Solving unsolved rare neurological diseases—a Solve-RD viewpoint

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Received: 13 October 2020 / Revised: 9 April 2021 / Accepted: 16 April 2021 / Published online: 10 May 2021
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Introduction

Rare genetic neurological disorders (RND; ORPHA:71859) are a heterogeneous group of disorders comprising >1700 distinct genetic disease entities. However, genetic discoveries have not yet translated into dramatic increases of diagnostic yield and indeed rates of molecular genetic diagnoses have been stuck at about 30–50% across NGS modalities and RND phenotypes [1, 2]. Existence of yet unknown disease genes as well as shortcomings of commonly employed NGS technologies and analysis pipelines in detecting certain variant types are typically cited to explain the low diagnosis rates.

To increase the diagnostic yield in RNDs - one of the four focus disease groups in Solve-RD - we follow two major approaches, that we will here present and exemplify: (i) systematic state-of-the art re-analysis of large cohorts of unsolved whole-exome/genome sequencing (WES/WGS) RND datasets; and (ii) novel-omics approaches. Based on the way Solve-RD systematically organizes researchers’ expertise to channel this approach [3], the European Reference Network for Rare Neurological Diseases (ERN-RND) has established its own Data Interpretation Task Force (DITF) within SOLVE-RD, which is currently composed of clinical and genetic experts from 29 sites in 15 European countries.

Systematic re-analysis of coding variation

Unsolved WES datasets (fastq) from 2048 families with RNDs were submitted by clinical sites of ERN-RND [4] to the RD-Connect Genome-Phenome Analysis Platform. Genomic data were processed and filtered as detailed [5]. The Solve-RD SNV/Indel working group reported back 74,456 variants in

Members of the Solve-RD-DITF-RND and The Solve-RD Consortium are listed in below Acknowledgements.

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Supplementary information
The online version contains supplementary material available at https://doi.org/10.1038/s41431-021-00901-1.

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2246 individuals, which were ranked according to their likelihood of being causative. One thousand nine hundred and forty-three variants in 1155 individuals (average 1.68 variants/individuum) were classified as rank 1 (genotype matches OMIM and variant (likely) pathogenic according to ACMG). Based on these results and the work of the RND DITF 44 cases could be solved by this systematic re-analysis approach, which equals 29% of the re-analysed cases for which feedback was available. Reasons for solving cases were firstly updates of the respective ClinVar entry of identified variants between the time of the initial genetic workup and the Solve-RD re-analysis due to now additional available evidence. One example is the re-classification of variants in highly variably genes like *ITPR1* between 2016 and 2020 [6] (Fig. 1A).

Second, use of human phenotype ontology-based phenotypes [7] rather than diagnostic categories as well as consideration of variant-specific rather than gene-specific phenotypes enabled detection of functionally relevant variants because initial analysis focused on disease-specific panels. Mis-classification of phenotypes in RNDs is a common problem due to the considerable overlap between diagnostic categories especially in phenotypes affecting more than one neurological system. This approach i.e. allowed identification of a causative variant in *EXOSC3* (c.395A>C) that is typically associated with a ‘milder’ clinical disease course and lacking the hallmark pontine atrophy characteristic for EXOSC3-associated disease (Fig. 1B).

**Analysis of non-coding variation**

The relative contribution of non-coding variation to RNDs has not been established yet and will be systematically explored by Solve-RD by combining WGS and RNA Seq. We will evaluate the added value of RNA Seq in early onset sporadic cases (Trio-WGS), multiplex recessive and dominant families.

In the meantime, the exon–intron boundaries commonly covered by WES already allow at least a glimpse into the realm of non-coding variants. Indeed, the systematic Solve-RD re-analysis top-listed a single heterozygous intronic POLR3A variant (NM_007055.3(POLR3A): c.1909+22C>T) that had recently been shown to lead to inclusion of the first 19 nucleotides from intron 14 into the final transcript and consequently to shift of the reading frame [8].
Finding novel variations through novel omics

Scientific rationale drives application of novel-omics technologies in Solve-RD. From the large variety of different omics technologies that will be used by SOLVE-RD, we here present the example of long-range WGS for ataxias, which has just been initiated. For ataxias >25% of all autosomal-dominant and >50% of all autosomal-recessive ataxia patients remain unsolved despite advanced WES analysis [9]. Ataxias are unique in so far as repeat expansions represent the most frequent disease cause. Seventy-five percent of all known autosomal-dominant ataxia cases and 50% of all known autosomal-recessive ataxia cases are caused by repeat expansions [10]. We thus hypothesize that a substantial share of repeat-expansion disorders is still to be found in the large share of still unsolved WES-negative ataxia cases. Therefore, in Solve-RD we will be using long-range WGS in family ‘triplets’ from autosomal-dominant ataxia families, which will be stringently enriched for novel repeat-expansion disorders: namely only families negative not only on WES and frequent SCA repeats, but also on short-read WGS and for which DNA from >2 affected and >2 non-affected family members are available. In a first round of submission, 20 families with 44 ‘slots’ have been submitted and we are awaiting data in 2021.

Conclusion

This viewpoint presents and exemplifies the approach being taken by Solve-RD to diagnostically solve unsolved RND. While re-analysis so far succeeded in 29% of cases, scientifically rational ‘beyond the exome’ approaches are being implemented to further unravel new RND causing genes.

Acknowledgements

We thank the patients and their families for supporting this study.

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Funding The Solve-RD project has received funding from the European Union’s Horizon 2020 research and innovation programme under Grant Agreement No. 779257. Data were analysed using the RD-Connect Genome-Phenome Analysis Platform, which received funding from EU projects RD-Connect, Solve-RD and EJP-RD (Grant Numbers FP7 305444, H2020 799257, H2020 825575), Instituto de Salud Carlos III (Grant Numbers PT13/00018044, PT17/00090019; Instituto Nacional de Bioinformática, INB) and ELIXIR Implementation Studies. The study was further funded by the Federal Ministry of Education and Research, Germany, through the TreatHSP network (01GM1905 to RS and LS), the National Institute of Neurological Diseases and Stroke (R01NS072248 to SZ and RS), the European Joint Program on Rare Diseases-EJP-RD COFUND-EJP N° 825575 through funding for the PROSPAX consortium (441409627 to MS, RS and BvW). CW was supported by the PATE program of the Medical Faculty, University of Tübingen. CEE received support from the Dutch Princess Beatrix Muscle Fund and the Dutch Spieren voor Spieren Muscle fund. Authors on this paper are members of the European Reference Network for Rare Neurological Diseases (ERN-RND, Project ID 739510). Open Access funding enabled and organized by Projekt DEAL.

Compliance with ethical standards

Conflict of interest HG receives/has received research support from the Deutsche Forschungsgemeinschaft (DFG), the Bundesministerium für Bildung und Forschung (BMBF), the Bundesministerium für Gesundheit (BMG) and the European Union (EU). He has received consulting fees from Roche. He has received a speaker honorarium from Takeda. The authors declare no competing interests.

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