Transcriptome-wide association study identifies new susceptibility genes for degenerative cervical spondylosis

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Research Article

Keywords: Degenerative cervical spondylosis, Transcriptome-wide association study, Differentially expressed genes, Pathway enrichment analysis, Gene ontology

Posted Date: April 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1400889/v2

Abstract

Objective

Degenerative cervical spondylosis (DCS) is a common musculoskeletal disease that encompasses a wide range of progressive degenerative changes and affects all the components of the cervical spine. DCS has brought a huge social and economic burdens to society. However, the genetic basis of DCS remains elusive now.

Methods

Firstly, by integrating the gene-expression prediction models of whole blood and skeletal muscle and genome-wide association study (GWAS) summary data of DCS (including 3739 DCS patients and 382326 controls), we conducted a transcriptome-wide association study (TWAS) to evaluate the associations of genetically predicted gene-expression with DCS risk using Functional Summary-based Imputation (FUSION) software. Secondly, to validate the genes identified by TWAS analysis, these genes were further compared with the differentially expressed genes (DEGs) detected by the RNA expression profiles of DCS acquired from the Gene Expression Omnibus database (GEO, accession number: GSE153761). Thirdly, we used Functional Mapping and Annotation of Genome-wide Association Study (FUMA) and Metascape tools to conduct gene functional enrichment and annotation analysis.

Results

TWAS identified 420 significant genes for DCS with a P value < 0.05 in the skeletal muscle, such as RPS15A (P_{TWAS} = 0.0003), and 110 genes in the whole blood, such as SELL (P_{TWAS} = 0.0028). After comparing with RNA expression profiling of DCS, we identified 12 common genes, such as APLNR (P_{TWAS} = 0.0013, P_{DEG} = 0.0254). We identified 148 GO terms enriched for DCS, such as mast cell degranulation (GO:0043303). We also identified 15 KEGG pathways enriched for DCS, such as sphingolipid signaling pathway (ko04071). By integrating the enrichment results of TWAS analysis and RNA expression profile, 9 common terms were identified such as Degradation of the extracellular matrix (R-HSA-1474228).

Conclusion

Our results identified putative susceptibility genes for DCS, providing new insights into the genetic mechanisms underlying DCS development.

Introduction

Degenerative cervical spondylosis (DCS) is a chronic, progressive deterioration of osseocartilaginous components of the cervical spine (i.e., intervertebral discs, facet joints, joints of Luschka, ligamenta flava, and laminae) [1]. The symptoms of DCS most commonly manifest as neck pain and can be accompanied by radicular symptoms when there is compression of neural structures [2]. The prevalence of neck pain ranges from 0.4–41.5% and the lifetime prevalence may be as high as 86.8% [3]. A review of the global burden of neck pain estimated that in 2015, more than a third of a billion people worldwide had mechanical neck pain of at least three months' duration, underscoring the global health implications of DCS [4]. The high prevalence and severe consequences of DCS bring enormous social and economic burdens to the society, so it is essential to explore the genetic mechanisms underlying DCS development.

With the development of bioinformatics, an increasing number of studies have focused on the pathogenesis of DCS, especially in genetic mechanisms. Through using genome-wide association study (GWAS), YF Zhang et al. have found several loci significantly associated with DCS such as BMP6, NIPAL1, and CNGA1 [5]. Several significant functional polymorphisms were also found between DCS patients and controls, such as BMP-4, COX-2, and HIF-1α [6]. Unfortunately, the specific genetic mechanisms of DCS are still unclear. GWAS is one of the primary tools for determining genetic links to diseases and it has been successfully applied for gene mapping of complex human diseases and traits. However, GWAS is limited in evaluating the risk of disease because most GWAS-identified SNPs are located in non-coding regions of the genome [7]. Some researchers found expression quantitative trait loci (eQTL) analysis is a way to identify genes related to variation in gene expression and identified genes with causal associations to disease more effectively by integrating with GWAS [8]. This new approach is called a transcriptome-wide association study (TWAS). TWAS combines the pre-computed gene expression weights with GWAS summary data to recognize novel causal genes of target diseases [9]. In addition, TWAS can drastically reduce the comparisons in statistical analysis and enhance the ability to detect the candidate genes of target diseases [10]. In recent years, an increasing number of researchers have identified genetic loci associated with complex disease by using TWAS analysis. For example, Wu et al. identified rheumatoid arthritis-associated susceptibility genes and pathways through integrating TWAS and RNA expression profiles data [11]. This study identified 692 genes associated with rheumatoid arthritis and provided novel clues for the genetic mechanism of rheumatoid arthritis [11]. However, the TWAS analysis of DCS is unavailable until now.

