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Increased Prevalence of Rare Copy Number Variants in Australian Children With Fetal Alcohol Spectrum Disorder: Experience in a State-Wide Diagnostic Service

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ABSTRACT

Background: Prenatal alcohol exposure (PAE) can result in fetal alcohol spectrum disorder (FASD), a permanent, heterogeneous condition characterized by severe neurodevelopmental impairments with or without microcephaly, characteristic facial dysmorphology, and congenital anomalies. Clinical and pre-clinical studies suggest that genetic factors, in the mother or fetus, influence the phenotypic outcomes following PAE. The objectives of this study were to evaluate the prevalence and types of DNA copy number variants (CNVs) in children (0–18 years) diagnosed with FASD and to identify genes known to be associated with neurodevelopmental disorders.

Methods: Chromosomal microarray (CMA) was performed in children diagnosed with FASD in the NSW CICADA FASD Service—a state-wide multidisciplinary diagnostic clinic in Australia. We performed a retrospective chart review of 175 patients diagnosed with FASD between 2015 and 2022 to obtain data on medical and family history, phenotype, and genotype. All CMA results were reviewed by a clinical geneticist.

Results: A rare CNV was identified in 38 of 157 (24.2%) patients who had CMA testing and 40 unique CNVs were identified. In four patients, a neurodevelopmental susceptibility syndrome was identified including Xq28 microduplication syndrome, 16p12.2 microdeletion syndrome, 17q11.2 microduplication syndrome, and an atypical variant of 22q11.2 deletion syndrome. Two children had incidental pathogenic variants. In 32 of 38 (84.2%) cases with a rare CNV, 34 variants of unknown significance (VOUS) were identified, of which 13 encoded a total of 14 genes associated with common neurodevelopmental disorders. Three variants of uncertain significance (VOUS) encoded different subunits of the voltage-dependent calcium channel complex protein (CACNB2, CACNA1A, CACNA2D3).

Conclusion: These novel results suggest that approximately one in four children diagnosed with FASD may harbor a rare CNV, of which 85.0% are currently of uncertain clinical significance. Genetic testing can support the FASD diagnostic process and may reveal additional diagnoses or sources of genetic vulnerability to FASD.

1 | Introduction

Alcohol is an established teratogen that readily crosses the placenta and may harm the developing embryo and fetus (Burd et al. 2012). Prenatal alcohol exposure (PAE) can result in fetal alcohol spectrum disorder (FASD), a permanent but heterogeneous condition characterized by severe neurodevelopmental impairments with or without microcephaly, characteristic facial dysmorphology, growth delay and other congenital anomalies (Popova et al. 2023). The FASD facial phenotype that is highly specific to PAE (short palpebral fissure length, a smooth philtrum and thin upper lip) is dependent on the timing of exposure and occurs in only a minority (~10%) of individuals diagnosed with FASD (Astley Hemingway 2020).

FASD is a common and preventable cause of developmental disability worldwide, with an estimated global prevalence of 7.7 per 1000 live births in the general population (Lange et al. 2017). Comorbid neurodevelopmental disorders associated with FASD include global developmental delay (GDD), intellectual disability (ID), autism spectrum disorder (ASD), and attention deficit hyperactivity disorder (ADHD) (Popova et al. 2023). Not all children with PAE develop FASD, and twin and animal studies provide convincing evidence that genetic risk factors and epigenetic responses to PAE may confer susceptibility to, or resistance against, the development of FASD (Astley Hemingway et al. 2018; Debelak and Smith 2000; Eberhart and Parnell 2016; Sambo and Goldman 2023).

In many human conditions, a proportion of the genetic risk is attributed to chromosomal variations called copy number variants (CNVs). A CNV is defined as a segment of DNA greater than 1 kilobase (Kb) that is present in a higher (duplication) or lower (deletion) copy number compared with a reference genome (Redon et al. 2006). These CNVs are below the detection threshold of a traditional karyotype test (limit of resolution ~5 million base pairs [5 Mb]). CNVs contribute to variability of the genome among individuals and may have no phenotypic effect (benign) or underpin pathogenic disease (pathogenic). Many are regarded as being of uncertain clinical significance (variant of unknown significance—VOUS) (Zarrei et al. 2015), due to a lack of existing confirmatory data regarding pathogenicity. CNVs are routinely identified by high-resolution chromosomal microarray analysis (CMA), utilizing comparative genomic hybridization (CGH) array technology and, more recently including single nucleotide polymorphism (SNP) microarray analysis. CMA is currently recommended as first-tier genetic testing for all children with developmental delay and/or congenital anomalies (Miller et al. 2010) and has therefore emerged as a first-line investigation for children undergoing diagnostic evaluation for FASD. However, it can be challenging for clinicians to interpret the relative contribution to the FASD phenotype of a positively identified CNV, particularly a VOUS, in the context of concurrent PAE.

Evidence supporting the clinical utility of CMA in patients with FASD has emerged from small cohorts of patients referred to genetic services with suspected FASD. Clinically significant CNVs have been reported with a frequency of 7.4% and 14.3% in a subset of cases that meet diagnostic criteria for FASD (Douzgou et al. 2012; Jamuar et al. 2018). This is consistent with the knowledge that the FASD phenotype may overlap with other genetic syndromes. The first case–control analysis of CNVs in a small cohort of 95 individuals diagnosed with FASD identified rare CNVs of potential significance to the FASD phenotype in 13% of cases (Zarrei et al. 2018). In that study, rare CNVs were defined as variants that encode for genetic deletion and duplication syndromes and/or novel genes with established roles in brain function, ASD, or other neurodevelopmental disorders. The potential mechanism(s) through which PAE may interact with neurodevelopmental genes to impact an FASD phenotype remain(s) poorly understood, and the clinical significance of harboring a gene of interest to FASD is yet to be elucidated.

Current evidence suggests that a pathogenic, or potentially pathogenic, CNV may be identified in up to one in seven patients diagnosed with FASD; however, validation of this finding in larger FASD cohorts is merited. The objective of our study is to define the clinical utility of CMA in a cohort of children diagnosed with FASD in a tertiary, state-based, multidisciplinary FASD diagnostic service in Australia. First, we aim to describe the type and frequency of rare CNVs in this cohort. Second, we aim to identify genes in CNVs that are known to be associated with neurodevelopmental disorders. Finally, we aim to compare our results to the established evidence and discuss the clinical implications of finding a pathogenic CNV, or CNV containing an FASD gene of interest, in a patient fulfilling FASD criteria.

2 | Methods

2.1 | Study Design and Patients

We performed a retrospective case review of all patients diagnosed with FASD between January 2015 and January 2022 through the Care and Intervention for Children and Adolescents affected by Drugs and Alcohol (CICADA) FASD Assessment Service within the Sydney Children's Hospitals Network at Westmead, New South Wales (NSW), Australia. The CICADA FASD Assessment Service comprises a state-wide, tertiary diagnostic clinic providing multidisciplinary assessment of infants, children, and adolescents aged 0–18 years, with confirmed PAE and/or in whom the diagnosis of FASD is suspected. A positive FASD case was defined using the diagnostic criteria outlined in the Australian Guide to the Diagnosis of FASD (Bower and Elliott 2016), which requires confirmed PAE, or the presence of the three sentinel facial features (SFF), and a severe impairment in at least three developmental domains for criteria to be met.

2.2 | Data Collection Including Original CMA Outcomes

The medical record of each child with FASD was reviewed to obtain the following information: demographic data, referral details, birth history, history of PAE, history of prenatal exposure to other toxins, family history, social history, medical history, physical examination findings, presence of major and minor congenital anomalies, the three sentinel facial features (SFF) associated with FASD, neurodevelopmental impairments and the results of biological investigations including genetic testing (Fragile X and CMA). Information collected from the original CMA report

included the year and site of testing, array methodology and reported outcome (no CNV identified or CNV identified). For each CNV, the type (duplication or deletion), genomic coordinates, size and classification (benign, pathogenic, incidental finding or VOUS) were recorded. If a CMA report was missing, an attempt was made to contact the referring pediatrician service and obtain a copy or to confirm the case status of 'CMA not completed'.

