PROJECT REPORT

Investigating the molecular cause of hypermobile Ehlers-Danlos syndrome

Cay Rahn
6255648

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1 Introduction

Ehlers-Danlos syndromes are a group of heritable connective tissue disorders that can be classified into multiple subtypes. The current classification describes 14 subtypes [1, 2], with hypermobile EDS (hEDS) being the most common form. It is also the only subtype with an unknown molecular cause, leading to a diagnosis based on clinical presentation. Identifying the molecular cause is crucial to improving the diagnosis process, understanding the disease and finding potential treatment options [3].

Although many studies investigated several genes, no clear molecular cause with a connection to connective tissue has been established yet [4]. While an ongoing study aims to find the genetic cause of hEDS by analysing the genes of around 1000 affected individuals, results are not expected before 2025 [5]. Until then, utilising already collected data is essential to understand more about hEDS, although a diagnosis criteria changed in 2017 results in data from before the change not being usable anymore [6, 7].

This project aims to investigate the molecular cause of hypermobile EDS by studying the influence of differentially expressed genes in hEDS patients. It mainly tries to find molecular functions and biological processes affected by differentially expressed genes that are similar to the ones affected by the molecular cause of other EDS types. This analysis eventually aims to find genes differentially expressed in hEDS that are candidates for being the molecular cause of hEDS and to relate the findings to existing research.

2 Methods

The following structured approach will be pursued to answer the research question:

Analysis of Differentially Expressed Genes (DEGs). A dataset of gene expression profiles from dermal fibroblasts from patients with hEDS and healthy controls is used, available at the NCBI GEO database with the accession number GSE218012 [3]. The analysis is performed with the R-packages DeSeq2 and limma based on the analysis from GEO2R to identify up-regulated and down-regulated genes [8, 9]. Genes with a log2-fold change $> \pm 0.5$ and a by the Benjamin-Hochberg procedure adjusted p-value < 0.05 were considered. The cut-offs were chosen based on similar research [10, 11].

Network Creation. The Protein-protein interaction (PPI) network is created in Cytoscape [12] by querying the before-identified DEGs from the STRING database with an interaction score > 0.4, which reflects medium confidence [13]. Since hEDS belongs to the family of Ehlers-Danlos syndromes, its molecular cause is most likely closely related to other EDS types. The PPI network of the DEGs is therefore expanded by additionally querying genes related to other EDS types. The genes are retrieved from Disease ontology (Disease Ontology ID 13359) [14] and queried similarly from STRING. The resulting network is annotated with the differential expression data of the genes.

Gene Ontology and Clustering. To gain insight into biological processes and molecular functions affected by DEGs, GeneOntology (GO) enrichment is performed using the R-package clusterProfiler [15, 16, 17]. Generally, results with $p < 0.05$ are considered as significant. The created network is clustered to attain more detailed insights into specific part. Two different algorithms, resulting in different cluster structures, are used: MCODE to analyse the molecular function and Community clustering to analyse biological processes and pathways. Only clusters of more than 15 genes are included in the analysis to ensure relevance and keep the analysis feasible in the project's scope. Further analysis on the resulting clusters includes investigating whether genes are clustered together with genes known to cause other EDS types and whether the resulting clusters consist of up-regulated or down-regulated genes or a combination of both.

MCODE MCODE is a clustering algorithm designed to find highly connected regions in PPI networks that might represent molecular complexes [18]. MCODE was applied in Cytoscape with the clusterMaker2 app using the default parameters [19].

Community Clustering To analyse the biological processes and pathways involved in the DEGs, larger clusters are required. GLay, a community clustering algorithm, was designed to be used for a functional interpretation of clusters in networks [20]. Analogous to the MCODE, clustering was performed with clusterMaker2 and Cytoscape using the default parameters [12, 19].

Heat Diffusion is applied to identify genes closely connected to genes causing other EDS types, starting with the EDS nodes using Cytoscape functionality [21]. [TODO: heat parameter]

3 Results and discussion

3.1 Differentially Expressed Genes and Network creation

Under the chosen thresholds discussed in 2 908 genes were found to be differentially expressed. Around half of them (495) are upregulated, the remaining 413 are downregulated. STRING was able to query 828 of them; using other ID types did not change this. After querying the additional EDS-related genes the resulting network consists of 847 genes and 6129 connections.

