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Systematic Collaborative Reanalysis of Genomic Data Improves Diagnostic Yield in Neurologic Rare Diseases

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Many patients experiencing a rare disease remain undiagnosed even after genomic testing. Reanalysis of existing genomic data has shown to increase diagnostic yield, although there are few systematic and comprehensive reanalysis efforts that enable collaborative interpretation and future reinterpretation. The Undiagnosed Rare Disease Program of Catalonia project collated previously

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inconclusive good quality genomic data (panels, exomes, and genomes) and standardized phenotypic profiles from 323 families (543 individuals) with a neurologic rare disease. The data were reanalyzed systematically to identify relatedness, runs of homozygosity, consanguinity, single-nucleotide variants, insertions and deletions, and copy number variants. Data were shared and collaboratively interpreted within the consortium through a customized Genome-Phenome Analysis Platform, which also enables future data reinterpretation. Reanalysis of existing genomic data provided a diagnosis for 20.7% of the patients, including 1.8% diagnosed after the generation of additional genomic data to identify a second pathogenic heterozygous variant. Diagnostic rate was significantly higher for family-based exome/genome reanalysis compared with singleton panels. Most new diagnoses were attributable to recent gene-disease associations (50.8%), additional or improved bioinformatic analysis (19.7%), and standardized phenotyping data integrated within the Undiagnosed Rare Disease Program of Catalonia Genome-Phenome Analysis Platform functionalities (18%). *(J Mol Diagn 2022, 24: 529—542; https://doi.org/10.1016/j.jmoldx.2022.02.003)*

Rare diseases collectively affect 3.5% to 5.9% of the worldwide population, and around 72% of them are of genetic origin.[1] Patients with rare diseases often undergo a years-long diagnostic odyssey characterized by multiple tests with little or no success. Health system costs ascribed to rare disease patients are an important public health issue, highlighting the need for improved access to early diagnosis and care coordination.[2,3] Reaching a molecular diagnosis in a timely manner shortens the diagnostic odyssey, and can guide therapeutic strategies, improve clinical management, and provide genetic counseling for patients and their families with respect to recurrence risk and prenatal options.[3]

Reanalysis of existing genomic data has emerged as an effective approach to increasing the diagnostic yield of previously undiagnosed patients, not only because of the rapid path of discovery of novel gene-disease associations, but also due to improvements in analytical workflows, reclassification of previously unrecognized variants, and/or availability of new phenotypic data.[4] However, systematic reanalysis coupled with reinterpretation of the results requires iterative communication between researchers, clinicians, and families as diagnoses can be made years after the initial sequencing and analysis were performed.[3] Systems like the RD-Connect Genome-Phenome Analysis Platform (GPAP; *https://platform.rd-connect.eu*, last accessed December 6, 2021) facilitate such communication and collaboration between clinicians and researchers within a trustworthy environment. The RD-Connect GPAP is an International Rare Diseases Research Consortium (IRDiRC)—recognized resource that brings together pseudonymized clinical/phenotypic and genomic data with tools and services to enable data sharing, analysis and interpretation for rare disease diagnosis, and gene discovery.[5,6]

The Undiagnosed Rare Disease Program of Catalonia (URD-Cat) aims to provide the Catalan Health System with personalized genomic medicine as a fully integrated service for patients with rare diseases, initially as a pilot project for rare diseases with neurologic involvement. This project involves the main groups that work in rare diseases in Catalonia: 15 consolidated research groups belonging to 7 Health Research Institutes in Barcelona Hospital del Mar Research Institute (IMIM); Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS); Institut d'Investigació Biomèdica de Bellvitge (IDIBELL); Hospital Sant Joan de Déu (HSJD); Vall d'Hebron Institut de Recerca (VHIR); Hospital de la Santa Creu i Sant Pau (HSP); Institut Germans Trias i Pujol (IGTP); the National Center for Genomic Analysis—Center for Genomic Regulation (CNAG-CRG); the Barcelona Institute for Global Health (ISGlobal); the National Supercomputing Center of Barcelona (BSC); and the Spanish Rare Disease Patient Federation (FEDER). A multidisciplinary team of >140 professionals, including clinicians, geneticists, bioinformaticians, biochemists, technicians, and software engineers, participate in the project. A customized version of the GPAP has been deployed for the URD-Cat project to meet the specific requirements needed to be integrated with a National Health System regarding data privacy, interoperability, availability, sustainability, and scalability, among others.

To date, the project has systematically collated clinical and phenotypic information from 928 undiagnosed index cases and their relatives (total of 1569 individuals) for which most of the available diagnostic tests had been performed without yielding a positive result. For 323 of those index cases (543 of those individuals, including relatives), previously existing good quality genomic data (panel, exome, or genome) were reanalyzed. Furthermore,

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additional sequencing data (exome, genome, or transcriptome) were generated in nine index cases with a single heterozygous pathogenic or likely pathogenic variant in an autosomal recessive gene identified through the reanalysis. This study describes the workflow applied and the diagnostic yield after reanalysis. The effect of the phenotype annotation quality and the sequencing approach as well as the reasons why diagnosed patients were not diagnosed in the original analysis are also explored.