In general, this study is divided into three steps. Firstly, by integrating a large-scale GWAS summary statistic of DCS and pre-computed gene expression weights of two specific tissues, we conducted a TWAS analysis to identify genes associated with DCS. Secondly, these genes were further validated by the RNA expression profiles of DCS. Thirdly, we used Functional Mapping and Annotation of genome-wide association study (FUMA) and Metascape tools to explore the functional relevance of identified genes. Our study may clarify the underlying genetic mechanisms of DCS.

Methods And Materials

GWAS summary datasets for DCS

A large-scale GWAS summary data set of DCS was obtained from the published study [12]. In short, this summary data set contains 3739 diagnosed DCS and 382326 controls of European from the UK Biobank [12]. Information about various phenotypes has been collected from every participant, and blood samples were taken when the subjects visited a UK Biobank assessment center [12]. DNA extraction and genotyping were performed in the Affymetrix Research Services Laboratory. After filtering, the data set contains 9,113,133 imputed variants [12]. The IMPUTE4 program was used to perform the imputation (http://jmarchini.org/software/). The detailed information on the subjects, genotyping, imputation, meta-analysis, and quality control can be found in the published study [12].

TWAS of DCS

The TWAS of DCS was carried out by using the Functional Summary-based Imputation software (FUSION http://gusevlab.org/projects/fusion/). FUSION is a software to identify genes whose expression is significantly associated with complex traits in individuals without directly measuring the expression level by integrating the GWAS summary data and pre-calculated gene expression weights of different tissues [13]. FUSION precomputed the gene expression weights of various tissues using a small set of individuals with both gene expression and genotype data. The expression was then imputed into much larger sets of phenotyped individuals according to SNP genotype data [13]. In this study, let w denotes the SNP expression weights. Z denotes the scores of DCS. L denotes the SNP-correlation matrix. The association testing statistics between predicted gene expression and each taxon was calculated as $ZTWAS=w'Z/(w',L,w)1/2$ [13]. Finally, the calculated tissue-related expression weights were integrated with summary-level GWAS results to impute the association statistics between target traits and the gene expression. The FUSION software and the gene expression weight panels for the skeletal-muscle and the whole blood were all downloaded from the FUSION website (http://gusevlab.org/projects/fusion/).

Validating TWAS results by RNA expression profiles of DCS

The RNA expression profiles of DCS were acquired from the Gene Expression Omnibus (GEO) Datasets (GSE153761, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE153761). In brief, DCS samples were sourced from 3 surgical patients with degenerative cervical myelopathy (DCM) undergoing discectomy [14]. For control, 3 samples were sourced from the patients undergoing decompressive surgery to correct cervical spinal trauma-related neuronal defects [14]. Total RNAs from 6 samples were extracted and purified using the RNeasy micro kit (Qiagen GmBH) and the RNase-Free DNase set (Qiagen GmBH). Specific in this study, the DEGs were analyzed by the GEO2R tool. GEO2R presents a simple interface that allows users to perform sophisticated R-based analysis of GEO data to help identify and visualize differential gene expression [15]. Genes were identified as differentially expressed when the following two conditions were met: P-value of < 0.05 by the moderated t statistic and $|\log_{FC}| > 1$ [14].

Gene Set Enrichment Analysis

The significant genes identified by TWAS and the DEGs were performed gene set enrichment analysis by using an online analysis tool: Gene Annotation & Analysis Resource (Metascape, https://metascape.org/gp/index.html). Metascape tool can make a functional exploration that included Gene Ontology (GO), pathway analysis. The function of significant genes was future annotated, prioritized, visualized, and interpreted by using the Functional Mapping and Annotation of Genome-wide Association Study (FUMA; https://fuma.ctglab.nl/) tool. The terms with FDR < 0.05 were considered significant. The top terms for TWAS results and DEGs were selected and plotted using GO plot package based on ggplot2 [16].

