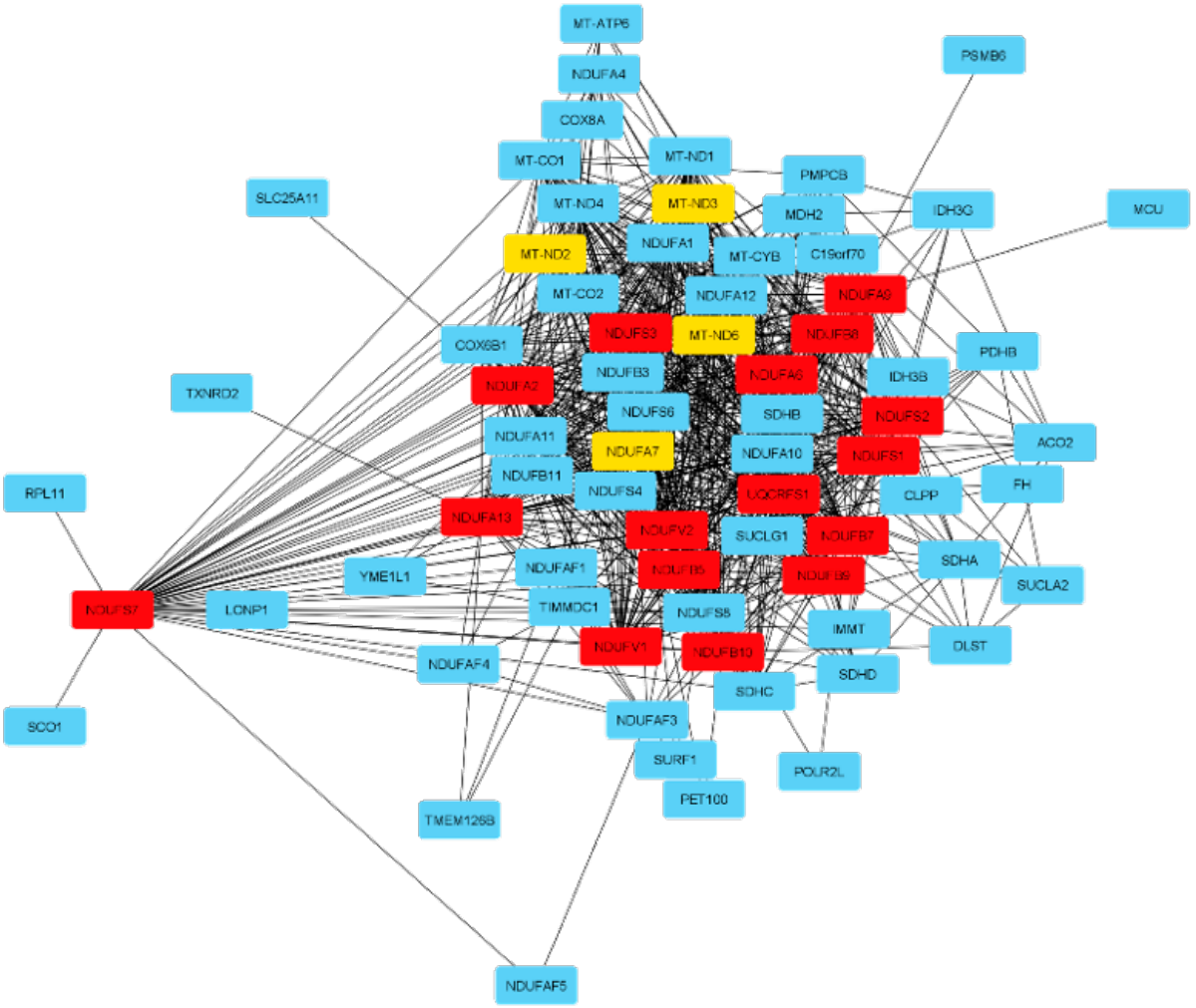

Figure 2. Hub gene network and the related expanded subnetwork. The MCC method ranks the genes with a red-yellow scale, when red genes are the most relevant in the top 20 genes in the network.

Table 2. Enriched pathways related to the hearing loss gene list.

ID	Term_Description	p_value	Genes
hsa00190	Oxidative phosphorylation	5.06E-46	*NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NDUFA1, NDUFA2, NDUFA4, NDUFA6, NDUFA7, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFB3, NDUFB5, NDUFB7, NDUFB8, NDUFB9, NDUFB10, NDUFB11, SDHA, SDHB, SDHC, SDHD, UQCRFS1, COX10, COX2, COX6B1, COX7B, COX8A, COX15, ATP6V1B1, ATP6V1B2, TCIRG1, ATP6V0A4, ATP6AP1*
hsa04714	Thermogenesis	1.22E-43	*GNAS, SMARCA2, SMARCA4, SMARCB1, SMARCC2, SMARCD1, SMARCE1, ACTG1, ACTB, ACTL6B, ARID1B, ARID1A, PNPLA2, FGFR1, SOS1, KRAS, NRAS, RPS6KA3, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NDUFA1, NDUFA2, NDUFA4, NDUFA6, NDUFA7, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFB3, NDUFB5, NDUFB7, NDUFB8, NDUFB9, NDUFB10, NDUFB11, NDUFAF1, NDUFAF3, NDUFAF4, NDUFAF5, SDHA, SDHB, SDHC, SDHD, UQCRFS1, COX2, COX6B1, COX7B, COX8A, COX10, COX15*
hsa03050	Proteasome	1.59E-42	*PSMD3, PSMD12, PSMD11, PSMD7, PSMD8, PSMD2, PSMD1, PSMC1, PSMC5, PSMC3, PSMC4, PSMA6, PSMA2, PSMA4, PSMA5, PSMA3, PSMB6, PSMB7, PSMB1, PSMB4*
hsa03010	Ribosome	4.93E-42	*MRPS2, MRPS7, RPS2, RPS3, RPS7, RPS9, RPS11, RPS12, RPS15A, RPS16, RPS21, RPS23, RPS26, RPS27A, RPS28, RPL3, RPL4, RPL7, RPL8, RPL10, RPL11, RPL13, RPL15, RPL18A, RPL19, RPL21, RPL27A, RPL35, RPL37, RPL38, RPLP1, RPLP2*