Materials and Methods

The URD-Cat Reanalysis Cohort

Pseudonymized clinical information from undiagnosed patients and their family members was collected from medical records by clinicians or geneticists from participating hospitals using PhenoTips.[7] A specific form was generated in collaboration with them to collect all relevant data: demographic data, disease category, family history and pedigree, personal history, clinical symptoms, biochemical analysis, and previous genomic and nongenomic tests performed. Clinical symptoms were collected using standardized Human Phenotype Ontology (HPO) terms.[8] Orphanet Rare Disease Ontology (*https://www.orpha.net/consor/cgi-bin/index.php*, last accessed December 6, 2021) and Online Mendelian Inheritance in Man (*https://www.omim.org*, last accessed December 6, 2021) codes were used to enter a clinical and molecular diagnosis, respectively.[9] Quality of the phenotypic data was assessed using the Monarch star rating system integrated within PhenoTips. The Monarch star rating system is an annotation sufficiency meter developed by the Monarch initiative that assesses the breadth and depth of the phenotype annotation profile for a given patient in the context of all curated human and model organisms using a five-star rating system: bad (0 to 1.4), fair (1.5 to 2.4), good (2.5 to 3.4), very good (3.5 to 4.4), and excellent (4.5 to 5).[10] All phenotypic entries were reviewed and approved by two clinical experts from another participating center. The reviewers were allowed to ask for clarifications or additional information to be included in the records during the review process.

Among all of the undiagnosed patients and relatives whose information was submitted to PhenoTips, 331 index cases (560 individuals including relatives) from seven hospitals were considered for reanalysis. The inclusion criteria were as follows: i) available and previously analyzed genomic data (genome, exome, or panel), ii) clinical suspicion of rare disease with neurologic involvement, iii) clinical suspicion of a genetic etiology, iv) availability of clinical information and disease progression (from the patients and the family members), and v) written informed consent of the patient or parents/guardians, enabling the use of the data in the URD-Cat project. This study was approved by the local ethics committees from each URD-Cat partnering institution.

Data Processing and Variant Detection

The URD-Cat GPAP, a customized version of the RD-Connect GPAP, was implemented for the URD-Cat project. Existing FASTQ files, all of them obtained with Illumina (San Diego, CA) sequencing platforms, and corresponding metadata (sequencing approach, capture kit, singleton or extended family analysis, and DNA source) were submitted for processing to the URD-Cat GPAP.

All of the samples were bioinformatically processed at the CNAG-CRG using the RD-Connect pipeline,[11] which is based on GATK best practices.[12] Briefly, sequencing reads were mapped to human genome build GRCh37d5 using BWA-MEM version 0.7.15.[13] The resulting BAM files were sorted, and duplicate reads were removed using Picard version 1.110 (*http://broadinstitute.github.io/picard*, last accessed September 6, 2021). Insertion and deletion realignment and base quality score recalibration were performed using GATK version 3.6.[14]

Single-nucleotide variants and short insertions and deletions were called using GATK version 3.6 HaplotypeCaller tool.[12] Single-nucleotide variants and insertions and deletions with a minimum depth of coverage of 8 and a minimum genotype quality of 30 were released to the URD-Cat GPAP. Copy number variants (CNVs) were detected with ExomeDepth for exomes and gene panels,[15] analyzing together all of the samples captured with the same kit. CNVs on the sex chromosomes were evaluated by comparison against samples from the same sex only. CNVs were called only for groups of samples in which there were at least 10 samples captured with the same kit (Supplemental Table S1). The CNV results were crossed with sets of common CNVs from Conrad et al[16] and Database of Genomic Variants Gold Standard data set (*http://dgv.tcag.ca*, last accessed May 21, 2021).[17] All of the CNVs obtained were released to the URD-Cat platform.

Additional Sequencing

Additional sequencing data were obtained for nine index cases with a single heterozygous pathogenic or likely pathogenic variant in an autosomal recessive gene identified through the reanalysis. Genome ($n = 5$) or transcriptome ($n = 2$) sequencing was performed if the existing data were an exome, whereas exome sequencing ($n = 2$) was performed if the existing data were a panel.

Relatedness and Consanguinity Analysis

Kinship between all individuals with a genome or an exome was computed on alignment files (BAM) with Somalier version 2.6[18] to identify putative duplicates and inconsistent family relationships. Runs of homozygosity (RoHs) were

Table 1 Characteristics of the 323 Probands Classified by the Main Disease Category

Disease category	Probands, *N*	Sex		Consanguinity (PhenoTips)		
		Male	Female	Yes	No	Unknown
Progressive neurodegenerative diseases	108	70 (64.8)	38 (35.2)	12 (11.1)	74 (68.5)	22 (20.4)
Neuromuscular diseases	56	31 (55.4)	25 (44.6)	6 (10.7)	41 (73.2)	9 (16.1)
Epilepsy/nonepileptic paroxysmal disorders	52	27 (51.9)	25 (48.1)	5 (9.6)	28 (53.8)	19 (36.5)
Inherited metabolic disorders	39	14 (35.9)	25 (64.1)	6 (15.4)	27 (69.2)	6 (15.4)
Intellectual disabilities/autism spectrum disorders	36	25 (69.4)	11 (30.6)	0 (0.0)	26 (72.2)	10 (27.8)
Movement disorders	23	12 (52.2)	11 (47.8)	2 (8.7)	13 (56.5)	8 (34.8)
Central nervous system malformations	5	4 (80.0)	1 (20.0)	1 (20.0)	4 (80.0)	0 (0.0)
Other diseases	4	3 (75.0)	1 (25.0)	1 (25.0)	3 (75.0)	0 (0.0)
Total	323	186 (57.6)	137 (42.4)	33 (10.2)	216 (66.9)	74 (22.9)

(*table continues*)

Data are given as number (percentage). Probands were classified into each disease category by the clinicians or researchers from the referring hospital. Other diseases included syndromic diseases that did not fit in any other group.

computed on the genetic variants from all genomic experiments using the PLINK software version 1.90[19] following previously suggested parameters.[20] The total length of the RoH from each individual was used to estimate if it was an offspring from a consanguineous couple, according to previously described thresholds.[21]

