Original Research Article

Importance of Phenotype-Genotype correlation for Next Generation Sequencing Data to diagnose Pediatric Neurological Disorders

N. Zainab Abbas[1], Prashant R Utage[2], Gayatri R Iyer[3], Muttaqi Ahmad Alladin[3,4], Qurratulain Hasan[3*]

[1]Department of Genetics & Molecular Medicine, Kamineni Hospitals, King Kothi, Hyderabad, India
[2]Utage Child Neuro Clinic and Development Centre, Narayanguda, Hyderabad,India
[3]Department of Genetics & Molecular Medicine, Kamineni Hospitals, LB Nagar, Hyderabad, India
[4]Department of Biotechnology, Chaitanya Bharathi Institute of Technology, Hyderabad, India

Received: 24-06-2020 / Revised: 28-08-2020 / Accepted: 05-09-2020

Abstract

Background: Pediatric neurological disorders can be mainly categorized into four areas such as movement disorders, epilepsy associated disorders, neuro-peripheral disorders and neuropsychiatric disorders. They can be identified from in-utero to 18 years. The interpretation of sequencing results based on phenotype-genotype correlation are important for the clinicians, patients and the family for further treatment and management.**Materials & Methods**: Sixteen patients were referred to the department of Genetics of a tertiary care hospital for post-test counselling along with clinical exome reports. In cases where there was no reported variants, reanalysis of raw data was performed using a freeware by Illumina. Variants identified were assessed for genotype-phenotype correlations and evaluated by segregation analysis wherever required to arrive at a molecular diagnosis.**Results:**Six patients had a report with a pathogenic sequence variant correlating with the phenotype, four patients were reported with a Variant of Unknown Significance, while the sequence data of remaining six patients was reanalyzed to establish diagnosis.**Conclusion:**Results indicate the important role that a genetic counselor plays in establishing the genotype-phenotype correlation and providing appropriate post-test genetic counselling to help pediatric neurologists to manage patients and assist patients to take informed reproductive/predictive/pre-natal decisions.

Keywords: neurological,patients,genetics,hospital

This is an Open Access article that uses a fund-ing model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Introduction

Pediatric neurology is one of the most complex areas of medical practice as diagnosing neonates and children with neurological symptoms is of prime importance both for appropriate management by early intervention, predicting prognosis and genetic counseling for recurrence risk to plan subsequent pregnancies. Neurological disorders can be caused due to infection, perinatal trauma or underlying genetic pathology [1]. It is important for clinicians to differentiate the cause to manage patients appropriately. Pediatric neurological disorders are a complex heterogenous group of diseases with overlapping symptoms and a subset of these have a well-defined genetic cause [2]. It is important to understand, recognize and record the progressive clinical manifestations observed in patients, as this helps in diagnosis using the most suitable genetic tests. Although neuroimaging, biochemical analyses of body fluids and other electrophysiological studies are helpful in diagnosis, accurate diagnosis can only be achieved after identifying a molecular cause for these complex

**Correspondence*
Dr. Qurratulain Hasan
Senior Consultant & HOD, Department of Genetics & Molecular Medicine, Kamineni Hospitals, LB Nagar, Hyderabad 500 068,
E- mail: qhasan2000@yahoo.com

disorders. Next generation sequencing (NGS) is a diagnostic modality which has recently gained a prominence in current day medical practice and has the capacity to sequence multiple genes, complete exomes or genomes, simultaneously. Hence it is a powerful tool for investigating pediatric neurological disorders [3].NGS plays an important role especially in pediatric practice for identifying DNA sequence variants and then prioritizing variants correlating with disease phenotype of the affected individual [4]. Role of Genetic counselors is indispensable in correlating phenotype-genotype along with using the pedigree information, as well as, databases available to diagnose the neurological disorder under investigation. Genetic counselors can also help clinicians by facilitating testing, understanding the genetic test reports and providing the appropriate genetic counselling to the patients/families adding value to patient care. In the present study, cases from paediatric neurology clinics referred to a single Genetic unit will be discussed. All the cases had a NGS based panel test report, which was carried out on the advice of a pediatric neurologist for disease diagnosis. The sequence variants reported or identified after reanalysis were correlated with the phenotype and diagnosis was established for patient management and reproductive counselling.

