Case Report

Genomic Exploration of Pediatric Neurological Diversity: Lessons from Clinical Exome Panel Sequencing

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Genomic Exploration of Pediatric Neurological Diversity: Lessons from Clinical Exome Panel Sequencing

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Abstract: We explore three cases of pediatric neurological diseases, *viz.* Arthrogryposis, congenital bilateral cataract and Autism by analyzing clinical exomes. As genetic variation attributing to pathogenesis is a significant bottleneck, we attempted to understand and validate them using Sanger validation. We further employ our CONVEX pipeline to infer pathogenic variants and discern the candidate genes for phenotype correlation.

Keywords: rare conditions; neurological disorders; Autism; Arthrogryposis; bilateral cataract

Introduction

Neurological disorders are quite common, with congenital/inborn disorders very difficult to diagnose or detect[1]. Intellectual disability, autism, and other associated disorders are known to affect 1-3% of the world population, with an incidence of 1 in 12,000 live births[2]. Although the NCBI's dbSNP (https://www.ncbi.nlm.nih.gov/snp/ last accessed on December 6, 2023) is validated, and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/ last accessed on December 6, 2023) has a compendium of bona fide and records, not all data is reviewed thoroughly. For example, several disease-causing genes that are known, with specific genetic diagnosis remain elusive in many cases[3], and as these are all heterogeneous groups of disorders, various mutations associated with developmental defects and dysfunction are nor properly reviewed and annotated. The last decade has led to a better understanding of disease diagnosis, owing to chromosomal breakpoint mapping, multi-omics integration, systems genomics approaches, whole-genome array-based copy-number analysis, and nanostring panels besides the emphasis on understanding the pedigree structure for molecular diagnosis[4]. In this work, we attempt to identify pathogenic mutations in three neurological diseases, *viz.* arthrogryposis, bilateral congenital cataracts, and Autism Spectrum Disorders (ASD). The autism spectrum is a neurodevelopmental condition that is visible at the beginning of early childhood and lasts throughout a person's life[5]. As it affects the nervous system, the affected person lacks cognitive, emotional, social and physical health. The severity and duration of symptoms might vary substantially with severe communication and social interaction issues with repetitive behavior patterns. Knowing the genetic origin of ASD could be one of the most critical aspects of future diagnosis and therapy. Likewise, arthrogryposis is a condition that causes a variety of joint contractures. It is a complicated aetiological illness that affects one in 3000 live babies, although the prenatal frequency is higher, signifying a high intrauterine mortality rate[6]. The disease's genetic diversity has been demonstrated by linking it to 400 distinct genes. Intrinsic/primary/fetal etiology is

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caused by abnormalities in different body sections, including the brain, nerve cells, muscles, bones, tendons, joints, etc. Amongst the 400 genes implicated, nine newly found genes including *CNTNAP1, MAGEL2, ADGRG6*, *ASXL3* and *STAC3* harbor pathogenic variants[7].

Summary of cases

The individuals were recruited from an outpatient clinic of Lifecare Hospitals, Amravati, Maharashtra, India. Before taking the patients' DNA samples and subjecting them to clinical exome sequencing, informed consent was duly obtained from their parents. A clinical exome panel was chosen to check the list of pathogenic variants with an in-house pipeline benchmarked by us to screen and filter the candidate variants[8]. We have recently established a consensus variant pipeline for exome analysis called CONVEX, and we used it to identify variants at stringency (Ujjwal and Ranjana et al. communicated 2023). The Fastq files (paired-end reads) containing the raw reads were retrieved and utilized for downstream processing. The variants filtered were obtained in variant calling format (VCF) file, and the SNP-nexus[9] yielding variants were thoroughly checked for ClinVar-mapped variations based on RefSeq (rs) IDs. GeneMANIA was used to find the genes most strongly associated with the gene responsible for these phenotypes and Phenolyzer was used to analyze distinct pathways[10,11]. The characteristics of the children with neurological disorders are discussed below.

