Case Report

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

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Case Report

Genomic Exploration of Pediatric Neurological Disorders: A Case Reports

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Abstract: Background: Pediatric neurological disorders include neurodegenerative disease causing cognitive impairment, vision loss to mention a few. They are one of the important causes of morbidity and mortality in children with diverse etiology. Diagnosis is difficult in spite of genetic work and in only 60% of cases final diagnosis can be achieved. **Case presentation:** We explore three cases of pediatric neurological diseases, *viz*. Arthrogryposis, congenital bilateral cataract and Autism by analyzing clinical exomes. In this work, we attempted to understand rare neurological disorders in an Indian pediatric cohort using exome studies. **Conclusions:** We used our benchmarked CONVEX pipeline for screening consensus variants, wherein *EIF2B2* was found to be inherently pathogenic. We map the association of variants and genes, disease correlation to neuroleptic malignant syndrome which is a matching phenotype to the cases.

Keywords: rare conditions; neurological disorders; autism; arthrogryposis; bilateral cataract; case report

1. Introduction

Neurological disorders are quite common, with congenital/inborn disorders very difficult to diagnose or detect[1]. Intellectual disability (ID), autism, and other associated disorders are known to affect 1%-3% of the world population, with an incidence of 1 in 12000 live births[2]. Although the NCBI's dbSNP (https://www.ncbi.nlm.nih.gov/snp/ last accessed on July 1, 2024) is validated, and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/ last accessed on July 1, 2024) has a compendium of *bona fide* 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 with global developmental delay (GDD) 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,

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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 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].

2. Case Presentation

Chief complaints

Case 1: Arthrogryposis congenita with delayed development.
Case 2: Small head and autism.
Case 3: Bilateral cataract, failure to thrive (FTT), and some developmental delay.

History of past illness

Case 1: In antenatal ultrasonography, he was found to have a short femur and humerus, and the mother had hyperemesis during the whole pregnancy. 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.
Case 2: Parents noticed developmental delay at 18 months of age and the development still lagged behind.
Case 3: 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, he was born preterm with low birth weight (1.7 kg), requiring neonatal intensive care unit admission for eight days.

Personal and family history

Case 1: Our further counseling in lieu of familial history revealed that his sister was similarly but only mildly affected than him.
Case 2: He was born from a non consanguineous marriage without significant family history.
Case 3: Born of a non-consanguineous marriage without a significant family history.

Physical examination

Case 1: He had 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.
Case 2: The clinical examination showed microcephaly, hypertonia, failure to recognize things, with significant speech delay.
Case 3: The clinical examination showed microcephaly, FTT and small anterior fontanelle with a prominent metopic suture. He had normal motor development but had significant speech delay (only cooning).

Laboratory examinations

Case 1: On referral to the previous investigation from a neurologist, an investigation showed normal karyotype, electromyography and nerve conduction study.
Case 2: Thyroid and routine blood investigations were normal.
Case 3: 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 negative, normal renal and liver function test except increased alkaline phosphatase level (567 U/L).

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Imaging examinations

Case 1: Magnetic resonance imaging (MRI) of the brain showed paucity of white matter.
Case 2: MRI brain was normal.
Case 3: The computed tomography 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.

FINAL DIAGNOSIS

Case 1

Patient was thought to be suffering from arthrogryposis congenita most probably due to neurological causes.

Case 2

Case of complex autism phenotype with microcephaly probably due to genetic cause.

Case 3

Case of GDD with bilateral cataract to rule out genetic or metabolic etiology.

TREATMENT

All of them were advised on physiotherapy and developmental and stimulation therapy but none of them on regular treatment.

OUTCOME AND FOLLOW-UP

Case 1

Able to walk with difficulty. No further deterioration or improvement from previous level.

Case 2

Had some improvement in speech and social communication but still significant lag present.

Case 3

Had more neurological deterioration. Not able to sit, stand or communicate.

3. DISCUSSION

Three variants in SMPD4 are known to be associated with Arthrogryposis

SMPD4 is the only known gene implicated in Arthrogryposis from our exome sequencing analyses and further, we found three variants derived from the same gene, *SMPD4* (Table 1)[8]. 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 Allele Frequency Aggregator (ALFA) database showed T = 0.000051/1 and A = 0./0. rs1391542283, GeneMANIA[9] 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 tumour necrosis factor-stimulated oxidant generation in skeletal muscle. Genomic research showed bi-allelic loss-of-function mutations in *SMPD4*, which codes for the neutral sphingomyelinase-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 endoplasmic reticulum (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[10].

