Received: 31 July 2020 | Revised: 14 September 2020 | Accepted: 27 September 2020

DOI: 10.1002/ajmg.c.31848

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART C Seminars in Medical Genetics

WILEY

The odyssey of complex neurogenetic disorders: From undetermined to positive

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Funding information
Ministry of Science and Technology of Argentina, Grant/Award Number: Grant; University of Cincinnati of the United States

Abstract

The genetic and phenotypic heterogeneity of neurogenetic diseases forces patients and their families into a "diagnostic odyssey." An increase in the variability of genetic disorders and the corresponding gene-disease associations suggest the need to periodically re-evaluate the significance of variants of undetermined pathogenicity. Here, we report the diagnostic and clinical utility of Targeted Gene Panel Sequencing (TGPS) and Whole Exome Sequencing (WES) in 341 patients with suspected neurogenetic disorders from centers in Buenos Aires and Cincinnati over the last 4 years, focusing on the usefulness of reinterpreting variants previously classified as of uncertain significance. After a mean of ±2years (IC 95:0.73–3.27), approximately 30% of the variants of uncertain significance were reclassified as pathogenic. The use of next generation sequencing methods has facilitated the identification of both germline and mosaic pathogenic variants, expanding the diagnostic yield. These results demonstrate the high clinical impact of periodic reanalysis of undetermined variants in clinical neurology.

KEYWORDS

diagnostic odyssey, mosaicism, targeted gene panel sequencing, variants of unknown significance, whole exome sequencing

1 | INTRODUCTION

Neurogenetic diseases encompass a vast group of entities with marked genetic and phenotypic heterogeneity. Nowadays, the process of establishing a diagnosis for this subset of neurological conditions requires extensive clinical, radiological, and genetic evaluations, often becoming a "diagnostic odyssey" for the patient and the family (Carmichael, Tsipis, Windmueller, Mandel, & Estrella, 2015).

Next Generation Sequencing (NGS) has become a widely used tool for obtaining genetic diagnosis in clinical medicine (Might & Wilsey, 2014). In particular, Whole-exome sequencing (WES) and Targeted Gene Panel sequencing (TGPS) have shown excellent cost/benefit ratios (Cohen et al., 2020; Córdoba et al., 2018; González-Morón et al., 2017; Perez Maturo et al., 2020) and are frequently used in the diagnostic workup of patients with neurogenetics disorders.

Valeria Salinas and Patricia Vega are the co-first authors of this article.

These NGS-based methods have diagnostic yields of 30–40%, substantially increasing the number of genetic diagnoses that may be amenable to disease-specific medical management and opening the doors for precision medicine (Córdoba et al., 2018; Yang et al., 2013). Potentially actionable diagnosis refers to variants that modify clinical and therapeutic management; about 12–23% of positive genetic tests by NGS assays were estimated to cause changes in patient care (Fogel, Satya-Murti, & Cohen, 2016; Yang et al., 2013). However, a major challenge is the interpretation of the pathogenicity of variants not previously reported, and thus considered of uncertain significance.

There has been a rapid increase in the knowledge of genetic diseases based on genomic data being generated from many corners of the world. New variant-disease or gene-disease associations continuously change the classification of variants from undetermined to pathogenic (Chisholm et al., 2018; el Mecky et al., 2019). This complex situation warrants a periodic review of new genes and phenotypes from medical databases and literature in an effort to evaluate whether the status of prior interpretations should be modified.

The aims of this study were to describe the results of a large series of diagnoses achieved by WES and TGPS in Argentina during the last 4 years in a heterogeneous bi-national cohort of patients with neurogenetic disorders, examining the clinical utility of reinterpreting variants classified as of uncertain significance.

2 | MATERIALS AND METHODS

2.1 | Clinical samples

An observational and descriptive cohort study was conducted, including a consecutive series of 341 adult and pediatric patients selected between 2016 and 2020 for WES and TGPS from centers in Buenos Aires, Argentina, and Cincinnati, USA. These patients were considered candidates for genomic studies because of findings raising the index of suspicion for neurogenetic diseases, including familial aggregation and absence of acquired pathology justifying the phenotype. We recorded perinatal and family history, likely pattern of inheritance, disease progression characteristics, comorbidities, and studies performed before NGS testing. All patients, or their parents if minors, provided informed consent through a form approved by the Ethics Committee of the respective institutions. The informed consent included the option to receive incidental findings according to the American College of Medical Genetics (ACMG) recommendations. All methods were performed in accordance with the relevant guidelines and regulations.

2.2 | Whole exome sequencing and targeted gene panel sequencing

Genomic DNA was isolated from different types of biological samples (predominantly blood) with the use of commercial systems, following the manufacturer's instructions. This was kept anonymized. DNA sequencing libraries were constructed mostly by chemical fragmentation using commercial preparation kits. The most frequent methods for enrichment were capture-based target and amplicon-based target for exome and gene panel. NGS sequencing runs were made in Illumina systems (Illumina, INC) as an outsourced service. Detection of variants was possible with an average sequence coverage of more than ×70, with more than 97% of the target bases having at least ×10 coverage. All standardized procedures were performed according to manufacturer's instructions, widely described in the literature (Kozarewa & Turner, 2011; Margraf et al., 2011). Clinically relevant variants, from the proband and parental samples (whenever available), were confirmed by Sanger sequencing (germinal variants) and ultra-high depth NGS sequencing (mosaic variants) in selected cases.