Results

TWAS results of DCS

In total, TWAS analysis identified 530 significant genes for DCS with P values < 0.05 (Fig. 1, Supplementary Table 1). In the skeletal muscle, TWAS analysis identified 420 genes with a P value < 0.05, such as NFU1 (P_{TWAS} = 0.0001), LA16c-OS12.2 (P_{TWAS} = 0.0001), and RPS15A (P_{TWAS} = 0.0003). TWAS analysis identified 110 genes with a P value < 0.05 in the whole blood, such as DCBLD1 (P_{TWAS} = 0.0003), FAM43A (P_{TWAS} = 0.0010), and SELL (P_{TWAS} = 0.0028). Table 1 presented the detailed information of the top 20 significant gene identified by TWAS, including heritability of genes (HSQ), rsID of the most significant GWAS SNP in the locus (BEST.GWAS.ID), number of SNPs in the locus (NSNP), and TWAS *P* value (P_{TWAS}).

Table 1
Top genes selected by transcriptome-wide association study (TWAS) analysis

Tissue	Gene	CHR	BEST.GWAS.ID	NSNP	TWAS.Z	TWAS.P
muscle skeleton	NFU1	2	rs7605572	397	4.8235	0.0001
	LA16c-OS12.2	16	rs35021117	305	3.8706	0.0001
	RGS11	16	rs35021117	344	-3.7930	0.0001
	RPS15A	16	rs2650611	219	3.6022	0.0003
	ARL6IP1	16	rs2650611	225	-3.5863	0.0003
	TLE6	19	rs951917	357	3.5592	0.0005
	ANKK1	11	rs11606008	518	-3.4671	0.0005
	STON1	2	rs13007591	478	-3.2538	0.0011
	APLNR	11	rs11606597	427	3.2162	0.0013
	FAM20B	1	rs12083856	459	-3.1996	0.0014
Whole Blood	DCBLD1	6	rs12083856	459	-3.5777	0.0003
	FAM43A	3	rs789862	512	3.2953	0.0010
	LL22NC03-86G7.1	22	rs9610216	400	3.1898	0.0014
	GPR146	7	rs4484581	364	-3.0953	0.0020
	RP11-449P15.1	7	rs4484581	364	-3.0953	0.0020
	TMEM80	11	rs10902194	449	3.0523	0.0023
	ARHGEF19-AS1	1	rs4661718	320	3.0366	0.0024
	SELL	1	rs3917751	532	-2.9870	0.0028
	TOR1A	9	rs3780697	450	-2.9594	0.0031
	CD93	20	rs4813479	566	2.9549	0.0031

Note: The large-scale Genome-Wide Association Study (GWAS) summary data for degenerative cervical spondylosis (DCS) acquired from a European cohort study, including 3739 diagnosed DCS and 382326 controls. The TWAS.P and TWAS.Z values were calculated by the FUSION approach (http://gusevlab.org/projects/fusion/)

TWAS: Transcriptome-Wide Association Study; GWAS: Genome-Wide Association Study; DCS: Degenerative cervical spondylosis; TWAS P: TWAS P value; TWAS Z: TWAS Z-score

Integrative the analysis of TWAS and RNA expression profiles of DCS

RNA expression profiles of DCS samples from 3 DCM patients and 3 controls found 1702 DEGs, among which 636 were downregulated and 1066 were upregulated. The distribution of gene expression for RNA expression profiles was visualized in the corresponding volcano plot (Fig. 2). After comparing with the RNA expression profiles of DCS, we found 12 common genes expressed both in TWAS analysis and RNA expression profiles, such as APLNR (P_{TWAS} = 0.0013, P_{DEG} = 0.0254), COL8A1 (P_{TWAS} = 0.0071, P_{DEG} = 0.0452), PLAC9 (P_{TWAS} = 0.0072, P_{DEG} = 0.0153) (Table 2).