2.3 | CNV Classification

A clinical geneticist (FC) was consulted to update each CNV classification independently between December 2021 and December 2022. The genomic coordinates of each CNV were entered into the Database of Genomic Variants (DGV) and the Database of Chromosomal Imbalance and Phenotype in Humans using Ensemble Resources (DECIPHER) based on the February 2009 assembly of the Genome Reference Consortium build 37 (GRCh37)/UCSC hg19 and the UCSC Genome Browser was used (Perez et al. 2024). This is an established method used by public clinical genetic services in NSW to interpret CNV results that provides data about the frequency of a particular CNV in the general population. It identifies RefSeq genes (genomic sequences to be used as reference standards for well-characterized genes) and Online Mendelian Inheritance in Man (OMIM) listed genes within the CNV. Each CNV was classified using the technical standards for the interpretation and reporting of constitutional copy-number variants joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (Riggs et al. 2020) and characterized into the following categories: (1) Benign—a well-documented variant in the normal population or a public database with no OMIM genes; (2) Pathogenic—known to cause well-recognized microdeletion or microduplication syndromes and contain morbid OMIM genes; (3) Incidental Finding—an unexpected pathogenic variant that may have health implications unrelated to the reason for testing; and (4) VOUS—variant of unknown significance that cannot be classified as pathogenic or benign due to insufficient evidence. Each VOUS was further classified as "likely benign" or contains a "FASD gene of interest." A "likely benign" VOUS is defined as a variant that either contains no genes or is described in a small number of cases in the general population but does not represent a common polymorphism (Kearney et al. 2011). In our study, a "FASD gene of interest" is defined as a gene with a published association with neurodevelopmental conditions that are often comorbid with FASD including GDD, ID, ASD, and ADHD. To identify genes of interest to FASD, a search was performed for each RefSeq and OMIM gene in the following databases: OMIM, PubMed, and the Simons Foundation Autism Research Initiative (SFARI) database. SFARI is a database dedicated to research involving genes of potential significance in ASD.

2.4 | Data Analysis

Data analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (Armonk, NY, USA: IBM Corp). Sociodemographic and clinical variables in children with FASD were summarized using descriptive measures, expressed as median (minimum–maximum) for continuous variables and frequency (percentage) for categorical variables. Sociodemographic and clinical characteristics were compared between children who completed genetic testing and those who did not, and between those identified with and without a rare CNV. The chi-square test was used to compare categorical variables, and the Mann–Whitney U test was used to compare continuous variables. Non-parametric tests were considered because preliminary checks indicated that the comparison groups had different distributional characteristics. While comparison groups were approximately distributed normally, based on Shapiro–Wilk tests, the assumption of equal distributional shape required for parametric tests (such as the independent samples *t*-test) was questionable. A statistically significant result was determined by $p < 0.05$.

2.5 | Study Approval

Approval for this study was obtained from the Sydney Children's Hospital Network Human Research Ethics (2021/ETH01225) and Governance (2021/STE02459) Committees.

3 | Results

A total of 175 children who fulfilled criteria according to the Australian Guide to the Diagnosis of FASD (Bower and Elliott 2016) were diagnosed with FASD between 2015 and 2022. CMA was completed for 157 of the 175 FASD cases (89.7%).

3.1 | Sociodemographic Description

Of the 175 participants with a FASD diagnosis, the mean age at diagnosis was 9.0 years (range 0.2 to 18.9) and 66.9% were male. Over half were Caucasian (53.4%) and 48.6% identified as Australian Aboriginal and/or Torres Strait Islander. At the time of assessment, over half of the children were living in foster care (56.9%) and only 14.4% were in the primary care of their biological parents. More than a quarter of participants (28.6%) had a biological sibling diagnosed with FASD. PAE was confirmed present by official records, direct witness and/or the biological mother in 173 of 175 cases (98.9%). For two participants with unconfirmed PAE, all three SFF were present.

The sociodemographic characteristics of the study cohort, including a comparison between 'CNV cohort' to the 'Non-CNV cohort', and 'Yes genetic testing' to the 'No genetic testing', are presented in Tables 1 and 2, respectively. No significant differences in sociodemographic variables were identified between children identified to harbor a CNV and those with a normal CMA result, or between children who underwent genetic testing and those who did not.

3.2 | Physical Characteristics

Of the 175 participants with a FASD diagnosis, 90.9% had fewer than three SFF and 9.1% had all three. The most common facial feature was a rank 4–5 smooth philtrum (45.3%) followed by a rank 4–5 thin upper lip (43.0%) and short palpebral fissure (23.4%) using > 2SD below the mean using Strömland

TABLE 1 | Sociodemographic characteristics of the total FASD cohort and CNV versus non-CNV cohorts.

Category	Frequency *n* (%)[a]			*p*[b] (Effect size (*φ*))[b] ((Correlation (*r*)))[b]
	Total FASD cohort (*n* = 175)	CNV cohort (*n* = 38)	Non-CNV cohort (*n* = 119)	CNV versus non-CNV
Sex				
Male	117/175 (66.9%)	29/38 (76.3%)	74/119 (62.2%)	0.110 (0.127)
Female	58/175 (33.1%)	9/38 (23.7%)	45/119 (37.8%)	
Age at diagnosis (years) Median (range)	9.0 (0.2–18.9)	8.4 (0.2–18.9)	9.1 (2.15–17.4)	0.161 ((−0.112))
Racial background (may include more than one)				
Caucasian	93/174 (53.4%)	21/38 (55.3%)	62/118 (52.5%)	0.770 (−0.023)
Aboriginal and/or Torres Strait Islander	83/174 (48.6%)	18/38 (47.4%)	58/118 (49.2%)	0.848 (0.032)
Pacific Islander	7/174 (4.0%)	2/38 (5.3%)	4/118 (3.4%)	0.634 (−0.042)
Asian	3/174 (1.7%)	1/38 (2.6%)	2/118 (1.7%)	0.570 (−0.029)
African	1/174 (0.6%)	1/38 (2.6%)	0 (0.0%)	0.244 (−0.142)
Unknown	25/174 (14.3%)	7/38 (18.4%)	15/118 (12.7%)	0.423 (−0.070)
Out of home care at any time[c]	141/173 (81.5%)	30/38 (78.9%)	96/117 (82.1%)	0.670 (−0.034)
Sibling(s) with FASD[c]	50/158 (28.6%)	10/36 (27.8%)	37/106 (34.9%)	0.461 (0.104)

Note: Statistical significance set at $p < 0.05$.

Abbreviations: CNV, copy number variant; FASD, fetal alcohol spectrum disorder.

[a]For variables where the data were missing, the frequency was calculated by dividing the number of valid responses recorded for that variable (numerator) by the total responses received for that category (denominator) and expressed as a percentage.

[b]Chi-square test was used to compare categorical variables (or Fisher's exact test where ≥ 20% of cells had expected count < 5) and was expressed as effect size phi (*φ*). Mann–Whitney U test was used to compare age (continuous variable) and expressed as correlation (*r*).

[c]"Don't know" responses were treated as missing values.

normative data (Strömland et al. 1999). Microcephaly (head circumference < 3rd percentile) was reported in 19.5% of the total cohort.