Method

Patients who had undergone a NGS test were referred by pediatric neurologists for genetic counselling [5]. The NGS test results were correlated with the phenotype and re-analysis was carried out in cases where a diagnosis was not achieved. This was done by procuring the raw sequence data in the form of variant caller file (.vcf). Using an in-silico software, Illumina Basespace, a cloud-based software tool used as a platform to interpret, report and analyse variants from the genomic data. Variants which were pathogenic, likely pathogenic or VUS were correlated with the phenotypic features of the proband, the organ systems affected, associated specific disease conditions and differential diagnosis for the same. Candidate genes and the significance of the relevant variants was established by searching the literature and databases such as ClinVar [6],OMIM [7], Face2Gene [8], Genetic home reference [9]. They were then assessed by *in-silico* methods like Mendelian Clinically Applicable Pathogenicity Score(M-CAP) [10], The scale-invariant feature transform (SIFT), Polymorphism Phenotyping v2(Polyphen) to confirm functional significance. Once the genotype -phenotype correlation was documented, the family was counselled forsanger confirmation and segregation analysis to further confirm the relevance of the identified variants in the patients.Informed Consent was taken from patients/parents/guardians prior to obtaining 2ml of peripheral blood in EDTA vacutainers as per the Institutional Ethics Committee of Kamineni Hospitals (Registration #ECR/58/Inst/AP/2013) guidelines. This study was carried out in accordance with the recommendations of the International Council of Harmonisation and Good Clinical Practice. All subjects/families gave written informed consent in accordance with Declaration of Helsinki.

Results

Sixteen patients which included seven females and nine males between the ages of 3 days and 16 years were referred for test based counseling with an NGS report from a commercial company. Patients were assessed for genotype–phenotype correlation with the sequence variants reported. Ten out of sixteen (62.5%) patients had a diagnosis from results given by the company (Table 1). Six of these patients (37.5%) were identified with a pathogenic variant, while four patients (25%) were identified with variants of unknown significance (VUS). Further confirmation of the variants was done by segregation analysis in the parents. Re-analysis of the raw sequence data was done for the remaining six patients, where the company did not report any clinically significant variants. Diagnosis could be established for all of them (Table 1). The different neurological disorders that were diagnosed were categorized into neuro-peripheral disorders (Case a.1-a.6), movement disorders (Case b.1-b.3), epilepsy associated disorders (Case c.1-c.5) and neuropsychiatric disorders (Case d.1-d.2) based on the review by Hung et al (2014).

Case no.	Age/Sex	Clinical details	Genotype	Disorder/Pattern of inheritance
Case a.1	1y/F	Global development delay, Krabbe disease, seizures, muscle weakness, vision disorder and hearing defect	*PIEZO2*- c.C1156T/p.R386W Heterozygous Known and pathogenic variant	Marden-Walker syndrome/Autosomal dominant (OMIM #248700)
Case a.2	3y11m/ M	Global development delay, seizures, muscle eye brain disease and suspicion of Fukuyama congenital muscular dystrophy	*ADGRG1* c.C1693T/p.Arg565T Homozygous Known and pathogenic variant	Bilateral frontoparietal polymicrogyria (BFPP)/Autosomal recessive (OMIM #606854)
Case a.3	1y/F	Intellectual disability, global development delay, Hypertonia	*AUTS2* c.G2521A/p.D841N Heterozygous Known and pathogenic variant	Mental Retardation, AD26/Autosomal Dominant (OMIM #607270)
Case a.4	1y/M	Development delay, strabismus, slight nystagmus, cerebellar atrophy	*EXOSC3* c.395A>C p.Asp132Ala Homozygous Pathogenic	Pontocerebellar hypoplasia, type 1B /Autosomal recessive
Case a.5	1y/M	Developmental delays/Refractory convulsions	*MMACHC* c.C394T/p.Arg132X Homozygous Known and pathogenic	Methylmalonic aciduria and homocystenuria, cb1C type Autosomal recessive (OMIM #609831)
Case a.6	16y/M	Global development delay with delayed milestones, No neck holding and no eye contact. Episodes of seizures	*NFU1* c.334G>A/p.Val112Ile Homozygous Likely Pathogenic	Multiple Mitochondrial dysfunctions syndrome 1 Autosomal Recessive (OMIM #605711)
Case a.7	1y8m/F	Global development delay with delayed milestones Neuro-regression from 6 months of age, no eye contact, no social smile	AGRN c.5645C>T p.(Thr1882Ile) Homozygous VUS	Myasthenic syndrome, congenital, 8, with pre- and postsynaptic defects Autosomal Recessive (OMIM #103320)