2.3 | Data analysis and annotation

Sequence data in FastQ format were aligned to the reference sequence of the human genome of the National Center for Biotechnology Information of the National Institutes of Health of the United States versions GRCh37 or GRCh38 using the Burrows-Wheeler Alignment Tool (BWA-MEM; Li, 2013). Variant calls were generated using GATK3.6 or GATK4.1 haplotype caller following the so called best practices (van der Auwera et al., 2013). The output VCF file was annotated at various levels using Annovar (Wang, Li, & Hakonarson, 2010), with information from several databases as previously described by our group (Cohen et al., 2020; Córdoba et al., 2018). We classified variants according to the ACMG recommendations (Richards et al., 2015). For the vast majority of exomes, virtual multigenic panels were built from a search of genes reported as pathogenic of disease. These virtual panels were central in filtering and in the clinical interpretation of annotated VCF files. In brief, variants were further prioritized in base to inheritance model(s) proposed in each case, population frequency, predicted molecular function and effects, and previous reports of pathogenicity in other patients with similar clinical diagnosis, using procedures and bioinformatics pathways developed by our group (Córdoba et al., 2018). Joining variant level and clinical features information, we classified each NGS study as ***positive*** if a pathogenic/likely pathogenic variant in a known disease gene was identified with *compatible* phenotypic and inheritance overlap; ***undetermined*** if a pathogenic/likely pathogenic variant in a putative candidate gene was identified *without* positive phenotypic and inheritance overlap; one pathogenic/likely pathogenic variant was identified with positive phenotypic overlap in a recessive disorder (*unable to be detected in the other allele*), one pathogenic/likely pathogenic variants was identified in a potential candidate gene not yet associated with disease; and ***negative*** in any other case. In addition, all genomic findings detected during sequencing analysis that could lead to a change in the patient management and/or a therapeutic opportunity not otherwise considered were classified as ***potentially actionable diagnosis***. Incidental

findings were informed according to ACMG recommendations. Counseling to patients was performed by trained professionals.

2.4 | Growth description of biomedical gene-disease and variant-disease annotations

Data from the Human Genome Mutation Database (HGMD), the OMIM database, MEDLINE, life science journals, and online books were used to quantify the growth in variant-disease, gene-disease and biomedical literature-neurogenetics disease associations. The number of OMIM gene-disease and HGMD variant-disease associations was established as previously described (Wenger, Guturu, Bernstein, & Bejerano, 2017).

2.5 | Re-interpretation of patient variants with undetermined genetic diagnoses

Between April and July 2020, a new literature and database review was performed for all variants identified in patients with undetermined genetic diagnoses. In this revision, a detailed phenotypic description and characterization of the respective variants was considered. The updated biomedical literature, medical databases, and web search engine as VarSome (The Human Genomic Variant Search Engine), PubMed and Google Scholar, were used for the interpretation of variants. Once a patient was reclassified, updated clinical reports were issued and patients received updated genetic counseling.

3 | RESULTS

3.1 | Whole exome and targeted gene panel sequencing

Approximately 90,000–130,000 single-nucleotide variants and small insertion and deletion changes were identified in each patient exome by aligning with the reference sequence of the human genome (versions GRCh37 or GRCh38). The same type of the variants with a range of 7,000–9,000 in each patient targeted gene panel were detected.

Population frequency filtering retained approximately 1,500 to 2,800 exome variants and 200 to 400 targeted gene panel variants of potential clinical usefulness per sample. Subsequently, we applied selective filters according to the phenotype and <7% of variants were selected as potential etiopathogenic factors. These possible causative agents were manually evaluated using genomic databases and reports available in the literature.

3.2 | Initial diagnostic yield

From a cohort of 341 cases accrued over 4 years, 194 males (57%) and 147 females (43%), 161 (47%) underwent WES and 180 (53%) TGPS. Of the total number of cases, 46 came from the United States and 295 from Argentina. Due to this difference between the number of American and Argentine patients selected, we did not have sufficient statistical power to estimate the differences in diagnostic yield between countries.