Case summary 1

NV (name acronymed, anonymised), an 8-year-old male child, is referred to as having a known case of focal arthrogryposis with delayed development. He was born full term by the vaginal route and had meconium-stained liquor along with crossed leg and contracture of the elbow and bilateral right hamstring and iliotibial band. During six days of hospitalization, no hypoglycemia and convulsion were noted. For contracture,he was operated on at the age of 3 years. In antenatal ultrasonography, he was found to have a short femur and humerus, and the mother had hyperemesis during the whole pregnancy. He had global developmental delay (GDD) and microcephaly (head circumference of 50 cm) and other examination findings indicate broad nose, long toes, laxity of fingers, with hyperextensibility of knees and elbow. Our further counseling in lieu of familial history revealed that his sister was similarly but only mildly affected than him. On referral to the neurologist, an investigation showed normal karyotype, electromyography (EMG), and nerve conduction study (NCV) with magnetic resonance imaging (MRI) of the brain showed paucity of white matter.

Case summary 2

AB (name acronymed, anonymised), aged 7, was presented with microcephaly and autism, born from a healthy labor. The clinical examination showed failure to recognize things, with significant speech delay. He was born from a non consanguineous marriage without significant family history. Previous studies revealed that chr 14:75094726 with the gene ID (*EIF2B2)* plays a very crucial role in pathogenesis and is found in various rare diseases like inclusion body myopathy with paget disease of bone and frontotemporal dementia, chronic progressive external ophthalmoplegia, mitochondrial encephalomyopathy, isolated atrial amyloidosis, narcolepsy, neuroleptic malignant syndrome, lactic acidosis, congenital central hypoventilation syndrome and congenital myasthenic syndrome.

Case summary 3

DB (name acronymed, anonymised), an 8-month-old male, was presented for genetic evaluation i/v/o bilateral cataract, failure to thrive, and some developmental delay. The mother had a fever without a rash during the first two months of pregnancy for 2-3 days of admission. While antenatal ultrasound showed signs of early onset intrauterine growth retardation (IUGR), he was born preterm with low birth weight (1.7 kg), requiring neonatal intensive care unit (NICU) admission for eight days. The clinical examination showed microcephaly, failure to thrive, and small anterior fontanelle (AF) with a prominent metopic suture. He had normal motor development but had significant speech delay (only cooning) and was born of a non-consanguineous marriage without a significant family

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history. The ophthalmic examination showed bilateral cataracts (right > left) with intraocular calcification. Other investigations showed normal to complete hemogram, toxoplasma and other, rubella, cytomegalovirus, and herpes simplex (TORCH) negative, normal renal and liver function test except increased alkaline phosphatase level (567 units/L). The computerized tomography (CT) of the brain showed generalized cerebral shrinkage with prominence of cortical sulci and cisternal spaces with bilateral periventricular volume loss and *ex vacuo* prominence of frontal horns.

Discussions

Three variants in SMPD4 are known to be associated with arthrogryposis

SMPD4 is the only known gene implicated in Arthrogryposis even as our exome sequencing and further analyses found three variants derived from the same gene, *SMPD4* (Table 1). These are found on chromosome 2 (rs766318490, rs780446128, and rs1391542283) with minor allele frequency (MAF) for the first two variants attributing to <=0.0001%, showing that they are extremely rare variants. While the MAF of rs766318490 and rs780446128 in the GnomAD attribute minor allele to T=0.000016/4 and A=0.000004/1, respectively, the ALFA database showed T=0.000051/1 and A=0./0. rs1391542283, GeneMANIA yielded distinct interactions for *SMPD4*, and many pathways, *viz.* transcription regulation, factors, cell adhesion, chromatin binding, and neurodegeneration are associated (Figure 1). *SMPD4* is associated with ceramide and is produced by sphingomyelinases as a secondary messenger in intracellular signaling pathways involved in the cell cycle, differentiation, or death. *SMPD4* mediates TNF-stimulated oxidant generation in skeletal muscle. Genomic research showed bi-allelic loss-of-function mutations in *SMPD4*, which codes for the neutral sphingomyelinase-3 (nSMase-3/*SMPD4*). However, we could not find any *SMPD4* variants attributing to pathogenesis from our CONVEX pipeline. Proteomics research on human Myc-tagged *SMPD4* overexpression demonstrated localization to both the outer nuclear envelope and the ER and interactions with multiple nuclear pore complex proteins. Fibroblasts from afflicted people had aberrant ER cisternae, suggesting enhanced autophagy, and were more vulnerable to apoptosis under stress circumstances, whereas *SMPD4* therapy slowed cell cycle progression. It has been demonstrated that *SMPD4* connects membrane sphingolipid homeostasis to cell fate by regulating the cross-talk between the ER and the outer nuclear envelope and that its absence indicates a pathogenic mechanism in Microcephaly[12].