ID	Term_Description	p_value	Genes
hsa05208	Chemical carcinogenesis - reactive oxygen species	1.43E-39	*GSTM1, GSTM2, NDUFV1, NDUFV2, NDUFA1, NDUFA2, NDUFA4, NDUFA6, NDUFA7, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFB3, NDUFB5, NDUFB7, NDUFB8, NDUFB9, NDUFB10, NDUFB11, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, SDHA, SDHB, SDHC, SDHD, UQCRFS1, COX2, COX6B1, COX7B, COX8A, SOD2, SLC25A4, SOD1, CAT, AHR, NCF1, HGF, MET, SOS1, KRAS, NRAS, BRAF, RAF1, MAP2K1, MAPK1, PIK3R1, PIK3CA, RAC1, AKT1, PTEN, IKBKG, NFKB1, PTPN11*
hsa03040	Spliceosome	2.92E-34	*DHX16, DHX38, SNRPB, SNRPD1, SNRPD2, SNRPD3, SNRPF, SNRPG, FUS, SF3B4, PUF60, PRPF3, PRPF4, PRPF31, SART1, EFTUD2, SNRNP200, PRPF6, PRPF8, SNRNP40, TXNL4A, PQBP1, RBM8A, THOC2, RBMX, HNRNPK*
hsa05205	Proteoglycans in cancer	2.78E-30	*ERBB2, KRAS, NRAS, RRAS2, MRAS, BRAF, RAF1, MAP2K1, MAPK1, RAC1, CDC42, ESR1, CCND1, ACTG1, ACTB, FLNA, FLNB, GAB1, PIK3CA, PIK3R1, AKT1, PPP1CB, STAT3, TWIST2, CAV1, TGFB1, TLR2, TNF, ERBB3, CTNNB1, MET, CBL, FAS, SMAD2, COL1A1, COL1A2, ITGB3, HGF, FZD4, RDX, FGF2, FGFR1, PTPN11, SOS1, PRKCB, SHH, PTCH1, SMO*
hsa04723	Retrograde endocannabinoid signaling	4.92E-30	*PRKCB, GNAI3, MAPK1, NDUFV1, NDUFV2, NDUFA1, NDUFA2, NDUFA4, NDUFA6, NDUFA7, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFB3, NDUFB5, NDUFB7, NDUFB8, NDUFB9, NDUFB10, NDUFB11, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8*
hsa04146	Peroxisome	1.33E-27	*PEX16, PEX3, PEX19, PEX1, PEX6, PEX26, PEX7, PEX5, PEX14, PEX13, PEX12, PEX10, PEX2, PEX11B, PHYH, ACOX1, HSD17B4, ABCD1, CAT, SOD1, SOD2*
hsa04110	Cell cycle	2.27E-25	*CCND1, HDAC2, TGFB1, SMAD2, SMAD3, SMAD4, CDKN2A, CDKN1C, SKP1, CDC6, CDC45, SMC1A, SMC3, STAG2, RAD21, BUB1, BUB1B, MAD2L1, MAD2L2, CDC14A, CREBBP, EP300, PRKDC, PCNA, ORC1, ORC2, ORC4, ORC5, ORC6, MCM2, MCM4, MCM5, MCM6, MCM7*
hsa04015	Rap1 signaling pathway	5.26E-23	*RAP1A, MRAS, GNAS, FGF2, FGF8, FGF9, FGF10, INS, PDGFB, KITLG, HGF, FGFR1, FGFR2, FGFR3, PDGFRB, KIT, MET, EPHA2, CRKL, CDH1, CTNNB1, PRKCB, GNAI3, RAC1, ITGA2B, ITGB3, ACTG1, ACTB, CDC42, CTNND1, BRAF, RAF1, MAP2K1, MAPK1, PIK3CA, PIK3R1, AKT1, KRAS, NRAS*
hsa04010	MAPK signaling pathway	6.56E-20	*PRKCB, NF1, RAP1A, FGF2, FGF8, FGF9, FGF10, BDNF, NTF3, INS, PDGFB, KITLG, HGF, ERBB2, ERBB3, FGFR1, FGFR2, FGFR3, PDGFRB, KIT, MET, EPHA2, SOS1, KRAS, NRAS, RRAS2, MRAS, BRAF, RAF1, MAP2K1, MAPK1, RPS6KA3, TNF, IL1A, IL1B, TGFB1, FAS, CD14, RAC1, CDC42, MYD88, MAP3K7, TAOK1, FLNA, FLNB, CRKL, MAX, AKT1, DUSP1, DUSP6, MECOM, IKBKG, NFKB1, NFKB2*
