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ADVANCES IN PEDIATRIC RESEARCH

What proportion of the brain structural and functional abnormalities observed among children with fetal alcohol spectrum disorder is explained by their prenatal alcohol exposure and their other prenatal and postnatal risks?

Susan J Astley Hemingway*, Julian K. Davies, Tracy Jirikowic, Erin M. Olson

University of Washington, Seattle, WA, USA

Abstract

Background: Individuals with prenatal alcohol exposure (PAE) often present with a myriad of other prenatal (e.g. exposure to tobacco and other illicit drugs, poor prenatal care) and postnatal risk factors (e.g. multiple home placements, physical/sexual abuse, low socio-economic status)-all of which are likely contributing to their adverse outcomes.

Methods: A comprehensive neuropsychological battery, coupled with magnetic resonance imaging, was administered to children with fetal alcohol spectrum disorders (FASD) in 2009. Study participants diagnosed with FASD by the University of Washington using the FASD 4-Digit Code were compared to typically-developing peers with no PAE. Data from this MRI study were used to explore the proportion of variance in brain structural and functional abnormalities explained by PAE and 14 other prenatal and postnatal risk factors.

Results: PAE was the dominant risk factor explaining the largest proportion of variance in regional brain size (total brain, frontal lobe, caudate, hippocampus and corpus callosum) and brain function (intellect, achievement, memory, language, executive-function, motor, adaptation, behavior-attention and mental health symptoms). Other prenatal and postnatal risk factors were 3 to 7-fold more prevalent than in the general population. Individually, each risk factor explained a statistically significant, but smaller proportion of variance in brain outcome compared to PAE. In combination, the proportion of variance explained by the presence of multiple prenatal and postnatal risks rivaled that of PAE.

Conclusion: A better understanding of the impact other prenatal and postnatal risk factors have on the neurodevelopmental outcomes of individuals with FASD can inform more effective prevention and intervention strategies.

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*E-mail: astley@uw.edu

Introduction

Individuals with prenatal alcohol exposure (PAE) often present with a multitude of other prenatal (e.g. exposure to tobacco and other illicit drugs, poor prenatal care, premature birth) and postnatal risk factors (e.g. multiple home placements, physical/sexual abuse, low socio-economic status (SES) [1,2]. Each of these risks individually is known to contribute to adverse neurodevelopmental outcomes [3-9].

To date, little research has examined the combined impact of these other prenatal and postnatal exposures on the neurodevelopmental outcomes of children with PAE. Interactions between PAE and prenatal cocaine exposure have been documented in a few studies [10-12]. Streissguth et al., [13] documented more impaired outcomes among individuals with FASD that experienced longer versus shorter durations of adverse postnatal experiences (abuse, violence, neglect). In a literature review, Price et al., [14] identified five studies that investigated the combined impact of trauma in children with PAE. In one of these studies, children with both PAE and trauma were more likely to have deficits in language, attention, memory, intelligence and more severe behavioral problems than children with only one of these adverse exposures [15]. Most recently, Uban et al., [16] assessed the impact of socioeconomic status (SES) on neurostructural development in children with and without PAE. As anticipated, typically developing youth without PAE exhibited increased subcortical volumes with increased SES. But unexpectedly, SES-brain associations were not observed among the youth with PAE.

Two key challenges to researching the adverse impacts of PAE and other prenatal and postnatal risk factors on neurodevelopment are 1) finding reliable documentation of these adverse exposures and experiences; and 2) having access to a study population that have all had the same comprehensive, standardized neuropsychological battery of assessments and MR imaging protocols. PAE along with other prenatal and postnatal risks are typically recorded in past medical, legal and social service records; records that are routinely obtained by FASD diagnostic clinics in preparation for a FASD diagnostic evaluation. Review and documentation of these other risks has always been a priority with the FASD 4-Digit Code, a systematic, validated diagnostic system. These risk factors are formally recorded in the FASD 4-Digit Code Diagnostic Form [17] and ranked on 4-point Likert scales (Prenatal Rank and Postnatal Rank) just like the growth, face, brain and alcohol components of the FASD 4-Digit Code. But FASD diagnostic clinics rarely have the ability to validly administer a single, standardized, comprehensive battery of neuropsychological assessments to all patients. Many factors come into play when selecting the most appropriate battery for an individual patient (their age, clinical presentation, previous participation in neuropsychological assessments, etc.). Comprehensive, standardized neuropsychological assessment batteries and MRI imaging protocols are the mainstay of controlled research studies, not diagnostic clinics.

The present study sought to capitalize on the combined strengths of our clinical and research programs. In 2009, a comprehensive FASD neuropsychological-magnetic resonance imaging study was completed at the University of Washington FAS Diagnostic & Prevention Network (FASDPN) [18-21]. Patients previously diagnosed across the full continuum of FASD were enrolled in the study. The combination of clinical and research protocols produced a study population with the full complement of exposure and outcome measures necessary to explore the proportion of variance in brain structural and functional abnormalities explained by PAE and other prenatal and postnatal risk factors. A better understanding of the impact other prenatal and postnatal risk factors have on the neurodevelopmental outcomes of individuals with FASD can inform more effective primary prevention and intervention strategies.

Research Methodology

Subjects and study groups

The current study is a retrospective exploratory analysis of data collected from a 2009 neuropsychological and magnetic resonance imaging (MRI) study [18-21] comparing central nervous system (CNS) structural and functional outcomes between children with FASD and typically developing peers with confirmed absence of PAE. In the original 2009 study, three FASD groups (defined below) were selected from among 1,200 patients previously diagnosed by an interdisciplinary team in the WA State FAS Diagnostic & Prevention Network (FAS DPN) of clinics using a comprehensive, validated diagnostic system called the FASD 4-Digit Code [17,22,23]. Briefly, the 4 digits of the FASD 4-Digit Code reflect the magnitude of expression of the 4 key diagnostic features of FASD, in the following order:

1. Growth deficiency,
2. FAS facial phenotype,
3. CNS structural/functional abnormalities, and
4. PAE

The magnitude of expression of each feature was ranked independently on a 4-point Likert scale, with 1 reflecting complete absence of the FASD feature and 4 reflecting a strong "classic" presence of the FASD feature. Each Likert rank is specifically case defined (Figure 1). 4-Digit Codes range from 1111 to 4444. Each 4-digit diagnostic code falls into 1 of 22 unique clinical diagnostic categories (labeled A through V). Seven of the 22 diagnostic categories (4-Digit Categories A–C and E–H) fall under the umbrella of FASD (A. FAS/ Alcohol Exposed, B. FAS/Alcohol Exposure Unknown, C. Partial FAS/Alcohol Exposed, E & F. Static Encephalopathy/Alcohol Exposed, and G & H. Neurobehavioral Disorder/Alcohol Exposed).