Case b.1	13y/F	Neuroregression, Lower limb weakness	NIPA1 c.45_47dupGGC p.(Ala16dup) Heterozygous VUS	Spastic paraplegia 6 Autosomal Dominant (OMIM #600363)
Case b.2	8y/M	Neurodegenerative disorder with suspicion of Alexander/vanishing white matter disease	ASPA c.237-1G>T Homozygous VUS	Canavan disease Autosomal recessive (OMIM #271900)`
Case c.1	1y/M	Seizures and Development Regression	*STXBP1* c.673A>T p.Lys22 Heterozygous Pathogenic	Epileptic Encephalopathy, early infantile 4, Autosomal Dominant (OMIM #612164)
Case c. 2	4m/F	Developmental delay, progressive dystonia, occular abnormalities	*TPP1* c.887-6delA Homozygous VUS	Ceroid lipofuscinosis, neuronal, 2 Autosomal Recessive (OMIM #204500)
Case c.3	3d/F	Antenatal scan showed IUGR with microcephaly, neonatal death on third day	*POMT1* c.123-4C>T & *POMT1* c.280+7_280+8d elGA Compound Heterozygous VUS	Walker-Walberg Syndrome Autosomal Recessive
Case c.4	2m/M	Tuberous Sclerosis	*TSC2* c.T4724C/p. Leu1575pro Heterozygous VUS	Tuberous Sclerosis – 2 Autosomal dominant (OMIM #613254)
Case c.5	6y4m/ M	Choreoathetosis and suspicion of Neurotransmitter disease, global development delay	MDH2 c.G916A/p.G306 S Homozygous VUS	Epileptic Encephalopathy early infantile, 51 Autosomal Recessive (OMIM #617339)
Case d.1	8y/F	Hyperactivity, Neurotransmitter deficiency, Development delay, Neuro regression	*GLI2* c.T4560G/p.D152 0E Heterozygo us Known and Pathogenic	Culler-Jones Syndrome Autosomal Dominant (OMIM #615849)

Case d.2	7y/M	Developmental Delay, ADHD, Autism, Nephrotic Syndrome, Myopia Microarray showed LOH in Chr19 q13.2 associated with renal issues.	*SYN1* c.1554delC p.(Ala519ArgfsTer148) *CACNA1H* c.1495G>A p.(Gly499Ser) VUS	Autism Spectrum Disorder (OMIM #300491) Nephrotic Syndrome