hsa04510	Focal adhesion	6.80E-19	*COL1A1, COL1A2, COL2A1, COL9A1, COL9A2, COL9A3, LAMB1, VWF, ITGA2B, ITGB3, ITGB6, PDGFB, HGF, PDGFRB, MET, ERBB2, DIAPH1, PPP1CB, ACTG1, ACTB, FLNA, FLNB, VCL, AKT1, CTNNB1, PRKCB, PIK3CA, PIK3R1, PTEN, RAC1, PAK3, CDC42, CRKL, RAP1A, BRAF, CAV1, SOS1, RAF1, MAP2K1, MAPK1, CCND1*
hsa00020	Citrate cycle (TCA cycle)	7.03E-19	*ACO2, IDH3B, IDH3G, IDH3A, OGDH, DLST, SUCLG1, SUCLA2, SDHA, SDHB, SDHC, SDHD, FH, MDH2, PDHA1, PDHB*
hsa04014	Ras signaling pathway	7.09E-19	*FGF2, FGF8, FGF9, FGF10, BDNF, NTF3, INS, PDGFB, KITLG, HGF, FGFR1, FGFR2, FGFR3, PDGFRB, KIT, MET, EPHA2, GAB1, PTPN11, SOS1, KRAS, NRAS, MRAS, RRAS2, NF1, RAC1, PAK3, PIK3CA, PIK3R1, AKT1, IKBKG, NFKB1, SHOC2, RAF1, MAP2K1, MAPK1, RAP1A, TBK1, CDC42, PRKCB*
hsa04917	Prolactin signaling pathway	4.57E-18	*JAK2, PIK3CA, PIK3R1, AKT1, SOS1, KRAS, NRAS, RAF1, MAP2K1, MAPK1, CCND1, STAT3, TNFSF11, TNFRSF11A, NFKB1, ESR1, INS*
hsa04012	ErbB signaling pathway	1.92E-17	*ERBB2, PRKCB, CBL, CRKL, PAK3, ERBB3, SOS1, KRAS, NRAS, BRAF, RAF1, MAP2K1, MAPK1, GAB1, PIK3CA, PIK3R1, AKT1*
hsa04810	Regulation of actin cytoskeleton	6.43E-17	*F2, INS, FGF2, FGF8, FGF9, FGF10, PDGFB, FGFR1, FGFR2, FGFR3, PDGFRB, ITGA2B, ITGB3, ITGB6, CRKL, SOS1, KRAS, NRAS, RRAS2, MRAS, PIK3CA, PIK3R1, BRAF, RAF1, MAP2K1, MAPK1, RAC1, CDC42, PAK3, PPP1CB, MYH9, DIAPH1, DIAPH3, ACTG1, ACTB, RDX, VCL, GSN, APC, APC2*
hsa04919	Thyroid hormone signaling pathway	3.89E-16	*ITGB3, KRAS, NRAS, RAF1, MAP2K1, MAPK1, ESR1, SIN3A, HDAC2, CREBBP, EP300, MED12, MED13L, MED13, CCND1, MYH7, MYH6, CTNNB1, NOTCH2, NOTCH3, PRKCB, ATP1A1, ATP1A2, ATP1A3, ATP1B1, PIK3CA, PIK3R1, AKT1, ACTG1, ACTB*
hsa04520	Adherens junction	1.49E-15	*CDC42, RAC1, VCL, CDH1, CTNND1, CTNNB1, ACTG1, ACTB, MET, ERBB2, FGFR1, MAPK1, SMAD3, SMAD4, CREBBP, EP300, MAP3K7*
hsa04722	Neurotrophin signaling pathway	3.95E-15	*BDNF, NTF3, SOS1, KRAS, NRAS, RAF1, BRAF, MAP2K1, MAPK1, RPS6KA3, CRKL, RAP1A, GAB1, PIK3R1, PIK3CA, AKT1, NFKB1, PTPN11, ARHGDIA, CDC42, RAC1, PSEN1*
hsa04068	FoxO signaling pathway	1.79E-14	*TGFB1, SMAD4, SMAD3, CREBBP, EP300, USP7, INS, PIK3CA, PIK3R1, PTEN, AKT1, STAT3, SOS1, KRAS, NRAS, BRAF, RAF1, MAP2K1, MAPK1, CCND1, CAT, SOD2*
hsa04390	Hippo signaling pathway	4.60E-14	*YAP1, CDH1, NF2, PPP1CB, DLG1, BTRC, FBXW11, GLI2, TGFB1, SMAD2, SMAD3, SMAD4, BMP2, FZD4, DVL1, CTNNB1, APC, APC2, CCND1, SOX2, ACTG1, ACTB*

