PROJECT REPORT

Investigating the molecular cause of hypermobile Ehlers-Danlos syndrome

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Course: MSB1014 Network Biology
Program: Master Systems Biology
Faculty: FSE
Academic Year: 2023/24

Date: October 20, 2023

1 Introduction

1.1 Background

- Ehlers-Danlos syndromes are a group of heritable connective tissue disorders that can be classified into multiple subtypes
- current classification describes 13 subtypes [1]
- most common form: hypermobile EDS (hEDS) - only subtype with unknown molecular cause
- diagnosis based on clinical presentation
- identify molecular cause for: diagnosis, understanding, potential treatment [2]
- current state: ongoing study with aim to do this by analysing genes of 1000 affected individuals, results not expected before 2025 [3]
- many studies investigated genes but no clear molecular cause with connection to connective tissue established yet [4]
- until then use data known
- problem with existing data: criteria change in 2017, earlier diagnosed people might not classify anymore [5] + there is a unclear overlap with Hypermobility Spectrum Disorder (HSD) and terms often used interchangeable and diagnoses often grouped together (unclear whether there is a difference) [5, 6]
- we know the clinical representation and how other eds types work (kind of)
- TODO: include information of what is affected, extracellular matrix, collagen, connective tissue

1.2 Aim

- aim: investigate moleculare cause of hypermobile EDS
- studying differentially expressed genes in hEDS patients
- which biological processes and pathways are affected
- similarities to pathways/processes/function affected by other EDS genes
- TODO: what do we want to do in the big picture: find candidate genes? is this to ambitious?

2 Methods

2.1 Analysis of Differentially Expressed Genes and Network Creation

- data accessible at NCBI GEO database with the accession number GSE218012 [2]
- analysis with DeSeq2 in R based on the analysis exported from GEO2R [7] to identily up-regulated and down-regulated genes
- |log2FoldChange| > 0.5, pValue < 0.05, pValue adjusted with Benjamini and Hochberg False Discovery Rate

- use Cytoscape to create network [8]
- query differentially expressed genes from string db [9] (confidence cut off 0.4)
- query eds genes related to other eds types additionally: 21 genes retrieved from Disease Ontology with Disease Ontology ID 13359 [10], queried from string, again with a medium confidence cut off of 0.4

- load data about differential expression into network, for EDS genes and differentially expressed genes

2.2 Enrichment analysis and clustering

- exploratory enrichment analysis on whole network
- use R-package clusterProfiler [11] for enrichment
- large network, thus cluster before to get better insights for specific parts
- two different cluster methods used with different resulting cluster structure
- create subnetworks for clusters with more than 15 nodes [TODO: why this threshold? to focus on more relevant because many in MCODE between 10 and 15 nodes, for community clustering smaller ones probably found by MCODE]

2.2.1 MCODE

- MCODE finds ”densely connected regions in large protein-protein interaction networks that may represent molecular complexes” [12]
- results in smaller clusters
- suited to analyse molecular function
- use default parameters
- analysis of molecular function with clusterProfiler with GO over-representation analysis
- do we see clusters with other molecular functions than expected
- are genes clustered together with other eds genes
- are clusters generelly mostly upregulated or downregulated

2.2.2 Community Clustering

- small cluster not helpful for biological processes and pathways
- therefore use second clustering method
- community clustering with GLay, ”more suitable for functional interpretation” [13]

3 Results

3.1 Differentially Expressed Genes and Network creation

- 908 differentially expressed genes with chosen thresholds section 2.1
- around half are upregulated, around half downregulated [TODO: get exact numbers]
- String was able to query 828 of them [TODO: provide table of not queried in supplementary material or at least github?]
- after querying additional genes that are known to be related to other eds types: resulting network with 847 nodes and 6129 edges
- position of known eds genes in network is mostly central (checked degree, clustering coefficient, betweenness centrality and closeness centrality)

3.2 Enrichment analysis and clustering

3.2.1 MCODE

- 3 clusters with more than 15 nodes (first with 66 nodes & 1953 connections, second with 44 nodes & 686 connections, third with 16 nodes & 114 connections)
- first and largest cluster upregulated only
- second one as well
- 3rd MCODE cluster, shown in figure 1, is mostly upgregulated with 2 downregulated nodes, some not relevantly differentially expressed, also only 9/16 are not known eds genes, 8 are
- the eds genes are all below the threshold of $|\text{log2FoldChange}| > 0.5$, 1 of them is 1 of the two downregulated genes
- quite interesting to see genes closely related to other eds genes, COL21A1 is also strongly upregulated (log2FoldChange > 2)
- all known EDS genes with ADAMTS2 as exception have a high Closeness Centrality
- regarding the enrichment of this cluster: not surprising to see extracellular

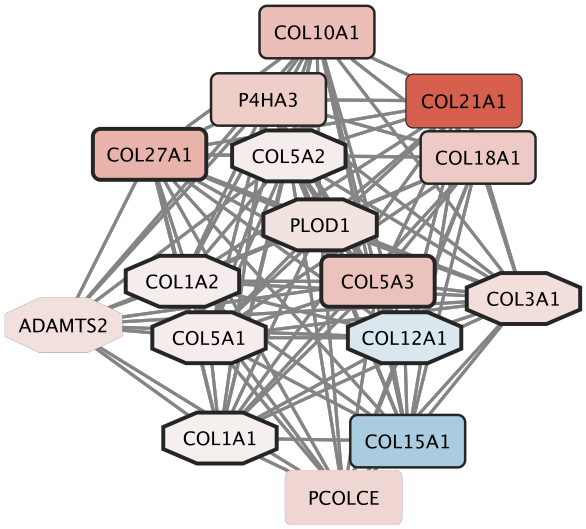

Figure 1: MCODE cluster 3 [TODO: add legend for shape and colour]

3.2.2 Community Clustering

- 6 clusters with more than 15 nodes
- 3 very small (18, 29, 29 nodes), two medium sized (76 and 105 nodes) and one very large cluster (363 nodes)
- especially medium sized clusters highly interconnected
- biggest one mix of up-regulated and down-regulated genes, contains all 21 genes known to cause other EDS types
- both medium sized clusters mostly upregulated $\rightarrow$ interesting!

4 Discussion

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