Table 1: showing the genotype and phenotype data of 16 neuropediatric case

a) **Neuro-peripheral disorder**

Five cases from this category had a diagnosis based on specific pathogenic variants, while one had a VUS. Pathogenic heterozygous variant c.C1156T in the PIEZO2 gene causative of Marden-Walker syndrome (OMIM #248700) and c.G2521 in the AUTS2 gene causative of Mental Retardation, AD26,(OMIM #607270), which are autosomal dominant disorders were identified in Case a.1 and Case a. 3 respectively, which correlated with phenotype of the probands (Table 1). Case a.2 and Case a.4 had homozygous pathogenic variants c.C1693T/p.Arg565T in ADGRG1gene causative of Bilateral frontoparietalpolymicrogyria, (OMIM #606854) and c.395A>C in the EXOSC3 gene consistent with Pontocerebellar Hypoplasia 1B, respectively. Case a.5 was referred in view of refractory convulsions and development delays and was diagnosed with a homozygous, pathogenic variant c. C394T/p.Arg132X in MMACHC gene causative of Methylmalonic aciduria and homocystenuria, cb1C type Autosomal recessive(OMIM #609831). Case a.6 was identified with homozygous VUS in NFU1 gene associated with Multiple Mitochondrial dysfunctions syndrome 1 (OMIM #605711) which is an autosomal recessive condition. Case a.7 was identified with homozygous VUS in the AGRN gene causative of Myasthenic syndrome, congenital, 8, with pre- and postsynaptic defects (OMIM #103320).

b) Movement disorders

The two movement disorders were identified with VUS: Case b.1 was identified with a heterozygous VUS c.45_47dupGGC in NIPA1 gene causative of Spastic paraplegia 6 (OMIM #600363) which is an autosomal dominant disorder. While Case b.2 was identified with homozygous VUS in the ASPA gene c.237-1G>T causative of Canavan disease (OMIM #271900), an autosomal recessive disorders.

c) Epilepsy associated disorders

All cases except one with seizure disorders had VUS in genes associated with various neurological disorders. Case c.1 was identified to have a novel heterozygous stop gain variant in STXBP1 gene responsible for Epileptic encephalopathy, early infantile, 4 (OMIM: 612164). Segregational analysis revealed that the variant was *denovo* in the proband. Case c.2 is a female child aged 4 months with non-consanguineous parents, and an intronic homozygous variant altering splice region was detected in TPP1 gene causative of Ceroid lipofuscinosis, neuronal, 2 (OMIM 204500). Segregation analysis, showed that the parents were heterozygous for the variant supporting its functional significance. Case c.4 was clinically diagnosed with Tuberous Sclerosis in view of multiple hypopigmented patches and sequencing reported a VUS in TSC2 gene correlating with Tuberous sclerosis 2 (OMIM #613254). Case c.5 had a homozygous VUS in MDH2 gene consistent with a diagnosis of Epileptic Encephalopathy early infantile, 51. It is an autosomal recessive disorder. (OMIM #617339). *Case c.3* is a neonatal autopsy sample from a 3 day old infant of a 3rd degree consanguineous couple (female - 23 years and her husband 30yrs) who also had a history of another neonatal death with features of Walker-Walberg Syndrome. Data analysis revealed two variants in POMT1 gene c.123-4C>T and POMT1 c.280+7_280+8delGA. Segregational analysis indicated that both the parents were heterozygous (carriers) for the c.280+7_280+8delGA variant, indicating that the c.123-4C>T variant is most likely a germline mosaicisim in one of the parents.

d) Neuropsychiatric disorders

There were two cases under this category Case d.1 with hyperactivity, Neurotransmitter deficiency, Development delay, Neuro regression who was identified with a pathogenic variant c.T4560G in the GLI2 gene associated with the Culler-Jones Syndrome (OMIM 615849). While *Case d.2* was referred in view of autism with nephrotic syndrome. CES revealed two heterozygous VUS: (i) c.1554delC p.(Ala519ArgfsTer148) in SYN1 gene and (ii) c.1495G>A p.(Gly499Ser) in CACNA1H gene associated with ASD (OMIM 300491 and 607904 respectively).

