Original Article

Copy Number Variants in 30 Saudi Pediatric Patients with Neurodevelopmental Disorders: From Unknown Significance to Diagnosis

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Abstract

Background: Structural variants (SVs), such as copy number variants (CNVs), insertions, deletions, inversions, and translocations, contribute significantly to genetic diversity and disease etiology. CNVs, which involve the duplication or deletion of DNA segments, are particularly impactful on genes crucial for biological functions and disease processes.

Objective: To reassess unclassified SVs that may be underlying unresolved neurodevelopmental disorders among Saudi patients.

Methodology: In this retrospective study conducted at King Saud Medical City, Riyadh, Saudi Arabia, 30 probands with neurodevelopmental disorders and congenital malformations were examined using next-generation sequencing methods—exome sequencing, gene panels, or SNP arrays (the Illumina platform). Reclassification was aided by online tools such as VarSome and ClinVar, with pathogenicity assessments using the ClinGen CNV Pathogenicity Calculator based on American College of Medical Genetics and Genomics criteria for CNV loss and gain, and dosage sensitivity.

Results: A total of 31 CNVs were analyzed, of which 2 were reclassified: one as benign and the other as pathogenic. The pathogenic CNV, [3p13p12.3 (70411134_75249376) x1], included a deletion of the FOXP1 gene and was associated with an intellectual developmental disorder, language impairment, possible autistic features, psychomotor impairment, developmental regression, and epilepsy.

Conclusion: This study underscores the importance of continuously documenting and revisiting unclassified CNVs in accessible databases to enhance the diagnosis and understanding of complex genotype–phenotype relationships. Reclassifying these CNVs not only accelerates diagnostic processes but also enriches our insight into their significant roles in health and disease.

Keywords: Array, copy number variant, exome sequencing, *FOXP1*, variant of unknown significance

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INTRODUCTION

The human genome is replete with structural variations (SVs), which include a broad range of alterations such as copy number variants (CNVs), insertions, deletions, inversions, and translocations. While many SVs are benign and have minimal impact on health, others are pathogenic and play a significant role in the etiology of genetic disorders. The prevalence and impact of these variations are crucial for understanding disease mechanisms.[1,2]

Genetic disorders can be diagnosed using various methods, each identifying different types of genetic abnormalities. Single nucleotide variants are the most common cause of genetic disorders, accounting for approximately 85% of known mutations.[3-5] In contrast, CNVs and other structural variants contribute to about 10%–15% of genetic conditions. Other significant genetic abnormalities include triple nucleotide repeat disorders, chromosome abnormalities, and imprinting disorders, each contributing variably to genetic pathology but are significant in specific contexts.[6,7]

Copy number variants, which involve gains or losses of DNA segments, can significantly influence gene function. Pathogenic CNVs typically affect genes that are critical in development and are highly conserved throughout evolution. Despite covering 12% to 16% of the human genome, only a fraction of CNVs are considered rare and clinically significant. These rare CNVs account for approximately 10% of the SVs associated with rare diseases.[8]

Challenges in diagnosing rare diseases often arise from unresolved cases with variants of unknown significance (VUS), which account for a substantial portion of genetic disorders. The complexity of phenotype–genotype correlations and limitations in variant annotation and bioinformatics can impede the accurate diagnosis of these conditions.[8,9] The American College of Medical Genetics and Genomics (ACMG) recommends revisiting undiagnosed cases periodically and reanalyzing data to uncover potential molecular causes.[10]

Furthermore, studies have shown that CNVs can also impact methylation sites across the genome, and thus further research exploring the role of methylation in CNVs is crucial as methylation patterns can influence gene expression and contribute to regulatory effects in various diseases. This deeper epigenetic layer of complexity may also affect the phenotypic outcome of a seemingly benign CNV.[11] This study aims to reassess unsolved cases with CNVs of unknown significance among Saudi patients, enhancing the diagnostic process and paving the way for future research that explores the intricate interplay between genetic alterations and epigenetic modifications.