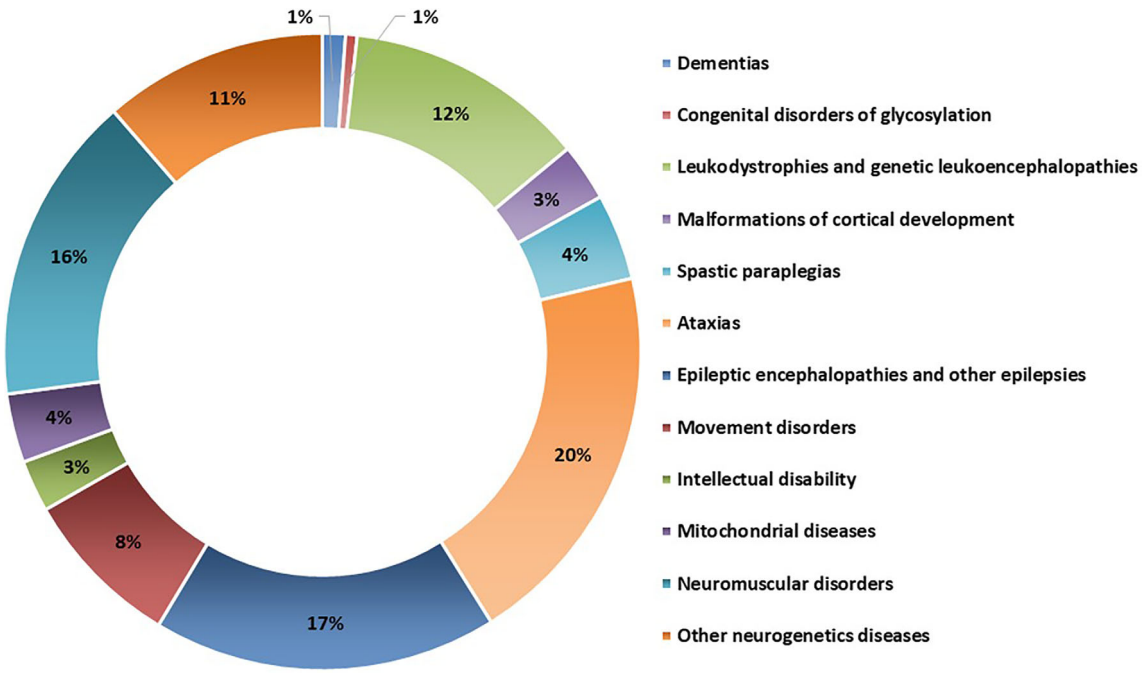

FIGURE 1 Types of neurogenetic diseases from adult and pediatric patients selected for whole exome sequencing (WES) and targeted gene panel Sequencing (TGPS)

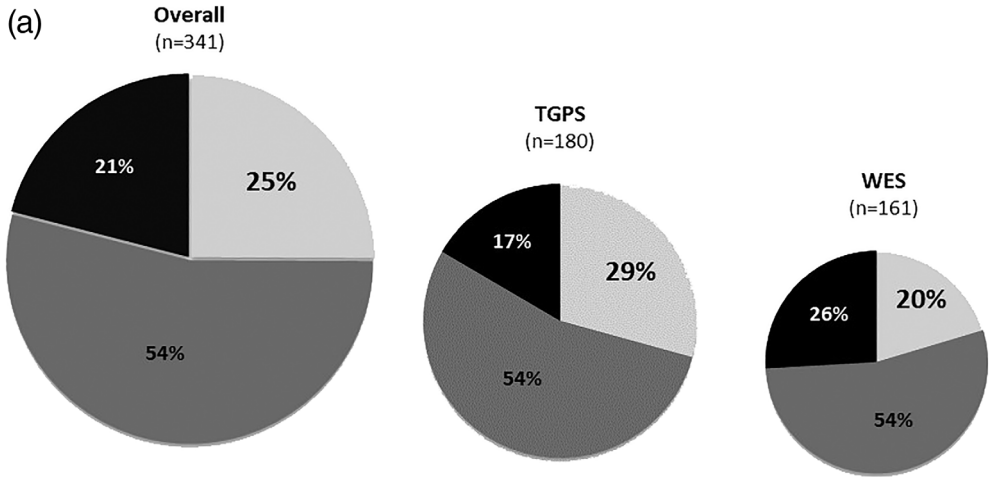

FIGURE 2 Diagnostic yield. (a) Diagnostic yield before re-analysis according to classification of patients diagnosed by Whole Exome Sequencing (WES) and Targeted Gene Panel Sequencing (TGPS; Overall), patients diagnosed by WES and patients diagnosed by TGPS. With 99% confidence, there is no significant difference between the diagnostic return of WES and TGPS (Statistical power = 60, 77% and *p* value = .9747). (b) Diagnostic Yield after re-interpretation according to classification of patients diagnosed by WES and TGPS (Overall), patients diagnosed by WES and patients diagnosed by TGPS. With 99% confidence, there is no significant difference between the diagnostic return of WES and TGPS (statistical power = 82, 16% and *p* value = .9928)

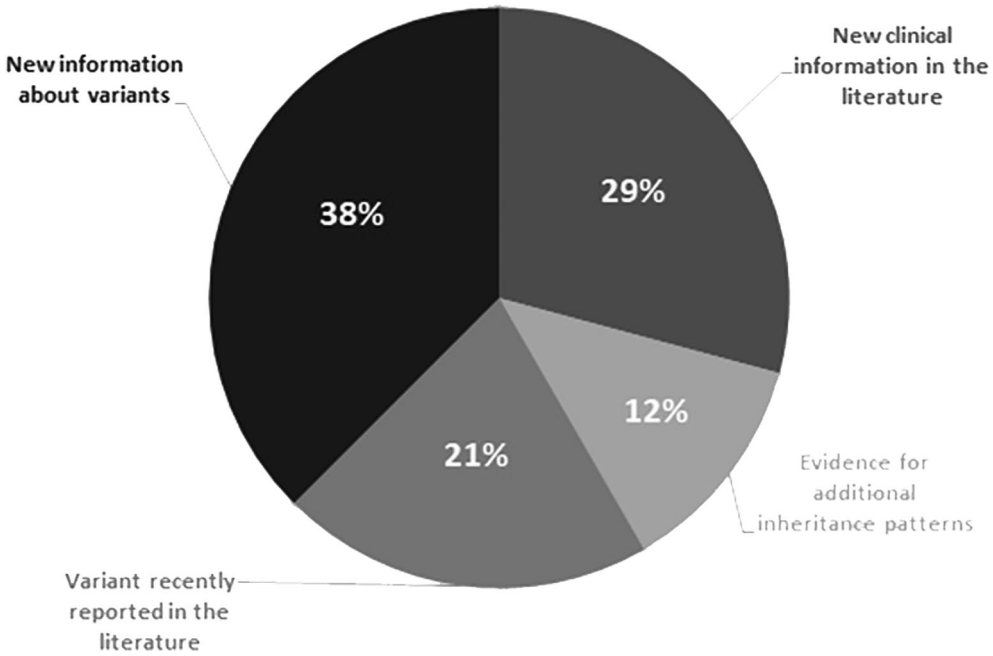

FIGURE 3 Reasons for reclassification of 24 patients with neurogenetics diseases

Ataxias (20%), epileptic encephalopathies and epilepsies (17%), neuromuscular disorders (16%), and leukodystrophies and genetic leukoencephalopathies (12%) were the most common disease categories (Figure 1).