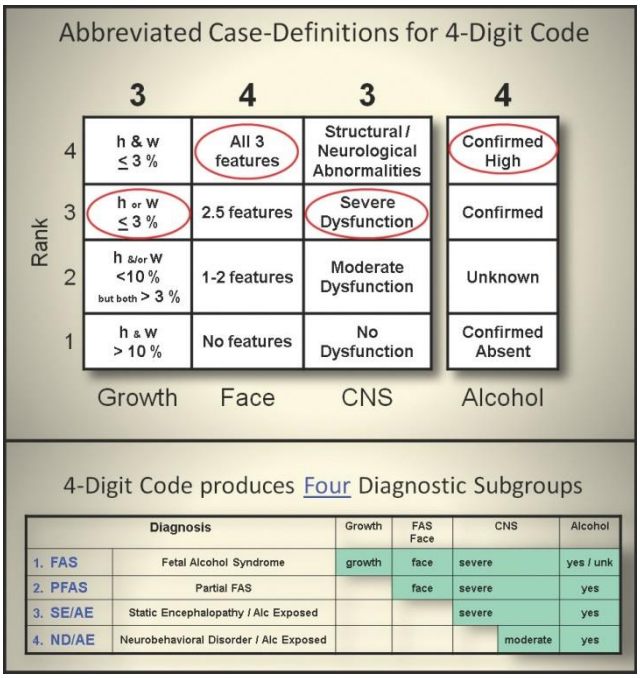

Figure 1. Abbreviated case-definitions of the FASD 4-Digit Code [17,23].
The 4-Digit Code 3434 is one of 12 Codes that fall under the diagnostic category FAS. The 4-Digit Code produces four diagnostic subgroups under the umbrella of FASD: FAS, PFAS, SE/AE, and ND/AE. Abbreviations: Alc alcohol; CNS central nervous system; h height; w weight; % percentile

The control population in the original 2009 study was selected from a large cohort of children enrolled at birth in a University of Washington study of typical development conducted through the Department of Speech and Hearing Sciences. This registry has been maintained over the years to serve as a source of typically developing controls for studies throughout the University. With the enrollment of each child in the FAS/PFAS group, a child matched on age (within 6 months), gender, and race was randomly identified and invited to enroll from the eligible SE/AE, ND/AE and Control populations.

The enrollment procedure for the original 2009 study produced a sample of 81 children. The age range (8 to 15.9 years) included the broadest age range of children that could be administered a comparable psychometric assessment battery and be reasonably capable of participating in the MR scanning. Each of the four study groups had 16-24 subjects successfully balanced on age, gender, and race. The 61 children with FASD in the original study were highly representative of the entire clinic sample of 2,828 from which they were drawn [1].

The diagnostic features specific to each study group were as follows:

1. Children in Group 1 had a 4-Digit diagnosis of FAS or Partial FAS (FAS/PFAS)/ Alcohol Exposed (e.g. 4-Digit Diagnostic Categories A,B,C: with Growth Ranks 1-4, Face Ranks 3-4, CNS Ranks 3 and/or 4, Alcohol Ranks 3-4). In summary, children in Group 1 had severe cognitive/behavioral dysfunction and the FAS facial phenotype.

2. Children in Group 2 had a 4-Digit diagnosis of Static Encephalopathy / Alcohol Exposed (SE/AE) (e.g. 4-Digit Diagnostic Categories E,F: with Growth Ranks 1-4, Face Ranks 1-2, CNS Ranks 3 and/or 4, Alcohol Ranks 3-4). In summary, children in Group 2 had severe cognitive/behavioral dysfunction, comparable to Group 1, but did not have the FAS facial phenotype.

3. Children in Group 3 had a 4-Digit diagnosis of Neurobehavioral Disorder / Alcohol Exposed (ND/AE) (e.g. 4-Digit Diagnostic Categories G, H: with Growth Ranks 1-4, Face Ranks 1-2, CNS Rank 2, Alcohol Ranks 3-4). In summary, children in Group 3 had PAE comparable to Groups 1 and 2, but in comparison to Groups 1 and 2 had only mild to moderate cognitive/behavioral dysfunction, and did not have the FAS facial phenotype.

4. Children in Group 4 (Typically Developing Controls / No Alcohol Exposure) were selected based on parental report that the child was typically developing, and no PAE (e.g. 4-Digit Diagnostic Category V: with Growth Ranks 1-2, FAS Face Ranks 1-4, CNS Ranks 1-2, Alcohol Rank 1). In

summary, these were non-exposed, average to high functioning controls.

Socio-demographic and clinical assessment

In the original 2009 study a comprehensive sociodemographic and health/medication history of each child was obtained by parent interview and record review. Information included birth data, growth, and all prenatal and lifetime exposures and adverse events. For subjects with FASD, most information was obtained at the time of their FASD diagnostic evaluation and recorded on the 4-Digit Code FASD Diagnostic Form [17].

Measures of PAE

The following measures of maternal alcohol consumption were collected retrospectively, with a focus on two time points (just before pregnancy and during pregnancy): a) average and maximum number of drinks per drinking occasion, b) average number of drinking days per week, c) type(s) of alcohol consumed (beer, wine, liquor), and d) trimester(s) of exposure.