Variant Filtering and Interpretation with the URD-Cat GPAP

Genomic data were analyzed by geneticists from participating centers using the URD-Cat GPAP, which has many functionalities, including standard filters and annotations (population databases and variant pathogenicity prediction tools), filters by clinical data (genes of interest, genes associated with patient's HPO terms entered in PhenoTips, Online Mendelian Inheritance in Man codes, and *in silico* panels), links to multiple external resources, and data sharing between authorized users (internal matchmaking by querying all of the data). Variant filtering and prioritization followed the guidelines established by geneticists in the URD-Cat project. Briefly, users selected the singleton, pair, trio, or quartet to analyze and applied all possible inheritance patterns to filter out the variants accordingly. Cutoffs for the population databases filters (GnomAD, 1000 Genomes, and internal database) were set on the basis of the inheritance: minor allele frequency <0.02 for autosomal recessive or X-linked in men; and minor allele frequency <0.01 for autosomal dominant or X-linked in women. Then, different filtering criteria were applied to look for the following: i) previously known variants: previously tagged by another user or reported in ClinVar database (*https://www.ncbi.nlm.nih.gov/clinvar/*, last accessed December 2, 2021); ii) variants in genes associated with patient's phenotype: filter by genes associated with at least one patient's HPO term, predefined or custom gene lists, or Online Mendelian Inheritance in Man clinical features; iii) variants predicted to be highly likely pathogenic based on SnpEff prediction[22]; and iv) candidate pathogenic CNVs based on Online Mendelian Inheritance in Man database (*https://www.omim.org*, last accessed November 22, 2021) and Database of Genomic Variants (*http://dgv.tcag.ca*, last accessed May 21, 2021).[17] The filtering settings can be saved and applied recurrently to further analyses to speed up the filtering process. Variants were prioritized by clinical researchers leveraging their expertise and the functionalities in the platform, including scored prioritization of variants according to patient's HPO terms with Exomiser.[23] In cases with suspected consanguinity, the RoH filter was used to narrow down the list of candidate variants to only those within RoH of at least 500 Kb. Specific chromosomal positions (such as known pathogenic variants) could be selected individually or through the upload of a BED file. Variant interpretation was done by geneticists and clinical experts based on variant classification following the American College of Medical Genetics and Genomics guidelines,[24,25] the clinical fit, and the familial segregation. Validation and segregation of the candidate pathogenic variants were performed by Sanger sequencing or comparative genomic hybridization arrays. Segregation with the disease was assessed for all patients unless otherwise indicated. Functional studies were performed when the variant and/or the gene had not been previously associated with the disease. Reporting of findings to diagnosed patients, or their families, was done according to the procedures of the corresponding managing hospital, which typically includes a genetic report and counseling.

Statistical Analysis

The Fisher exact test was used to evaluate whether phenotype annotation quality and sequencing approaches were significantly different between diagnosed and undiagnosed groups of patients. Nonparametric Kruskal-Wallis test was done to compare the median number of HPO terms between diagnosed and undiagnosed patients. To remove annotation

Table 1 (*continued*)

Sequencing strategy			Family analysis			
Panel	Exome	Genome	Singletons	Pairs	Trios	Quads
2 (1.9)	103 (95.4)	3 (2.8)	73 (67.6)	18 (16.7)	16 (14.8)	1 (0.9)
28 (50.0)	28 (50.0)	0 (0.0)	49 (87.5)	4 (7.1)	2 (3.6)	1 (1.8)
2 (3.8)	50 (96.2)	0 (0.0)	17 (32.7)	3 (5.8)	30 (57.7)	2 (3.8)
9 (23.1)	30 (76.9)	0 (0.0)	21 (53.8)	3 (7.7)	13 (33.3)	2 (5.1)
6 (16.7)	30 (83.3)	0 (0.0)	17 (47.2)	2 (5.6)	12 (33.3)	5 (13.9)
13 (56.5)	10 (43.5)	0 (0.0)	18 (78.3)	1 (4.3)	4 (17.4)	0 (0.0)
2 (40.0)	3 (60.0)	0 (0.0)	5 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
1 (25.0)	3 (75.0)	0 (0.0)	3 (75.0)	0 (0.0)	1 (25.0)	0 (0.0)
63 (19.5)	257 (79.6)	3 (0.9)	203 (62.8)	31 (9.6)	78 (24.1)	11 (3.4)

redundancy, only the most specific HPO terms were considered by counting only terms from leaf nodes or nodes without selected parent or child nodes. $P < 0.05$ was considered statistically significant for all analyses.

Results

Systematic Evaluation of the Reanalysis Cohort

Available genomics data from a total of 331 patients with neurologic diseases (560 individuals including relatives) were uploaded to the URD-Cat GPAP. Data were processed as indicated in *Materials and Methods*. The mean depth of coverage was 78.9× for panels, 74.1× for exomes, and 31.2× for genomes (Supplemental Table S2). Genomic relatedness was computed between 491 individuals for which there was a genome or an exome with average coverage >10×. Six exomes with average coverage ≤10× and 63 panels were not included in the relatedness analysis. Predicted kinship was compared with the reported family relationships. Inconsistencies were found in seven families, including duplicated individuals ($n = 3$), true siblings reported as individuals belonging to different families ($n = 2$), and true siblings reported as the same individual ($n = 2$). These inconsistencies were corrected in the URD-Cat platform. After complete evaluation of the cohort, a total of 17 individuals were excluded because they were duplicates ($n = 3$), no proband was available ($n = 4$), the proband was affected by a nonneurologic disease (Munchausen syndrome; $n = 3$), or they were solved by another ongoing project ($n = 7$). Therefore, reanalysis of genomic data and variant interpretation was finally performed in a total of 323 probands (543 individuals including relatives) between 1 and 8 years (median, 4 years) after the data were originally generated.