METHODOLOGY

Sample size and data collection

This retrospective study was conducted at King Saud Medical City, Riyadh, Kingdom of Saudi Arabia, between September 2020 and December 2021. We included 30 pediatric patients diagnosed with neurodevelopmental disorders (NDDs) or congenital malformations, or both. The cohort comprised 26 probands aged 1 month–5 years, three probands aged 6–10 years, and one proband aged 12 years. In total, 31 CNVs classified as VUS were identified, with 1 patient exhibiting two distinct CNVs. Genetic testing was performed in a CAP-accredited commercial laboratory using next-generation sequencing methods, including exome sequencing (ES), ES-based gene panels, and SNP arrays on the Illumina platform, aligned to the reference genome GRCh37/hg19. The collected data encompassed patient demographics, family history, consanguinity, phenotypic presentation, clinical investigations, and genetic results, all documented confidentially in Excel for analysis.

Study design

The study was designed to reevaluate cases of NDDs and congenital malformations that lacked a molecular diagnosis. We included patients presenting with CNVs of uncertain significance and excluded those with confirmed CNVs (pathogenic or likely pathogenic) where the genotype matched the phenotype. Using online tools such as VarSome and ClinVar,[12] we revisited significant and previously unclassified CNVs for potential reclassification. In addition, the ClinGen CNV Pathogenicity Calculator was used to assess each CNV for pathogenicity following the ACMG criteria for CNV loss and gain,[13] as well as dosage sensitivity.[14] The phenotypes of the patients were evaluated in terms of their relationship to the identified CNVs, and categorized as related, potentially explanatory, partially explanatory, uncertain, or unrelated.

Databases used

For each CNV, we utilized VarSome version 16.1 to determine if the variation had been previously observed and in other patients.[10] The ClinGen CNV Interpretation Calculator was applied to reclassify each CNV for gains and losses of function.[15] We also utilized DECIPHER version 11.16[16] and ClinVar Structural Variants report through DECIPHER to identify matching patients with the same CNVs and to explore other features such as dosage sensitivity.[17,18] Additional resources included Database of Genomic Variants (DGV)

Gold Standard and gnomAD databases for gain-and-loss of function research. For gains, we considered the pTriplo score (>0.94) from DECIPHER, triplosensitivity scores from ClinGen, and regulatory features. For losses, we evaluated population CNV, pHaplo score (>0.86) from DECIPHER, haploinsufficiency scores from ClinGen, probability of loss of function intolerance (pLI > 0.9) from gnomAD, observed/expected upper bound fraction (LOEUF < 0.35) from gnomAD, and regulatory features [Figure 1].

Ethical considerations

The study was approved by the Institutional Review Board of King Saud Medical City Research Centre and King Abdullah International Medical Research Center. Informed consent was obtained from the parents/guardians of all patients, ensuring compliance with ethical standards for research involving human subjects.

RESULTS

The targeted phenotypes are related to central nervous system manifestations, including NDDs, psychomotor impairment, developmental regression, and epilepsy [Figure 2]. Other cases involved a variety of health

