Utility of Genetic Testing in Fetal Alcohol Spectrum Disorder

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Objective To study the utility of genetic evaluation and testing in patients with suspected fetal alcohol spectrum disorder (FASD).

Study design We performed a retrospective chart review of all patients (n = 36) referred for evaluation for suspected FASD to the genetics clinic at Boston Children's Hospital between January 2006 and January 2013. Records of all patients were reviewed to obtain the medical history, family history, examination findings, and investigations, including genetic testing.

Results Of the 36 patients, definite prenatal exposure was documented in 69%. Eight patients did not fulfill clinical criteria for FASD. Chromosomal microarray analysis (CMA) detected 19 copy number variants (CNVs) in 14 patients. Among patients who fulfilled criteria for FASD and underwent CMA, pathogenic CNVs were detected in 3 patients (2q37del, 22q11.22dup, and 4q31.21del syndromes), giving a yield of 14.3%. All 3 patients had overlapping features between FASD and the genetic syndrome.

Conclusion Genetic testing, especially CMA, should be considered in patients referred for evaluation of FASD, as a significant proportion have a clinically significant CNV even when they fulfill diagnostic criteria for FASD spectrum. *(J Pediatr 2017;■■:■■-■■).*

Fetal alcohol spectrum disorder (FASD) is one of the commonest developmental disorders, with an estimated global prevalence of 7.7 per 1000 live births.[1] It is a clinical diagnosis and is based on the presence of growth retardation (pre- or postnatal onset), microcephaly, neurologic dysfunction, and distinct craniofacial features.[2-7] Diagnostic categories for FASD have been proposed by Stratton et al in 1996[8] and updated by Hoyme et al in 2005[9] and in 2016.[10] These include fetal alcohol syndrome (classical FAS) with confirmed maternal alcohol exposure, FAS without confirmed maternal alcohol exposure, partial fetal alcohol syndrome (pFAS) with confirmed maternal alcohol exposure, pFAS without confirmed maternal alcohol exposure, alcohol-related neurodevelopmental disorders (ARNDs), and alcohol-related birth defects (ARBDs). In addition, individuals with FASD are at risk of multiple comorbidities, including conduct disorder, receptive and expressive language disorders, and abnormal results of function studies of peripheral nervous system and special senses.[11]

Diagnosis of FASD in the newborn or infant remains a challenge, as neuropsychological assessment is age and development dependent and may be difficult in infancy.[3,12] A facial scoring system can be helpful in the assessment of newborn infants at risk of FASD.[4] A history of prenatal exposure to alcohol, although useful, is not necessary for those with classical FAS but is required for pFAS or ARND subtypes. Conversely, not all babies with prenatal exposure to alcohol develop FASD. Only approximately 10%-15% of pregnancies exposed to alcohol result in a child with FASD, suggesting a role of modifying factors, either genetic or environmental.[13]

In view of the evolving understanding of FASD, in 2016, the Canada Fetal Alcohol Spectrum Disorder Research Network had suggested a revised diagnostic criteria, using FASD as a diagnostic term: this would consist of either FASD with sentinel facial features (classical FAS) or without sentinel facial features (pFAS and ARND), and add a new "at-risk" category for individuals who do not meet diagnostic criteria but are still at risk of FASD.[14] However, adoption of these revised guidelines is not universal, and training programs are under development to aid dissemination.[10,14]

At the same time, with updates in genetic testing technologies, such as chromosomal microarray analysis (CMA), 10%-15% of patients with developmental delay and/or multiple congenital anomalies have been diagnosed to have submicroscopic chromosomal aberrations that explain their clinical phenotype.[15] However, the role of a genetics evaluation and testing in the diagnostic process in a child with developmental delay and/or craniofacial anomalies with suspected FASD is not fully

ARBD	Alcohol-related birth defect
ARND	Alcohol-related neurodevelopmental disorder
CMA	Chromosomal microarray analysis
CNV	Copy number variant
FAS	Fetal alcohol syndrome
FASD	Fetal alcohol spectrum disorder
pFAS	Partial fetal alcohol syndrome
VUS	Variants of uncertain significance

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established.[16,17] Through this study, we aim to understand the utility of genetic evaluation and testing in patients with suspected FASD referred to our genetics clinic.

Methods

We included all patients who were referred for evaluation for suspected FASD to the genetics clinic at Boston Children's Hospital (Boston, Massachusetts) between January 2006 and January 2013. Approval was obtained from the institutional review board at Boston Children's Hospital for this study.