The 323 probands included 186 males (57.6%) and 137 females (42.4%) (sex ratio, 1.4). They were classified into eight disease categories, according to the main clinical features: progressive neurodegenerative diseases (33.4%), neuromuscular diseases (17.3%), epilepsy/nonepileptic paroxysmal disorders (16.1%), inherited metabolic disorders (12.1%), intellectual disabilities/autism spectrum disorders (11.2%), movement disorders (7.1%), central nervous system malformations (1.6%), and other diseases (1.2%) (Table 1).

Genetic variant-derived RoH analysis to predict the absence or presence of consanguinity (consanguinity status)

Table 2 Consanguinity Status Comparison between the Medical Records and the Runs of Homozygosity Analysis for the 257 Probands with Exome Sequencing Data

	Runs of homozygosity analysis		
Variable	Consanguineous/likely consanguineous ($n = 24$)	Nonconsanguineous ($n = 183$)	Uncertain ($n = 50$)
Medical records (PhenoTips)			
Consanguineous ($n = 23$)	15	3	5
Nonconsanguineous ($n = 164$)	4	126	34
Unknown ($n = 70$)	5	54	11

Four consanguinity ranges were established on the basis of the accumulated length of the runs of homozygosity (RoHs), as described in Matalonga et al[21]: consanguineous (total RoH size > 123 Mb), likely consanguineous (79 Mb < total RoH size < 123 Mb), uncertain (22 Mb < total RoH size < 79 Mb), and nonconsanguineous (total RoH size < 22 Mb).

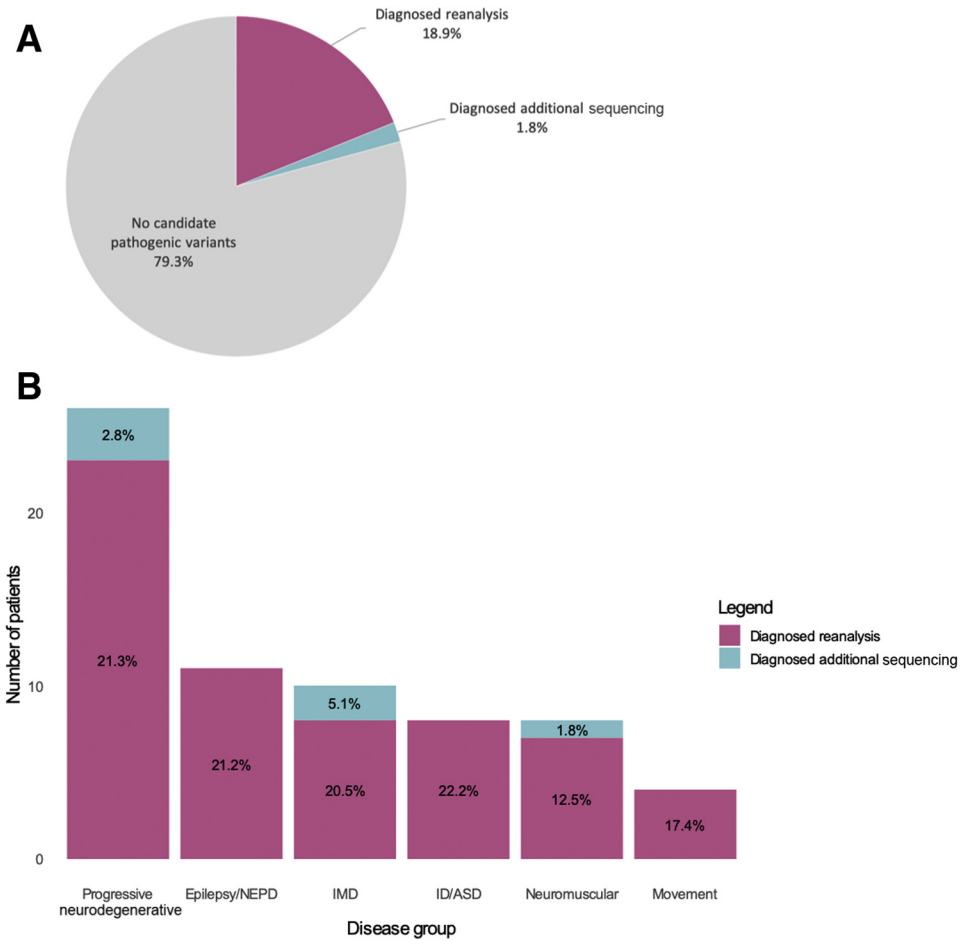

Figure 1 Molecular results. **A:** Results overview from the reanalysis of 323 index cases. **B:** Results of the reanalysis by disease group. ID/ASD, intellectual disabilities/autism spectrum disorders; IMD, inherited metabolic disorders; NEPD, nonepileptic paroxysmal disorders.

was performed in the 257 probands with exome data available. A total of 24 probands were predicted to be part of a consanguineous ($n = 18$) or likely consanguineous ($n = 6$) family. In the medical records, the consanguinity status reported by the patient/family was available for 249 probands, 33 of which were tagged as consanguineous (Table 1). The consanguineous status predicted through the RoH analysis was compared with that reported in the medical records for the 257 probands with exome data available (Table 2). Consanguinity was confirmed in 15 probands. Discrepancies between the predicted and the reported consanguinity were found in seven probands: four cases predicted to be consanguineous had been reported as nonconsanguineous, and three cases predicted to be nonconsanguineous had been reported as consanguineous. The predicted consanguinity of 59 probands with no available information in the medical records indicated 5 would be consanguineous ($n = 3$) or likely consanguineous ($n = 2$) and 54 would be nonconsanguineous.