Other prenatal and postnatal risk factors

Measures of other prenatal and postnatal adverse exposures and experiences were collected from a caregiver interview and/or review of records. Prenatal risks included: maternal use of tobacco, marijuana, cocaine, any illicit drugs and no prenatal care (all measured on a yes/no scale). While illicit drugs other than marijuana and cocaine were reported, the prevalence of each individual drug was too low in this study population to include as separate risk factors. Postnatal risks included: not living with either birth parent, number of foster placements, physical abuse, sexual abuse, SES of current caregiver (e.g. years of education attained, occupation prestige, and gross annual family income level). All SES measures were based on the subject's current primary caregiver participating in the study. Education and occupation were codified in accordance with the Hollingshead Four-Factor Index of SES [24] as follows. The parent's education was rated on a 7-point scale with 1 equal to less than a 7th grade education; 4 equal to a high school education and 7 equal to graduate/ professional training. The parent's occupational code was rated on a 9-point scale from 1 equal to farm laborers, menial service workers, students, housewives; 6 equal to technicians, semi-professionals, small business owners, to 9 equal to higher executive, proprietor of large businesses, major professionals. The scale score for education is multiplied by a weight of three; the scale score for occupation is multiplied by a weight of 5. Annual income was coded < $50,000 reflecting roughly twice the U.S. Health and Human Services Poverty Guidelines for a family of four in 2009 [25].

All children had a standardized digital facial photograph taken at the time of enrollment in the original 2009 study. The facial photographs were analyzed using the FAS Facial Analysis Software [26] to generate two measures of the magnitude of expression of the FAS facial phenotype: 1) the 4-Digit Code Facial Rank (1 to 4) [23] and 2) the continuous FAS facial D-score [27]. The D-score documents the severity of the FAS facial phenotype on a continuous scale. The higher the D-score, the more FAS-like the facial phenotype. A D-score > 0.8 is equivalent to a Rank 4 FAS facial phenotype.

Neuropsychological / Psychiatric assessments

In the original 2009 study, a comprehensive, standardized neuropsychological battery was administered to each child and their primary caregiver by a psychologist masked to group assignment (Table 1). Based on an extensive review of the prior literature, the assessment battery was designed to capture the domains of potential neuropsychological deficit and mental health conditions seen as the result of the typically diffuse brain damage arising from alcohol teratogenesis [28-30]. Key outcome measures (composite and subtest scores) from the battery of assessments were selected in the original study to represent the different domains of deficit (Figure 2). These same outcome measures served as the primary dependent variables for brain function in the current study [31-75].

Table 1. Assessment battery administered to the four study groups.

Soft Neurological Signs	Quick Neurological Screening Test II (QNST-II) [60]
General Intellectual Function	Wechsler Intelligence Scale for Children-Third Edition (WISC-III) [33]
Academic Achievement	Wechsler Individual Achievement Test (WIAT) Basic Reading subtest) [61]
	KeyMath Revised/NU: A Diagnostic Inventory of Essential Mathematics [62]
Visuospatial Skills, Visual Memory, and Organization	Beery Buktenica Developmental Test of Visual-Motor Integration (VMI) [63]
	Rey Complex Figure Test (RCFT) [64]
Executive Function	Delis-Kaplan Executive Function System (D-KEFS) Trail Making Test [65]
	Delis-Kaplan Executive Function System (D-KEFS) Tower Test [65]
	Delis-Kaplan Executive Function System (D-KEFS) Color-Word Interference Test [65]
	Delis-Kaplan Executive Function System (D-KEFS) Verbal Fluency Test: Standard [65]
	Wisconsin Card Sorting Test: Computer Version 3 (WCST) Research Edition [66]
Verbal Memory	California Verbal Learning Test-Children's Version (CVLT-C) [67]
Attention	Integrated Visual and Auditory Continuous Performance Test (IVA CPT) [68]
Receptive and Expressive Language	Test of Language Development-Intermediate: Third Edition (TOLD-I:3) [69] • Sentence Combining subtest (subjects aged 8 to 10 years)
	Test of Language Competence-Expanded Edition (TLC-1-Expanded) Level 1 [70] • Oral Expression: Recreating Speech Arts subtest (subjects aged 8 to 9 years)
	Test of Language Competence-Expanded Edition (TLC-2-Expanded) Level 2 [70] • Oral Expression: Recreating Sentences subtest (subjects aged 10 to 15.9 years)
	Test of Word Knowledge (TOWK) [71] • Conjunctions and Transition Words subtest (subjects aged 11 to 15.9 years)
Adaptive Behavior	Vineland Adaptive Behavior Scales (VABS) Interview Edition, Survey Form[72]
Behavior Problems and Social Competence	Child Behavior Checklist for Ages 6-18 (CBCL/6-18) [73]
Caregiver Report of Behaviors Related to Executive Function	Behavior Rating Inventory of Executive Function (BRIEF) [74]
Psychiatric Conditions	Computerized Diagnostic Interview Schedule for Children: Parent Form (C-DISC) [75]

Magnetic resonance evaluation

The MRI components of this study are reported separately [19]. Briefly, all scans were acquired using a General Electric 1.5 Tesla scanner in the Diagnostic Imaging Sciences Center at the University of Washington. MRI was used to measure the size (volumes and/or midsagittal areas) of the following structures: total brain, frontal lobe, caudate, putamen hippocampus, corpus callosum, and cerebellar vermis. These outcomes served as the primary dependent variables for brain structure.

Statistical analyses

Descriptive statistics (means, SDs, proportions) were used to summarize the sociodemographic and clinical profiles of the four study groups (Table 1). For comparisons between groups, chi-square was used for categorical variables and ANOVA was used for continuous variables. When ANOVA was employed, the overall f- statistic was used to test if differences existed among the four group means. When the overall f-statistic was statistically significant, the Duncan post hoc range test was used to identify which group means differed. The

