A multilayer network approach to elucidate severity in Congenital Myasthenic Syndromes

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EXTENDED ABSTRACT

A. Introduction

Congenital Myasthenic Syndromes (CMS) conform a group of diverse rare diseases characterized by neuromuscular junction (NMJ) dysfunctions resulting in muscle weakness, which is a common hallmark in these conditions. While causative genes have been previously described , a molecular explanation of the observed large range of phenotypic severity remains unclear.

In this work we undertook a Personalized Medicine approach with the goal of elucidating the molecular processes causing severity in CMS. Personalized Medicine is defined as the integration of molecular research with clinical data in order to deliver better diagnoses and treatments tailored to the individual characteristics of each patient.

We jointly analysed DNA and RNA from 20 samples from an isolated region in Eastern Europe (Bulgaria), integrating RNA sequencing (RNAseq) from fibroblast culture and Whole Genome Sequencing (WGS) from blood, identifying a set of candidate genes. Since none of these genes' features (annotations, mutations, etc.) were able to explain severity , we performed a multilayer network analysis of several aspects (interactome, reactome and metabolome) involving the damaged genes of each CMS patient in our cohort.

B. Methods

1 – RNAseq & WGS

Whole genome sequencing (WGS) data have been obtained from blood, while RNA sequencing (RNA-seq) data have been obtained from fibroblasts. All analyses have been performed using RD-Connect project (https://rd-connect.eu/) specific pipeline and the human genome GRCh37d5 as reference.

Copy Number Variants (CNVs) have been extracted using ClinCNV (https://github.com/imgag/ClinCNV) by employing a set of Eastern European samples as background control group. Heterozygous compound mutations have been obtained by phasing the WGS and RNA-seq data, removing variants with allele frequency > 3%, outsde exonic and splicing regions (Ensembl annotation), synonymous, and with read depth (coverage) smaller than 8.

2 – Multilayer Community Detection

We generated a multilayer network using reactome [2], the metabolome [3], and the interactome [4] layers. The multilayer community detection analysis was performed by using MolTi software [5]. To study the relationship between disease-associated genes multilayer network communities' membership, we analyzed the curated gene-disease associations of the DisGeNET database (http://www.disgenet.org/).

C. Results

Albeit all affected individuals shared the very same causal mutation (a deletion within the acetylcholine receptor -AChR- ε subunit, CHRNE c.1327delG), the severity of symptoms across this cohort varies considerably regardless of age and sex, pointing towards causes to the disease phenotype other than the causal variant. In this work, we performed an in-depth characterization of 20 CMS samples from patients from this cohort by analyzing multi-omics data (see Methods: *RNAseq & WGS*). Distinct CMS severity levels have been classified by specialized physicians , namely severe (8 patients) and not-severe (2 intermediate and 10 mild patients) disease phenotypes. In particular, the clinical phenotyping reports the outcomes of medical tests such as swallowing, speech, respiratory dysfunction, as well as the prescription of pharmacological treatments.

We jointly analyzed DNA and RNA from 20 patients, with the objective of validating whether the severity was determined by the accumulation of damaging mutations hampering the neuromuscular activity, on top of the CHRNE damaging mutation. By analysing segregating single nucleotide polymorphisms (SNPs) and copy number variations (CNVs), we could not find any unique cause of severity. Nevertheless, we observed that heterozygous compound mutations are enriched in pathways related to the extracellular matrix (ECM) receptor, which has been recently proposed as a target for CMS therapy [1]. As a result, we used CNVs and heterozygous compound mutation gene sets in the multilayer network analysis (**Figure 1**).

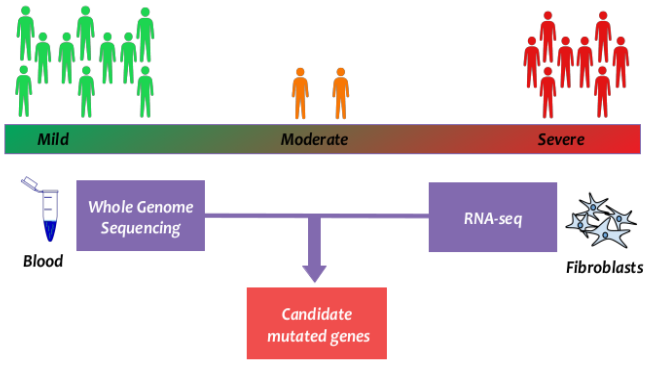

Fig. 1: Phenotypic severity spectrum and genomic work performed on the CMS cohort.

Once assessed that compound heterozygous variants are associated with functionally related pathways, we sought to analyze the relationships among the affected genes at different scales . We generated a multilayer network, a complex system representation in which distinct features of nodes and edges are described by several layers, containing different facets of molecular biology **(Figure 2)**, namely the reactome [2], the metabolome [3], and the interactome [4].

We performed a multilayer community analysis using MolTi, a software that adapts classical Louvain clustering analysis into multilayer networks [5] and looked for topological relationships between the affected genes and previously known CMS causal genes [6].

We identified a largest component module of damaged genes that is specific of the severe group of patients, composed of 15 genes **(Figure 2)**. 6 out of these 15 are previously described CMS causal genes, while the other genes are mutated with damaging compound heterozygous mutations (9 out of 15, as agrin, a well-known CMS causal gene present this type of mutations in one of the patients) or CNVs (1 out of 15). The mutated genes are involved in a varied spectrum of functions at the neuromuscular junction, such as neuromuscular synapsis development and AChR clustering at the skeletal muscle fiber. Our results show that alterations in proteoglycans (AGRN, HSPG2, VCAN, COL15A1), tenascins (TNC, TNXB), and chromogranins (CHGB) are specific of the severe group. Strikingly, no proteoglycans are damaged in the not-severe group, suggesting a direct involvement of ECM in CMS severity.