Different sequencing strategies (panels, exomes, and genomes) and different family analyses (singleton cases, pairs, trios, or quartets) were included in the reanalysis (Table 1). Fifteen different sequence capture kits were included: 3 targeted panels and 12 whole-exome kits (Supplemental Table S1). DNA was mostly obtained from blood (535 individuals), although in some samples, the DNA was extracted from muscle (5 individuals), fibroblasts (3 individuals), or liver (1 individual).

Molecular Diagnosis

Reanalysis of existing genomic data provided a conclusive genetic diagnosis for 67 probands (20.7%), including the identification of six novel gene-disease associations[26–29] (V. Salpietro et al, unpublished data; and A. Pujol, unpublished data). Sixty-one of the diagnoses (18.9%) were achieved with just the reanalysis of existing data. Six of the diagnoses (1.8% of the total probands with existing data) were possible after identifying a single heterozygous candidate pathogenic variant in an autosomal recessive gene through the reanalysis and the second causative variant through additional sequencing (Figure 1A).

The outcomes of the reanalysis for the different disease groups are depicted in Figure 1B. Intellectual disabilities/autism spectrum disorders had the highest diagnostic rate (22.2%, 8 of 36), followed by progressive

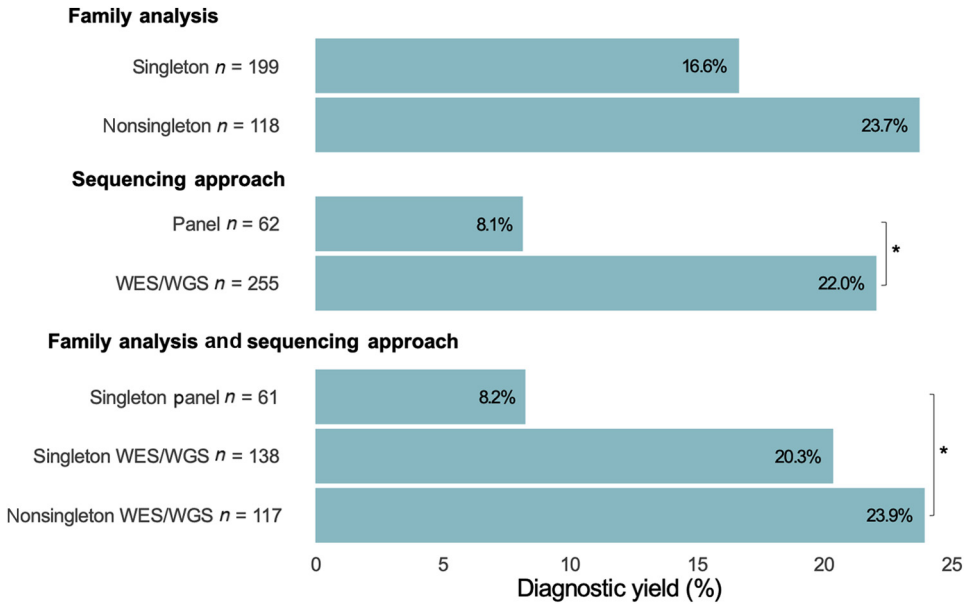

Figure 2 Diagnostic yield by type of family analyses and sequencing strategy. The diagnostic yield of the different family analysis (singleton and nonsingletons) and sequencing approaches (panels, exomes, and genomes) was compared for the 61 probands fully diagnosed through reanalysis and the 256 undiagnosed probands. Patients diagnosed after additional sequencing were not considered for this analysis. Nonsingleton panel category is not shown because only one undiagnosed patient fell into this category. **Asterisk** denotes a statistically significant difference. *$P < 0.05$. WES, whole exome sequencing; WGS, whole genome sequencing.

neurodegenerative diseases (21.3%, 23 of 108), epilepsy/nonepileptic paroxysmal disorders (21.2%, 11 of 52), inherited metabolic disorders (20.5%, 8 of 39), movement disorders (17.4%, 4 of 23), and neuromuscular diseases (12.5%, 7 of 56). None of the patients classified within the central nervous system malformations group ($n = 5$) or the other diseases group ($n = 4$) were diagnosed. Additional sequencing increased the diagnostic rate of inherited metabolic disorders, progressive neurodegenerative diseases, and neuromuscular diseases by around 5% (two patients), 3% (three patients), and 2% (one patient), respectively.

Supplemental Table S3[26–62] provides further details on the 67 probands fully diagnosed through reanalysis ($n = 61$) and diagnosed through reanalysis plus additional sequencing ($n = 6$). Autosomal dominant conditions accounted for 22 of the 67 (32.8%) diagnosed patients, 14 (63.6%) with a *de novo* variant, 4 (18.2%) inherited, 1 (4.6%) inherited from a mosaic-unaffected parent, and 3 (13.6%) undetermined because of unavailability of one or both parental samples. Autosomal recessive conditions were found in 42 of the 67 (62.7%) diagnosed patients: 20 (47.6%) compound heterozygous cases and 22 (52.4%) homozygous cases. X-linked inheritance was reported in 3 cases of the 67 (4.5%) diagnosed patients, being dominant in 1 of them and recessive in the remaining 2 cases. CNVs accounted for 8.9% of all of the diagnosed patients (6 of 67 cases) and included four deletions (two homozygous, one X-linked hemizygous, and one heterozygous) and two heterozygous duplications, ranging from 114 bp to almost 2 Mb. Altogether, a total of 81 different pathogenic variants in 61 different genes were detected through reanalysis in the 67 diagnosed patients, consisting of 59 single-nucleotide variants, 16 insertions and deletions, and 6 CNVs. The variants identified by additional sequencing were deep intronic variants ($n = 4$) or variants located in poorly covered regions in the reanalyzed panel or exome ($n = 2$).