Duncan test makes pairwise comparisons using a stepwise procedure. Means are ordered from highest to lowest, and extreme differences are tested first. The Duncan test identifies homogeneous subsets of means that are not different from one another. For example, when the mean head circumference was compared across the 4 study groups, the outcome was depicted 1,23,4 (group 1 was significantly smaller than groups 2 and 3, and group 4 was significantly larger than groups 1, 2 and 3. SPSS [31] linear regression with forward entry (probability of F to enter <0.05) was used in this exploratory analysis to identify which risk factor(s) explained a statistically significant proportion of the variance in CNS structural and functional outcomes. With forward entry, independent variables are added to the equation one at a time. At each step, the variable not in the equation with the smallest probability of F is entered if the value is smaller than .05. The order in which independent variables are entered into the equation provides insight about the quality of the predictor variables. Separate regressions were conducted for each CNS brain region and each neuropsychological measure within each functional domain (Figure 2). These separate regressions were conducted, in part, to explore if similar patterns of risk factors explained the different neuropsychological outcomes within a domain of function (e.g. were similar patterns of risk identified across the five WISC-III subtests within the General Intellectual Function domain) (Figure 2). Also of interest was whether similar patterns of risk were identified between brain regions and functions often attributed to those brain regions (e.g. hippocampus and memory, caudate and cognition). Only cases with valid data across all variables were included in each regression analysis (SPSS missing = list wise procedure). All regressions met the following goodness of fit and collinearity parameters: dependent variables normally distributed; independent variables: tolerance >0.1, Variance Inflation Factor <10, variance decomposition proportions: no two variables >0.90 and Condition Index <50. Partial and standardized residual plots were used to validate assumptions of normality, linearity and equality of variances. The proportion of variation in the dependent variable (brain region sizes and neuropsychological assessment scores) attributable to each risk factor is reported as the adjusted R^2. The adjusted R^2 is a modified version of R^2 that has been adjusted for the number of predictors in the model. P-values were not adjusted for multiple comparisons in this exploratory analysis, thus should be interpreted accordingly.

Results

Study population

Of the 81 subjects enrolled in the original MRI study, 50 presented with complete data across all independent and dependent variables needed for this current study. Although presence or absence of PAE was reliably documented for all subjects; more detailed information such as quantity, frequency, and duration of PAE was only available on 53 of the 65 alcohol-exposed subjects. This is not atypical, as accurate, detailed alcohol histories are frequently unavailable on patients presenting to a FASD diagnostic clinic. The regression analyses described below confirmed that the more detailed measures of quantity, frequency and timing of PAE (not just the presence or absence of PAE) were necessary to detect the correlations between PAE and brain outcomes. It is for this reason the study sample was restricted to those with this level of detail available. All controls had a reported absence of PAE per birth mother report. This subset of 50 subjects (11 FAS/PFAS, 12 SE/AE, 11 NE/AE and 16 Controls) (Table 2) was highly representative of the larger study group of 81 subjects in the original 2009 study [19,20] as well as the entire clinical population of 2,828 patients evaluated to date in the FASDPN clinic [1,32] from which they were selected.

Table 2. Sociodemographic and FASD 4-Digit Diagnostic Code profiles of the four study groups.

Characteristic	Groups				Statistics		
					ANOVA		Chi^2
	1. FAS/PFAS[A,B]	2. SE/AE	3. ND/AE	4. Control	Overall	PostHoc	Chi
	N = 11	N = 12	N = 11	N = 16	F (p)[C]	Duncan	(p)
Gender: female, n (%)	5 (45.5)	9 (75.0)	6 (54.5)	8 (50.0)			2.5 (.48)
Age in years at enrollment, mean (SD)	12.9 (2.4)	12.5 (2.7)	11.8 (2.7)	12.4 (2.7)	.33 (.81)		
Race, n (%)							
Caucasian	6 (54.5)	4 (33.3)	7 (63.6)	13 (81.3)			6.1 (.11)[D]
African American	3 (27.3)	3 (25.0)	3 (27.3)	2 (12.6)			
Native American	2 (18.2)	5 (41.7)	1 (9.0)	0 (0.0)			
Asian	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.3)			
Growth Rank, 4-Digit Code, n (%)							
1. None	4 (36.4)	9 (75.0)	7 (63.6)	15 (93.7)			10.5 (.01)[E]
2. Mild	2 (18.2)	2 (16.7)	3 (27.3)	1 (6.3)			
3. Moderate	4 (36.4)	0 (0.0)	0 (0.0)	0 (0.0)			
4. Severe	1 (9.1)	1 (8.3)	1 (9.1)	0 (0.0)			
Face Rank, 4-Digit Code, n (%)							
1. None	0 (0.0)	3 (25)	3 (27.3)	10 (62.5)			
2. Mild	0 (0.0)	9 (75)	8 (72.7)	6 (37.5)			
3. Moderate	3 (27.3)	0 (0.0)	0 (0.0)	0 (0.0)			
4. Severe [F]	8 (72.7)	0 (0.0)	0 (0.0)	0 (0.0)			
FAS facial D-score [G], mean (SD)	1.16 (1.0)	-0.6 (1.0)	-0.8 (0.7)	-1.5 (0.9)	17.8 (.000)	1,23,34	
CNS Ranks 1-3, 4-Digit Code, Functional impairment level, n (%)							
1. None	0 (0.0)	0 (0.0)	0 (0.0)	16 (100)			
2. Moderate	0 (0.0)	2 (16.7) [H]	11 (100)	0 (0.0)			
3. Severe	11 (100)	10 (83.3)	0 (0.0)	0 (0.0)			
CNS Rank 4, 4-Digit Code Structural/Neurologic Abnormality, n (%)	10 (90.9)	3 (25.0)	0 (0.0)	0 (0.0)			10.1 (.001)[I]
Current OFC percentile, mean (SD)	10.9 (29.2)	51.6 (34.6)	53.6 (8.7)	82.7 (18.1)	19.7 (.000)	1,23,4	
Microcephaly (OFC $\leq$ -2 SD), n (%)	10 (90.1)	2 (16.7)	0 (0.0)	0 (0.0)			12.7 (.000)[J]
Alcohol Rank, 4-Digit Code, n (%)							
1. Confirmed absent	0 (0.0)	0 (0.0)	0 (0.0)	16 (100)			
2. Unknown exposure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
3. Confirmed: Level moderate or Unk	1 (9.1)	1 (8.3)	2 (18.2)	0 (0.0)			
4. Confirmed: Level high	10 (90.9)	11 (91.7)	9 (81.8)	0 (0.0)			
Alcohol prior to pregnancy							
Days/week, mean (SD)	5.3 (1.8)	4.4 (2.0)	5.4 (2.1)	0.9 (1.0)	20.8 (.000)	123,4	
Most drinks/occasion, mean (SD)	23.1 (24.8)	23.0 (28.3)	13.5 (7.7)	1.7 (1.5)	4.2 (.01)	123,4	
Alcohol during pregnancy							
Days/week, mean (SD)	5.5 (1.7)	3.4 (2.1)	5.3 (2.1)	0 (0.0)	11.5 (.000)	13,2,4	
Most drinks/occasion, mean (SD)	11.6 (7.1)	14.1(8.9)	12.6 (7.8)	0 (0.0)	35.9 (.000)	1,234	
Drank all 3 trimesters, n (valid%)	9 (81.8)	8 (66.7)	4 (36.4)	0 (0.0)			21.9 (.000)