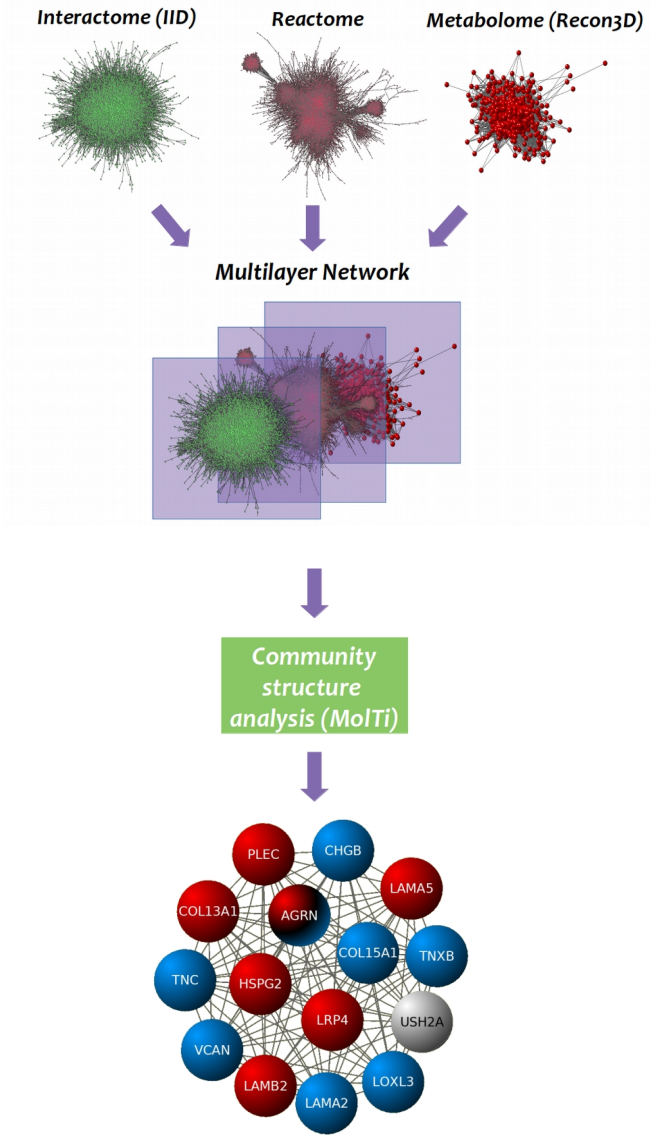

Fig. 2: Multilayer network components, community analysis workflow and the identified largest component module of genes functionally related to neuromuscular junction function.

In red, previously known CMS genes. In blue, genes mutated with compound heterozygous mutations. In white, genes mutated with copy number variants (CNVs).

D. Discussion

In this work, we tested the hypothesis that the severity of a rare disease with a known causative mutation was determined by the accumulation of additional damaging alterations hampering a specific impaired functional process. In particular, we performed an in-depth analysis of 20 CMS patients, from a narrow and geographically isolated population, who share the same causative mutation in the acetylcholine receptor ε subunit (CHRNE), presenting different levels of disease severity, as assessed by expert physicians.

Our results show that CMS severity can be ascribed to the personalized impairment of specific classes and localizations of NMJ activities, namely extracellular matrix components (proteoglycans, tenascins, chromogranins) and postsynaptic modulators of AChR clustering. Moreover, this work shows that coupling multilayer network analysis with personalized omics information helps give a molecular explanation of the phenotypic severity of rare diseases.

Finally, as our results suggest that severity is related to AChR clustering at the AGRN-PLEC-LRP4-Laminins axis level, severe affected patients may potentially benefit from pharmaceutical interventions enhacing the AChR clustering process. For example, beta-2 adrenergic receptor agonists like ephedrine and salbutamol have been documented as capable of enhancing AChR clustering and proved to be successful in the treatment for severe AChR deficiency syndromes [7].

References

[1] Ito M & Ohno, K. *Protein-Anchoring Therapy to Target Extracellular Matrix Proteins to Their Physiological Destinations.* Matrix Biol. 2018, 68-69, 628-636.

[2] Fabregat et al. *The Reactome Pathway Knowledgebase.* Nucleic Acids Res. 2018 46 (D1): D469-55

[3] Brunk et al. *Recon3D Enables a Three-Dimensional View of Gene Variation in Human Metabolism.* Nat. Biotechnol 36 (3): 272-81

[4] Kotlyar et al. *Integrated Interactions Database: Tissue-Specific View of the Human and Model Organism Interactomes".* Nucleic Acids Res 2016. 44 (D1): D536-541

[5] Didier et al. . *Identifying communities from multiplex biological networks.* PeerJ 2015. 3, e1525

[6] Bonne et al. *Diagnostic Approach to the Congenital Muscular Dystrophies.* Neuromuscul. Disord. 2017. 27(12): 1152-83

[7] Rodríguez Cruz et al. *Salbutamol and Ephedrine in the Treatment of Severe AChR Deficiency Syndromes.* Neurology 85(12): 1043-47

Author biography

Iker Núñez was born in Valladolid, Spain, in 1995. He received the BSc degree in Biology from Universidad de Alcalá (UAH), Spain, in 2017, and the MSc degree in Biomedical Research from Universidad de Valladolid (UVA) and Molecular Biology and Genetics Institute (IBGM) of Valladolid, Spain, in 2018. Since October 2018, he is a Ph.D. student at Computational Biology Group within the Life Sciences Department of Barcelona Supercomputing Center (BSC), Spain. His current main research interests includes Network Analysis, Deep Learning and Personalized Medicine.