The position of the known EDS genes in the network is on average more central than expected by chance based on degree, clustering coefficient, betweenness centralitiy and closeness centrality. This supports the close relationship between hEDS and other EDS types.

3.2 Enrichment analysis and clustering

- Cellular Component: Nuclosome, collagen-containing extracellular matrix, chromatin, chromosamal region [TODO: dot plot or graph?]
- Biological Progress: nucleosome assembly & organization, protein-DNA assembly & organization, cell clycle signaling, regulation & transition, chromosome separation regulation
- Molecular function difficult to analyse on large graph, but we see structural constituent of chromatin, protein heterodimerization activity and extracellular matrix structural constituent

TODO

3.2.1 MCODE

Running MCODE on the created networks finds 3 clusters with more than 15 nodes, one with 66 nodes and 1953 connections, one with 44 nodes and 686 connections and one with 16 nodes and 114 connections with the second two clusters containing upregulated genes only. The two bigger clusters contain no genes known to cause other EDS types.

MCODE cluster with EDS genes

The third, smaller cluster, shown in figure1, contains mostly upregulated but also two downregulated genes. Some do not show a strong differential expression but are genes known to cause other EDS types. In total, the cluster contains eight EDS genes, all having a $|\text{log2FoldChange}| < 0.5$ and nine differentially expressed genes. One of the EDS genes is also one of the two down-regulated genes. All known EDS genes besides ADAMTS2 have a high Closeness Centrality, consistent with the findings of EDS genes being more central in the complete network. COL21A1 shows the strongest differential expression (log2FoldChange > 2), more than twice as high as the other genes while being less central in the cluster.

GO-Enrichment testing overrepresentation of molecular functions of the cluster returns extracellular matrix in two terms, GO:0005201 and GO:0030020 with the second one being a subterm of the first. The first describes the action of a molecule that contributes to the structural integrity of the extracellular matrix, the second is a constituent of the extracellular matrix that enables the matrix to resist longitudinal stress. Both GO-terms contain the same EDS genes and seven respectively six other genes with

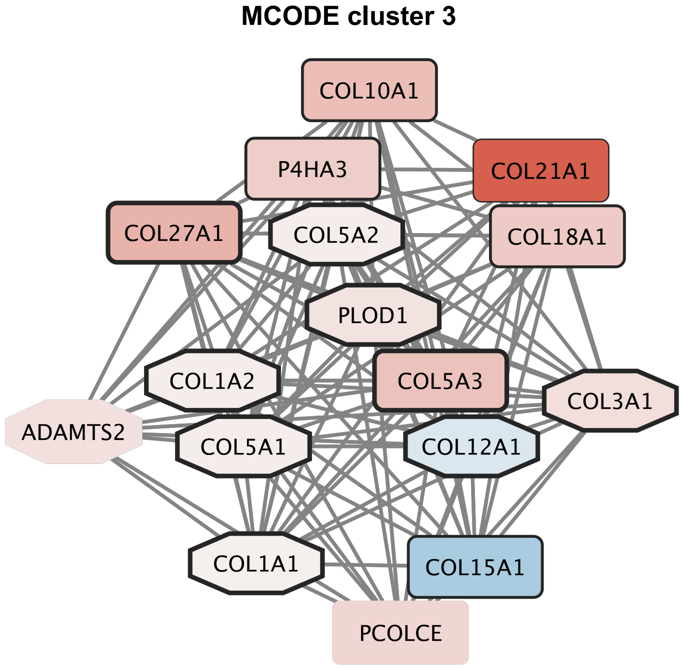


Figure 1: The MCODE cluster contains many genes known to cause other types of EDS. [TODO: add legend for shape, border and colour]

PCOLE being the only gene not present in the GO subterm. These genes are investigated in more detail in terms of their centrality, differential expression and what is known about them. COL27A1, a fibrillar collagen gene has a central position in the cluster and relatively strong differential expression. The same applies for COL5A3, another gene related to collagen. The gene COL21A1 is very strongly differentially expressed, as mentioned before. It encodes the alpha chain of XXI collagen, which maintains the integrety of ECM and is a paralog to COL5A1, a known EDS gene [22].