Table 1. List of identified pathogenic variants and the ones indicated with * are seen in the IndiGen database.

Sample	Gene	Chr Pos	RS ID	Variation	Clinical significance	Phenotypes	MAF	Database
NV	SMPD4	2:130155268	rs766318490	C>T	Pathogenic	Neurodevelopmental disorder with microcephaly,arthrogryposis, and structural brain anomalies	0.000016/4	GnomAD_exomes
NV	SMPD4	2:130173278	rs780446128	C>A	Pathogenic	Neurodevelopmental disorder with microcephaly,arthrogryposis,and structural brain anomalies	0.000004/1	GnomeAD_exomes
AB	*ZFYVE26	14:67816033	rs17192296	T/A	Conflicting interpretations of pathogenicity	Spastic Paraplegia Recessive	0.02	Qatari
AB	*DRD3	3:114128842	rs3732791	G/A	Conflicting interpretations of pathogenicity	Hereditary Essential Tremor	0.015	Korean
AB	*DNAH5	5:13900212	rs115004914	G/T	Conflicting interpretations of pathogenicity	Primary ciliary dyskinesia	0.002	Korean 1K
DB	OBSCN	1:228211913	rs371783634	G/A	NA	NA	0.000007/1	Extremely rare variant In GenomeAD

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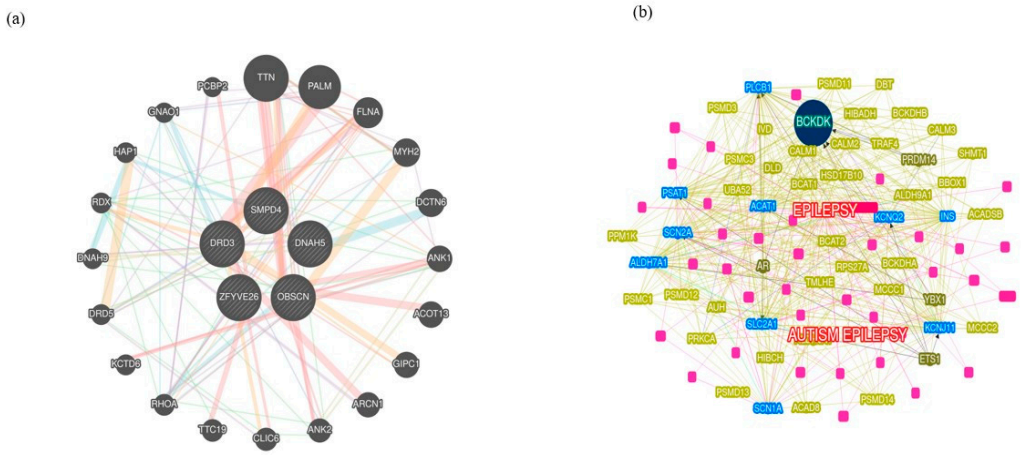
Pathogenic variants associated with microcephaly and autism