ID	Term_Description	p_value	Genes
hsa04550	Signaling pathways regulating pluripotency of stem cells	5.30E-14	*JAK2, STAT3, SOX2, MAP2K1, MAPK1, PIK3CA, PIK3R1, AKT1, SMAD2, SMAD3, ACVR1, SMAD4, FZD4, DVL1, APC, APC2, CTNNB1, FGF2, FGFR1, FGFR2, FGFR3, KRAS, NRAS, RAF1, REST, HOXB1, HOXA1*
hsa05100	Bacterial invasion of epithelial cells	6.30E-14	*CDH1, CTNNB1, MET, GAB1, PIK3R1, PIK3CA, CRKL, CDC42, RAC1, ACTG1, ACTB, CBL, CAV1, MAD2L2, VCL*
hsa04623	Cytosolic DNA-sensing pathway	2.87E-13	*POLR3A, POLR3B, POLR1C, POLR1D, POLR3H, POLR3GL, POLR2F, POLR2K, POLR2L, NFKB1, TBK1, RIPK1, IKBKG, IL1B*
hsa03420	Nucleotide excision repair	4.93E-13	*DDB2, XPC, ERCC8, ERCC6, ERCC3, ERCC2, GTF2H5, GTF2H2, ERCC5, XPA, ERCC4, ERCC1, POLD1, PCNA, RFC1, RFC2*
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer	5.73E-13	*KRAS, NRAS, RAF1, MAP2K1, MAPK1, PIK3R1, PIK3CA, PTEN, AKT1, IKBKG, NFKB1, JAK2, STAT3, TLR2, MYD88, PTPN11*
hsa04935	Growth hormone synthesis, secretion and action	8.06E-13	*GNAS, GNAI3, CREBBP, EP300, GH1, PRKCB, JAK2, SOS1, KRAS, NRAS, RAF1, MAP2K1, MAPK1, STAT3, PIK3R1, PIK3CA, AKT1, CRKL*
hsa05417	Lipid and atherosclerosis	1.41E-12	*NOS3, PIK3CA, PIK3R1, AKT1, SOD2, NCF1, RAC1, JAK2, STAT3, CDC42, KRAS, NRAS, MAPK1, NFKB1, FAS, IL1B, TNF, HSPD1, HSPA4, TLR2, CD14, MYD88, MAP3K7, IKBKG, TBK1, RAP1A, XBP1*
hsa04310	Wnt signaling pathway	2.06E-12	*FZD4, DVL1, CTNNB1, APC, APC2, CTBP1, CREBBP, EP300, SMAD4, SMAD3, MAP3K7, CCND1, PSEN1, SKP1, TBL1X, TBL1XR1, BTRC, FBXW11, RAC1, PRKCB*
hsa04933	AGE-RAGE signaling pathway in diabetic complications	3.02E-12	*TGFB1, SMAD2, SMAD3, SMAD4, COL1A1, COL1A2, PRKCB, MAPK1, IL1A, IL1B, TNF, NFKB1, DIAPH1, RAC1, CDC42, KRAS, NRAS, PIK3CA, PIK3R1, AKT1, NOS3, JAK2, STAT3, CCND1*
hsa04062	Chemokine signaling pathway	3.07E-12	*JAK2, STAT3, GNAI3, SOS1, KRAS, NRAS, RAF1, BRAF, MAP2K1, MAPK1, PIK3CA, PIK3R1, AKT1, IKBKG, NFKB1, RAC1, CDC42, CRKL, RAP1A, PRKCB, NCF1*
hsa04660	T cell receptor signaling pathway	3.39E-12	*PAK3, CDC42, DLG1, SOS1, KRAS, NRAS, RAF1, MAP2K1, MAPK1, MAP3K7, IKBKG, NFKB1, PIK3R1, PIK3CA, AKT1, TNF*
hsa04072	Phospholipase D signaling pathway	3.83E-12	*PDGFB, KITLG, INS, PDGFRB, KIT, GAB1, PTPN11, SOS1, KRAS, NRAS, MRAS, RRAS2, PIK3CA, PIK3R1, AKT1, RAF1, MAP2K1, MAPK1, F2, GNAS*
hsa04664	Fc epsilon RI signaling pathway	5.35E-12	*BTK, PIK3CA, PIK3R1, AKT1, RAC1, TNF, SOS1, KRAS, NRAS, RAF1, MAP2K1, MAPK1*
hsa04662	B cell receptor signaling pathway	2.20E-11	*BTK, RAC1, SOS1, KRAS, NRAS, RAF1, MAP2K1, MAPK1, PRKCB, IKBKG, NFKB1, PIK3R1, PIK3CA, AKT1*
hsa03440	Homologous recombination	3.02E-11	*NBN, BRCA1, BRIP1, PALB2, BRCA2, RAD51, RAD51C, XRCC2, POLD1, TOP3A*
hsa04650	Natural killer cell mediated cytotoxicity	3.27E-11	*HLA-A, HLA-B, PTPN11, RAC1, MAP2K1, MAPK1, TNF, PIK3CA, PIK3R1, SOS1, KRAS, NRAS, BRAF, RAF1, PRKCB, FAS*
hsa04370	VEGF signaling pathway	3.33E-11	*PRKCB, KRAS, NRAS, RAF1, MAP2K1, MAPK1, CDC42, PIK3CA, PIK3R1, RAC1, AKT1, NOS3*
hsa04910	Insulin signaling pathway	7.11E-11	*INS, PIK3R1, PIK3CA, AKT1, PPP1CB, CBL, CRKL, SREBF1, SOS1, KRAS, NRAS, BRAF, RAF1, MAP2K1, MAPK1*
hsa04218	Cellular senescence	1.95E-10	*TGFB1, SMAD2, SMAD3, CCND1, PIK3CA, PIK3R1, HLA-A, HLA-B, KRAS, NRAS, RRAS2, MRAS, AKT1, PTEN, CDKN2A, BTRC, FBXW11, PPP1CB, RAF1, MAP2K1, MAPK1, NBN, SQSTM1, NFKB1, IL1A, SLC25A4, MCU*
hsa04140	Autophagy - animal	2.03E-10	*INS, PIK3CA, PIK3R1, AKT1, PTEN, KRAS, NRAS, MRAS, RRAS2, RAF1, MAP2K1, MAPK1, IGBP1, SQSTM1, TBK1, MAP3K7*
hsa04066	HIF-1 signaling pathway	3.43E-10	*STAT3, NFKB1, INS, ERBB2, MAP2K1, MAPK1, PIK3CA, PIK3R1, AKT1, VHL, CREBBP, EP300, PRKCB, NOS3, PDHA1, PDHB*
hsa04540	Gap junction	3.46E-10	*GNAI3, PDGFB, PDGFRB, SOS1, KRAS, NRAS, RAF1, MAP2K1, MAPK1, TUBB2A, TUBB3, GNAS, PRKCB*
hsa04611	Platelet activation	5.10E-10	*F2, PPP1CB, RAP1A, ITGA2B, ITGB3, GNAI3, GNAS, ACTG1, ACTB, COL1A1, COL1A2, PIK3CA, PIK3R1, BTK, VWF, GP1BA, GP1BB, AKT1, NOS3, MAPK1*
hsa05162	Measles	1.39E-09	*TBK1, IKBKG, NFKB1, MYD88, TLR2, MAP3K7, IL1A, IL1B, FAS, FADD, CCND1, PIK3R1, PIK3CA, AKT1, STAT3*
hsa04926	Relaxin signaling pathway	1.48E-09	*GNAI3, GNAS, NFKB1, PIK3CA, PIK3R1, RAF1, MAP2K1, MAPK1, AKT1, NOS3, TGFB1, SMAD2, COL1A1, COL1A2, KRAS, NRAS, SOS1*
hsa05131	Shigellosis	2.10E-09	*CRKL, RAC1, ACTG1, ACTB, DIAPH1, VCL, CDC42, PIK3CA, PIK3R1, AKT1, IL1B, TNF, RIPK1, MAP3K7, IKBKG, NFKB1, CD14, SKP1, BTRC, FBXW11, MYD88, TBK1, MAPK1, CBX3, C3, SQSTM1, RPS27A, UBB*
hsa04613	Neutrophil extracellular trap formation	3.06E-09	*MAP3K7, RAF1, MAP2K1, MAPK1, NCF1, RAC1, ACTG1, ACTB, SLC25A4, PRKCB, PIK3CA, PIK3R1, AKT1, NFKB1, C3, TLR2, ITGA2B, ITGB3, GP1BA, VWF, HDAC2, HDAC4*