FASD 4-Digit Code [K] (n)	1434 (1) 1444 (3) 2444 (2) 3343 (1) 3344 (2) 3444 (1) 4444 (1)	1134 (3) 1234 (5) 1244 (1) 2233 (1) 2244 (1) 4244 (1)	1123 (1) 1124 (2) 1224 (4) 2224 (3) 4223 (1)	1111 (5) 1121 (5) 1211 (1) 1221 (4) 2221 (1)			
Other Prenatal Risk Factors							
Prenatal Rank, 4-Digit Code, n (%)							
1. No risk	0 (0.0)	0 (0.0)	0 (0.0)	16 (100)			
2. Unknown risk	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
3. Some risk	11 (100)	12 (100)	11 (100)	0 (0.0)			
4. High risk	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
Cigarette smoking, n (%)	10 (90.9)	9 (75.0)	8 (72.7)	0 (0.0)			28.5 (.000)
Any illicit drug use, n (%)	6 (54.5)	7 (58.3)	6 (54.5)	0 (0.0)			14.5 (002)
Marijuana use, n (%)	5 (45.5)	4 (33.3)	4 (36.4)	0 (0.0)			8.7 (.03)
Cocaine use, n (%)	3 (27.3)	4 (33.3)	2 (18.2)	0 (0.0)			4.6 (.03)
Poor or no prenatal care, n (%)	4 (36.4)	7 (63.6)	6 (54.5)	0 (0.0)			14.1 (.003)
Gestational age in weeks, mean (SD)	38.7 (1.9)	37.9 (1.3)	39.9 (1.9)	39.3 (1.7)			2.3 (.09)
Other Postnatal Risk Factors							
Postnatal Rank, 4-Digit Code, n (%)							
1. No risk	0 (0.0)	0 (0.0)	0 (0.0)	15 (94.0)			
2. Unknown risk	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
3. Some risk	4 (36.)	7 (58.3)	5 (45.5)	1 (6.0)			
4. High risk	7 (63.6)	5 (41.7)	6 (54.5)	0 (0.0)			
Not living with birth parent, n (%)	8 (72.7)	9 (75.0)	8 (72.7)	1 (6.2)			19.7 (.000)
Number home placements, mean (range)	5.1 (1-27)	3.5 (1-6)	4.3 (1-9)	1.1 (1-2)	2.9 (.04)	123,4	
Age (years) at 1st foster placement mean (SD)	4.7 (4.4)	3.6 (2.8)	3.1 (2.3)	3 (0.0)	.04 (.74)		
Annual household income less than $50,000, n (%)	6 (54.5)	6 (50.0)	2 (18.2)	1 (6.3)			10.4 (.02)
Caregiver education (years), mean (range)	12.4 (11-18)	12.8 (7-18)	14.4 (12-18)	16.3 (7-18)	7.1 (.001)	123,34	
Caregiver occup.: executive level [L] n (%)	1 (9.0)	1 (8.3)	6 (54.5)	8 (50.0)			10.7 (.01)
Physical abuse, n (%)	5 (45.5)	2 (16.7)	3 (27.3)	0 (0.0)			16.2 (.01)
Sexual abuse, n (%)	4 (36.4)	3 (25.0)	5 (45.5)	0 (0.0)			17.7 (.007)

Notations: **A**. Four of the 11 subjects in the FAS/PFAS group had full FAS using the 4-Digit Code. **B**. One subject with PFAS had agenesis of the corpus callosum. **C.** Between groups degrees of freedom = 3; within groups df = total sample size minus 4. **D**. Caucasian versus not Caucasian. **E**. No growth deficiency versus mild to severe growth deficiency. **F**. Definition of Rank 4 FAS Face: palpebral fissure lengths 2 or more SDs below the mean, and lip and philtrum are Rank 4 or 5 on University of Washington Lip-Philtrum Guide. **G**. No child had hypo-or hypertelorism that could impact the validity of the D-score. **H**. Both children with moderate functional impairment had structural evidence of brain abnormality (microcephaly). **I.** Chi-square for FAS/PFAS versus SE/AE. **J**. Chi-square for FAS/PFAS versus SE/AE. **K.** The 4 digits represent the rank for growth, face, brain and alcohol, in that order. **L** Reflects Level 9 Hollingshead occupation: higher executive/proprietor of large businesses.

Abbreviations: Chi2: chi-square test across the four study groups, unless otherwise specified. Duncan: The Duncan multiple comparison range test is reported if the overall ANOVA is statistically significant; commas separate groups with homogeneous means at $p < 0.05$. F: F statistic. FAS/PFAS: FAS/partial FAS. L: left. ND/AE: Neurodevelopmental Disorder/Alcohol Exposed; Occup. Occupation; OFC: occipital frontal circumference. Overall: Overall assessment of between-group means using ANOVA. p: p-value. R: right. SD: standard deviation. SE/AE: Static Encephalopathy/Alcohol Exposed. Unk: unknown. Z-score: number of standard deviations above/below the population-based mean. $: United States dollars.

Prevalence of risk factors

Among the subjects with FASD, all had confirmed PAE (88% with high Rank 4 alcohol exposures) (Table 2). Seventy-nine percent had prenatal tobacco exposure and 56% had prenatal exposure to illicit drugs (marijuana 38%, cocaine, 27%, heroine 6%, quaaludes 3%, methamphetamines 3%). Seventy-four percent were not living with their birth parents and had on average 4.3 out-of-home placements with the first placement starting at 3.8 years of age on average. Twenty-nine percent were physically abused; 35% were sexually abused. Household annual income was less than $50,000 US dollars in 2009 for 42%. Forty-six percent of their primary caregivers had a high-school degree or less. Although the presence of other risk factors was not restricted for enrollment of controls, the prevalence of other prenatal and postnatal risk factors was significantly lower among the Controls (no prenatal exposure to tobacco or illicit drugs, only one subject (6%) had one out-of-home placement, 19% had a primary caregiver with a high-school degree or less, 6% had an annual household income less than $50, 000, and no reported physical or sexual abuse)..