Table 2
The common genes identified by both transcriptome-wide association study (TWAS) and differentially expressed genes (DEGs) for cervical spondylosis

Tissue	Gene	Chromosome	P_{TWAS}	P_{DEG}	Log_{FC}
skeletal muscle	COL8A1	3	0.0071	0.0452	2.0268
	PLAC9	10	0.0072	0.0153	1.0039
	APLNR	11	0.0013	0.0254	1.5325
	LMO1	11	0.0469	0.0318	2.0237
	SCN4B	11	0.0102	0.0436	-2.4215
	LRRC45	17	0.0253	0.0193	1.0843
	ARHGAP28	18	0.0332	0.0466	1.0364
	CAPS	19	0.0059	0.0059	1.7147
	RSPH1	21	0.0351	0.0196	1.0144
whole blood	NGF	1	0.0120	0.0483	1.0391
	SLC26A1	4	0.0440	0.0032	1.0786
	BSPRY	9	0.0187	0.0130	-1.2037

Note: Each P_{TWAS} value was calculated by transcriptome-wide association study (TWAS) analysis. Each P_{DEG} value was the differential expressed gene (DEG) derived from the published studies. TWAS, Transcriptome-Wide Association Study; DEG, Differential Expressed Gene; P_{TWAS}, $P_{Transcriptome-Wide\ Association\ Study}$ value; P_{DEG}, $P_{Differential\ Expressed\ Gene}$ value.

Go ontology and pathway enrichment analysis

Through using FUMA, we found these candidate genes were differentially expressed in muscle-skeletal and whole blood (-log 10 P-value > 20, Fig. 3). The total 530 TWAS-identified genes in the two tissues were successfully submitted to Metascape to perform GO enrichment analysis. Metascape identified 148

GO terms enriched for DCS, such as mast cell degranulation (GO: 0043303), organelle localization (GO: 0051640), and membrane lipid biosynthetic process (GO: 0046467). We also identified 15 KEGG pathways enriched for DCS, such as sphingolipid signaling pathway (ko04071), pyrimidine metabolism (ko00240) and prion diseases (ko05020). The GO Chord plot and Sankey diagram shows the top overrepresented GO terms and related genes belonging for TWAS analysis was shown in Fig. 4a,4b. By integrating the results of enrichment analysis of DEGs, 9 terms were identified such as Degradation of the extracellular matrix (R-HSA-1474228) (Table 3).

Table 3
Common terms identified by both TWAS analysis and RNA expression profile

GO	Description	Log_p value TWAS	Log_p RNA expression
GO:0060627	regulation of vesicle-mediated transport	-2.1919	-2.0554
hsa6798695	Neutrophil degranulation	-3.4544	-5.4593
GO:0097435	supramolecular fiber organization	-2.4973	-2.6788
hsa375165	NCAM signaling for neurite out-growth	-2.7783	-2.4737
hsa419037	NCAM1 interactions	-2.6016	-2.0239
hsa1442490	Collagen degradation	-2.7478	-3.0926
hsa1474228	Degradation of the extracellular matrix	-2.5064	-2.3514
M160	PID AVB3 INTEGRIN PATHWAY	-2.4708	-3.3229
M198	PID SYNDECAN 1 PATHWAY	-2.4563	-3.2903

Discussion

DCS is a common musculoskeletal disease that is caused by a combination of multiple risk factors, including genetic, psychological, biological and social factors. Although recent GWASs have successfully identified multiple DCS risk loci, the biological interpretations and functional understanding of these associations remain poorly understood. TWAS is a powerful approach to identify associated genes by combining the GWAS results and expression data. TWAS can identify genes whose cis-regulated expression is associated with complex traits. TWAS has been widely applied to multiple degenerative diseases, yet the first time for DCS in this study.

By integrating the TWAS analysis and RNA expression profiles of DCS, we identified apelin receptor (APLNR), the receptor for apelin receptor early endogenous ligand (APELA) and apelin (APLN) hormones coupled to G proteins that inhibit adenylate cyclase activity [17]. The Apelin/APLNR system participates in many basic activities of multiple cells with autocrine or paracrine and Apelin promotes cell proliferation, maturation, and induces mitochondrial autophagy [18]. Liu W et al. demonstrated

Apelin/APLNR system plays a key role in intervertebral disc degeneration by reducing the degradation of the extramedullary matrix of nucleus pulposus, promoting the proliferation of nucleus pulposus cells, reducing the level of apoptosis and inflammation [18]. This may provide a new direction for the pathogenesis of the DCS.