Initially, 85 WES and TGPS satisfied criteria for a positive genetic diagnosis, thus our overall diagnostic baseline yield was 25%. There were no significant differences in the diagnostic yields between WES and TGPS (Figure 2a and Tables S1 and S2). The

highest overall diagnosis success rate corresponds to leukodystrophies and genetic leukoencephalopathies (45%), spastic paraplegias (40%), epileptic encephalopathies and epilepsies (30%) and ataxias (28%; Table S3). For patients with movement disorders (excluding ataxias), congenital disorders of glycosylation and mitochondrial diseases the diagnostic yield was <10% (Table S3). Twenty-five patients with positive genetic tests had a potentially actionable diagnosis (Tables S1 and S2).

TABLE 1 Categories of neurogenetic diseases in our cohort and genes identified

Neurogenetic disease	Genes
Leukodystrophies and genetic leukoencephalopathies	***GFAP*** *(3),* ***EIF2B5*** *(3), EIF2B3 (2), GJC2 (2), RNASEH2B (1), RNASEH2C (1), HEPACAM (1), POL3A (1), PLP1 (1), MLC1 (1), CSF1R (1), TUBB4A (1)*
Dementias	*PSEN2 (1)*
Malformations of cortical development	*L1CAM (1), TSC1 (1), COL4A1 (1), PTEN (1), RHEB (1)*
Spastic paraplegias	***SPG11*** *(3), SPAST (2), ATL1 (1), SPG7 (1), DDHD2 (1), SPG7 (1)*
Ataxias	***ATM*** *(4), SETX (2), SYNE1 (2),* CACNA1A (2), AFG3L2 (2), *STUB1 (2), SACS (2), ITPR1 (2), OPA1 (2), SPTBN2 (2), CAMTA1 (1), TPP1 (1), KCNJ10 (1)*
Epileptic encephalopathies and other epilepsies	***SCN1A*** *(4), SCN2A (3), KCNQ2 (2), STXBP1 (2), MEPC2 (2), GABRA1 (1), PCDH19 (1), SCN1B (1), CACNA1A (1), SCN8A (1), EPM2A(1), SLC6A1 (1), CHD2 (1), HNRNPU (1)*
Movement disorders	*PRKN (1), CC2D2A (1), C19orf12 (1),PARK8 (1), WDR45 (1), GNAO1 (1)*
Intellectual disability	*NEXMIF (1), TCF4 (1), PPP2R5S (1), CHD8 (1), UBTF (1)*
Mitochondrial diseases	*TRMU (1), BOLA3 (1)*
Neuromuscular disorders	*DMD (2), NEB (1), LMNA (1), SCN4A (1), GJB1 (1), MTM1 (1), TOR1A (1), SCN11A (1), TPM3 (1), IGHMBP2 (1)*
Other neurogenetic diseases	*KMT2D (1)*

Note: The parentheses indicate the number of patients with etiopathogenic variants detected in the respective genes mentioned. The most frequently associated genes with the conditions are indicated in bold.

3.3 | Diagnostic yield after re-interpretation

After a mean period of 28 months (SD 12.33) since the original analysis, 72 patients with indeterminate variants were re-interpreted. Among these, 10 WES and 14 TGPS were reclassified from undetermined to positive (Tables S1 and S2). The most frequent reasons for reclassification of patients were "New information about variants" and "New clinical information in the literature" (Figure 3). The overall diagnostic yield increased from 25% (85 patients) to 32% (109 patients). The diagnostic yield for WES and TGPS improved to 26% (42 cases) and 37% (67 cases), respectively (Figure 2b).

The final positive genetic diagnoses included 65 individuals with autosomal dominant inheritance, 38 autosomal recessive and six X-linked (Tables S1 and S2). Different types of variations were detected including frameshift, nonsense, missense and splice sites variants. Of note, 35% of the variants were novel, according to the ClinVar database and biomedical literature. *GFAP*, *EIF2B5*, *SPG11*, *ATM*, and *SCN1A* were the genes most frequently identified as causing neurogenetic diseases in our series (Table 1).

The re-evaluation increased the overall diagnostic success rate in spastic paraplegias, malformations of cortical development, leukodystrophies and genetic leukoencephalopathies, epileptic encephalopathies and epilepsies, ataxias, neuromuscular disorders, and movement disorders (Table S4). The number of patients with potentially actionable diagnosis also increased by 32 subjects, 29% of the final positive genetic tests (Tables S1 and S2).

3.4 | Genomic databases are continually growing

In December 2015, OMIM listed the molecular basis on 4,724 phenotypes. By December 2019, the list had grown to 5,709 Mendelian disorders with molecular basis documented. Between 2016 and December 2019, the number of OMIM phenotypes with known molecular basis increased at an average rate of 246 per year (Figure 4a). The number of variant-disease associations in the HGMD database also increased. Since January 2016, the number of