Prenatal alcohol exposure explained the highest proportion of variance in brain structure and function. Other risk factors explained a significant, but smaller proportion of variance

Prenatal alcohol exposure accounted for up to 34% of the variance (adjusted R^2) in regional brain volumes, up to 52% of the variance in CNS function and up to 51% of the variance in mental health symptoms (Figure 2). Of the various measures of quantity, frequency and timing of PAE available for entry into the regressions, "days per week of drinking during pregnancy" and "drank all three trimesters" demonstrated the strongest, significant correlations with brain outcomes. Other prenatal and postnatal risk factors that met criteria for entry into the regression equations each explained an additional 5-15% of the variance (Figure 2). All correlations between risk factors and brain outcomes were in the direction anticipated (the more severe the risk factor, the more severe the brain outcome). When patient gender entered the equation, being male was associated with more severe functional outcomes and larger brain volumes relative to females.

The order of entry of each risk factor into the regression equation (depicted by the numbers 1, 2, 3 or 4 in the boxes in Figure 2) documents which risk factors explained the greatest proportion of variance in the brain structural, functional or mental health outcomes. For example: of all 14 prenatal and postnatal risk factors available for entry into the regression equation (Figure 2), the number of days/ week of drinking during pregnancy explained the greatest proportion of variation (46%) in the WISC-III Full Scale Intelligence Quotient (FSIQ) [33] score and thus was the first statistically significant risk factor ($p<.05$) to enter into the regression equation. Caregiver's years of education explained the 2nd greatest and statistically significant proportion of variance of the FSIQ (an additional 8% of variance). These two risk factors together explained 54% of the variance in the FSIQ. Maternal drinking through all three trimesters was the third and final statistically significant risk factor to enter the equation, explaining an additional 4% of variance. The three risk factors together explained a total of 58% of the variance in the FSIQ. The regression equation produced was WISC-III FSIQ standard score = 86.9 -2.75 (average number of days/week drinking during pregnancy) +1.7 (Hollingshead's Score for caregiver's years of education (3 through 21 with <7th grade = 3 and post graduate = 21) – 12.4 (drank all 3 trimesters (yes = 1, no = 0)). Higher levels of prenatal alcohol use were correlated with lower FSIQ scores. Higher parental education levels were correlated with higher FSIQ scores.

				Prenatal Risks						Postnatal Risks						
	Brain Outcome		Proportion of variance explained: Adjusted R^2	Alc: days/ wk	Alc: all 3 Tri	gender	cigs	cocaine	mj	any illicit drugs	# homes	not w/ birth parent	SES: educ	SES: occup	SES: low income	phys abuse	sex abuse
BRAIN STRUCTURE	Brain:	Total brain volume (cm3)	.17 .31	2		1											
		Total brain midsaggital area (cm2)	. 20	1													
		Current OFC (cm)	0.26	1													
	Frontal Lobe:	Frontal lobe gray matter volume (cm3)	.20 .29 .35 .43	1		2			3							4	
		Frontal lobe white matter volume (cm3)	. 13	1													
		Frontal lobe volume (cm3)	.21 .28	1		2											
	Caudate:	R. Caudate volume (cm3)	.30 .39		1					2							
		L. Caudate volume (cm3)	.29 .38		1					2							
		Total Caudate volume (cm3)	.30 .40		1					2							
	Putamen:	R. Putamen volume (cm3)	. 08							1							
		L. Putamen volume (cm3)	. 12									1					
		Total Putamen volume (cm3)	. 10									1					
	Hippocampus:	R. Hippocampus volume (cm3)	. 34	1													
		L. Hippocampus volume (cm3)	.28 .32	1								2					
		Total Hippocampus volume (cm3)	. 33	1													
	Cerebellar Vermis:	Total CV: midsagittal area (cm2)	. 10													1	
		CV: lobules I–V midsagittal area (cm2)	.10 .18 .29			3		2		1							
		CV: lobules VI–VII midsagittal area (cm2)	. 09													1	
		CV: lobules VIII–X midsagittal area (cm2)	. 15				1										
	Corpus Callusom:	CC: midsagittal area (cm2)	.13 .23												2		1
		CC: Region 1 (genu) (cm2)	.16 .26												1		2
		CC: Region 2 (cm2)	. 10														1
		CC: Region 3 (cm2)	. 10														1
		CC: Region 4 (cm2)	. 11												1		
		CC: Region 5 (splenium) (cm2)	. 10							1							
		CC: Length (cm)*	.20 (.26)	1													
BRAIN FUNCTION	Soft Neurologic Signs:	QNST-II: Total Score (raw)	. 24	1													
	General Intellectual Function:	WISC III Full Scale IQ (ss)	.46 .54 .58	1	3								2				
		WISC III Verbal IQ (ss)	.43 .56 .62	1	3								2				
		WISC III Performance IQ (ss)	.40 .47 .51	1								3			2		
		WISC III Freedom fromDistractability (ss)	.42 .49	1	2												
		WISC III Processing Speed (ss)	.30 .40		1		2										
	Academic Acheivement:	WIAT Basic Reading (ss)	. 37		1												
		KeyMath Total (ss)	.42 .53		1								2				
	Visuospatial Skills, Visual Memory, Organization:	VMI: Total (ss)	.28 .34		1		2										
		RCFT: Copy (raw)	.19 .25 .32	1							3			2			
		RCFT: Immediate Recall (T)	.28 .38	1										2			
		RCFT: Delayed Recall (T)	.44 .52	1										2			
	Executive Function:	DKEFS: Tower, Total Achievement (ss)	. 20										1				
		D-KEFS: Tower, Total RuleViolation (cumulative %tile Rank)	.22 .30 .36											3	1	2	
		D-KEFS: Verbal Fluency Conds 1-3 % Switch Accuracy (ss)	.22 .29			2							1				
		D-KEFS: Color Word Inhibit/Switch Complete Time (ss)	. 12		1												
		D-KEFS: Trails, #/Letter Switch Complete Time (ss)	.31 .42		2		1						1				
		WCST: Total Errors (ss)	.22 .34		1									2			
	Visual Memory:	CVLT-C: List A, Total Trials #Correct (T)	.35 .45 .50 .53	1		3							2	4			
		CVLT-C: List A, Trial 1, Free Recall (T)	. 17	1													
	Attention:	IVA: Full Response Control Quotient (ss)	.14 .21				1							2			
	Language:	TOWK & TOLD (ss)	.40 .48 .55	1	2								3				
		TLC 1 & 2 (ss)	.41 .55		1		2										
	Adaptive Behavior:	VABS: Adaptive Behav. Composite (ss)	.46 .52 .56	2	1	3											
		VABS: Socialization (ss)	.46 .50	2	1												
	Behavioral Problems:	CBCL: Social Problems (T)	.24 .31		1											2	
		CBCL: Attention Problems (T)	.49 .56	1											2		
		CBCL: Internalizing Problems (T)	.31 .39	2												1	
		CBCL: Externalizing Problems (T)	.29 .34	1						2							
		CBCL: Total Competence (T)	.40 .46 .51	1						3						2	
	Caregiver Report of Behavior:	BRIEF: Gen.Execut. Composite (T)	.44 .48	1	2												
		BRIEF: Behavioral Regulation Index (T)	.39 .43	1								2					
	Mental Health: DISC # symptoms	Panic Disorder	none														
		Social Phobia	.36 .46			2										1	
		Obsessive Compulsive Disorder	. 20													1	
		Post Traumatic Stress Disorder	. 19													1	
		Schizophrenia	.22 .28										2			1	
		Mania / Hypomania	.41 .47 .52	1									2			3	
		Generalized Anxiety Disorder	.34 .45 .49	2						3			1				
		Attention Deficit/Hyperactivity Disorder	.47 .51	1	2												
		Separation Anxiety Disorder	. 17	1													
		Conduct Disorder	.30 .35	1												2	
		Oppositional Defiant Disorder	. 13	1													