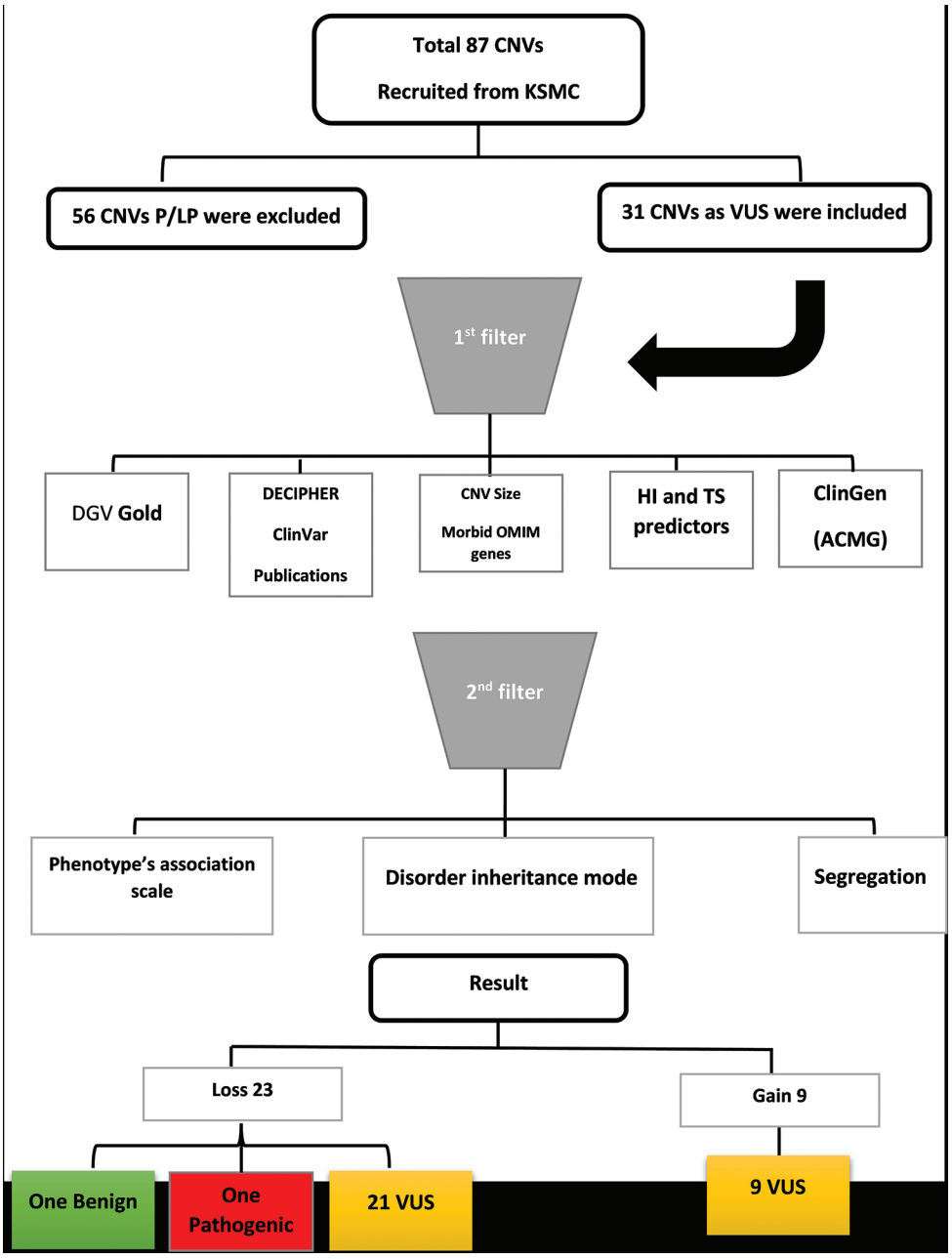

Figure 1: Schematic diagram representing the analysis pipeline for copy number variant analysis. CNV: Copy number variant, KSMC: King Saud Medical City, VUS: Variants of unknown significance, DGV: Database of genomic variant

conditions. The CNVs were classified based on genetic dosages into two groups: nine CNVs with duplications and 23 CNVs with deletions.

All nine CNVs with duplications remained of unknown significance. One CNV with a deletion was reclassified as benign, located at [1p33 (49917287_49997674) x1] with a size of 80 kb, spanning two genes: *AGBL4* (intragenic) and *AGBL4-IT1* (terminal deletion). This CNV has been observed three times in the normal population and in two patients in DECIPHER, where it was classified as a VUS. It was also found in 128 of 18,066 cases (0.71%) in the DGV gold standard.

One CNV with a deletion was reclassified as a pathogenic variant. This variant is located at [3p13p12.3 (70411134_75249376) x1], with a CNV size of 4838 kb, spanning the following 19 genes: *FOXP1, FOXP1-AS1, MIR1284, EIF4E3, GPR27, PROK2, LINC00877, LINC00870, RYBP, LOC105377162, SHQ1, GXYLT2, PPP4R2, EBLN2, PDZRN3, LOC101927296, PDZRN3-AS1, LINC02005,* and *CNTN3*. Three of these genes—*FOXP1, PROK2,* and *SHQ1*—are considered morbid OMIM genes. Although this CNV was observed in the normal population, it was detected twice in ClinVar and DECIPHER, where it was classified as pathogenic.