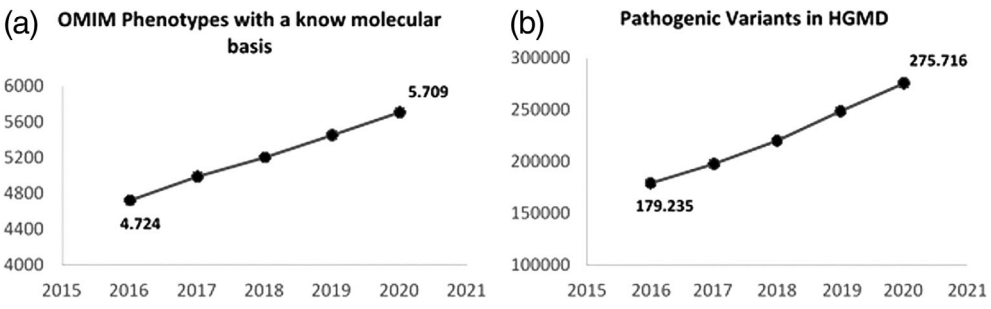

FIGURE 4 Growth in gene–disease and variant–disease associations. (a) The number of phenotypes or diseases with a known molecular basis reported in OMIM. (b) The number of pathogenic variants causing a disease reported in HGMD

pathogenic variants in HGMD has increased at an average growth rate of 24,120 entries per year (Figure 4b).

4 | DISCUSSION

NGS proved to be an efficient and cost-effective diagnostic tool, allowing the elucidation of the molecular basis of heterogeneous and complex phenotypes (Córdoba et al., 2018; Frank, Prenzler, Eils, & Graf von der Schulenburg, 2013).

The reinterpretation of previously undetermined variants increased the diagnostic yield from 25% to 32%, highlighting the value of periodic re-interpretation in an era of rapid and constant accrual of genomic knowledge. The reclassification from undetermined to positive for 24 variants over 24 months was possible due to the constant growth of genomic databases (OMIM and HGMD) and biomedical literature in the last 4 years. Furthermore, these results impacted management or therapeutic decisions in 30% of the patients. Our reanalysis showed a performance similar to that reported by Liu et al; who re-evaluated 2000 patients with Mendelian disorders achieving a growth in diagnostic performance of approximately 25–37% (Liu et al., 2019).

Furthermore, the final diagnostic yield for TGPS and WES were also similar to previous reports on neurogenetic diseases utilizing the same technologies (Jones et al., 2013; Martínez et al., 2017; Segal et al., 2016). We previously reported a diagnostic yield slightly higher using WES in a small series of patients with neurogenetic disorders (Córdoba et al., 2018). However, the cohort reported here is more heterogeneous, including entities with a lower expected rate of positive findings according to the experience of other groups (Gorcenco et al., 2020).

On the other hand, the yield obtained in patients with genetic white matter abnormalities (leukodystrophies and genetic leukoencephalopathies) and epileptic encephalopathies and other epilepsies was higher than previously reported (Segal et al., 2016; Vanderver et al., 2016).

Overall, 54% of the study population was classified as genetically undiagnosed. It is possible that the neurogenetic disease in some of these patients is caused by a genetic alteration located in areas not covered by WES or TGP sequencing, such as intronic regions. Additionally, it is likely that at least some of these individuals have disease-causing variants in genes that have not previously been associated with the clinical phenotype of the patients under study. Furthermore, WES and TGPS are not standard methods for the detection of structural alterations (deletions and duplications) that might be causative in at least some of our undiagnosed patients.

Currently, arriving at a definitive diagnosis is a challenging and complex situation due to the constant increase in genetic knowledge. The growing role of mosaic variants in some entities such as epilepsies (de Lange et al., 2018), epileptic encephalopathies (Myers et al., 2018) and neuromuscular disorders (Perez Maturo et al., 2019) require the implementation of innovative sequencing and bioinformatic analysis methods for their detection and validation.

Noteworthy, we identified pathogenic mosaic variants in addition to germinal ones. The *PCDH19*-related epileptic encephalopathy was initially reported affecting only heterozygous women carrying germline variants. Mechanisms of cellular interference, requiring diploidy in this locus, may be involved in the development of symptoms (Romasko et al., 2018). Mosaic male patients with pathogenic variants in *PCDH19* have been rarely reported and we identified a similar genotype–phenotype association in a 2-year-old boy (Depienne et al., 2009; Perez, Hsieh, & Rohena, 2017; Figure 5a). Much remains to be known about the association between the degree of peripheral blood mosaicism and the severity of symptoms. The identification of X chromosome mosaicisms in boys with seizures in the first year of life is likely to increase as the use of NGS becomes more massive in the genetic diagnosis of epilepsies. These genetics changes have been underdiagnosed for years due to the limitations of the Sanger technique for the detection of low allele frequency variants and the lack of adequate bioinformatic analysis tools.

Furthermore, for the first time a mosaic pathogenic variant was detected in the *RHEB* gene in a girl with hemimegalencephaly in brain tissue sample (Salinas et al., 2019; Figure 5b). In this patient, hyperactivation of the Mammalian Target of Rapamycin (mTOR) pathway was evidenced by immunohistochemistry, highlighting the complementarity of NGS-based assays with other classic techniques in affected tissues. The mTOR pathway has proven to be a pharmacological target in some pathologies with phenotypic similarities to those in our case (Lee et al., 2012; Reijnders et al., 2017). These results allowed *RHEB* to be included among the genes that are analyzed in