ID	Term_Description	p_value	Genes
hsa04371	Apelin signaling pathway	3.06E-09	*GNAI3, NOS3, KRAS, NRAS, RRAS2, MRAS, RAF1, MAP2K1, MAPK1, AKT1, JAG1, NOTCH3, CCND1, HDAC4, SMAD2, SMAD3, SMAD4, CDH1*
hsa04120	Ubiquitin mediated proteolysis	3.30E-09	*UBB, RPS27A, UBA1, UBE2A, TRIP12, NEDD4L, HUWE1, STUB1, CBL, BRCA1, FANCL, SKP1, BTRC, FBXW11, VHL, DDB2, ERCC8*
hsa04150	mTOR signaling pathway	3.99E-09	*ATP6V1B1, ATP6V1B2, TELO2, FZD4, DVL1, TNF, INS, SOS1, KRAS, NRAS, BRAF, RAF1, MAP2K1, MAPK1, RPS6KA3, PIK3R1, PIK3CA, PTEN, AKT1, PRKCB*
hsa04916	Melanogenesis	4.59E-09	*GNAS, CREBBP, EP300, FZD4, DVL1, CTNNB1, KITLG, KIT, KRAS, NRAS, RAF1, MAP2K1, MAPK1, TYR, GNAI3, PRKCB*
hsa04625	C-type lectin receptor signaling pathway	5.03E-09	*KRAS, NRAS, MRAS, RRAS2, RAF1, IL1B, PTPN11, NFKB2, MAPK1, IKBKG, NFKB1, TNF, PIK3CA, PIK3R1, AKT1*
hsa03022	Basal transcription factors	5.14E-09	*TAF1, TAF7, TAF3, TAF10, TAF4, TAF12, GTF2E2, GTF2H2, ERCC3, ERCC2, GTF2H5, GTF2I*
hsa04350	TGF-beta signaling pathway	5.39E-09	*BMP2, FBN1, TGFB1, ACVR1, SMAD2, SMAD3, SMAD4, CREBBP, EP300, TGIF1, PITX2, SKP1, MAPK1, TNF*
hsa04330	Notch signaling pathway	8.37E-09	*DLL1, JAG1, NOTCH2, NOTCH3, DVL1, PSEN1, CREBBP, EP300, CTBP1, HDAC2*
hsa05418	Fluid shear stress and atherosclerosis	1.21E-08	*NOS3, CAV1, GSTM1, GSTM2, SQSTM1, CTNNB1, PIK3CA, PIK3R1, AKT1, ITGA2B, ITGB3, ACTG1, ACTB, DUSP1, MAP3K7, IKBKG, NFKB1, TNF, IL1A, IL1B, PDGFB, ACVR1, NCF1, RAC1*
hsa04620	Toll-like receptor signaling pathway	1.24E-08	*TLR2, CD14, RAC1, PIK3CA, PIK3R1, AKT1, MYD88, FADD, MAP3K7, IKBKG, NFKB1, MAP2K1, MAPK1, TNF, IL1B, RIPK1, TBK1*
hsa04929	GnRH secretion	2.15E-08	*PRKCB, KCNJ11, KRAS, NRAS, RAF1, MAP2K1, MAPK1, PIK3R1, PIK3CA, AKT1*
hsa04668	TNF signaling pathway	2.61E-08	*TNF, RIPK1, MAP3K7, IKBKG, NFKB1, MAP2K1, MAPK1, FADD, FAS, JAG1, IL1B, LTA, TNFRSF1B, PIK3CA, PIK3R1, AKT1*
hsa04071	Sphingolipid signaling pathway	2.94E-08	*SPTLC1, SPTLC2, TNF, AKT1, PPP2R3C, PIK3CA, PIK3R1, GNAI3, MAPK1, SGPL1, KRAS, NRAS, RAF1, MAP2K1, NOS3, RAC1, PRKCB, PTEN, NFKB1*
hsa04659	Th17 cell differentiation	2.94E-08	*IL1B, TGFB1, SMAD2, SMAD3, SMAD4, JAK2, STAT3, AHR, HLA-DPA1, HLA-DPB1, HLA-DRB1, IKBKG, NFKB1, MAPK1*
hsa04360	Axon guidance	3.69E-08	*NTN1, DCC, RAC1, CDC42, PAK3, PTPN11, EPHA2, KRAS, NRAS, MAPK1, GNAI3, MET, RAF1, SHH, PTCH1, SMO, PIK3CA, PIK3R1*
hsa04210	Apoptosis	4.65E-08	*FAS, FADD, TNF, ACTG1, ACTB, LMNA, LMNB1, LMNB2, RIPK1, IKBKG, NFKB1, PIK3CA, PIK3R1, AKT1, KRAS, NRAS, RAF1, MAP2K1, MAPK1*
hsa04915	Estrogen signaling pathway	5.97E-08	*ESR1, GNAS, SOS1, KRAS, NRAS, RAF1, MAP2K1, MAPK1, PIK3CA, PIK3R1, AKT1, GNAI3, NOS3*
hsa04920	Adipocytokine signaling pathway	8.50E-08	*TNF, TNFRSF1B, IKBKG, NFKB1, AKT1, JAK2, STAT3, PTPN11*
hsa04630	JAK-STAT signaling pathway	8.76E-08	*GH1, PDGFB, MPL, PDGFRB, JAK2, STAT3, CCND1, CREBBP, EP300, PTPN11, SOS1, RAF1, PIK3CA, PIK3R1, AKT1*
hsa04024	cAMP signaling pathway	1.18E-07	*ADCYAP1, GNAS, GNAI3, CNGA1, CNGB1, RRAS2, RAP1A, RAC1, PIK3CA, PIK3R1, AKT1, BRAF, RAF1, MAP2K1, MAPK1, PPP1CB, CREBBP, EP300, BDNF, GLI3, PTCH1, NFKB1, ACOX1, TNNI3, ATP1A1, ATP1A2, ATP1A3, ATP1B1*
hsa04730	Long-term depression	2.30E-07	*KRAS, NRAS, BRAF, RAF1, MAP2K1, MAPK1, GNAI3, GNAS, PRKCB*
hsa04726	Serotonergic synapse	2.67E-07	*PRKCB, MAPK1, GNAS, GNAI3, DUSP1, KRAS, NRAS, BRAF, RAF1, MAP2K1*
hsa04912	GnRH signaling pathway	3.14E-07	*GNAS, PRKCB, CDC42, SOS1, KRAS, NRAS, RAF1, MAP2K1, MAPK1*
hsa04658	Th1 and Th2 cell differentiation	3.15E-07	*DLL1, NOTCH3, NFKB1, JAK2, HLA-DPA1, HLA-DPB1, HLA-DRB1, IKBKG, MAPK1, JAG1, NOTCH2*
hsa00600	Sphingolipid metabolism	3.23E-07	*SPTLC1, SPTLC2, SGPL1, GLB1, GALC, ARSA, NEU1, PSAP*
hsa04931	Insulin resistance	4.05E-07	*INS, RPS6KA3, PPP1CB, PTPN11, PIK3CA, PIK3R1, AKT1, PRKCB, STAT3, TNF, NFKB1, NOS3, SREBF1, PTEN*
hsa04666	Fc gamma R-mediated phagocytosis	4.62E-07	*PIK3CA, PIK3R1, AKT1, RAF1, MAP2K1, MAPK1, PRKCB, NCF1, GSN, CDC42, RAC1, CRKL*
hsa04657	IL-17 signaling pathway	5.13E-07	*FADD, NFKB1, TBK1, MAP3K7, IKBKG, MAPK1, TNF, IL1B*
hsa04621	NOD-like receptor signaling pathway	6.42E-07	*IKBKG, NFKB1, IL1B, TNF, MAP3K7, MAPK1, TBK1, MCU, MFN2, RIPK1, FADD, MYD88*
hsa04020	Calcium signaling pathway	7.23E-07	*GNAS, PDGFB, FGF2, FGF8, FGF9, FGF10, HGF, GDNF, ERBB2, ERBB3, PDGFRB, FGFR1, FGFR2, FGFR3, MET, RET, MCU, SLC25A4, TNNC1, NOS3, PRKCB*