Effect of the Sequencing Strategy on the Diagnostic Rate

The most prevalent sequencing approaches in the cohort were exome sequencing of singletons (42.7%, 138 of 323 probands) and nonsingletons (36.9%, 119 of 323 probands). Panels and genomes accounted for 19.5% (63 of 323) and 0.9% (3 of 323) of the probands, respectively, and all cases but one (a panel) were sequenced in singletons.

Analysis of the diagnostic rate for the different sequencing approaches revealed a significantly higher diagnostic rate for exome or genome reanalysis compared with panels (22.0% and 8.1%, respectively; $P = 0.0116$). Reanalysis of more than one family member (pair, trio, or quartet) also resulted in a higher diagnostic rate compared with singleton analysis (23.7% and 16.6%, respectively; $P = 0.1406$), although no statistical significance was achieved. Taken together, the highest diagnostic rate was achieved for exome or genome reanalysis with more than one family member (23.9%) and the lowest for panel reanalysis in single cases (8.2%) ($P = 0.0137$) (Figure 2).

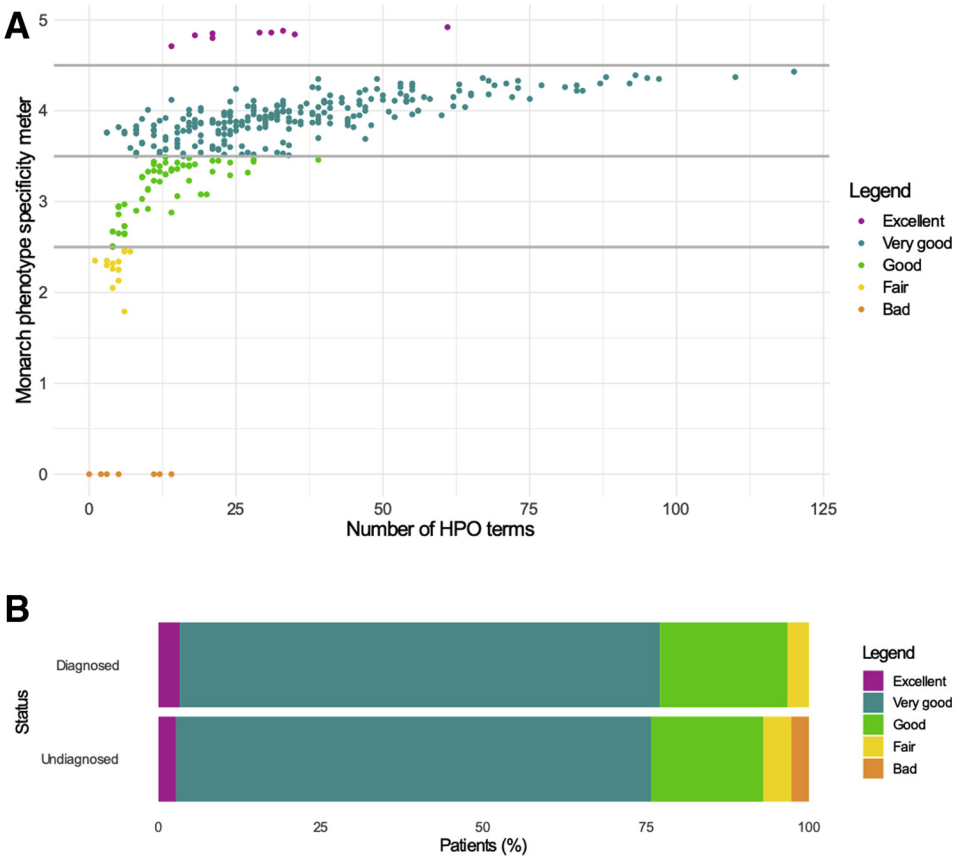

Figure 3 Phenotypic quality. **A:** Monarch score distribution according to the number of Human Phenotype Ontology (HPO) terms for the 323 probands in the cohort. The Monarch score categories are as follows: bad (0 to 1.4), fair (1.5 to 2.4), good (2.5 to 3.4), very good (3.5 to 4.4), and excellent (4.5 to 5) phenotypic quality. **B:** Monarch category distribution for diagnosed and undiagnosed probands. The Monarch score categories for the 61 patients fully diagnosed through the reanalysis are excellent ($n = 2$), very good ($n = 45$), good ($n = 12$), and fair ($n = 2$). The Monarch score categories for the 256 undiagnosed patients are excellent ($n = 7$), very good ($n = 187$), good ($n = 44$), fair ($n = 11$), and bad ($n = 7$). Patients diagnosed after additional sequencing were not considered for this analysis. $n = 61$ diagnosed probands (**B**); $n = 256$ undiagnosed probands (**B**).

Quality of Standardized Phenotyping

Evaluation of the standardized HPO-coded phenotypic data rated 93.8% of the probands (303/323) as having good (17.6%, 57/323), very good (73.4%, 237/323), or excellent (2.8%, 9/323) quality, according to the Monarch score. The median Monarch score was 3.9 (very good category), ranging from 0 to 4.92. The Monarch score gradually increased with the number of HPO terms per patient until it reached a plateau at approximately 25 HPO terms (Figure 3A). The median number of nonredundant HPO terms per patient was 24, ranging from 0 to 120.