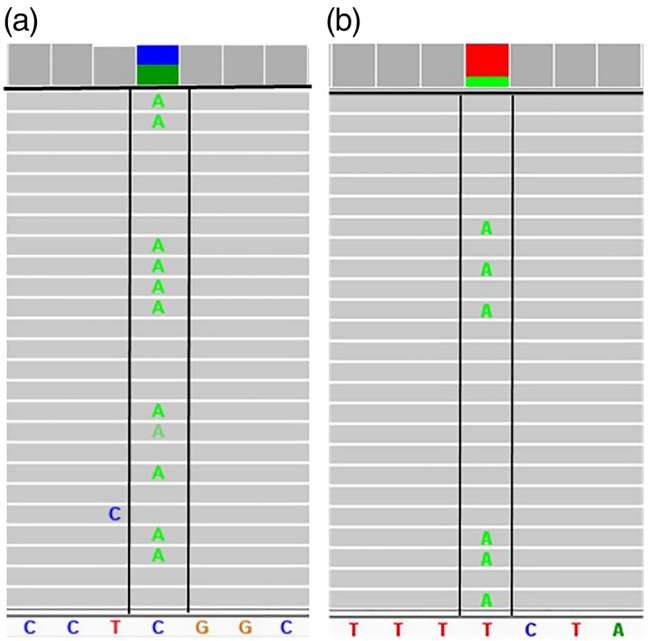

FIGURE 5 Genetic analysis of mosaic variants. (a) TGPS of blood from *patient 9*, showing the mosaic variant 1720 G>T in *PCDH19* gene located on chromosome X in 66 of 128 reads (variant allele frequency of 52%). (b) WES of brain tissue from *patient 99*, showing the somatic variant 119A>T in the *RHEB* gene in 10 of 49 reads (variants allele frequency of 22%)

the context of the genetic study of malformations of cortical development throughout the world. Our findings illustrate the utility of WES and TGPS beyond the recognition of germinal variants.

The positive reinterpretation of former uncertain clinical reports depended not only on recent discoveries of new disease-causing genes but on new inheritance mechanisms for well-known disease-causing genes. Such is the case of a 24-year-old man with a heterozygous frameshift variant in the last exon of *C19orf12* detected in 2017 by WES. He presented a complex movement disorder and evidence of iron accumulation in the basal ganglia. A most detailed visual examination of brain MRI showed pallidal hypointensity with hyperintense streaking in the region of the medial medullary lamina, which suggested Mitochondrial Membrane Protein Associated Neurodegeneration (MPAN; Yoganathan, Sudhakar, Thomas, Dutta, & Danda, 2016). At the time of the original report, the only known mechanism of inheritance for Mitochondrial Membrane Protein Associated Neurodegeneration (MPAN) was autosomal recessive. But, after 2018 a number of cases with dominant MPAN were reported in subjects with a clinical-radiological phenotype indistinguishable from the recessive ones but carrying variants located in the last exon of *C19orf12* (Gregory et al., 2019; Monfrini et al., 2018). So, the new evidence in the literature served to reclassify the result of the exome of this patient from uncertain to positive, facilitating genetic counseling for the proband and the family.

The diagnostic yields estimated in this work could be influenced by the disproportionate distribution of our heterogeneous cohort in each category of neurogenetic disease studied. This limitation may be explained by differences in population frequency and referral for genetic consultation observed for these neurological conditions.

Through this study, we describe our first steps in establishing a collaboration between health care centers located in Argentina and the United States. It is difficult to find similar multicenter and binational reports in the literature.

Furthermore, in Argentina there are no other studies involving such a high number of patients with as large a neurogenetic range as ours.

In summary, we reported here the findings of a large series of patients with neurogenetic disorders in which the use of NGS-based assays proved useful to identify not only germline but also mosaic pathogenic variants and solve the diagnostic odysseys in more than a third of the cohort. These data demonstrate the high clinical impact that periodic reanalysis can have, supporting the value of widely disseminating genomic information as it becomes available.

ACKNOWLEDGMENTS

We thank the patients and families for their support and collaboration. This study was funded by a grant from the Ministry of Science and Technology of Argentina and University of Cincinnati of the United States.

CONFLICT OF INTEREST

Josefina Perez Maturo and Valeria Salinas have received scholarship support from Argentinean National Science Council (CONICET). Patricia Vega has received scholarship support from the Government of the Autonomous City of Buenos Aires. Marcelo A. Kauffman has received grant support from the Ministry of Health of Buenos Aires City, Argentinean National Science Council (CONICET) and Argentinean Ministry of Science and Technology. He serves as Associate Editor of the journal of Neurology from Argentina. Alberto J. Espay has received grant support from the NIH and the Michael J Fox Foundation; personal compensation as a consultant/scientific advisory board member for Abbvie, Neuroderm, Neurocrine, Amneal, Adamas, Acadia, Acorda, InTrance, Sunovion, Lundbeck, and US WorldMeds; publishing royalties from Lippincott Williams & Wilkins, Cambridge University Press, and Springer; and honoraria from US WorldMeds, Acadia, and Sunovion. The rest of the authors declare that they have no conflict of interest.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee of the Hospital JM Ramos Mejía of Buenos Aires, Argentina and the Institutional Review Board of the University of Cincinnati, United States. All patients or, as appropriate, parents provided written informed consent for genetic analyses and use of their anonymized data. All experiments and methods were carried out in accordance with the relevant guidelines and approved. All clinical investigations have been conducted in accordance with the 1964 Helsinki Declaration and its later amendments.