Each individual had undergone a standard genetic assessment in the genetics clinic. Records of all patients were reviewed to obtain the following information: demographic data, referral characteristics, birth history, history of prenatal alcohol exposure, history of exposure to other toxins, family history, medical history, examination findings, including presence of major and minor malformations, and investigations, including genetic testing.

The investigators performed craniofacial examination and scoring. Two investigators then reviewed the data independently and classified the cases into 1 of the following diagnostic categories[10]: (1) FAS: presence of all of the following (with or without documented prenatal alcohol exposure): growth retardation, craniofacial features, deficient brain growth, and neurodevelopmental delay; (2) pFAS with documented prenatal exposure to alcohol and presence of craniofacial features and neurodevelopmental delay, with or without 1 of the following: growth retardation or deficient brain growth and/or unknown prenatal exposure to alcohol and presence of craniofacial features and neurodevelopmental delay with 1 of the following: growth retardation or deficient brain growth; (3) ARND: definite prenatal exposure to alcohol with neurodevelopmental delay, no growth retardation, deficient brain growth, or craniofacial features; (4) ARBD: definite prenatal exposure to alcohol and 1 or more specific major malformations associated with prenatal alcohol exposure; and (5) not FASD: does not fulfill any of the aforementioned criteria. Data analysis and scores were compared to ensure agreement.

CMA was performed in the clinical laboratory at Boston Children's Hospital. It was performed with either oligoarray (before 2010) or 180K SNP arrays (2010 onwards). Abnormal copy number variants (CNVs) detected through CMA were reinterpreted independently and classified on the basis of the American College of Medical Genetics and Genomics guidelines[18] into the following categories: (1) pathogenic: associated with a well-described phenotype and/or included a gene in which abnormality in copy number is a known mechanism of disease causation; (2) familial variant: inherited from a phenotypically normal parent; and (3) variants of uncertain significance (VUS): when parental samples were unavailable and/or variant included genes that had potential relevance to the phenotype but had yet to be associated with human disease.

All data were entered on a standard study pro forma followed by an MS Excel spreadsheet (Microsoft, Redmond, Washington). The data were analyzed via frequency analysis to identify common findings and significant differences within the study cohort.

Results

In the 7-year study period, 36 patients were referred with a suspicion of FASD. The median age at initial evaluation was 3.5 years, ranging from newborn to 17 years. There were 20 (56%) female patients. Primary care physicians (75%) were the main source of referrals.

Among the 36 patients, 25 (69%) had definite exposure to alcohol during the pregnancy. In the remaining, prenatal exposure to alcohol was suspected but could not be verified, as the biological parents were unavailable (**Table I**). The patients with definite exposure were referred for evaluation at a younger age (median age 1.9 years) compared with those with unknown exposure (median age 9.0 years, $P = .021$). There were no other significant differences between the 2 groups. The majority of patients in both groups had coexposure to other toxins or substances of abuse (amphetamines, cocaine, tobacco, barbiturates, citalopram, clonazepam, methadone, clonidine, opiates, hydrocodone, heroin, and oxycodone).

Clinical Features

Speech delay was the commonest developmental problem, affecting 22 (61%) patients (**Table I**). Twenty (56%) had behavioral problems, and 10 (28%) had intellectual disability and/or learning difficulties.

Fourteen (39%) had a family member with a psychiatric disorder, such as bipolar disorder, schizophrenia, or attention deficit–hyperactivity disorder (**Table I**). Seven (19%) had a family history of learning disorder, and 7 (19%) had a family history of FASD.

Twelve (33%) patients had growth retardation, defined as weight below the third percentile for age and sex (**Table I**). Nine (25%) patients had microcephaly, defined as a head circumference below the third percentile for age and sex. Twenty-seven (75%) patients had craniofacial features suggestive of FASD, with a smooth philtrum being observed most commonly in 25 (69%) of these cases. Depressed midface, thin upper lip, and short palpebral fissures were the other features commonly observed in 20 (56%), 19 (53%), and 18 (50%) of the patients, respectively. One individual had Pierre Robin sequence, which was determined to be unrelated to alcohol exposure as the child did not fit clinical criteria for FASD.

Clinical Classification

Seven patients fulfilled criteria for classical FAS. Sixteen patients were given the diagnosis of pFAS, 5 were classified as ARND, and none were classified as ARBD. Eight patients did not fulfill criteria for FAS.

Genetic Testing

A karyotype was performed on 20 (56%) patients—1 individual with a definite alcohol exposure also had an interstitial

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