DISCUSSION

Our findings affirm that significant CNVs, especially large ones, play a pivotal role in NDDs.[1] Advanced analytical technologies and *in silico* tools have enhanced our ability to identify and predict the regulatory effects of these CNVs, facilitating a faster diagnostic journey for patients by linking findings to national and international databases.[19,20] This linkage not only speeds up the pathogenic or benign classification of new variations but also supports the reclassification of previously unclassified variations.[1]

In this study, we screened 31 CNVs with unknown significance and revealed new classifications for two variants. One was a deletion in [1p33 (49917287_49997674) x1], which was reclassified as benign; thus, it was eliminated as the cause of the patient's phenotype. The other CNV loss, in heterozygous status, at 3p13p12.3 (70411134_75249376) x1, was reclassified as a pathogenic variant. This CNV has three OMIM morbid genes, two of which were excluded from being phenotype related because one is H/ACA ribonucleoprotein assembly factor (*SHQ1*) gene and its inheritance requires biallelic involvement. The other gene, prokineticin 2 (*PROK2*), was excluded because it is not known to cause a similar phenotype]. The aforementioned CNV encompasses deletion of Forkhead Box P1 (*FOXP1*), which is well described as being associated with an autosomal dominant intellectual developmental disorder with language impairment, with or without autistic features (OMIM: 613670).[15] The gene belonging to the transcription factor family *FOXP1* is a transcriptional suppressor that interacts with several genes and plays an essential role in regulating organ development.[21,22] This gene does not tolerate loss of function, as indicated by several HIT predictors[15,16] Several previous probands harboring different types of pathogenic or likely pathogenic variant in *FOXP1*, including CNV loss spanning part or the entire gene, frameshift, nonsense and splice variants, demonstrated the presence of a neurological disorder as a common finding.[22,23] The phenotype of individuals with a pathogenic variant in *FOXP1* includes congenital heart disease, pulmonary stenosis, diaphragmatic hernia, liver disorder, antenatal and postnatal growth retardation, genital abnormalities, and non-specific dysmorphic features.[16,17,23,24] The CNV deletion that harbored *FOXP1* was detected by ES, and the patient did not harbor other potential variants that could account for their phenotype.

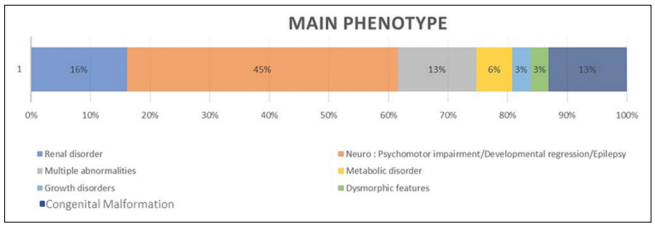

Figure 2: Phenotypes associated with each patient included in the study

The result was conducted using ES and SNP arrays, both of which have certain limitations in identifying SV. Most of our data were analyzed using an SNP array, knowing that there are certain limitations, such as genome-wide SNP data related to a certain population. The result includes ascertainment bias due to pre-ascertained SNPs that are commercially available for a specific population.[25-27] The rest of the data were analyzed using ES with limited-read 150–300 bp with a low detection rate compared with long-read sequencing of >10 kb, with a detection rate reaching 80%.[28,29]

Reinvestigating CNVs classified as a VUS is of major interest for at least two reasons: (1) if it is classified as benign, it is possible to close the loop of investigation of the variant, to consider other leads, and to reassure the patient about the lack of pathogenicity of the specific CNV, or (2) if the VUS is ultimately pathogenic, it is possible to name the disorder for the patient, to specify genetic counselling, to avoid further delay and expensive techniques, and to propose a plan of treatment.[30,31] In 2010, 67 individuals with intellectual disabilities were investigated

with high-resolution arrays: 301 CNVs were found and analyzed, of which 19% were classified as pathogenic, 6% as benign, and 75% as a VUS. The CNVs were reanalyzed in 2012, and there was a statistically significant difference in the assessment of CNVs ($P < 0.0001$). More than eight patients were reclassified as having a pathogenic CNVs, and several additional susceptibility or modifier CNVs were identified.[31]