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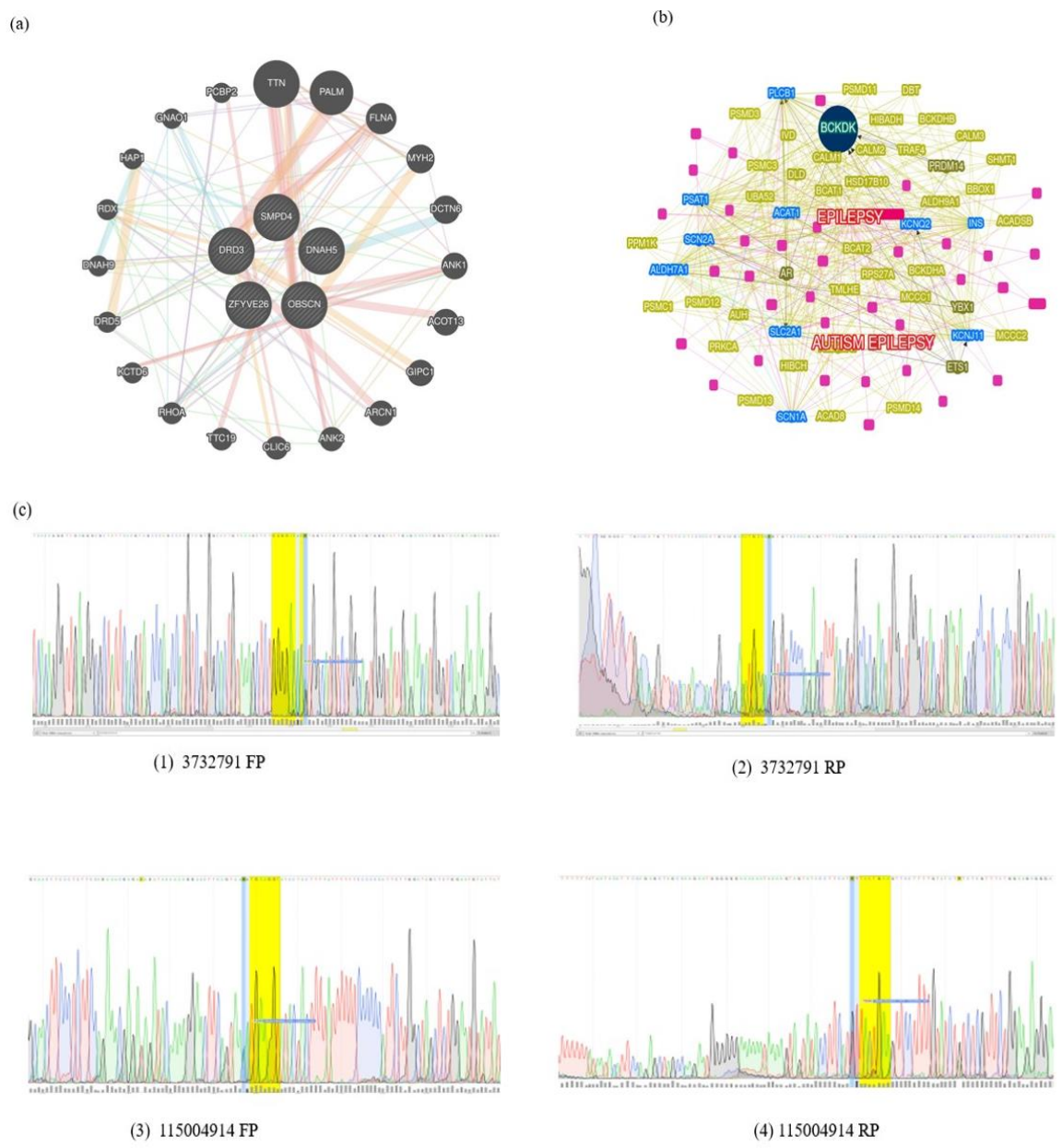

Figure 1. Gene networks and validation of single nucleotide polymorphisms. A: GeneMANIA-interaction network (all interactions) with physical interaction network as indicated with pink edges; B: Phenolyzer map of all genes associated with distinct pathways. The colored genes match epilepsy. The most disease-relevant 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 disorders, epilepsy, neuroleptic malignant syndrome, and neuro abnormalities; C: Sanger validation results.