Nerve growth factor (NGF) also identified by both TWAS analysis and RNA expression profiles of DCS. NGF has been shown to play an important role in many degenerative diseases such as osteoarthritis and Alzheimer's Disease [19, 20]. By perturbing NGF-TrkA signaling could strongly enhance human chondrocyte matrix calcification, Yangzi J et al. found a novel NGF-mediated chondrocyte calcification, which may provide new insights regarding the pathologic mechanism in early OA [19]. In summary, our results demonstrated the potential roles of NGF in DCS by TWAS analysis and RNA expression profiles. Although more experiments are needed to further confirm the biological mechanism of NGF.

RPS15A was identified in the weight of muscle-skeleton by TWAS analysis. Ribosomal protein S15A (RPS15A) is a member of the RPS family, maps to human chromosome 16p12.3 locus and encodes a highly conserved 40S ribosomal protein [21]. RPS15A has also exhibited various extra-ribosomal functions, such as cell division, tumorigenesis, and progression. For example, RPS15A encodes Ribosomal protein S15a. It is noteworthy that the NF-κB pathway is activated in the process of intervertebral disc degeneration [22]. To sum up, RPS15A could provide new clues for the pathogenesis of DCS by regulating the NF-κB pathway.

SELL (L-selectin) was identified in the weight of whole blood by TWAS analysis. L-selectin is a cell adhesion molecule consisting of a large, highly glycosylated, extracellular domain, a single spanning transmembrane domain and a small cytoplasmic tail [23]. L-selectin can express on most circulating leukocytes and contribute to adhesion, migration, and signal transduction in various diseases. In recent years some studies found L-selectin can regulate the expression of ICAM1(CD54) and the activation of ICAM1 is part of specific pathophysiology in intervertebral degeneration [24]. We have already introduced the pathogenesis of DCS is the degeneration of the intervertebral discs, so L-selectin may provide novel clues for understanding the genetic mechanism of DCS.

We used FUMA and Metascape tools to perform gene functional enrichment and annotation analysis. Several GO terms and KEGG pathways were detected to explore the functions of candidate genes and how they are distributed in DCS. Degradation of the extracellular matrix (R-HSA-1474228) was identified by both TWAS analysis and RNA expression profiles. The degeneration of intervertebral disc can cause the breakdown of the extracellular matrix [25]. By using the protein gel electrophoresis analysis, another study found gradually increased extracellular matrix fragmentation in the model of degenerative intervertebral discs, which also confirms our result [26]. In all, the degradation of the extracellular matrix pathway may be a powerful therapeutic prospect for DCS.

Mast cell degranulation (GO:0043303), mast cell activation involved in immune response (GO:0002279), and mast cell mediated immunity (GO:0002448) were identified as enriched GO terms for TWAS analysis. A study shows mast cells play a significant role in degenerative musculoskeletal diseases especially as

they secrete several pro-inflammatory, neurovascular, and catabolic factors [27]. During aging and degeneration, mast cells are recruited into the intervertebral disc by up-regulation of the mast cell chemoattractant stem cell factor, and then mast cells can be activated through several different mechanisms, one of them likely being cellular interactions with intervertebral disc cells [28]. Once activated, inflammatory cytokines are released into the immediate microenvironment, inducing a catabolic/pro-inflammatory phenotype in the disc cells which then secrete factors that further promote mast cell activation [28]. This degenerative cycle can lead to a state of chronic inflammation in the intervertebral disc and result in DCS. Here we demonstrated that mast cells are present in the intervertebral disc, and they play potential roles in the pathogenesis of DCS.

Sphingolipid signaling pathway (ko04071) is significantly identified for DCS. Sphingolipid is an important part of the plasma membrane and implicated in a multitude of cellular processes [29]. Its function has been investigated in the muscle skeleton system. For example, sphingomyelin phosphodiesterase 3 (SMPD3), an important regulator of sphingolipid metabolism in the skeleton. SMPD3 cleaves sphingomyelin to generate ceramide and phosphocholine and the deficiency of SMPD3 can impair the mineralization in both cartilage and bone extracellular matrices leading to severe skeletal deformities [30]. In addition, Navone SE et al found sphingosine-1-phosphate is a metabolic product of cell membrane sphingolipids which are involved as a microenvironmental signal in the intervertebral disc degeneration process by inducing chemotaxis, migration, and secretion of pro-inflammatory cytokines [31]. We demonstrated sphingolipid plays potential roles in the pathogenesis of muscle skeleton diseases, further experiments are needed to prove our results.