Discussion

Pediatric neurological disorders have overlapping but distinct features, hence molecular analysis is important to diagnose the disorders for proper management and appropriate genetic counseling, for prevention in the future generations. They are generally categorized into four clinical types such as movement disorders, neuro-peripheral disorders, epilepsy associated disorders, and neuropsychiatric disorders . The study was carried out in sixteen patients who were tested by NGS - Neurological gene panel available in commercial companies and by reanalysis assessing all gene variants reported.Seven were diagnosed as neuroperipheral disorders, five of these cases had a pathogenic variant (Case a.1 - a.5), one had a likely pathogenic variant (Case a.6) indicating that the neurological panel of genes evaluated in companies are mostly associated with this category of disorders. Re-analysis followed by genotype-phenotype correlation identified a de-novo pathogenic variant in the STXBP1 gene helping in the diagnosis of Case c.1 as Epileptic Encephalopathy, early infantile 4. This was confirmed by Segregation analysis of parents and helped in future reproductive counseling. Another pathogenic variant T4560G/p.D1520E was reported in the GLI1 gene associated with Culler-Jones Syndrome (Case d.1) under Neuro-psychiatric disorders.

Commercial testing also reported four VUSs, one diagnosed as a neuroperipheral disorder (Case a.7), one as a movement disorder (Case b.2) and two as epilepsy disorders (Cases c.4, c.5). All these require further functional analysis and maybe novel variants unique to our population. Reanalysis found VUS in genes associated with movement disorders (Cases b.1 and b.2), epilepsy disorders (Cases c.2 and c.3) and neuropsychiatric disorders (Case d.2). Case d.2 was diagnosed with two causative gene variants c.1554delC / p.(Ala519ArgfsTer148) in the SYN1 gene and c.1495G>A / p.(Gly499Ser) variants in the CACNA1H gene associated with Autism Spectrum Disorder (OMIM 300491). Segregation analysis in the parents confirmed that SYN1 gene was causative of the disorder.

This study demonstrates that NGS panel testing can help in exact molecular diagnosis of patients with similar clinical phenotypes in a clinical setting. The current neurological panel of genes available with companies needs to be expanded to cover genes responsible for other categories of pediatric neurological disorders apart from the neuro-peripheral disorders. Since the NGS provides a powerful platform to sequence multiple genes simultaneously, it is important to design and develop the right panel of multiple genes that cover and target the total spectrum of pediatric neurological disorders.Our study also highlights the importance of genetic counseling sessions during the diagnostic process. Since the setup is pediatric, it is presumable that the patients and their immediate families are in their reproductive ages. Five cases in addition to syndromic counseling about the diagnosis also received comprehensive reproductive counseling to guide and empower them to take informed decisions. Three of which planned their subsequent pregnancies after the counseling and also underwent prenatal testing. In other two cases, adequate pre-natal counseling was offered for future pregnancies. It is important for clinicians to analyze not only the clinical features but also the complex genetic aspect of these neurological conditions to be able to offer the most appropriate management/treatment to the patients. Incorporating pre and post genetic counseling sessions before advising a genetic test can be helpful for both the clinicians and the patients. This will help in making the diagnosis much easier and help to establish the correct genotype-phenotype correlation. The value added by genetic counselor can be further enhanced if advanced in-silico and functional studies along with the right genetic tests are carried out to validate the variants to establish the appropriate diagnosis. This emphasizes the need for genetic counselors to be part of a core team involved with both the diagnosing physician and the molecular testing laboratories to achieve accurate diagnosis.

Acknowledgement

We thank the staff, students and management of the Department of Genetics & Molecular Medicine, Kamineni Hospitals and Utage Child Neuro Clinic and Development Centre, Narayanguda, Hyderabad. We also thank all our patients and families who have agreed to be the part of the study.