Phenotype quality based on Monarch score categories was compared between probands fully diagnosed through reanalysis ($n = 61$) and undiagnosed probands ($n = 256$). The median Monarch score was 3.9 for both diagnosed and undiagnosed patients, and the median number of nonredundant HPO terms per patient was 25 for both groups. A slightly better phenotype quality was observed in diagnosed patients, with 96.7% of them classified as having excellent, very good, or good phenotype quality compared with 93.0% of undiagnosed patients falling into those categories (Figure 3B). However, these results are not statistically significant ($P = 0.3863$).

Reasons for Increasing the Diagnostic Rate with Data Reanalysis

The geneticists from the participating hospitals were asked to indicate why new diagnoses were achieved in the 61 probands fully diagnosed through reanalysis (Supplemental Table S3). Novel gene-disease associations explained 50.8% of the diagnoses ($n = 31$): 41% ($n = 25$) being in genes published in the literature after the original analysis and 9.8% ($n = 6$) in genes discovered within this study. Additional or improved bioinformatics analyses were responsible for 19.7% of the diagnoses ($n = 12$): 8.2% ($n = 5$) due to CNV detection, 8.2% ($n = 5$) thanks to improved variant calling, and 3.3% ($n = 2$) due to consanguinity assessment, as the RoH filter could be used in these two consanguineous cases to narrow down the list of candidate pathogenic variants. The integration of standardized HPO-coded phenotypic information with genomic data was claimed to be crucial for 18% of the diagnoses

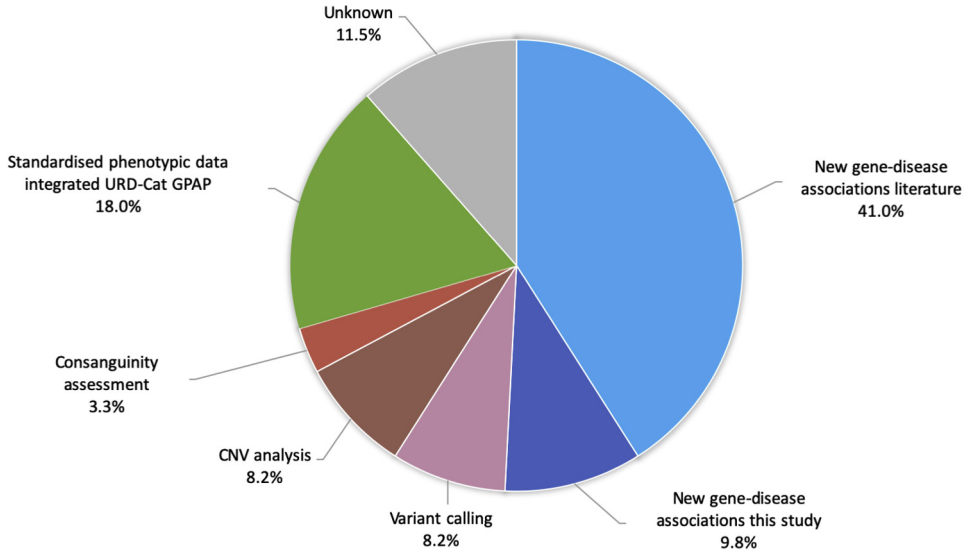

Figure 4 Reasons for increasing diagnostic rate with data reanalysis. The chart indicates reasons for which cases were diagnosed with the reanalysis according to the clinicians or researchers who submitted the data. The figure only includes the 61 probands who were diagnosed exclusively with reanalysis of previously existing data. CNV, copy number variant; GPAP, Genome-Phenome Analysis Platform; URD-Cat, Undiagnosed Rare Disease Program of Catalonia.

($n = 11$). In the remaining 11.5% of diagnoses ($n = 7$), the reason why the patient was not diagnosed in the original analysis was unknown (Figure 4).

Discussion

The URD-Cat data reanalysis contributed to ending the diagnostic odyssey of 67 patients with a neurologic disease. According to their referring clinicians, the genetic diagnosis was important to improve patients' medical management, to enable genetic counseling for parents, and to diagnose other affected family members. In addition, families were able to contact patients' associations, which made them feel more supported.

The diagnostic rate of 18.9% obtained in this reanalysis study (without including additional data) is similar to that reported in the literature, with 15% being the median diagnostic rate for reanalysis studies.[63] Most new diagnoses resulted from novel gene-disease associations (50.8%), either published after initial analysis or discovered with the reanalysis[26–29] (V. Salpietro et al, unpublished data; and A. Pujol, unpublished data). Because approximately 250 novel gene-disease associations are reported each year, this was quite expected.[64] New molecular diagnoses were also possible due to additional or improved bioinformatics analyses (19.7%), supporting the idea that reanalysis studies should include consanguinity assessment, updated pipelines, and analysis of single-nucleotide variants and CNVs. In fact, CNVs accounted for 8.9% of the diagnosed patients. The standardized high-quality phenotyping promoted within the URD-Cat project, and its integration with the genomic data, was the reason for the new diagnoses in 18% of the diagnosed patients, highlighting the valuable role of the URD-Cat GPAP system for an efficient and comprehensive genomic analysis. Similarly, accurate and updated phenotypic information was the main contributing factor to diagnosing patients in several studies.[65–68]