The physical characteristics of the study cohort including a comparison between 'CNV cohort' to the 'Non-CNV cohort' and 'Yes genetic testing' to the 'No genetic testing' are presented in Tables 3 and 4, respectively. No significant differences in physical variables were identified between children identified to harbor a CNV and those with a normal CMA result. Of children who completed genetic testing, 88.8% were identified to have one or more minor congenital abnormalities, which was significantly higher than in the non-tested group (46.7%) ($p = 0.001$, $\varphi = 0.331$).

3.3 | Neurodevelopmental Domains

The number of neurodevelopmental domains identified with a severe impairment in children with FASD ranged from three to nine (median four). Approximately two-thirds of the cohort had severe impairment in either three (34.8%) or four (35.4%) neurodevelopmental domains. Problems with attention (83.6%) and adaptive behavior, social skills and/or communication (73.5%) were most common. A similar rate of impairment was diagnosed in the domains of cognition and academic achievement (61.7%).

The neurodevelopmental characteristics of the study cohort including a comparison between 'CNV cohort' to the 'Non-CNV cohort' and 'Yes genetic testing' to the 'No genetic testing' are presented in Tables 3 and 4, respectively. No significant differences in neurodevelopmental function were identified between children with a CNV and those with a normal CMA result. Of the children who did complete genetic testing, 14.2% reported a significant impairment in disruptive, impulse control, and conduct disorder which was borderline significantly lower than reported in the non-tested group (33.3%), ($p = 0.046$, $\varphi = -0.168$).

TABLE 2 | Sociodemographic characteristics of the total FASD cohort and those who completed genetic testing versus the non-tested group.

Category	Frequency *n* (%)[a]			p[b] (Effect size (φ))[b] ((Correlation (r)))[b]
	Total FASD cohort ($n=175$)	Genetic testing, yes ($n=157$)	Genetic testing, no ($n=18$)	Genetic testing (yes versus no)
Sex				
Male	117/175 (66.9%)	103/157 (65.6%)	14/18 (77.8%)	0.299 (−0.079)
Female	58/175 (33.1%)	54/157 (34.4%)	4/18 (22.2%)	
Age at diagnosis (years) Median (range)	9.0 (0.2–18.9)	9.23 (0.2–18.66)	10.57 (4.53–17.19)	0.279 ((−0.008))
Racial background (may include more than one)				
Caucasian	93/174 (53.4%)	83/156 (53.2%)	10/18 (55.6%)	0.850 (0.014)
Aboriginal and/or Torres Strait Islander	83/174 (48.6%)	42/89 (47.2%)	1/6 (16.7%)	0.146 (0.149)
Pacific Islander	7/174 (4.0%)	6/156 (3.8%)	1/18 (5.6%)	1.000 (0.026)
Asian	3/174 (1.7%)	3/156 (1.9%)	0 (0%)	0.719 (−0.045)
African	1/174 (0.6%)	1/156 (0.6%)	0 (0%)	0.897 (−0.026)
Unknown	25/174 (14.3%)	22/156 (14.1%)	3/18 (16.7%)	1.000 (0.022)
Out of home care at any time[c]	141/173 (81.5%)	126/155 (81.3%)	15/18 (83.3%)	0.874 (−0.015)
Sibling(s) with FASD[c]	50/158 (28.6%)	47/98 (48.0%)	3/9 (33.3%)	0.400 (0.081)

Note: Statistical significance set at $p<0.05$.

Abbreviations: CNV, copy number variant; FASD, fetal alcohol spectrum disorder.

[a]For variables where the data were missing the frequency was calculated by dividing the number of valid responses recorded for that variable (numerator) by the total responses received for that category (denominator) and expressed as a percentage.

[b]Chi-square test was used to compare categorical variables (or Fisher's exact test where ≥20% of cells had expected count < 5) and expressed as effect size phi (φ). Mann–Whitney U test was used to compare age (continuous variable) and expressed as correlation (r).

[c]"Don't know" responses were treated as missing values.

3.4 | Comorbid Neurodevelopmental Diagnoses Associated With FASD

ADHD was reported in two-thirds (67.1%) of the cohort and ASD was reported in one-fifth (19.5%). ID was reported in 20 children and classified as mild (9), moderate (1), severe (1), or not classified (9). In children who had microarray testing completed and were less than 6 years of age, GDD was reported in half (52.9%). No significant differences were identified between children identified to harbor a CNV and those with a normal CMA result, or between children who underwent genetic testing and those who did not.

3.5 | CMA Reports and Methodology

The original laboratory report was available for 153 of the 157 participants who completed CMA, and a specialist geneticist's letter confirmed the outcome for the remaining four participants. CMA was performed in NSW government health pathology laboratories for 146 of the 157 children (93.0%) undergoing genetic testing with either oligonucleotide CGH array or, more recently, utilizing a combination of oligonucleotide and SNP arrays. Eleven children had genetic testing completed prior to or after the FASD assessment in a private service using the method of SNP array. Since 2016, the average resolution of the CMA has been approximately 130 Kb for CNV detection. Nine patients with "normal reports" were investigated prior to 2016. The authors acknowledge that the variable sensitivity of CMA methodologies in this study may have resulted in very small-sized CNVs being missed, leading to an underestimation of the true CNV yield. Parental segregation of the CNVs was reported for only two of 157 patients (one sibling pair) because many children did not live with their biological parents. A total of 137 of 157 patients completed Fragile X testing, and all recorded a normal result (less than 45 CGG repeats).

3.6 | CMA Outcomes

Figure 1 illustrates the CMA outcomes identified in 175 patients who fulfilled diagnostic criteria for FASD. Of the 157

TABLE 3 | Physical and neurodevelopmental characteristics of the total FASD cohort and CNV versus non-CNV cohorts.

	n (%)[a]			*p*[b] (Effect size (*φ*))[b]
Category	**Total cohort (*n* = 175)**	**CNV cohort (*n* = 38)**	**Non-CNV cohort (*n* = 119)**	**CNV versus non-CNV**
FASD diagnosis				
FASD with 3 SFF	16/175 (9.1%)	4/38 (10.5%)	12/119 (10.1%)	1.000 (0.006)
FASD < 3 SFF	159/175 (90.9%)	34/38 (89.5%)	107/119 (89.9%)	
Number of sentinel facial features identified				
None	62/172 (36.0%)	12/38 (31.6%)	39/116 (33.6%)	0.692 (0.097)
One	44/172 (25.6%)	13/38 (34.2%)	29/116 (25.0%)	
Two	50/172 (29.1%)	9/38 (23.7%)	36/116 (31.0%)	
Three	16/172 (9.3%)	4/38 (10.5%)	12/116 (10.3%)	
Sentinel facial feature identified				
Smooth philtrum	78/172 (45.3%)	16/38 (42.1%)	57/116 (49.1%)	0.451 (−0.061)
Thin upper lip	74/172 (43.0%)	20/38 (52.6%)	47/116 (40.5%)	0.191 (0.105)
Short palpebral fissure	40/171 (23.4%)	7/38 (18.4%)	33/116 (28.4%)	0.221 (−0.099)
Brain structure/neurology[c]				
Microcephaly (head circumference ≤ 3rd percentile for age)	34/174 (19.5%)	6/35 (17.1%)	25/112 (22.3%)	0.512 (−0.054)
Other clinically significant structural brain abnormality	11/148 (6.5%)	3/37 (8.1%)	7/111 (6.3%)	0.711 (0.031)
Clinically significant neurological abnormality	15/157 (9.6%)	5/36 (13.9%)	8/103 (7.8%)	0.321 (0.092)
Global developmental delay (age < 6 years)[d]	18/31 (52.9%)	6/11 (54.5)	11/20 (55.0%)	1.000 (−0.004)
Co-morbidities[c]				
Attention Deficit Hyperactivity Disorder	104/155 (67.1%)	24/35 (68.6%)	65/101 (64.4%)	0.651 (0.039)
Trauma, stress-related or attachment disorder	38/72 (52.8%)	11/15 (73.3%)	20/40 (50.0%)	0.120 (0.210)
Anxiety disorder	64/167 (38.3%)	9/34 (26.5%)	48/109 (44.0%)	0.068 (0.153)
Communication disorder	25/73 (34.2%)	5/14 (35.7%)	16/45 (35.6%)	1.000 (0.001)
Sleep disorder	53/167 (31.7%)	9/35 (25.7%)	37/110 (33.6%)	0.380 (0.073)
Vision impairment	25/79 (31.6%)	6/13 (46.2%)	18/49 (36.7%)	0.535 (−0.079)