The strength of our study is that we conducted TWAS analysis by using the latest GWAS summary data of DCS. On the one hand, TWAS analysis is a creative method that can predict the gene expression in DCS and avoid confounding from environmental differences caused by the trait that may influence expression. On the other hand, the large sample size of GWAS summary data ensures the accuracy of our results. In addition, we verified the candidate genes by comparing them with the RNA expression profiles. This study also has some limitations. The GWAS summary data are based on European ancestry and may not apply to other ancestry studies. Therefore, it should be cautious to apply our results to other populations. Further TWAS analysis on other populations are needed to prove our results. In addition, our results lacked sufficient mechanism-based experiments. More experiments are warranted to further confirm the biological rationality and clarify the biological mechanism of our study results.

Conclusion

To be conclude, TWAS analysis is the key to understand disease etiology, facilitate biological interpretation of GWAS results, and prioritize follow-up functional studies. We performed TWAS analysis and identified multiple candidate genes and GO terms/KEGG pathways of DCS. Our study attempted to provide clues into the genetic mechanisms underlying DCS development. Further studies are needed to demonstrate specific biological mechanisms in the future.

Abbreviations

APLNR
Apelin Receptor
DCS
Degenerative Cervical Spondylosis
eQTL
Expression Quantitative Trait Loci
FUSION
Functional Summary-Based Imputation
FUMA
Functional Mapping and Annotation of Genome wide Association Study
GEO
Gene Expression Omnibus
GO
Gene ontology
GWAS
Genome-wide association study
KEGG
Kyoto Encyclopedia of Genes and Genomes
RPS15A
Ribosomal protein S15A
TWAS
Transcriptome-wide association study

Declarations

Ethics approval and consent to participate

All the database obtained from the internet, so not applicable

Consent for publication

Not applicable

Availability of data and supporting materials

The datasets analyzed during the current study are available from the Gene Expression Omnibus database(https://www.ncbi.nlm.nih.gov/gds) accession number: GSE153761, the UK biobank (http://geneatlas.roslin.ed.ac.uk/) fields: 20002

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (grant number 81974347 and 81802210), the Department of Science and Technology of Sichuan Province (grant number 2021YFS0122). Financial support had no impact on the outcomes of this study.