Figure 2. Proportion of variance in brain structure and function explained by prenatal and postnatal risks. The order of entry of each risk into the regression equation (depicted by the numbers 1,2,3 or 4 in the colored boxes) documents which risks explained the greatest proportion of variance in the brain structural, functional or mental health outcomes. For example: of 14 prenatal and postnatal risks available for entry into the regression equation, the number of days/week of drinking during pregnancy explained the greatest proportion of variation (46%) in the WISC-III FSIQ score and thus was the first significant risk factor ($p < .05$) to enter into the regression equation. Caregiver's years of education explained the 2nd greatest and statistically significant proportion of variance of the FSIQ (8% additional variance). These two risks together explained 54% of the variance in FSIQ. Maternal drinking all three trimesters was the third and final statistically significant risk to enter the equation, explaining an additional 4% of variance. The three risk factors together explained 58% of the variance in the FSIQ. To further aid interpretation, prenatal and postnatal risks were collapsed into 5 categories depicted by colored boxes: (PAE black; gender green, other prenatal exposures brown, postnatal home environment and caregiver SES blue, and trauma orange). Using this color scheme, one can quickly see how often a particular category of risk explained a significant proportion of variation in brain outcomes. *One subject with PFAS and agenesis of the corpus callosum was removed from the analysis in parentheses. **Abbreviations**: cm centimeters; edu education; L left; mj marijuana; occup occupation; OFC occipital frontal circumference; R right; SES socioeconomic status; ss standard or scaled score; T t-score; Tri trimesters; wk week; # homes number of home placements. See Table 1 for test names.

To aid in the interpretation of Figure 2, the 14 prenatal and postnatal risk factors were collapsed into 5 categories of risk depicted by the following color scheme: prenatal alcohol is depicted with black boxes; gender with green boxes, other prenatal exposures with brown boxes, postnatal home environment and caregiver SES with blue boxes, and postnatal trauma with orange boxes. Using this color scheme, one can quickly see how often a particular category of risk explained a significant proportion of variation in brain outcomes. Prenatal alcohol exposure explained the greatest proportion of variance (was the first risk factor to be entered into the regression equation) 31% of the time across all brain outcome measures. In other words, 43 of the 138 cells under the two alcohol measures in Figure 2 are black boxes with a number 1 inside the box (43/138 = 31%). In contrast, the remaining risk factor categories (depicted by green, brown, blue and orange boxes) were first to enter the equation as follows: gender 1.4% of the time; other prenatal exposures risks 2.2%; postnatal home environment and SES risks 2.6% and postnatal trauma 7.2%. Prenatal alcohol exposure was also the most common risk factor to enter into a regression equation, irrespective of the order of entry into the equation. In other words, a measure of PAE entered into a regression equation 40% of the time (55 of the 138 cells for PAE in Figure 2 have black boxes). In contrast, the remaining risk factor categories (depicted by green, brown, blue and orange boxes) entered into the equations as follows: gender 11.6% of the time; other prenatal exposures risks 6.2%; postnatal home environment and SES risks 8.7% and postnatal trauma 12.3%.

Discussion

Individuals with PAE often present with a multitude of other prenatal and postnatal risk factors. Based on the outcomes of this study:

1. PAE was the dominant risk factor explaining the largest proportion of variance across the greatest number of brain structural and functional measures. PAE was not the only risk factor influencing brain outcomes.

2. Other prenatal and postnatal risk factors were prevalent and contributed significantly to the adverse brain outcomes. Individually, each risk factor explained a statistically significant, but smaller proportion of variance in brain outcome compared to PAE. In combination, however, the proportion of variance explained by the presence of multiple prenatal and postnatal risks rivaled that of PAE.

These findings pose important implications for prevention and intervention. The proportion of variance explained by each risk factor can help guide which risk factors (when prevented) will have the greatest positive impact on outcome. Clearly, the greatest positive impact on brain outcome is achieved through prevention of both prenatal and postnatal risk factors. But when prevention of prenatal risks does not occur, Figure 2 illustrate the clear benefits of an early FASD diagnosis; maximizing the opportunity to mitigate postnatal risks. The outcomes of this study also illustrate the importance of documenting and addressing the multitude of other prenatal and postnatal risk when diagnosing FASD. The prenatal and postnatal risk factors used to conduct this study were collected during the child's FASD diagnostic evaluation using the FASD 4-Digit Code. These risk factors are formally documented in the electronic 4-Digit Code FASD Diagnostic Form [17] and ranked on 4-point Likert scales (Prenatal Rank and Postnatal Rank) just like the growth, face, brain and alcohol components of the FASD 4-Digit Code.