(Continues)

TABLE 3 | (Continued)

Category	*n* (%)[a]			*p*[b] (Effect size (φ))[b]
	Total cohort (*n* = 175)	CNV cohort (*n* = 38)	Non-CNV cohort (*n* = 119)	CNV versus non-CNV
Intellectual disability	20/76 (26.3%)	2/15 (13.3%)	15/47 (31.9%)	0.200 (−0.178)
Hearing impairment	16/82 (19.5%)	5/14 (35.7%)	10/49 (20.4%)	0.291 (−0.149)
Autism spectrum disorder	15/77 (19.5%)	2/15 (13.3%)	11/47 (23.4%)	0.493 (−0.106)
Disruptive, impulse control, conduct disorder	27/166 (16.3%)	4/34 (11.8%)	17/107 (15.9%)	0.556 (−0.050)
Mood disorder (depression)	19/166 (11.4%)	1/32 (3.1%)	15/106 (14.2%)	0.118 (−0.145)
Motor disorder	8/73 (11%)	0/14 (0%)	7/45 (15.6%)	0.181 (−0.205)
Specific learning disorder	7/70 (10.0%)	3/14 (21.4%)	2/40 (5.0%)	0.103 (0.068)
Congenital anomalies[c]				
Minor	126/149 (84.6%)	30/34 (88.2%)	89/99 (89.9%)	0.785 (−0.024)
Major	17/88 (19.3%)	7/20 (35.0%)	8/54 (14.8%)	0.100 (0.223)
Growth impairment[c]				
Birth weight ≤ 3rd percentile for gestation	22/171 (12.9%)	5/22 (22.7%)	14/74 (18.9%)	0.762 (0.040)
Birth length ≤ 3rd percentile for gestation	15/171 (8.8%)	3/15 (20.0%)	12/66 (18.2%)	1.000 (0.018)
Postnatal weight ≤ 3rd percentile for age	34/173 (19.7%)	6/20 (30.0%)	26/72 (36.1%)	0.792 (−0.053)
Postnatal height ≤ 3rd percentile for age	22/173 (12.7%)	3/19 (15.8%)	18/70 (25.7%)	0.544 (−0.096)

Abbreviations: CNV, copy number variant; FASD, fetal alcohol spectrum disorder.
[a]For variables where the data were missing the frequency was calculated by dividing the number of valid responses recorded for that variable (numerator) by the total responses received for that category (denominator) and expressed as a percentage.
[b]Chi-square test was used to compare categorical variables (or Fisher's exact test where ≥ 20% cells had expected count < 5) and expressed as effect size phi (φ). Mann–Whitney U test was used to compare age (continuous variable) and expressed as correlation (*r*). *Statistical significance set at $p < 0.05$.
[c]"Don't know" responses were treated as missing values.
[d]This analysis only included those who had microarray testing done and were aged < 6 years.

patients who completed CMA testing, a normal CMA outcome was reported for 119 cases (75.8%). Rare CNVs were identified in 38 children who completed CMA testing (24.2%), and a total of 40 unique CNVs were identified. Of these CNVs, 17 were microdeletions, 22 were microduplications, and one was a combined X1,Yq11.223 variant. The genetic description, classification, and genes encoded within each rare CNV are identified in Table 5.

For two participants with unconfirmed PAE and all three SFF, one had a normal CGH array result. In the second case, the mother denied PAE, although evidence of PAE was found in medical records. In that child, a 1q43 deletion (including the *RYR2* gene) was recorded and classified by a clinical geneticist as a VOUS (likely benign).

Pathogenic CNVs were identified in four children and represented four known genetic neurodevelopmental susceptibility syndromes: Xq28 microduplication syndrome, 16p12.2 microdeletion syndrome, 17q11.2 microduplication syndrome, and an atypical variant of 22q11.2 deletion syndrome. This resulted in a comorbid genetic diagnostic yield of 2.5% (4/157) for individuals with FASD who underwent CMA testing. Two 'incidental' pathogenic findings were detected which would be expected

TABLE 4 | Physical and neurodevelopmental characteristics of the total FASD cohort and those who completed genetic testing versus the non-tested group.

	n (%)[a]			*p*[b] (Effect size (*φ*))[b]
Category	**Total cohort (*n* = 175)**	**Genetic testing yes (*n* = 157)**	**Genetic testing no (*n* = 18)**	**Yes versus No**
FASD diagnosis				
FASD with 3 SFF	16/175 (9.1%)	16/157 (10.2%)	0 (0%)	0.227 (0.107)
FASD < 3 SFF	159/175 (90.9%)	141/157 (89.8%)	18/18 (100%)	
Number of sentinel facial features identified				
None	62/172 (36.0%)	53/156 (34.0%)	11/18 (61.1%)	0.083 (0.196)
One	44/172 (25.6%)	42/156 (26.9%)	2/18 (11.1%)	
Two	50/172 (29.1%)	45/156 (28.8%)	5/18 (27.8%)	
Three	16/172 (9.3%)	16/156 (10.3%)	0 (0%)	
Sentinel facial feature identified				
Smooth philtrum	78/172 (45.3%)	73/156 (46.8%)	5/18 (27.8%)	0.948 (−0.006)
Thin upper lip	74/172 (43.0%)	67/156 (42.9%)	7/18 (38.9%)	0.488 (−0.062)
Short palpebral fissure	40/171 (23.4%)	40/156 (25.6%)	0 (0%)	0.077 (0.171)
Brain structure/neurology[c]				
Microcephaly (head circumference ≤ 3rd percentile for age)	34/174 (19.5%)	31/156 (19.9%)	3/18 (16.7%)	0.769 (0.034)
Other clinically significant structural brain abnormality	11/148 (6.5%)	10/151 (6.6%)	1/18 (5.6%)	1.000 (0.015)
Clinically significant neurological abnormality	15/157 (9.6%)	13/139 (9.4%)	2/18 (11.1%)	1.000 (0.019)
Global developmental delay (age < 6 years age)[d]	18/31 (52.9%)	17/31 (54.8%)	1/3 (33.3%)	0.591 (0.122)
Co-morbidities[c]				
Attention Deficit Hyperactivity Disorder	104/155 (67.1%)	89/137 (65.0%)	15/18 (83.3%)	0.128 (−0.123)
Trauma, stress-related or attachment disorder	38/72 (52.8%)	31/61 (50.8%)	7/11 (63.6%)	0.421 (−0.100)
Anxiety disorder	64/167 (38.3%)	57/149 (38.3%)	7/18 (38.9%)	0.937 (0.006)
Communication disorder	25/73 (34.2%)	21/62 (33.9%)	4/11 (36.4%)	1.000 (−0.006)
Sleep disorder	53/167 (31.7%)	46/150 (30.7%)	7/17 (41.2%)	0.432 (−0.062)
Vision impairment	25/79 (31.6%)	24/89 (27.0%)	1/6 (16.7%)	1.000 (−0.023)
Intellectual disability	20/76 (26.3%)	17/34 (26.6%)	3/12 (25.0%)	1.000 (0.020)
Hearing impairment	16/82 (19.5%)	15/89 (16.9%)	1/6 (16.7%)	0.435 (0.105)
Autism spectrum disorder	15/77 (19.5%)	13/65 (20.0%)	2/12 (16.7%)	1.000 (0.039)