This study contemplates a broad spectrum of sequencing strategies—singleton and familial analysis in gene panel, exome, and genome sequencing—giving us the opportunity to evaluate the diagnostic yield of the different approaches. The diagnostic rate progressively increased as a wider approach was considered, from reanalysis of panels in single cases (8.2%), through exome/genome reanalysis in singletons (20.3%), to exome/genome reanalysis in more than one family member (23.9%). Similarly, trio exome analysis has been associated with an approximately 7% to 9% higher molecular diagnostic rate compared with proband-only analysis.[69,70] Besides the higher diagnostic rate, data interpretation in trio exome sequencing is more straightforward as segregation can be used to prioritize variants.[64,71] However, the higher cost derived from sequencing more than one family member still hampers its routine use in diagnostic settings.[72] Thus, singleton exome reanalysis is one of the most prevalent sequencing strategies reported in reanalysis studies.[64,66,71,73,74] The limited number of genome samples in the cohort makes it difficult to draw any conclusions about the utility of genome sequencing compared with exome sequencing, but several studies have highlighted the importance of exome reanalysis before performing genome sequencing.[67,75,76]

The reanalysis approach presented herein is innovative for at least three reasons. First, the systematic evaluation of the data proved to be effective to predict and check familial relationships and consanguineous status. The large-scale and highly collaborative nature of the URD-Cat project, with samples coming from the main groups that attend and

investigate patients with undiagnosed diseases in Catalonia, implied an increased risk of duplicated samples or incorrect familial relationships that could hamper the identification of the pathogenic variant. In fact, three duplicated patients were excluded, and four inconsistent family relationships were corrected thanks to the relatedness analysis. Consanguinity estimation through RoH analysis was used to predict the consanguinity status in patients with no previous information (59 cases) and to detect discrepancies between the consanguinity status reported in the medical records and the estimated one (7 cases). This information was useful to guide the filtering strategy and to focus the analysis in the homozygous regions for the consanguineous cases. However, all of the inheritance patterns were assessed in all patients because *de novo* variants can also cause a disease in consanguineous families, as illustrated by cases URD-Cat_06 (likely *de novo* variant in the *SPAST* gene) and URD-Cat_35 (*de novo* duplication in the *HNRNPH1* gene). Two cases in the cohort were diagnosed with a homozygous pathogenic variant despite being reported in the medical record as nonconsanguineous (Patient URD-Cat_21) or with unknown consanguinity status (Patient URD-Cat_41). In light of this, conducting a systematic evaluation of the data through relatedness and consanguinity analysis to ensure a correct characterization of the cohort is highly recommended.

Second, standardized high-quality phenotyping was achieved in the cohort through collection of clinical symptoms in the form of HPO terms. HPO terms have been used to collect phenotypic data in some reanalysis studies.[4,64,71,76] However, the phenotype quality achieved was not evaluated. Herein, 93.8% of patients had good or higher phenotype quality based on Monarch score. The specificity of the phenotype gradually increased with the number of HPO terms per patient until it reached a plateau at approximately 25 HPO terms. Diagnosed patients had a higher proportion of good, very good, or excellent Monarch scores (96.7%) compared with undiagnosed (93%) ones, although no statistical significance was obtained, likely because of the high phenotype quality achieved for the whole cohort. Similarly, Wright et al[4] did not find phenotypic differences between diagnosed and undiagnosed in terms of number of HPO terms per patient, similarity by most informative term, or similarity by Jaccard index.

Third, the URD-Cat GPAP allows efficient and continuous reanalysis of genomic data and is extremely useful for collaborative efforts as it can be used by clinicians and technicians thanks to its user-friendly interface. Periodic reanalysis and reinterpretation of sequencing data coupled with updates in patients' phenotype is recommended by several studies, including the American College of Medical Genetics and Genomics.[4,24,64,77,78] However, bioinformatics infrastructures must be able to cope with the organizational and communicative challenge that iterative reanalysis require.[4,71,77] The URD-Cat platform is a key tool for continuous communication and data sharing among all of the professionals implicated in a patient's genetic diagnosis, bringing together otherwise scattered expertise. Clinical information can be updated at any time, and reinterpretation of genomic variants can efficiently be done through all functionalities included in the system. Data sharing is necessary to advance faster in rare disease research and diagnosis because of the low prevalence (<5/10,000 in the European Union), high genetic heterogeneity, and phenotypic variability of these diseases.[79] International data-sharing platforms have been reported to be relevant for the diagnosis of reanalysis patients.[63,72] Herein, genomic data sharing among all centers involved in the URD-Cat project was crucial to diagnose some patients, such as Patient URD-Cat_02, who was diagnosed by a researcher from another center by internal matchmaking. The platform is periodically updated with disease and population databases, and new functionalities are included on the basis of geneticists' needs in a nice example of close collaboration among clinicians, geneticists, bioinformaticians, and software engineers. A model based on continuous interaction between all of the parties implicated in a patient's genetic diagnosis has been proposed for automated iterative reanalysis.[77]

In conclusion, the systematic collaborative reanalysis of genomic data within the URD-Cat project diagnosed 20.7% of probands, thanks to standardized high-quality phenotyping, reprocessing of genomic data, reinterpretation of the results, and data sharing. The URD-Cat GPAP has been key for systematic collation, analysis, and interpretation of the data in a collaborative manner.

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Author Contributions

G.B., L.M., G.P., and D.O. processed and analyzed data; M.P. coordinated the Undiagnosed Rare Disease Program of Catalonia (URD-Cat) project; D.P., A.Pa., and C.Lu. developed and released data to the URD-Cat platform; S.L., R.T., and D.O. implemented the analysis pipelines; R.A., P.G., G.G., J.R.G., D.G., M.G., C.La., R.M., M.M., A.Pu., E.T., A.M., F.P., A.R., and L.A.P.-J. recruited patient clinical and genomic data and coordinated molecular diagnosis feedback; S.B. and G.B. designed the study and wrote the manuscript; all authors revised the article and read and approved the final manuscript. S.B. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2022.02.003*.

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