References

1. Ashwal, S., Rust, R. Child Neurology in the 20th Century. Pediatr Res 53, 345–361 (2003). [https://doi.org/10.1203 /01.PDR .000004 7655. 66475.52](https://doi.org/10.1203/01.PDR.0000047655.66475.52)
2. Valente EM, Ferraris A, Dallapiccola B. Genetic testing for paediatric neurological disorders. Lancet Neurol. 2008 Dec;7(12):1113-26. doi:10.1016/S1474- 4422(08)70257-6. Review. PubMed PMID:19007736.
3. Huang, Yue, et al. "Genetics of hereditary neurological disorders in children." Translational pediatrics 3.2 (2014):108.
4. Abou Tayoun AN, Krock B, Spinner NB. Sequencing-based diagnostics for pediatric genetic diseases: progress and potential. Expert Rev Mol Diagn. 2016; 16(9) :987-999.
5. Fonda Allen J, Stoll K, Bernhardt BA. Pre- and post-test genetic counselling for chromosomal and Mendelian disorders. SeminPerinatol. 2016 Feb;40(1):44-55.
6. Landrum MJ, Lee JM, Riley GR, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 2014;42 (Database issue): D980-D985.
7. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), {date}. World Wide Web URL: https://omim.org/
8. Pantel JT, Zhao M, Mensah MA, et al. Advances in computer-assisted syndrome recognition by the example of inborn errors of metabolism. J Inherit Metab Dis. 2018;41(3):533-539.
9. Home page: National Library of Medicine (US). Genetics Home Reference [Internet]. Bethesda (MD): The Library; 2013 Sep 16 [cited 2013 Sep 19]. Available from: https://ghr.nlm.nih.gov/.
10. Jagadeesh, Karthik& Wenger, Aaron & Berger, Mark &Guturu, Harendra& Stenson, Peter & Cooper, David & Bernstein, Jonathan &Bejerano, Gill. (2016). M-CAP eliminates a majority of variants of uncertain significance in clinical exomes at high sensitivity. Nature genetics. 48. 10.1038/ng.3703
11. Rudnik-Schöneborn S, Senderek J, Jen JC, et al. Pontocerebellar hypoplasia type 1: clinical spectrum and relevance of EXOSC3 mutations. Neurology. 2013;80(5):438–446.
12. Peter E. Davis, Rajna Filip-Dhima, Georgios Sideridis, Jurriaan M. Peters, Kit Sing Au, Hope Northrup, E. Martina Bebin, Joyce Y. Wu, Darcy Krueger, Mustafa Sahin and on behalf of the Tuberous Sclerosis Complex Autism Center of Excellence Research Network
13. Pediatrics.2017, 140 (6) e20164040; DOI: https://doi.org/10.1542/peds.2016-404
14. Vajsar J, Schachter H. Walker-Warburg syndrome. Orphanet J Rare Dis. 2006;1:29. Mahal DG, Matsoukas IG. The Geographic Origins of Ethnic Groups in the Indian Subcontinent: Exploring Ancient Footprints with Y-DNA Haplogroups. *Front Genet*. 2018;9:4.
15. Ahmed P H, V V, More RP, Viswanath B, Jain S, Rao MS, Mukherjee O; ADBS Consortium. INDEX-db: The Indian Exome Reference Database (Phase I). J Comput Biol. 2019 Mar;26(3):225-234. doi: 10.1089/cmb.2018.0199. Epub 2019 Jan 7. PubMed PMID: 30615482; PubMed Central PMCID: PMC6441288.
16. Sinha S, Black ML, Agarwal S, Das R, Bittles AH, Bellgard M. ThalInd, a β- thalassemia and hemoglobinopathies database for India: defining a model country- specificanddisease-centricbioinformaticsresource.HumMutat.2011Aug;32(8):887- 93. doi: 10.1002/humu.21510. Epub 2011 Jun 23. PubMedPMID:21520336.
17. Thiffault I, Lantos J. The Challenge of Analyzing the Results of Next-Generation Sequencing in Children. Pediatrics. 2016 Jan;137Suppl 1:S3-7.
18. Engel AG, Shen XM, Selcen D, Sine SM. Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment [published correction appears in Lancet Neurol. 2015 May;14(5):461]. Lancet Neurol. 2015;14(4):420–434

Source of Support:Nil
Conflict of Interest: Nil