(Continues)

TABLE 4 | (Continued)

Category	*n* (%)[a] Total cohort (*n* = 175)	Genetic testing yes (*n* = 157)	Genetic testing no (*n* = 18)	*p*[b] (Effect size (*φ*))[b] Yes versus No
Disruptive, impulse control, conduct disorder	27/166 (16.3%)	21/148 (14.2%)	6/18 (33.3%)	**0.046*** (−0.168)
Mood disorder (depression)	19/166 (11.4%)	16/148 (10.8%)	3/18 (16.7%)	0.700 (−0.050)
Motor disorder	8/73 (11%)	7/62 (11.3%)	1/11 (91%)	1.000 (0.032)
Specific learning disorder	7/70 (10.0%)	5/59 (8.5%)	2/11 (18.2%)	0.592 (−0.108)
Congenital anomalies[c]				
Minor	126/149 (84.6%)	119/134 (88.8%)	7/15 (46.7%)	**0.001*** (0.331)
Major	17/88 (19.3%)	15/75 (20.0%)	2/13 (15.4%)	0.736 (0.044)
Growth impairment[c]				
Birth weight ≤ 3rd percentile for gestation	22/171 (12.9%)	19/154 (12.3%)	3/17 (17.6%)	0.707 (−0.043)
Birth length ≤ 3rd percentile for gestation	15/171 (8.8%)	15/154 (9.7%)	0 (0%)	0.201 (0.163)
Postnatal weight ≤ 3rd percentile for age	34/173 (19.7%)	32/155 (20.6%)	2/18 (11.1%)	0.329 (0.123)
Postnatal height ≤ 3rd percentile for age	22/173 (12.7%)	21/55 (13.5%)	1/18 (5.6%)	0.295 (0.120)

Abbreviations: CNV, copy number variant; FASD, fetal alcohol spectrum disorder.
[a]For variables where the data were missing the frequency was calculated by dividing the number of valid responses recorded for that variable (numerator) by the total responses received for that category (denominator) and expressed as a percentage.
[b]Chi-square test was used to compare categorical variables (or Fisher's exact test where ≥ 20% of cells had expected count < 5) and expressed as effect size phi (*φ*).
[c]"Don't know" responses were treated as missing values.
[d]This analysis only included those who had microarray testing done and were aged < 6 years.
*Statistical significance set at $p < 0.05$.

to result in additional clinical features. One female case had a Turner Syndrome variant with partial X monosomy, and one male child had the *PMP22* gene duplication associated with peripheral neuropathy (Charcot Marie–Tooth disease, Type 1A -CMT1A). In the remaining 32 cases, 34 VOUS were identified.

One sibling pair was identified to have an identical paternally inherited CNV (11q22.3) encoding the *ELMOD1* gene of interest to FASD. For all other patients who had a sibling with FASD, the sibling(s) were diagnosed with FASD outside of our service, and we did not have access to any additional medical or neurodevelopmental information for those patients, including the status of CMA testing and result(s).

Three cases were identified that each harbored two unique CNVs. Twenty-one VOUS had no published evidence to support a pathogenic role at this time and were interpreted as 'likely benign'. Thirteen VOUS identified in 14 individuals (identical CNV in one sibling pair) were classified to encode 14 different genes of interest associated with one comorbid neurodevelopmental disorder seen in FASD. Each gene of interest and its published neurodevelopmental association(s) are described in Table 6. The yield for individuals with FASD who underwent CMA testing and were identified to have either a pathogenic or 'FASD gene of interest' CNV was 11.5% (18/157).

4 | Discussion

We conducted a study in an Australian state-wide diagnostic FASD service in children who both fulfilled FASD criteria and underwent CMA between 2015 and 2022. Approximately a quarter (24.2%) of cases had a rare CNV. Among those with a rare CNV, 50% had either a pathogenic variant or FASD gene of interest, which required genetic consultation to support the diagnostic formulation and inform clinical management. These results show an increased prevalence of rare CNVs in individuals diagnosed with FASD when compared to normal control populations (9%–12%) (Zarrei et al. 2015) and other children with developmental delay and/or congenital abnormalities (10%–15%) (Miller et al. 2010).

This study represents the first Australian data on the frequency of rare CNVs in a large cohort of children (0–18 years) with a confirmed diagnosis of FASD. Our study is one of only three internationally conducted studies in a relatively large

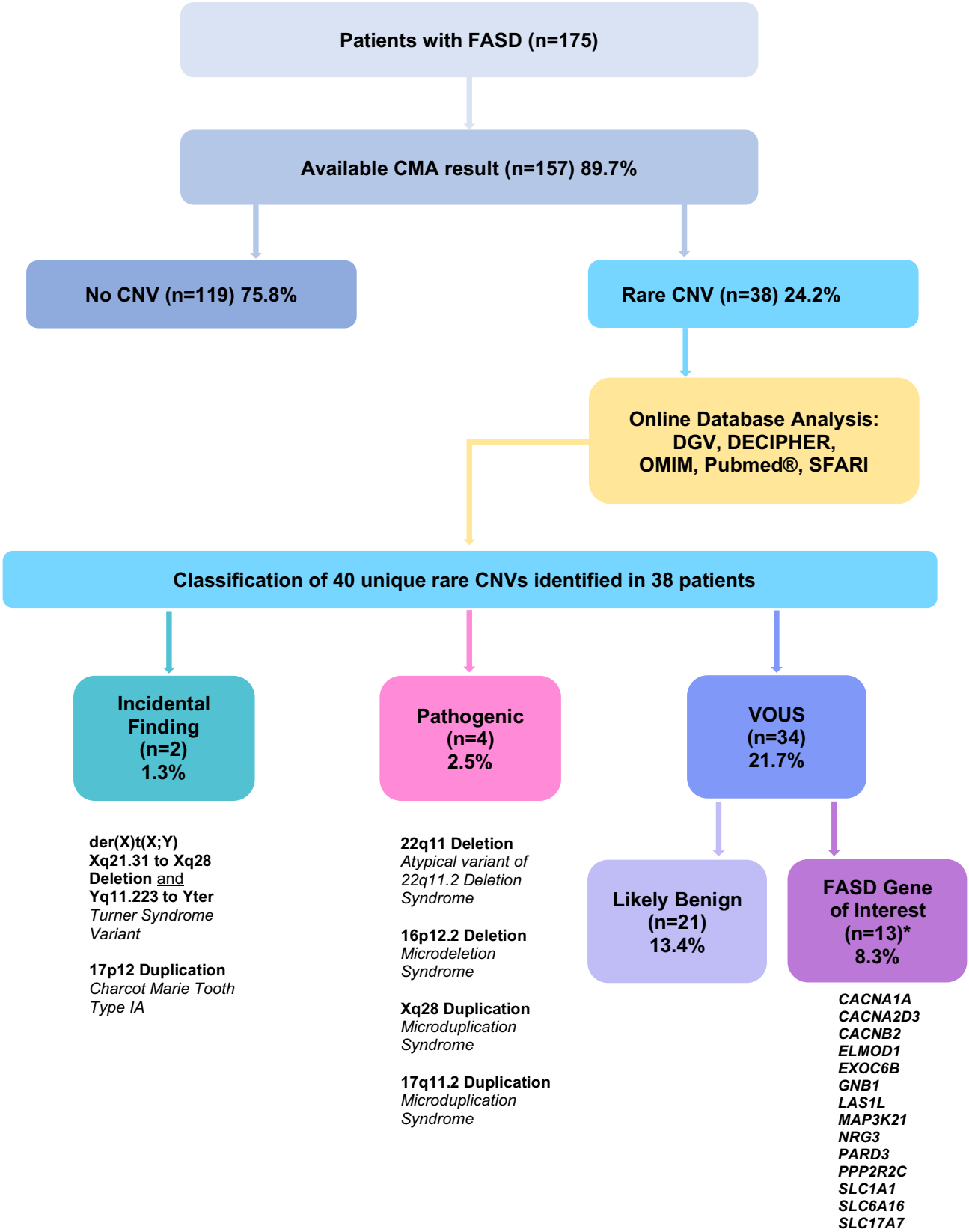

FIGURE 1 | CMA outcomes in a cohort of 175 children (0–18 years) diagnosed with FASD in an Australian state-wide diagnostic service between 2015 and 2022. CMA, chromosomal microarray; CNV, copy number variant; DECIPHER, database of chromosomal imbalance and phenotype in humans using ensemble resources; DGV, database of genomic variants; FASD, fetal alcohol spectrum disorder; OMIM, online Mendelian inheritance in man; SFARI, Simons Foundation Autism Research Initiative; VOUS, variant of unknown significance. *Thirteen VOUS identified in 14 individuals (identical CNV in one sibling pair) were classified to encode 14 different genes of interest to FASD.