The pathogenic variants were screened for AB, and a final list of 15 variants was filtered across subpopulation databases and specific phenotype matches based on their MAF, clinical significance, and phenotypes. The four pathogenic variants, *viz.* NM_001104.4(ACTN3):c.1729C>T(p.Arg577Ter); NM_015346.4(ZFYVE26):c.-70A>T; NM_000796.6(DRD3):c.1077C>T(p.His359=); NM_001369.3(DNAH5):c.2253C>A (p.Asn751Lys). All the variants mentioned above were found on chromosome positions 11,14,3,5 respectively, and were linked with neurodevelopmental disorders like Schizophrenia and structural brain anomalies. A few extremely rare variants with MAF <=0.001 were identified for the filtered set matching the index case. These variants were further searched in the Indian genome variant database, Indigen (https://clingen.igib.res.in/indigen last accessed on December 6, 2023) in addition to ALFA and GnomAD_exomes reporting these variants (Indicated with * in Table 1), however, from the latest ClinVar mapping, we found them to be benign.

Further shortlisting to three pathogenic variants was done and were probed to check whether they are associated with inherent pathways, The genes harboring mutations are *ACTN3* (Alpha-actinin-3), *DRD3* (Dopamine receptor D3), *DNAH5* (Dynein axonemal heavy chain 5) and *ZFYVE26* (Zinc finger five types containing 26) were the candidates inherent to CBC even as we obtained D3 subtype receptor proteins inhibiting adenylyl cyclase pathways (Figure 1). The receptor is localized to the limbic areas of the brain, associated with cognitive, emotional, and endocrine function. Several literature studies show that this gene has some association with ASD[13]. A SNP of the *DRD3* gene (rs167771) was recently associated with ASD with different polymorphisms corresponding to varying degrees of behavior. In contrast, the other two genes are the normal genes unrelated to neurological disease. We further sought to check whether or not Phenolyzer pathways revealed the diagnosis of PWS and how four genes, *viz. DRD3, DNAH5, ZFYVE26*, and *ACTN3* are associated with the phenotypes represented by HPO terms. The branched-chain ketoacid dehydrogenase kinase *(BCKDK*) is a seed gene, on chromosome 16 associated with mitochondrial protein kinases family and regulates the catabolic pathways for valine, leucine, and isoleucine. By searching two related types of syndromic ASD-one caused by mutations in branched-chain ketoacid dehydrogenase kinase and the other by mutations in branched-chain ketoacid dehydrogenase kinase. Lower BCAA levels may also be detrimental to brain development, as evidenced by the discovery of BCKDH mutations in families with ASD, ID, and seizures. Other genes *ACAT1, SLC2AL, ALDH7A1, SCN2A, PSAT1, SCNIA,* and *KCNJ11* are all connected and may likely cause some neurological disorders.



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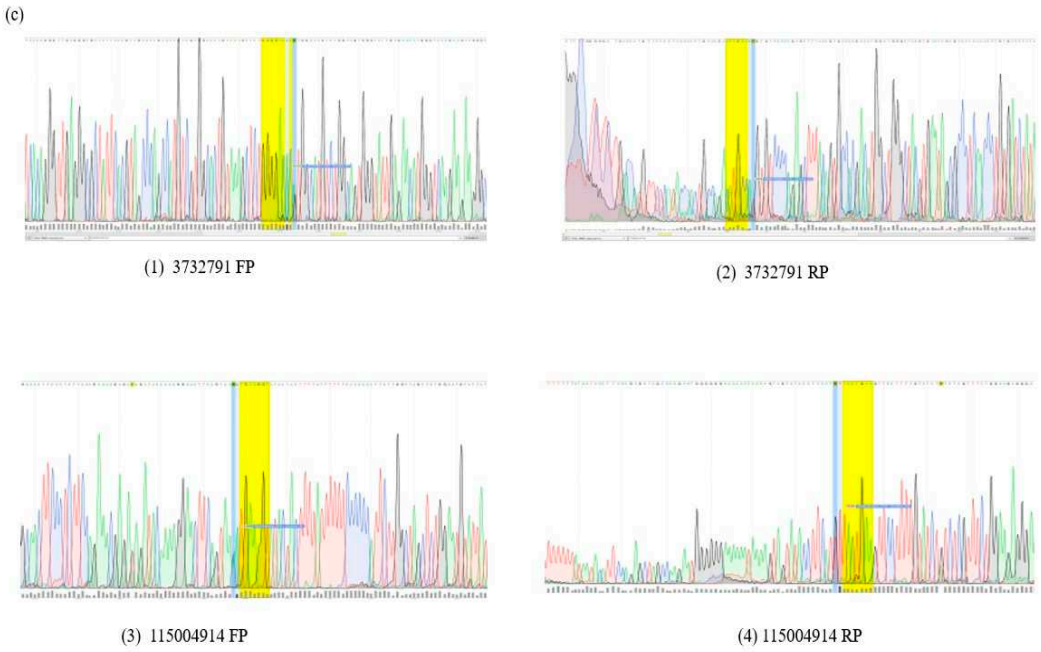