To find connections to ECM in the enrichment analysis is consistent with findings of similar research [7]. [Todo: there was other ressearch, find] The affection of ECM with particular disorganization of collagen and fibrocencting was found in hEDS and two other EDS types [23]. [TODO: point out what my new findings are, COL21A1 etc]

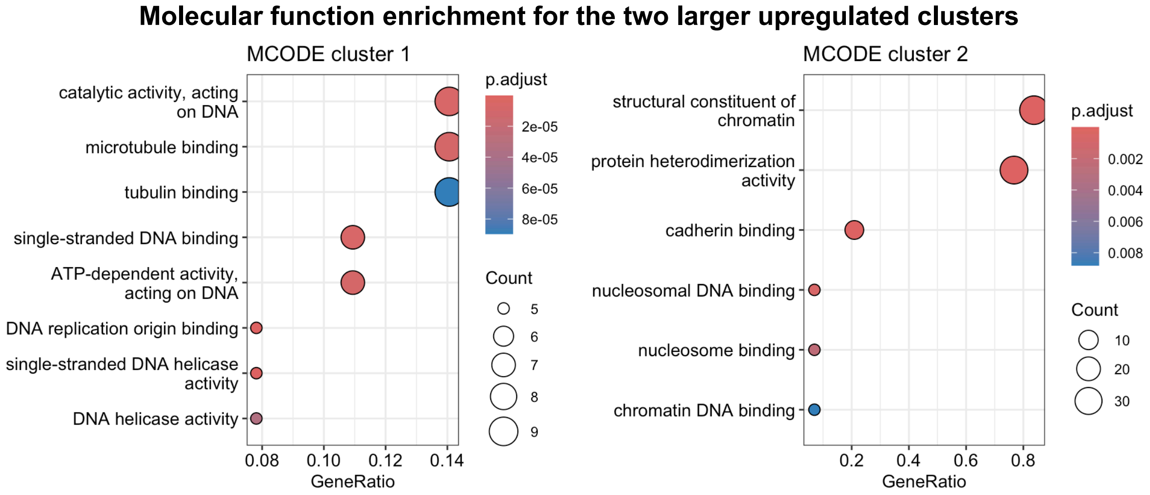
Upregulated MCODE cluster

The two larger, upregulated MCODE clusters show no over-representation in ECM-terms, as is shown in figure 2. It is noticable, that the enrichment of the first cluster shows, that not many genes are part of the enriched terms. For the second cluster, the gene ratio is much higher, with up to around 80 % of the genes being involved in the second tow terms. Therefore the enrichment of the first cluster does not provide much insight in terms of molecular function in hEDS patients.

On the other hand, the up-regulation seen in the second cluster for the GO-term GO:0030527, the structural constituent of chromatin is interesting, because earlier findings found down-regulated genes involved in processes related to chromatin in vEDS [23].

3.2.2 Community Clustering

Community Clustering results in six clusters with more than 15 genes. Three of them are very small and loosely connected clusters, containing 18 to 29 genes and approximately the same amount of connections as genes. Since clusters of less than 20 genes are not large enough for analysis of biological



(a) First MCODE cluster

(b) Second MCODE cluster

Figure 2: The results of the GO-enrichment for molecular function of the two upregulated, larger MCODE clusters

processes, smaller clusters are ommited from the analysis. Additionally there are two medium-sized highly connected clusters with respectively 76 genes and 100 connections and 105 genes and 2330 connectinos and one very large cluster with 363 gens and 1661 connections. Especially the medium-sized clusters are highly interconnected and contain mostly upregulated genes.

Largest Community Cluster

The largest cluster contains a mix of up-regulated and down-regulated genes and also includes all 21 genes known to cause other EDS types.

4 Conclusion

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