TABLE 5 | Description and classification of copy number variants identified in 38 children diagnosed with FASD.

Case ID/ Gender	Chromosome location and type	Genomic coordinates size (base pairs)	Encoded gene(s) identified in DGV, DECIPHER and OMIM	CNV classification	DECIPHER genomic disorder, OMIM-listed Syndrome or gene of interest
79/Male	17p12 Duplication	14099504_15482833 (2.38 Mb)	*PMP22, COX10*	Incidental Finding	Charcot–Marie–Tooth Disease, Type 1A (CMT1A) and Hereditary Liability to Pressure Palsies (HNPP)
39/Female	der(X)t(X;Y) - Xq21.31 to Xq28 Deletion and Yq11.223 to Yter	X:86880570_155246643(68 Mb) Y:24657861_59349649 (35 Mb)	*KLHL4, IL9R, DIAPH2, PRY, WASIR1*	Incidental Finding	Turner Syndrome variant with Partial X Monosomy
53/Male	Xq28 Duplication	154109681_154560346 (451 Kb)	*CLIC2, RAB39B, H2AFB3, FUNDC2, MTCP1, BRCC3, VBP1, F8A1, CMC4, MIR1185*	Pathogenic	Xq28 Duplication Syndrome
84/Male	22q11 Deletion	19749541_24749540 (5 Mb)	Multiple genes distal to *TBX1* (not containing) including *LZTR1, MAPK1, SMARCB1 and* part of *SPECC1L*	Pathogenic	22q11.2 Deletion Syndrome Atypical variation
97/Male	16p12.2 Deletion	21959891_22430592 (471 Kb)	*EEF2K, CDR2, UQCRC2, POLR3E, PDZD9, VWA3A*	Pathogenic	16p12.2 Microdeletion Syndrome
138/Male	17q11.2 Duplication	Genetic Clinic Letter	Not reported	Pathogenic	17q11.2 Microduplication Syndrome
12/Male	19q13.33 Duplication	49792961_49954993 (162 Kb)	*SLC6A16, CD37, TEAD2, MIR4324, DKKL1, CCDC155, PTH2, PIH1D1, LOC100507003, SLC17A7*	VOUS – FASD gene of interest	*SLC6A16 and SLC17A7*
20/Male	1p36.33 Duplication	834,131 _1723681 (890 Kb)	45 genes from *SAMD1* to *GNB1* in part	VOUS - FASD gene of interest	*GNB1*

(Continues)

TABLE 5 | (Continued)

Case ID/ Gender	Chromosome location and type	Genomic coordinates size (base pairs)	Encoded gene(s) identified in DGV, DECIPHER and OMIM	CNV classification	DECIPHER genomic disorder, OMIM-listed Syndrome or gene of interest
25/Female	10p12.32–31 Duplication	18623463_18740632 (281 Kb)	*CACNB2*	VOUS - FASD gene of interest	*CACNB2*
26/Male	9p24.2 Duplication	4232824_4555573 (323 Kb)	*SLC1A1, GLIS3*	VOUS - FASD gene of interest	*SLC1A1*
44/Male	19p13.2 Deletion	13497073_13578909 (162 Kb)	*CACNA1A*	VOUS - FASD gene of interest	*CACNA1A*
49/Female	4p16.1 Duplication	6429844_6924462 (495 Kb)	*PPP2R2C, MAN2B2, BLOC1S4, S100P, KIAA0232, TBC1D14, MRFAP1, LOC93622*	VOUS - FASD gene of interest	*PPP2R2C*
72/Male	10q23.1 Deletion	84481967_84530117 (48 Kb)	*NRG3* -intragenic deletion	VOUS - FASD gene of interest	*NRG3*
73/Male	3p14.3 Deletion and 3q14.3 Duplication	54547284_54637769 (90 Kb) 51808952_51914960 (106 Kb)	*CACNA2D3*— intragenic deletion *FAM123A*	VOUS—FASD gene of interest VOUS—likely benign	*CACNA2D3*
98/Female	Xq11.2-q12 Duplication and 5q34 Deletion	64327208_64752951 (426 Kb) 162523592_162790605 (267 Kb)	*LAS1L, ZCH12B* No genes encoded	VOUS - FASD gene of interest VOUS—likely benign	*LAS1L*
155/Male	1q42.2 Duplication	232989850_233503053 (513 Kb)	*MAP3K21, NTPCR, PCNXL2*	VOUS - FASD gene of interest	*MAP3K21*
160/Male	10p11.2-p11.1 Deletion	34644592_39150257 (4.5 Mb)	Includes 23 genes from *PARD3* (in part) to *ACTR3BP5*	VOUS - FASD gene of interest	*PARD3*
166/Male	2p13.2 Deletion	72573969_72738902 (165 Kb)	*EXOC6B* gene in part only	VOUS - FASD gene of interest	*EXOC6B*
175/Male 176/Male	11q22.3 Deletion	107470714_107711269 (240 Kb)	*ELMOD1, SLN, SLC35F2*	VOUS - FASD gene of interest	*ELMOD1*
2/Male	10q11.2 Duplication	46609948_47410453 (800 Kb)	No genes encoded	VOUS—likely benign	
4/Male	1p32.3 Duplication	55355819_55504715 (149 Kb)	*DHCR24, BSND, TMEM61*	VOUS—likely benign	

(Continues)

TABLE 5 | (Continued)

Case ID/ Gender	Chromosome location and type	Genomic coordinates size (base pairs)	Encoded gene(s) identified in DGV, DECIPHER and OMIM	CNV classification	DECIPHER genomic disorder, OMIM-listed Syndrome or gene of interest
11/Male	11q23.1 Deletion	111680460_111745960 (66 Kb)	*ALG9, FDXACB1* in part	VOUS—likely benign	
17/Male	16q14.3 Deletion	Genetic Clinic Letter	No genes encoded	VOUS - likely benign	
42/Male	6p12.3 Deletion	50025642_50518874 (493 Kb)	No genes encoded	VOUS - likely benign	
45/Male	7p12.2 Duplication	49708644_50427714 (719 Kb)	*IKZFI, VWC2, ZPBP*	VOUS - likely benign	
51/Female	6q13 proximal and distal Duplication	Genetic Clinic Letter	*MTO1, SLC17A5*	VOUS - likely benign	
60/Male	4q26 Duplication	1117862498_118905372 (1.04 Mb)	*TRAM1L1*	VOUS - likely benign	
61/Male	Xp22.23 or Yp11.32 Duplication	2270123_2443157 (173 Kb)	*DHRSX, ZBED1*	VOUS - likely benign	
74/Female	2q34 Deletion	211332509_212469202 (1.14 Mb)	*CPS1, LANCL1, ERBB4* in part	VOUS - likely benign	
82/Male	1p21.1 Duplication	Genetic Clinic Letter	No genes encoded	VOUS - likely benign	
89/Male	21q22.3 Duplication	42806167_43036634 (230 Kb)	*MX1, TMPRSS2*	VOUS - likely benign	
93/Male	Xp22.2 Duplication	15624439_15790203 (166 Kb)	*TMEM27, CA5B, CA5BP1*	VOUS - likely benign	
110/Female	12p12.3 Duplication	17046854_17194269 (162 Kb)	*SKP1P2*	VOUS - likely benign	
122/Female	19p13.3 Duplication	782854_1008505 (226 Kb)	*ELANE, KISS1, PTBP1, AZU1, PRTN3, GRIN3B, WDR18, MIR4745, LPPPR3, MIR3187, R3HDM4, AR1D3A*	VOUS - likely benign	
132/Male	16q21 Deletion	61500159_61591628 (92 Kb)	No genes encoded	VOUS - likely benign	
161/Male	9p21.3 Deletion	22212553_22416608 (204 Kb)	No genes encoded	VOUS - likely benign	
184/Male	1q43 Deletion	Genetic Clinic Letter	*RYR2*	VOUS - likely benign	