Table 1. List of identified pathogenic variants.

Sample	Gene	Chr:position	Rs ID	Variation	Clinical significance	Phenotypes	MAF	Database
NV	*SMPD4*	2:130155268	rs766318490	C>T	Pathogenic	Neurodevelopmental Disorder With Microcephaly,	0.000016/4	GnomAD_Exomes

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						Arthrogryposis, Structural Brain Anomalies, and Microcephaly		
NV	*SMPD4*	2:130173278	rs780446128	C>A	Pathogenic	Neurodevelopmental Disorder With Microcephaly, Arthrogryposis, Structural Brain Anomalies, and Microcephaly	0.000004/1	GnomAD_Exomes
AB	*ZFYVE261*	14:67816033	rs17192296	T/A	Conflicting interpretations of pathogenicity	not specified,Spastic Paraplegia, Recessive	0.02	Qatari
AB	*DRD31*	3:114128842	rs3732791	G/A	Conflicting interpretations of pathogenicity	Hereditary Essential Tremor,not provided	0.015	Korean
AB	*DNAH51*	5:13900212:	rs115004914	G/T	Conflicting interpretations of pathogenicity	Primary ciliary dyskinesia,not specified,not provided	0.002	Korea1K
DB	*OBSCN*	1:228211913	rs371783634	G/A	NA		0.000007/1	Extremely rare variant

1 The ones are seen in the IndiGen database.

Pathogenic variants associated with microcephaly and autism

The pathogenic variants were screened for case 2, 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 July 1, 2024) in addition to ALFA and GnomAD_exomes reporting these variants (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 alpha-actinin-3 (*ACTN3*), dopamine receptor D3 (*DRD3*), dynein axonemal heavy chain 5 (*DNAH5*) and zinc finger five types containing 26 (*ZFYVE26*) were the candidates inherent to congenital bilateral cataract even as we obtained D3 subtype receptor proteins inhibiting adenylyl cyclase pathways (Figure 1). The receptor is localized to the limbic areas

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of the brain, associated with cognitive, emotional, and endocrine function. Several literature studies show that this gene has some association with ASD[11,12]. A single nucleotide polymorphism of the *DRD3* gene (rs167771) was recently associated with ASD with different polymorphisms corresponding to varying degrees of behavior[13]. 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 human phenotype ontology terms[14]. 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 *BCKDK* and the other by mutations in *BCKDK*. 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. ASD is a complex neurodevelopmental condition characterized by social communication deficits and repetitive behaviors[10,11]. Recent research has highlighted the involvement of molecular factors in ASD, with particular attention to the glycoprotein Reelin. Encoded by the *RELN* gene, Reelin plays a crucial role in neuronal migration and synaptic plasticity during brain development, notably in the cerebral cortex and cerebellum, influencing neural circuit formation. Altered Reelin expression in individuals with autism suggests its potential contribution to atypical neural connectivity. The downstream effects of Reelin on molecular pathways, including the modulation of GABAergic interneurons, further enhance its role in autism pathogenesis. Recent findings underscore the importance of considering Reelin's role in the biological etiology of autism, emphasizing the intricate interplay of genetic and environmental factors[15]. These insights not only advance our understanding of autism's biological underpinnings but also offer promising avenues for targeted therapeutic interventions,shaping the future of diagnostic and therapeutic strategies for individuals with autism

4. 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. The CONVEX pipeline did not yield pathogenic variants for consensus variant calling tools, nevertheless, *EIF2B2* is inherently pathogenic. 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*. (1) 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 attributed to the entire population; and (2) 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. We argue that 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. Further 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[16]. As genomics is a dynamic field,

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new genes and variant classifications might develop, affecting study findings' relevance and accuracy over time. In conclusion, 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.

Author Contributions: Tayade N and Krishna A A contributed equally to this work as co-first authors; Tayade N and Suravajhala P conceptualized the work and proofread the manuscript; Suravajhala P, Krishna A A, Manoj G and Kewat A performed the analyses; Devulapalli R, Kumar S, Polipalli SK, Nair BG and Bandapalli OR contributed to write-up figures and writing the manuscript.

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