Acknowledgements

Not applicable

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Figures

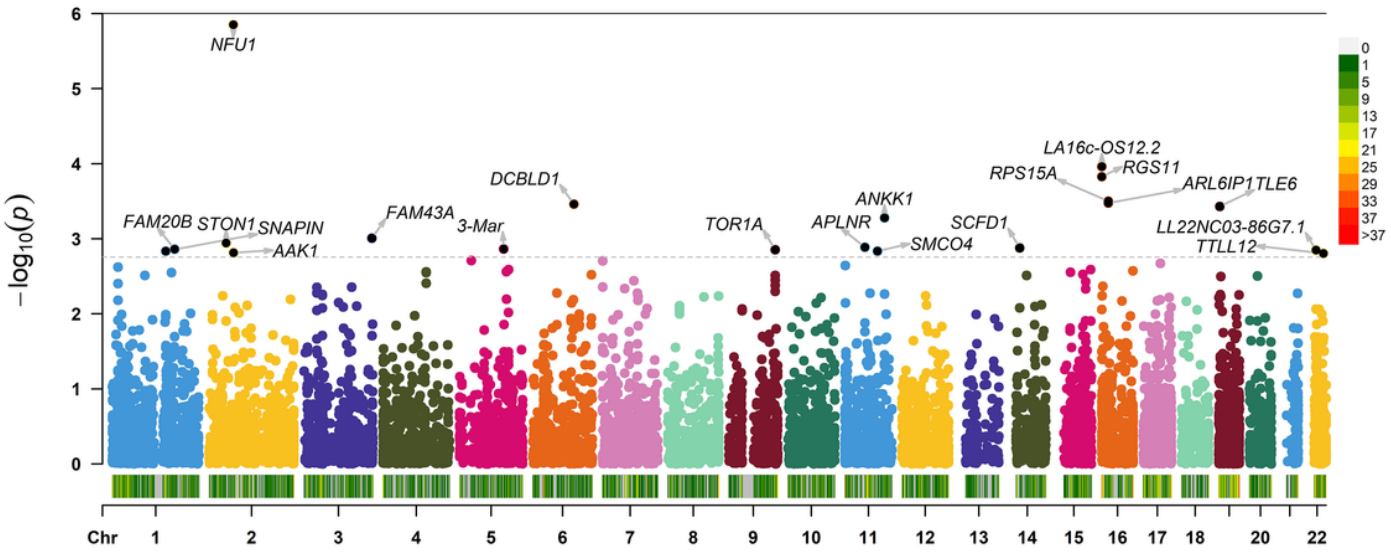

Figure 1

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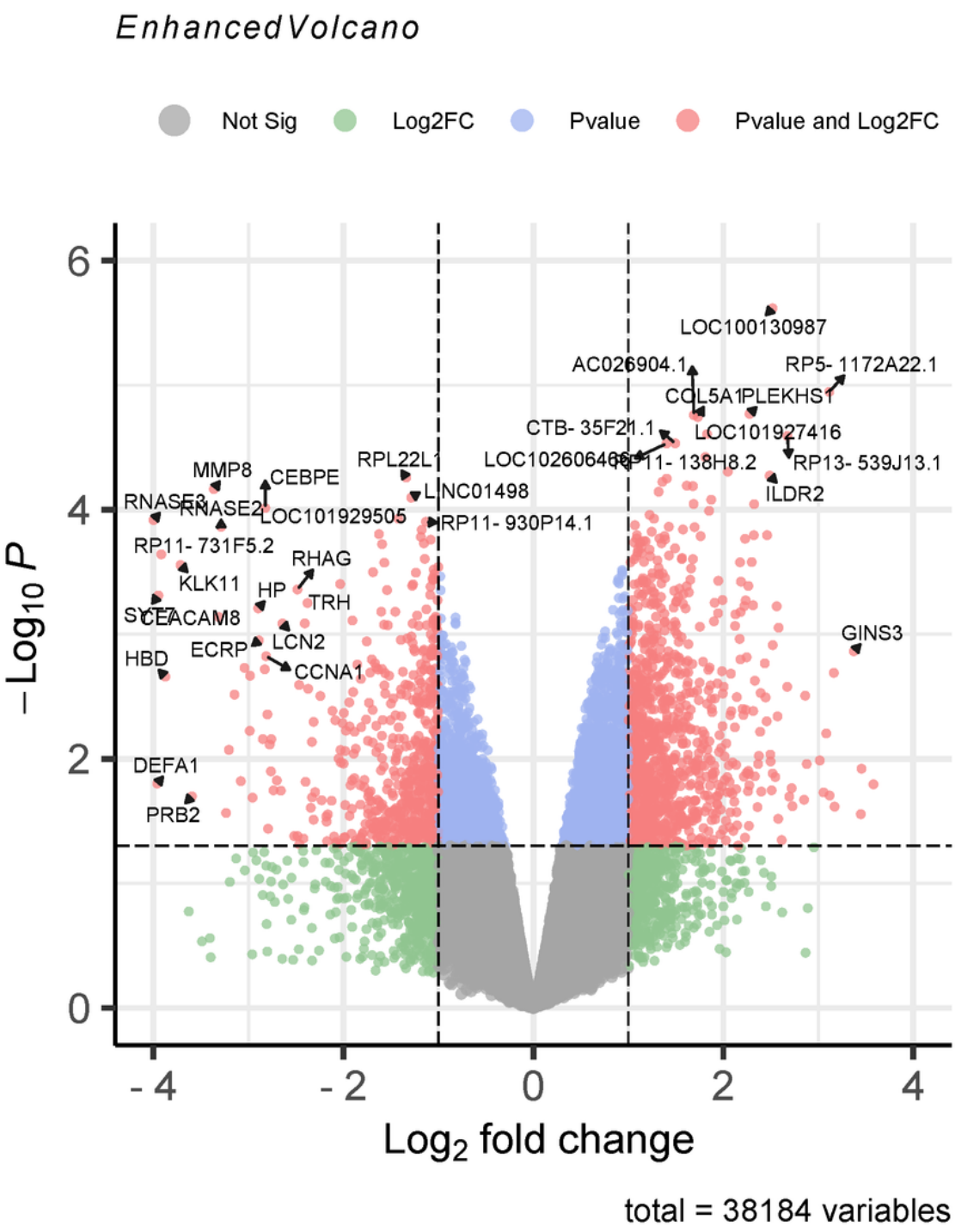