ID	Term_Description	p_value	Genes
hsa04670	Leukocyte transendothelial migration	7.78E-07	*ACTG1, ACTB, PIK3CA, PIK3R1, RAC1, NCF1, CTNNB1, CTNND1, PTPN11, PRKCB, RAP1A, VCL, GNAI3, CDC42*
hsa04064	NF-kappa B signaling pathway	9.11E-07	*BTK, PRKCB, IL1B, MYD88, TNF, RIPK1, CD14, TNFSF11, TNFRSF11A, LTA, MAP3K7, IKBKG, NFKB1, NFKB2*
hsa04973	Carbohydrate digestion and absorption	9.14E-07	*ATP1A1, ATP1A2, ATP1A3, ATP1B1, PIK3R1, PIK3CA, AKT1, PRKCB*
hsa04921	Oxytocin signaling pathway	9.35E-07	*KRAS, NRAS, RAF1, MAP2K1, MAPK1, CCND1, PRKCB, EEF2, NOS3, ACTG1, ACTB, PPP1CB, GNAS, GNAI3*
hsa04960	Aldosterone-regulated sodium reabsorption	9.41E-07	*NEDD4L, KRAS, ATP1A1, ATP1A2, ATP1A3, ATP1B1, INS, PIK3R1, PIK3CA, PRKCB, MAPK1*
hsa05202	Transcriptional misregulation in cancer	1.21E-06	*HDAC2, SIN3A, CD14, DUSP6, PBX1, KMT2A, RUNX2, JMJD1C, KDM6A, SIX1, EYA1, MYCN, MAX, MEN1, ARNT2, FUS, NFKB1, PAX3, MET, DDB2*
hsa04725	Cholinergic synapse	1.43E-06	*CHAT, PRKCB, KCNQ1, GNAI3, KRAS, NRAS, MAP2K1, MAPK1, JAK2, PIK3CA, PIK3R1, AKT1*
hsa04530	Tight junction	1.63E-06	*CDC42, RAC1, NF2, DLG1, NEDD4L, PCNA, CCND1, ERBB2, HSPA4, RDX, ACTG1, ACTB, MYH9, RAP1A*
hsa04961	Endocrine and other factor-regulated calcium reabsorption	2.53E-06	*GNAS, VDR, ESR1, ATP1A1, ATP1A2, ATP1A3, ATP1B1, PRKCB*
hsa04728	Dopaminergic synapse	3.84E-06	*PRKCB, GNAS, PPP1CB, GNAI3, PPP2R3C, AKT1*
hsa04022	cGMP-PKG signaling pathway	3.94E-06	*MYH7, MYH6, ATP1A1, ATP1A2, ATP1A3, ATP1B1, GTF2I, PPP1CB, CNGA1, CNGB1, GNAI3, INS, AKT1, NOS3, SLC25A4, RAF1, MAP2K1, MAPK1*
hsa04137	Mitophagy - animal	7.37E-06	*RPS27A, UBB, MFN2, SQSTM1, TBK1, KRAS, NRAS, MRAS, RRAS2*
hsa04152	AMPK signaling pathway	7.89E-06	*MAP3K7, CCND1, EEF2, SREBF1, PPP2R3C, INS, PIK3CA, PIK3R1, AKT1*
hsa04928	Parathyroid hormone synthesis, secretion and action	8.00E-06	*GNAI3, VDR, FGFR1, MAPK1, BRAF, RAF1, MAP2K1, PRKCB, GNAS, TNFSF11, RUNX2*
hsa04142	Lysosome	8.45E-06	*TCIRG1, ATP6V0A4, ATP6AP1, GLB1, GALC, NEU1, ARSA, GALNS, IDS, PSAP, SUMF1, AP1B1, AP1S1, AP1S2*
hsa03430	Mismatch repair	1.54E-05	*RFC1, RFC2, PCNA, POLD1*
hsa04964	Proximal tubule bicarbonate reclamation	1.57E-05	*ATP1A1, ATP1A2, ATP1A3, ATP1B1*
hsa04261	Adrenergic signaling in cardiomyocytes	2.65E-05	*GNAS, ATP1A1, ATP1A2, ATP1A3, ATP1B1, KCNQ1, KCNE1, PPP2R3C, PPP1CB, TNNC1, TNNI3, TNNT2, TPM1, TPM2, ACTC1, MYH7, MYH6, MAPK1, GNAI3, AKT1*
hsa04340	Hedgehog signaling pathway	3.28E-05	*PTCH1, SMO, GLI2, GLI3, SUFU, CCND1, BTRC, FBXW11, SHH, CDON, LRP2, SPOP*
hsa04750	Inflammatory mediator regulation of TRP channels	3.84E-05	*PPP1CB, IL1B, PIK3CA, PIK3R1, GNAS, PRKCB*
hsa04612	Antigen processing and presentation	0.00151481	*TNF, HSPA4, HLA-A, HLA-B, B2M, HLA-DPA1, HLA-DPB1, HLA-DRB1*
hsa04710	Circadian rhythm	0.001542239	*SKP1, BTRC, FBXW11*
hsa04918	Thyroid hormone synthesis	0.001543667	*GNAS, PRKCB, ATP1A1, ATP1A2, ATP1A3, ATP1B1, DUOX2, LRP2*
hsa04217	Necroptosis	0.001609144	*TNF, RIPK1, FADD, SLC25A4, IL1B, IL1A, FAS, JAK2, STAT3, SQSTM1*
hsa04713	Circadian entrainment	0.001793336	*MAPK1, GNAI3, ADCYAP1, GNAS, PRKCB*
hsa00630	Glyoxylate and dicarboxylate metabolism	0.002728933	*MDH2, ACO2, CAT*
hsa03410	Base excision repair	0.002760112	*PCNA, POLD1, FEN1*
hsa04115	p53 signaling pathway	0.002978801	*CDKN2A, CCND1, FAS, DDB2, RRM2B, PTEN*
hsa04911	Insulin secretion	0.003048253	*ATP1A1, ATP1A2, ATP1A3, ATP1B1, ABCC8, KCNJ11, ADCYAP1, GNAS, INS, SNAP25, PRKCB*
hsa04070	Phosphatidylinositol signaling system	0.003420739	*PIK3CA, PIK3R1, PTEN, PIK3C2A, PRKCB*
hsa04724	Glutamatergic synapse	0.003448637	*PRKCB, MAPK1, GNAS, GNAI3*
hsa00620	Pyruvate metabolism	0.003573158	*PDHA1, PDHB, MDH2, FH*
hsa04925	Aldosterone synthesis and secretion	0.005890451	*PRKCB, ATP1A1, ATP1A2, ATP1A3, ATP1B1, GNAS*
hsa03060	Protein export	0.006962103	*SRP54, SRP72, SRP68*