A study by Hollenbeck *et al*. (2017) demonstrated the need for careful clinical interpretation and for including small (<500 kb) nonrecurrent CNVs during clinical investigations. These small CNVs can also facilitate the detection of new genes implicated in the pathogenesis of disorders and malformations.[32] The diagnostic workflow for unresolved patients with CNVs also needs to be established, relying on the identification of rare CNVs, determining their inheritance patterns and understanding the contribution of CNVs to genomic disorders not only via *de novo* occurrence but also via X-linked and recessive inheritance patterns, as well as models that account for mosaicisms, imprinting, and digenic inheritance.[33]

Our findings highlight the pivotal role of CNVs in NDDs and advocate for more in-depth phenotypic correlations to bolster our assertions. Specifically, the reclassification of CNVs such as the pathogenic [3p13p12.3 (70411134_75249376) x1], which impacts the *FOXP1* gene, underscores the necessity for detailed analysis of CNV-related clinical presentations and their developmental consequences. Enhancing our dataset with longitudinal studies tracking the progression of identified phenotypes would provide valuable insights into the variable expression of traits associated with these CNVs. This approach could significantly aid in achieving personalized healthcare interventions.

However, our study is limited by a relatively small cohort of 30 probands, which may affect the statistical power and limit the general application of our findings. While expanding the sample size would enhance the robustness of our conclusions, the specific and rare nature of the CNVs studied poses challenges in recruiting larger cohorts. Future studies should consider collaborative or multicentric efforts to gather a more extensive dataset, which could offer deeper insights into the genomic architecture and its implications in neurodevelopmental and congenital disorders.

Moreover, integrating more detailed phenotypic correlations would strengthen the assertions regarding the impact of specific CNVs. By documenting and analyzing these correlations, our research not only contributes to the existing body of knowledge but also sets a precedent for future studies, ensuring comprehensive genotype–phenotype mapping is achieved.

The relationship between genetic and epigenetic variations, particularly the interaction between CNVs and methylation patterns, is an area of active research. Methylation, often occurring in CpG islands, is influenced by CNVs and may regulate gene expression by affecting noncoding regulatory elements such as promoters and enhancers. Understanding these methylation dynamics could elucidate new mechanisms by which CNVs contribute to disease phenotypes, particularly in complex disorders such as cancer, where altered methylation patterns frequently accompany somatic copy number alterations.[11]

CONCLUSION

This study highlights the necessity of establishing a comprehensive genetic database for the Saudi population to enhance CNV diagnosis and treatment strategies. Although the reclassification of only one benign and one pathogenic variation may seem modest, such findings are vital for resolving VUS, thus accelerating the classification and diagnostic processes. It is crucial to revisit and document unclassified variations to aid the diagnosis of unsolved cases. In addition, exploring the methylation effects in duplicated CNVs could further elucidate their biological and clinical impacts.

Ethical considerations

The study was approved by the Institutional Review Board of King Saud Medical City Research Centre (Protocol Approval No: H1R1-01-Aug21-01; date: August 2021), and King Abdullah International Medical Research Center (Approval no.: SP 19.161.R). Informed consent was obtained from the parents/guardians of all patients. The study adhered to the principles of the Declaration of Helsinki, 2013.

Peer review

This article was peer-reviewed by two independent and anonymous reviewers.

Data availability statement

Data have been submitted to ClinVar database (Submission ID: SUB13005929).

Author contributions

Conceptualization: A.A.Q.; Methodology: A.A.Q.; Data analysis: T.A.O., K.K.N., and M.J.A.S.; Writing–original draft preparation: R.S.A and M.M.A.E; Writing – review

and editing: A.A.Q., R.S.A and M.M.A.E., T.A.O., K.K.N., and M.J.A.S.; Supervision: A.A.Q., R.S.A., and M.M.A.E.

All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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