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REFERENCES

Carmichael, N., Tsipis, J., Windmueller, G., Mandel, L., & Estrella, E. (2015). "is it going to hurt?": The impact of the diagnostic odyssey on children and their families. *Journal of Genetic Counseling*, *24*(2), 325–335. https://doi.org/10.1007/s10897-014-9773-9

Chisholm, C., Daoud, H., Ghani, M., Mettler, G., McGowan-Jordan, J., Sinclair-Bourque, L., … Jarinova, O. (2018). Reinterpretation of sequence variants: One diagnostic laboratory's experience, and the need for standard guidelines. *Genetics in Medicine*, *20*(3), 365–368. https://doi.org/10.1038/gim.2017.191

Cohen, L., Manín, A., Medina, N., Rodríguez-Quiroga, S., González-Morón, D., Rosales, J., … Vega, P. (2020). Argentinian clinical genomics in a leukodystrophies and genetic leukoencephalopathies cohort: Diagnostic yield in our first 9 years. *Annals of Human Genetics*, *84*(1), 11–28. https://doi.org/10.1111/ahg.12345

Córdoba, M., Rodriguez-Quiroga, S. A., Vega, P. A., Salinas, V., Perez-Maturo, J., Amartino, H., … Kauffman, M. A. (2018). Whole exome sequencing in neurogenetic odysseys: An effective, cost- and time-saving diagnostic approach. *PLoS One*, *13*(2), e0191228. https://doi.org/10.1371/journal.pone.0191228

de Lange, I. M., Koudijs, M. J., van 't Slot, R., Gunning, B., Sonsma, A., van Gemert, L., … Koeleman, B. (2018). Mosaicism of de novo pathogenic SCN1A variants in epilepsy is a frequent phenomenon that correlates with variable phenotypes. *Epilepsia*, *59*(3), 690–703. https://doi.org/10.1111/epi.14021

Depienne, C., Bouteiller, D., Keren, B., Cheuret, E., Poirier, K., Trouillard, O., … Leguern, E. (2009). Sporadic infantile epileptic encephalopathy caused by mutations in PCDH19 resembles Dravet syndrome but mainly affects females. *PLoS Genetics*, *5*(2), e1000381. https://doi.org/10.1371/journal.pgen.1000381

el Mecky, J., Johansson, L., Plantinga, M., Fenwick, A., Lucassen, A., Dijkhuizen, T., … van Langen, I. (2019). Reinterpretation, reclassification, and its downstream effects: Challenges for clinical laboratory geneticists. *BMC Medical Genomics*, *12*(1), 170. https://doi.org/10.1186/s12920-019-0612-6

Fogel, B. L., Satya-Murti, S., & Cohen, B. H. (2016). Clinical exome sequencing in neurologic disease. *Neurology Clinical Practice*, *6*(2), 164–176. https://doi.org/10.1212/CPJ.0000000000000239

Frank, M., Prenzler, A., Eils, R., & Graf von der Schulenburg, J. M. (2013). Genome sequencing: A systematic review of health economic evidence. *Health Economics Review*, *3*, 29. https://doi.org/10.1186/2191-1991-3-29

González-Morón, D., Vishnopolska, S., Consalvo, D., Medina, N., Marti, M., Córdoba, M., … Kauffman, M. A. (2017). Germline and somatic mutations in cortical malformations: Molecular defects in Argentinean patients with neuronal migration disorders. *PLoS One*, *12*(9), e0185103. https://doi.org/10.1371/journal.pone.0185103

Gorcenco, S., Ilinca, A., Almasoudi, W., Kafantari, E., Lindgren, A. G., & Puschmann, A. (2020). New generation genetic testing entering the clinic. *Parkinsonism & Related Disorders*, *73*, 72–84. https://doi.org/10.1016/j.parkreldis.2020.02.015

Gregory, A., Lotia, M., Jeong, S. Y., Fox, R., Zhen, D., Sanford, L., … Hayflick, S. J. (2019). Autosomal dominant mitochondrial membrane protein-associated neurodegeneration (MPAN). *Molecular Genetics & Genomic Medicine*, *7*(7), e00736. https://doi.org/10.1002/mgg3.736

Jones, M. A., Rhodenizer, D., da Silva, C., Huff, I. J., Keong, L., Bean, L. J., … Hegde, M. R. (2013). Molecular diagnostic testing for congenital disorders of glycosylation (CDG): Detection rate for single gene testing and next generation sequencing panel testing. *Molecular Genetics and Metabolism*, *110*(1–2), 78–85. https://doi.org/10.1016/j.ymgme.2013.05.012

Kozarewa, I., & Turner, D. J. (2011). Amplification-free library preparation for paired-end Illumina sequencing. *Methods in Molecular Biology (Clifton, N.J.)*, *733*, 257–266. https://doi.org/10.1007/978-1-61779-089-8_18

Lee, J. H., Huynh, M., Silhavy, J. L., Kim, S., Dixon-Salazar, T., Heiberg, A., … Gleeson, J. G. (2012). De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. *Nature Genetics*, *44*(8), 941–945. https://doi.org/10.1038/ng.2329

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997.

Liu, P., Meng, L., Normand, E. A., Xia, F., Song, X., Ghazi, A., … Yang, Y. (2019). Reanalysis of clinical exome sequencing data. *The New England Journal of Medicine*, *380*(25), 2478–2480. https://doi.org/10.1056/NEJMc1812033

Margraf, R. L., Durtschi, J. D., Dames, S., Pattison, D. C., Stephens, J. E., & Voelkerding, K. V. (2011). Variant identification in multi-sample pools by illumina genome analyzer sequencing. *Journal of Biomolecular Techniques*, *22*(2), 74–84.