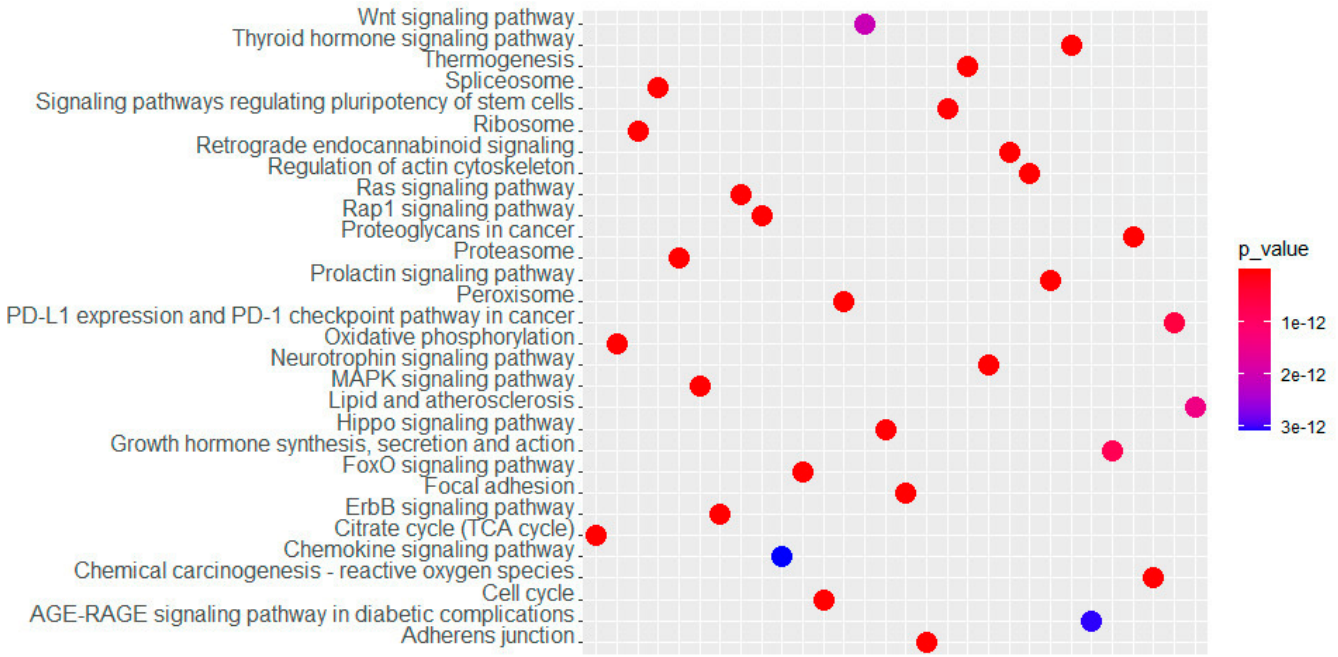

Figure 3. Pathway enrichment results using the KEGG database.

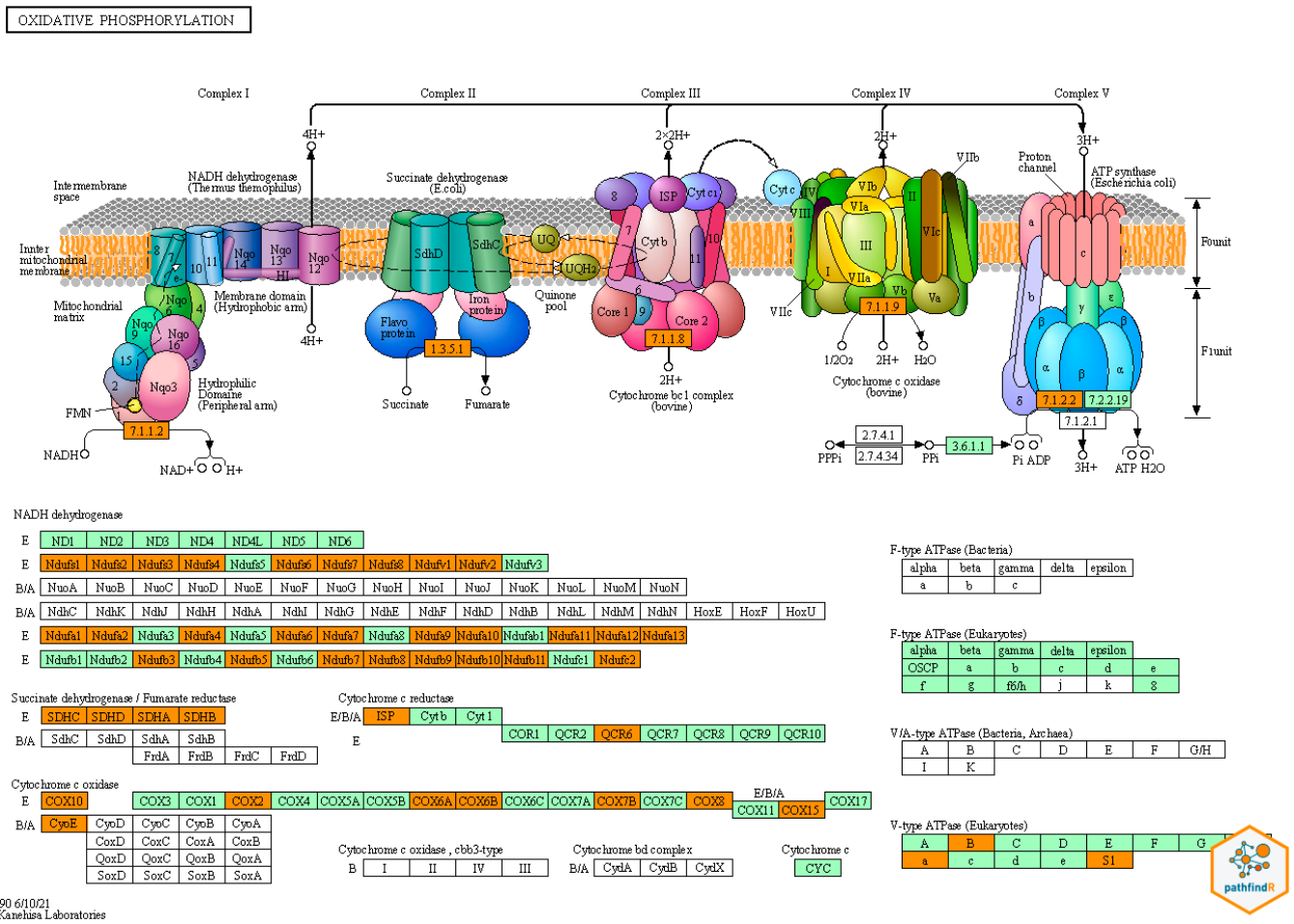

Figure 4. KEGG map of the oxidative phosphorylation pathway. Green genes are the most important genes in the pathway. In orange, the genes that appear in our network.

Table 3. Gene expression analysis of hub genes and most frequent genes of the pathway analysis.