Figure 1. (a): GeneMANIA- Interaction network (all interactions) with physical interaction network as indicated with pink edges and (b) Phenolyzer map of all genes associated with distinct pathways. The colored genes match epilepsy. The most disease-relevant genes are shown as seed genes alongside predicted genes in the deletion regions. Green lines show that the two node genes belong to the same gene family, whereas yellow lines indicate that they belong to the same biosystem. This pink node represents the diseased phenotypes that we enter regarding our disease. It includes autism spectrum disorder, epilepsy, neuroleptic malignant syndrome, and neuro abnormalities. (c) Sanger validation results.

Conclusions

Genetic variation attributing to pathogenesis is a significant bottleneck. In this work, we attempted to understand rare neurological disorders in an Indian pediatric cohort using exome studies. While we used both pipelines, the CONVEX pipeline did not yield pathogenic variants for consensus variant calling tools. Nevertheless, *EIF2B2* is inherently pathogenic, and the interpretations match the two pipelines. While these are the diseased correlations, we find that neuroleptic malignant syndrome may cause brain damage, which is a matching phenotype. Our study has certain limitations, viz. (a) parental genotyping or family exome analyses was not done, which could bring candidate germline mutations associated with these disorders. Despite the lack of parental data and the potential for misdiagnosis, this study identifies the inherent pathogenicity of *EIF2B2* and its association with neuroleptic malignant syndrome and brain damage. However, we acknowledge that our findings are specific to a particular region in India and may not be attributable to the entire population. (b) The potential presence of intronic variants and the limited scope of exome sequencing necessitate further investigation with whole-genome sequencing and functional studies. Identifying *EIF2B2* as a disease-causing gene offers a promising avenue for future research and development of targeted therapies for these rare neurological disorders. Small sample sizes may not be typical for the general population, especially in rare diseases. If the research population is not diverse, the results may not be applied to other ethnic or demographic groups. The clinical exome panel may not cover all crucial genes or areas, and variation categorization might be complex and subjective. Exome sequencing may overlook regulatory regions and non-coding variations that may play a role in illness, and variant identification methods may yield false positives or false negatives. Using ClinVar alone to evaluate variants has limits, and prediction methods may not always adequately represent *in vivo* biological importance. Finally, as monogenic disorders are rare and poorly understood, it hints at a general restriction in our understanding of rare diseases. Because the

CONVEX pipeline is considered a consensus variant pipeline, it is critical to confirm its performance by comparing it to known standards and datasets. Because genomics is a dynamic field, new genes and variant classifications might develop, affecting study findings' relevance and accuracy over time. This research lays the groundwork for further explorations into the genetic landscape of rare neurological disorders in the Indian population, paving the path for improved diagnosis, treatment, and, ultimately, a brighter future for those affected by these debilitating conditions.

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Conflicts of Interest: None.

Ethics clearance: Informed consent and ethical approvals were taken duly before carrying out the clinical exome panel.

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