Note: Re-analysis of the original chromosomal microarray (CMA) report for each patient in the DGV of Human Genome and DECIPHER build 37 (GRCh37)/UCSC hg19.
Abbreviations: CNV, copy number variant; DECIPHER, Database of Chromosomal Imbalance and Phenotype in Humans using Ensemble Resources; DGV, database of genomic variants; FASD, fetal alcohol spectrum disorder; OMIM, Online Mendelian inheritance in man; VOUS, variant of unknown significance.

TABLE 6 | FASD genes of interest identified in 13 children diagnosed with FASD and published neurodevelopmental association(s).

Gene of interest	Description, OMIM-listing, SFARI database-listing and potential clinical significance to FASD
CACNA1A	**Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit** OMIM-Disease-listed (601011) encoding the pore-forming alpha-1A subunit predominantly expressed in neuronal tissue. SFARI-listed as a 'high confidence' gene with evidence of heterogeneous loss of function variants associated with ID, ADHD, ASD, epilepsy and executive dysfunction (Damaj et al. 2015, Li et al. 2015). Gene mutations are also associated with cognitive impairment (Humbertclaude et al. 2020)
CACNA2D3	**Calcium channel, voltage-dependent, alpha 2/delta subunit 3** OMIM-listed (606399) encoding voltage-dependent calcium channel complex protein and regulating P/Q-type calcium channels (CACNA1A-D). SFARI-listed 'high confidence' gene with enrichment of CACNA2D3 deletions in ASD subjects compared to controls (De Rubeis et al. 2014, Girirajan et al. 2013). Established susceptibility gene for ASD (Liao and Li 2020).
CACNB2	**Calcium channel, voltage-dependent, beta 2 subunit** OMIM-listed (600003) member of the voltage-gated channel superfamily with SFARI-listed evidence of three rare CACNB2 missense mutations in ASD probands (Breitenkamp et al. 2014)
ELMOD1	**ELMO Domain-containing protein 1** OMIM-listed (615456) encoding a GTPase activating protein (GAP) with a role in regulating ciliogenesis and ciliary protein trafficking overexpressed in brain tissue and with evidence of a mutation associated with ASD and cognitive impairment (Miryounesi et al. 2019).
EXOC6B	**Exocyst complex component 6B** OMIM-listed (607880) encoding a GTPase protein necessary for exocytosis postulated to have an important role in the molecular pathogenesis of intellectual disability (Evers et al. 2014). SFARI-listed with one de novo translocation associated with ID, ASD features and minor dysmorphism (Frühmesser et al. 2013). A second de novo deletion was reported in an individual with DD, ASD, epilepsy and aggressive behavior (Evers et al. 2014).
GNB1	**Guanine nucleotide -binding protein subunit-1 GB1** OMIM-listed (139380) and OMIM-disease listed (616973) as heterozygous missense mutations result in autosomal dominant intellectual developmental disorder-42 (MRD42). Evidence of heterozygous missense mutations characterized by DD and ID, and including comorbidities of ASD and ADHD (Petrovski et al. 2016, Da Silva et al. 2021). Variable features of other genetic variants including hypotonia, seizures, poor growth, strabismus, cortical visual impairment, and ASD (Lohmann et al. 2017).
LAS1L	**Lasi-Like ribosome biogenesis factor** OMIM-listed (300,964 and 309,585) protein-coding gene with evidence of missense mutations resulting in Wilson-Turner X-linked syndromic intellectual developmental disorder (Wilson et al. 1991, Hu et al. 2016). SFARI-listed with evidence of de novo missense mutations in ASD probands with ID and DD and a maternally inherited in-frame deletion variant in a male with ASD (Tran et al. 2020).
MAP3K21	**Mitogen-activated protein kinase kinase kinase 21** OMIM-listed (614793) with evidence of RAS/MAP signaling pathway disorders associated with neurocognitive impairments and ASD (Vithayathil et al. 2018, Kang and Lee 2019)
NRG3	**Neuregulin 3** OMIM-listed (605533) protein of the ***neuregulin family*** protein family with evidence of important functioning in the developing nervous system (Carteron et al. 2006) and evidence of structural and polymorphic variations in NRG3 associated with neurodevelopmental disorders including DD, ID, and ASD (Balciuniene et al. 2007, Kao et al. 2010).
PARD3	**PAR3 family cell polarity regulator** OMIM-listed (606745) as critical for subcellular compartmentalization and involved in neural development and maintenance regulation (Hakanen et al. 2019). Genetic variations associated with intelligence (Davies et al. 2018), educational attainment (Lee et al. 2018) and ASD (Pinto et al. 2014, Özaslan et al. 2021). The paralog of *PARD3, PARD3B*, is reported as a candidate gene for ASD in a study that reported altered learning and memory with increased repetitive behaviors simulating behaviors of ASD in a PARD3 knockout mouse model (Voglewede et al. 2024).

(Continues)

TABLE 6 | (Continued)

Gene of interest	Description, OMIM-listing, SFARI database-listing and potential clinical significance to FASD
PPP2R2C	**Protein phosphatase 2 regulatory subunit B gamma** OMIM-listed (605997) with evidence of mutations associated with autosomal dominant mild ID and ASD (Backx et al. 2010).
SLC1A1	**Solute carrier family 1** OMIM-listed (133550) encoding a high-affinity glutamate transporter expressed selectively by neurons in the central nervous system. SFARI-listed evidence of deletions associated with anxiety and restrictive repetitive behaviors in ASD (Gadow et al. 2010).
SLC6A16	**Solute carrier family 6 member 16** OMIM-listed (607972) encoding Na + and Cl- dependent neurotransmitter transporter (NNT5) expressed in brain. Mutations in the SLC6A family, including SLC6A1, associated with cognitive impairment, seizures and mild to moderate ID (Bhat et al. 2021).
SLC17A7	**Solute carrier family 7 member 7** OMIM-listed (605208) encoding the vesicular glutamate co-transporter (VGLUT1) which transports glutamate into synaptic vesicles in neurons in the brain, with a role in synaptic vesicle organization, glutamate release and neurotoxicity. Association established in Alzheimer's disease, glioblastoma, dystonia and concussion severity and duration (Bhat et al. 2021). Unpublished evidence of potential links to ID and Parkinson's disease (Jensenlab, n.d.).