Gene name	Symbol	MPS type	Expression	P value
Gene Hub				
	NDUFB7	MPS IIIB	11.49 (Up- regulated)	1.45E-6
	NDUFS7	MPS I	-1.60 (Down- regulated)	0.03
		MPS IIIB	7.43 (Up- regulated)	4.29E-5
	NDUFB8	MPS IIIB	9.92 (Up- regulated)	3.64E-8
	NDUFA13	MPS IIIB	8.33 (Up- regulated)	1.87E-7
	NDUFS2	MPS IIIB	9.80 (Up- regulated)	6.30E-8
	NDUFV1	MPS IIIB	9.39 (Up- regulated)	3.39E-8
	NDUFV2	Not identified	as differentially	expressed
	NDUFS3	Not identified	as differentially	expressed
	NDUFA9	MPS IIIB	9.61 (Up- regulated)	4.04E-8
	NDUFA2	MPS IIIB	10.23 (Up- regulated)	3.39E-8
Gene pathway				
	MAPK1	MPS IIIB	10.99 (Up- regulated)	1.45E-6
	PIK3CA	MPS IIIB	5.36 (Up- regulated)	8.68E-6
	PIK3R1	MPS IIIB	4.93 (Up- regulated)	3.51E-5
	AKT1	MPS IIIB	9.63 (Up- regulated)	2.00E-6
	KRAS	MPS IIIB	8.35 (Up- regulated)	3.40E-8
	MAP2K1	MPS IIIB	8.39 (Up- regulated)	1.55E-6
	NRAS	MPS IIIB	10.69 (Up- regulated)	4.22E-8
	PRKCB	MPS I	-2.13 (Down- regulated)	0.008
	RAF1	MPS IIIB	8.77 (Up- regulated)	1.45E-6
	NFKB1	MPS IIIB	7.34 (Up- regulated)	2.17E-6

Gene expression data retrieved from https://www.ufrgs.br/mpsbase/

Discussion

Several pathways involved in cell adhesion, proliferation and differentiation were enriched in our analysis. The Wnt signaling is the most enriched pathway, as it controls cellular events related to the formation of sensory hair cells during development [15], and in cochlear formation and hair cell differentiation and polarization [16–17]. Other pathways, like PI3K/Akt, MAPK/ERK and EGFR and ERBB signaling were also enriched. Given that these pathways are related to formation, maintenance and regeneration activity of specialized cells, it is not surprising that these pathways are deranged in progressive degenerative diseases, as MPS.

Another set of differentially expressed genes in our analysis were related to mitochondrial function. This organelle has a role in oxidative phosphorylation, oxidative stress control, and apoptosis. The relationship between hearing loss and mitochondrial diseases has been discussed previously in the literature [18–20]. Zwirner and Wilichowski demonstrated that there is a high incidence of 42% of sensorineural hearing loss in childrens with mitochondrial encephalomyopathies [21]. Besides, it was shown that causative mitochondrial DNA mutations appear in 5–10% of patients with post-lingual nonsyndromic hearing loss [22].

Mitochondrial defects in MPS were also described in the literature. Martins and collaborators observed structurally abnormal mitochondria and impaired mitochondrial energy metabolism in a 5-month-old mouse model of MPS III C [23]. In another study, light microscopy of brain sections of 6-months-old mice with MPS III B showed the accumulation of mitochondrial ATP synthase subunit c in the brain [24]. Alterations in mitochondria and lysosomes lead to neurological dysfunction and oxidative stress [25], observed in some MPS types. Interestingly, Baixauli et al. [26] showed that mitochondrial deficiency impairs lysosome function, and disrupts endolysosomal trafficking pathways and autophagy, thus linking a primary mitochondrial dysfunction to a lysosomal disturbance. Mitochondrial dysfunction is emerging as a significant contributor to the pathophysiology of lysosomal storage disorders, like MPS [27].

The NADH:ubiquinone oxidoreductase subunits gene family appeared several times in our hub analysis. The

NADH:ubiquinone oxidoreductase (complex I) is part of the respiratory complex and is a major source of reactive oxygen species (ROS) and an essential contributor to cellular oxidative stress. Moreover, ROS production has a relationship with several apoptotic and necrotic cell death pathways in auditory tissues [28]. The subsequent apoptosis induction and elevated ROS formation are involved in developing several hearing loss impairments. In parallel, the involvement of ROS in MPS IVA [29–30] and MPS IIIB [31] has been shown, but the role of the NDUF family in any type of MPS is not yet demonstrated.

Several genes related to complex I are up-regulated in MPS III B and down-regulated in MPS I – both of which present hearing loss in a significant portion of patients [32–36]. In MPS III, previous studies demonstrated rates of hearing loss of 87% in MPS III A, 100% in MPS III B, 75% in MPS III C, and 25% in MPS IIID [37–40]. MPS III B's higher proportion can be related to *NDUF* expression results, although more studies are needed to understand and validate this relationship.

Other genes found in our network analysis are related to succinate dehydrogenase, fumarate reductase, cytochrome c oxidase and reductase, and V-ATPase, which are all involved in the oxidative phosphorylation (Figure 4). A3, one of the four isoforms of subunit A V-ATPase, is required for secretory lysosome trafficking to the plasma membrane. It is also necessary to maintain the ionic concentration and pH of the endolymph that bathes the mechanosensory hair cells of the Corti organ in the inner ear [41–44]. The V-ATPase is also present in the cochlea, and interdental cells are especially V-ATPase-rich [44]. Besides that, mutations in the subunit A4 or B1 are associated with sensorineural hearing loss [43]. Moreover, Santra and Amack, 2021 [45] have shown a specific role for V-ATPase inducing caspase-independent necrosis-like cell death in mechanosensory hair cells in neuromasts. Patients with mutations in specific V-ATPase subunits can develop sensorineural deafness. The mechanism involves modulation of the mitochondrial permeability transition pore, which regulates mitochondrial membrane potential, thus improving hair cell survival.

The majority of young MPS patients present mixed hearing loss (32%) and 16% sensorineural [46]. Many studies exploring the mechanisms of hearing loss in MPS suggest a combination of conductive and sensorineural processes [3]. In this model, GAG accumulation leads to copious secretion and recurrent ear infection that, in conjunction with bone and cartilage deformities, contribute to conductive hearing loss. In addition, sensorineural hearing loss is caused by the death of hair cells. Our study sheds light into this second mechanism, suggesting a role for mitochondrial and V-ATPase dysfunction in the loss of hair cells [47]. Even though we analyzed data from *in vitro* neural stem cells and not from the inner ear, one can suppose that the same mechanisms that lead to lysosomal storage-derived disturbance of mitochondrial function and V-ATPases in the brain are also present in other cells, such as cochlear hair cells, but in that case with specific consequences. Therefore, experimental studies in MPS animal models could test this hypothesis directly in the involved cells.

Conclusions

We identified several genes and biological pathways involved in ear development and hearing loss. These genes and biological pathways may serve as potential candidates for clinical and experimental studies to better understand hearing impairment mechanisms in lysosomal storage diseases, like MPS.

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Declaration of Conflicting Interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Supplementary Material

The following online material is available for this article: Supplementar File 1 - KEGG maps of the top enriched pathways.

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