Martínez, F., Caro-Llopis, A., Roselló, M., Oltra, S., Mayo, S., Monfort, S., & Orellana, C. (2017). High diagnostic yield of syndromic intellectual disability by targeted next-generation sequencing. *Journal of Medical Genetics*, *54*(2), 87–92. https://doi.org/10.1136/jmedgenet-2016-103964

Might, M., & Wilsey, M. (2014). The shifting model in clinical diagnostics: How next-generation sequencing and families are altering the way rare diseases are discovered, studied, and treated. *Genetics in medicine: official journal of the American College of Medical Genetics*, *16*(10), 736–737. https://doi.org/10.1038/gim.2014.23

Monfrini, E., Melzi, V., Buongarzone, G., Franco, G., Ronchi, D., Dilena, R., … Di Fonzo, A. (2018). A de novo C19orf12 heterozygous mutation in a patient with MPAN. *Parkinsonism & Related Disorders*, *48*, 109–111. https://doi.org/10.1016/j.parkreldis.2017.12.025

Myers, C. T., Hollingsworth, G., Muir, A. M., Schneider, A. L., Thuesmunn, Z., Knupp, A., … Mefford, H. C. (2018). Parental mosaicism in "De novo" epileptic encephalopathies. *The New England Journal of Medicine*, *378*(17), 1646–1648. https://doi.org/10.1056/NEJMc1714579

Perez, D., Hsieh, D. T., & Rohena, L. (2017). Somatic mosaicism of PCDH19 in a male with early infantile epileptic encephalopathy and review of the literature. *American Journal of Medical Genetics. Part A*, *173*(6), 1625–1630. https://doi.org/10.1002/ajmg.a.38233

Perez Maturo, J., Vega, P., Medina, N., Salinas, V., Pauni, M., Agosta, G., … Kauffman, M. (2019). Inferring parental gonadal mosaicism in LMNA-associated muscular dystrophy by ultra-deep next generation sequencing: A sensitive approach providing valuable information for genetic counseling. *American Journal of Medical Genetics. Part A*, *179*(6), 1074–1076. https://doi.org/10.1002/ajmg.a.61135

Perez Maturo, J., Zavala, L., Vega, P., González-Morón, D., Medina, N., Salinas, V., … Kauffman, M. A. (2020). Overwhelming genetic heterogeneity and exhausting molecular diagnostic process in chronic and progressive ataxias: Facing it up with an algorithm, a gene, a panel at a time. *Journal of Human Genetics*, *65*, 895–902. https://doi.org/10.1038/s10038-020-0785-z

Reijnders, M., Kousi, M., van Woerden, G. M., Klein, M., Bralten, J., Mancini, G., … Brunner, H. G. (2017). Variation in a range of mTOR-related genes associates with intracranial volume and intellectual disability. *Nature Communications*, *8*(1), 1052. https://doi.org/10.1038/s41467-017-00933-6

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., … ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30

Romasko, E. J., DeChene, E. T., Balciuniene, J., Akgumus, G. T., Helbig, I., Tarpinian, J. M., … Tayoun, A. . (2018). PCDH19-related epilepsy in a male with Klinefelter syndrome: Additional evidence supporting PCDH19 cellular interference disease mechanism. *Epilepsy Research*, *145*, 89–92. https://doi.org/10.1016/j.eplepsyres.2018.06.008

Salinas, V., Vega, P., Piccirilli, M. V., Chicco, C., Ciraolo, C., Christiansen, S., … Kauffman, M. (2019). Identification of a somatic mutation in the RHEB gene through high depth and ultra-high depth next generation sequencing in a patient with Hemimegalencephaly and drug resistant epilepsy. *European Journal of Medical Genetics*, *62*(11), 103571. https://doi.org/10.1016/j.ejmg.2018.11.005

Segal, E., Pedro, H., Valdez-Gonzalez, K., Parisotto, S., Gliksman, F., Thompson, S., … Fertig, E. (2016). Diagnostic yield of epilepsy panels in children with medication-refractory epilepsy. *Pediatric Neurology*, *64*, 66–71. https://doi.org/10.1016/j.pediatrneurol.2016.06.019

van der Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., del Angel, G., Levy-Moonshine, A., … DePristo, M. A. (2013). From FastQ data to high confidence variant calls: The genome analysis toolkit best practices pipeline. *Current Protocols in Bioinformatics*, *43*(1110), 11.10.1–11.10.33. https://doi.org/10.1002/0471250953.bi1110s43

Vanderver, A., Simons, C., Helman, G., Crawford, J., Wolf, N. I., Bernard, G., … Taft, R. J. (2016). Whole exome sequencing in patients with white matter abnormalities. *Annals of Neurology*, *79*(6), 1031–1037. https://doi.org/10.1002/ana.24650

Wang, K., Li, M., & Hakonarson, H. (2010). ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Research*, *38*(16), e164. https://doi.org/10.1093/nar/gkq603

Wenger, A. M., Guturu, H., Bernstein, J. A., & Bejerano, G. (2017). Systematic reanalysis of clinical exome data yields additional diagnoses: Implications for providers. *Genetics in Medicine*, *19*(2), 209–214. https://doi.org/10.1038/gim.2016.88

Yang, Y., Muzny, D. M., Reid, J. G., Bainbridge, M. N., Willis, A., Ward, P. A., … Eng, C. M. (2013). Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *The New England Journal of Medicine*, *369*(16), 1502–1511. https://doi.org/10.1056/NEJMoa1306555

Yoganathan, S., Sudhakar, S. V., Thomas, M., Dutta, A. K., & Danda, S. (2016). "eye of tiger sign" mimic in an adolescent boy with mitochondrial

membrane protein associated neurodegeneration (MPAN). *Brain & Development*, *38*(5), 516–519. https://doi.org/10.1016/j.braindev.2015.10.017

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Salinas V, Vega P, Marsili L, et al. The odyssey of complex neurogenetic disorders: From undetermined to positive. *Am J Med Genet Part C*. 2020;1–9. https://doi.org/10.1002/ajmg.c.31848