Figure 2

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Figure 3

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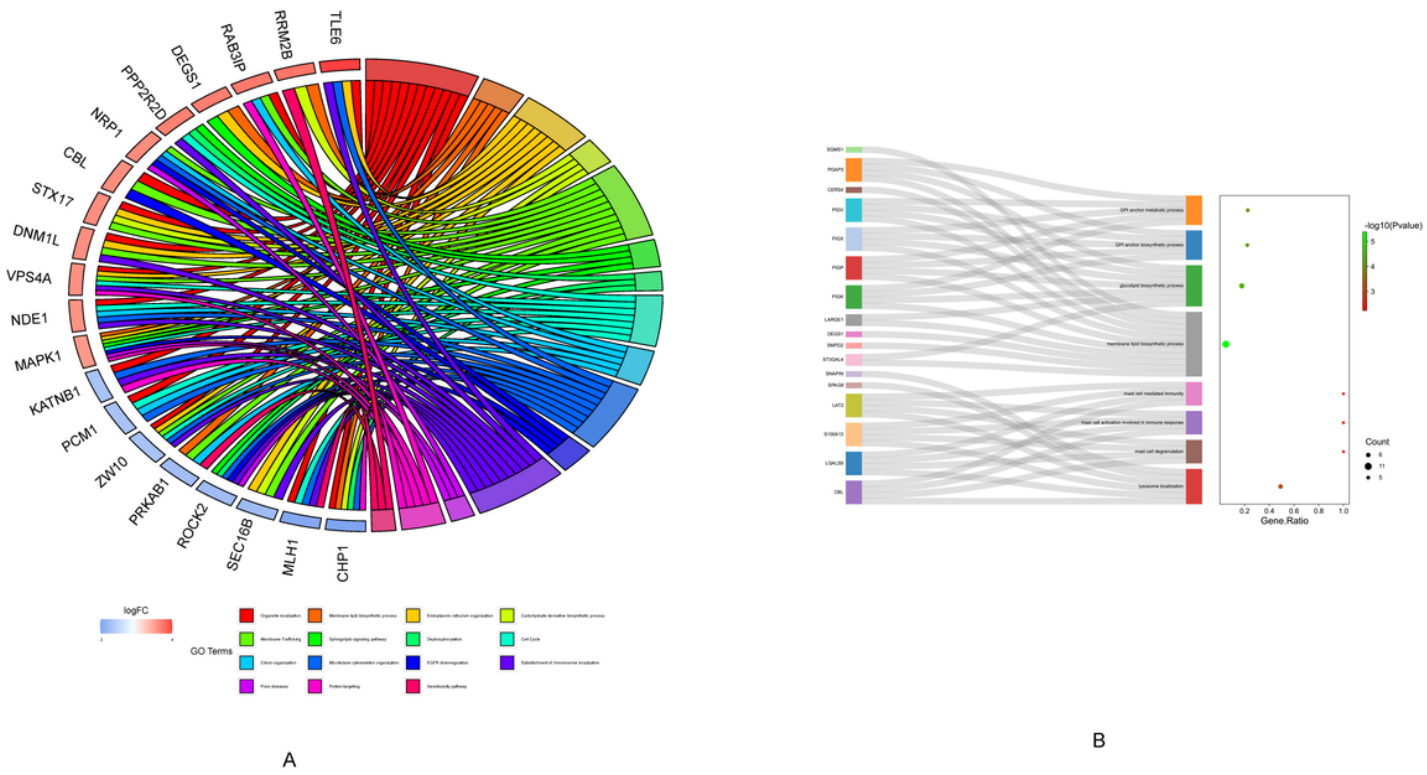

Figure 4

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Supplementary Files

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- SupplementaryTable1.docx