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; CNV, copy number variant; DD, developmental delay; FASD, fetal alcohol spectrum disorder; ID, intellectual disability; OMIM, Online Mendelian inheritance in man; SFARI, Simons Foundation Autism Research Initiative.

cohort of children with confirmed FASD who also underwent genetic testing and includes the highest caseload ($N = 157$) (Sennsfelder et al. 2023; Zarrei et al. 2018). The rare CNV frequency recorded in our study (24.2%) is comparable with that in a recently published study in French children and adolescents diagnosed with FASD that employed similar methodology and reported a CNV rate of 20.8% (21/101) (Sennsfelder et al. 2023). In a study among 95 Canadian children with FASD, the frequency of rare CNVs, including CNVs encoding genes implicated in brain function and neurodevelopmental disorders, was 14.7% (14/95) (Zarrei et al. 2018). Due to significant methodological differences between that study and ours, it is challenging to draw conclusions about the differing CNV frequency rates.

Our study is differentiated from four small cohort studies that were limited to patients referred for specialist genetic consultation with an unconfirmed diagnosis of FASD (Abdelmalik et al. 2013; Douzgou et al. 2012; Jamuar et al. 2018; Lam et al. 2021). The yield of pathogenic CNVs (2.5%) recorded in our study was lower than reported in all four studies (range 3.6%–14.3%) and can potentially be explained by selection bias where individuals referred to a genetic service for consideration of FASD are more likely to exhibit a severe phenotype and have pathogenic CNVs.

In our study, one patient was identified to have an atypical variation of 22q11.2 deletion syndrome and similar cases have been reported in three studies exploring CNV frequency in FASD cohorts (Douzgou et al. 2012; Jamuar et al. 2018; Zarrei et al. 2018). 22q11.2 deletion syndrome is commonly misdiagnosed as FASD because of the similar facial phenotype and this child demonstrated physical features of both PAE and 22q11.2 deletion syndrome. Our study is the first to report the findings of three separate pathogenic CNVs: 16p12.2 deletion; Xq28 duplication and 17q11.2 duplication in a FASD cohort.

Three patients in our study had a CNV that could potentially impact the expression of different subunits of voltage-dependent calcium channel complex proteins (*CACNA1A, CACNA2D3, CACNB2*), which each play a role in intracellular calcium homeostasis. This finding is consistent with the established finding that loss or gain of function in these proteins is associated with conditions of cognitive impairment, including GDD and ID (Kessi et al. 2021). Three of our patients had CNVs that could potentially impact the expression of neurotransmitter proteins involved in central nervous system function (*SLC6A16, SLC1A1, NRG3*), and one Xq11.2-q12 duplication included a novel X-linked intellectual disability gene (*LAS1L*). For individuals with FASD who have an abnormal CNV encoding a neurodevelopmental susceptibility gene, it remains to be elucidated as to whether PAE may cause teratogenic effects on the central nervous system by independent or potentially synergistic mechanisms. Future research is required to understand if the presence of a neurodevelopmental susceptibility gene modulates fetal susceptibility to the teratogenic impacts of alcohol.

The concept that there is a genetic vulnerability that sensitizes an individual to develop FASD in the presence of PAE is supported by the findings of three recently published studies, which highlight the potential role of rare genetic variants in neurodevelopmental disorders (Carter et al. 2024; de la Morena-Barrio et al. 2018; McKay et al. 2024). A Canadian study in 23 children with FASD investigated variants in retinoic acid signaling network genes and reported novel FASD risk and resilience alleles that were nominated as potential future biomarkers for determining the risk of FASD following PAE (McKay et al. 2024). In a cohort of 25 Spanish children with fetal alcohol syndrome (FAS), rare missense/splice site variants were more frequent compared to controls (84% vs. 50%), and three patients carried variants with functional effects similar to those identified in patients with congenital disorders of glycosylation (de la Morena-Barrio et al. 2018). In a study of an ancestrally mixed South African cohort of 280

children and 308 mothers, both maternal and child ancestry profiles were associated with higher FASD risk, and PAE effects on child cognition were informed by maternal and child genetics (Carter et al. 2024). Collectively, these findings suggest that rare genetic variants in a fetus may increase the risk of FASD when a mother consumes alcohol during pregnancy. Our results in a large Australian FASD cohort study have reproduced similar findings to those reported in three very different world populations and further highlight the significant role that genetic variants may play in the evolution of neurodevelopmental disorders including FASD.

Our study identified that children who underwent genetic testing following a FASD diagnostic assessment were more likely than not to have a minor congenital anomaly. This finding suggests that clinicians may demonstrate a bias toward advocating for the undertaking of genetic investigations in the presence of significant phenotypical features. The borderline significant finding of a decreased frequency of disruptive, impulsive control, and conduct disorder in children who completed genetic testing, compared to those who did not, may be attributable to limited statistical power rather than a true group difference.

Our study demonstrates multiple strengths. Firstly, it includes the largest published cohort of children with both a diagnosis of FASD and CMA testing. Secondly, internal validity of the FASD diagnosis was ensured by requiring case congruency with the Australian Guide to the diagnosis of FASD (Bower and Elliott 2016). Thirdly, each CNV was independently processed by a senior clinical geneticist using internationally recognized criteria and genetic databases to characterize and code. Finally, these data represent 89.7% of all children diagnosed with FASD through our service in the study period.

The retrospective nature of our study has inherent methodological limitations including the absence of a control sample (e.g., children with neurodevelopmental disorders but without a FASD diagnosis). Secondly, the inclusion of CMA test results from a variety of laboratories using heterogeneous techniques over time may have underestimated the CNV rate. Further, microarray platforms have a resolution limitation and may not detect small variants and point mutations (Zarrei et al. 2015). Finally, we acknowledge the inability to determine the mode of CNV inheritance in our study cohort, because 82.5% of the children resided in out-of-home care, which is consistent with information reported in other international FASD cohorts.

Our study has a number of implications for clinical practice. For example, the high frequency of rare CNVs identified in our cohort of children with FASD suggests we should advocate for the inclusion of CMA as a first-tier genetic investigation for all patients diagnosed with FASD as routine care. This is to ensure a comprehensive diagnostic formulation process and support clinical management. This practice would have the potential to identify both comorbid genetic abnormalities and incidental conditions that may benefit from medical management. In addition, screening for abnormal CNVs could identify clinically important neurosusceptibility genes that may support an improved understanding of the individual's presentation. Further, when a genetic abnormality is identified, we would recommend that genetic consultation be sought to inform the clinical recommendations and support potential family planning decisions. Consultation may result in a recommendation for whole-exome sequencing, additional medical screening investigations and/or referral to other clinical subspecialties.

In our clinical experience, it is not uncommon for children to experience wait times of up to three years to secure a genetic consultation after receiving a diagnosis of FASD. Within our service, a direct consequence of our study was the establishment of a bi-monthly multidisciplinary FASD and Genetics teams meeting to expediate this process. Using this mechanism, we have successfully case conferenced 28 cases of FASD between 2023 and 2024. This practice enables robust debate regarding the congruency of the identified abnormal genotype with the presenting phenotype and neurodevelopmental profile, resulting in a comprehensively informed diagnostic formulation and clinical recommendations.

A similar approach could be executed nationally and internationally to decrease wait times for genetic consultation and expediate the interpretation and implications of abnormal genetic results. In Australia, any child under 10 years of age with a GDD, ID, ASD, or at least two congenital abnormalities is eligible for CMA under the Medicare Benefits Schedule at no charge to the patient. We acknowledge, however, that access to genetic testing is neither universally available nor financially accessible in many other countries.

In conclusion, the results of our study suggest that all children with a diagnosis of FASD should be offered a CMA because a significant proportion have a comorbid clinically significant CNV. When a rare CNV is identified, seeking a genetic opinion is merited to inform the diagnostic formulation and support clinical recommendations. If the CNV result is normal, due consideration should be given to using alternative options for genetic testing, including whole-exome sequencing (WES), particularly in the context of a patient with an established intellectual disability or unrecognized dysmorphology.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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