The prevalence of PAE and other prenatal and postnatal risk factors in a FASD diagnostic clinical population is substantially greater than in the general population. The reported prevalence of these risk factors in the general U.S. population, current study population, and the entire clinical population of 2,461 individuals with PAE evaluated at the University of Washington FASDPN clinic to date, respectively, are as follows: PAE (15%, 100%, 100%), PAE all three trimesters (8%, 62%, 48%); prenatal tobacco exposure (25%, 79%, 71%); marijuana exposure (7%, 38, 36%); cocaine exposure (0.3%, 26%, 34%); any illicit drug exposure (6%, 56%, 42%); foster placement (0.6%, 74%, 64%); physical abuse (8%, 32%, 31%) sexual abuse (10%, 35%, 31%) [34-38].

The patterns of risk that significantly influenced brain structure and function observed in the current exploratory study (Figure 2) present with interesting corollaries in the FASD, trauma, illicit drug exposure and SES literature. These corroborative findings represent an important incremental step toward supporting/validating the outcomes observed in this study. Of particular note was an unexpected inverse correlation observed in the current study between corpus callosum size and

sexual abuse (Figure 2). Interestingly, this finding is well documented in the trauma literature. Both Rinne-Albers et al., [5] and Teicher et al., [39] found that sexual abuse was the strongest factor influencing reduced corpus callosum size; a correlation observed only among girls. Upon further evaluation of our data, we too found the inverse correlation between corpus callosum size and sexual abuse was only among females (Figure 3). It is theorized that early traumatization is likely to have a major influence on the corpus callosum, as the process of myelinization and selective pruning are typically influenced by stress hormones [6,39,40]. The prevalence of sexual abuse in the current FASD study population is 4-fold higher (35%) than in the general population (10%) [41].

Tobacco is the most commonly used substance during pregnancy-in 2004, up to 25% of U.S. women smoked cigarettes during pregnancy [38]. Carbon monoxide and nicotine from tobacco smoke may interfere with the oxygen supply to the fetus. Nicotine also readily crosses the placenta, and concentrations in the blood of the fetus can be as much as 15% higher than in the mother [3].

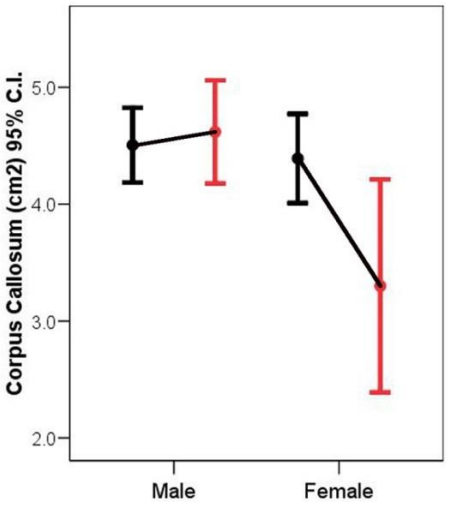

Figure 3. Sexual abuse was the strongest factor influencing reduced corpus callosum size (midsagittal area). As documented in the literature (Rinne-Albers et al., [5], the association in the current study was observed only among females. The mean midsagittal area of the corpus callosum was significantly smaller (3.3, 1.2 SD) among females that experienced sexual abuse compared to females (4.4, 0.9 SD) that did not experience sexual abuse (T = 2.5; p = .03). Key: Error bars reflect the mean and 95% confidence interval. Black: no sexual abuse; Red: sexual abuse

Maternal smoking during pregnancy has been associated with numerous adverse outcomes among offspring including reduced birth weight, inattention, hyperactivity and impulse and emotion control [42,43]. Ekblad, et al., [44] report prenatal smoking exposure was associated with significantly smaller frontal lobe and cerebellar volumes in brains of preterm infants. ADHD has been shown to be related to decreased brain volumes, especially cerebellar volume [45,46]. Consistent with these findings, maternal smoking during pregnancy in the current study was significantly correlated with inattention, reduced cerebellar volume and low birth weight among the offspring. Prenatal tobacco was the strongest significant factor influencing visual and auditory attention and the midsagittal size of cerebellar vermal lobules VIII-X (Figure 2). And although birth weight was not a focus of the current study, we recently found that tobacco exposure (not PAE) was the single strongest factor influencing birth weight centile in a large sample of individuals with FASD (n = 1,814) from our clinical dataset [2]. When we repeated the analysis in the current dataset (using the same array of prenatal and postnatal risk factors and regression model parameters used for the current study),tobacco exposure, once again was the single strongest factor influencing birth weight centile (adjusted R^2 = .17, F 10.2, p =.003). Maternal smoking during pregnancy was 7-fold more prevalent (79%) among the current FASD study population than in the general population of pregnant women (7%).

Child physical abuse is the second most common form of child maltreatment (second to neglect), being reported by 8% of the U.S. adult population [47]. The deleterious effects of child physical abuse on later mental health have been extensively recognized. A history of child physical abuse has been associated with an increased risk of emotional and behavioral problems [7,48], and several psychiatric disorders, including major depression, posttraumatic stress disorder (PTSD), conduct disorder, oppositional defiant disorder, agoraphobia, and generalized anxiety disorder [7,49]. Physical abuse was 4-fold more prevalent (32%) among the current FASD study population than in the general population (8%). In the current study, physical abuse was often the primary factor explaining behavioral problems and six of the eleven childhood psychiatric disorders.

According to a national survey conducted in the United States in 2012 [50], 5.8% of pregnant women used illicit drugs. Marijuana is generally the most commonly used drug during pregnancy, followed by cocaine and opiates. Associations have been found between marijuana use during pregnancy and developmental and hyperactivity disorders among

children [51-54] and evidence of low birth weight [55]. Prenatal cocaine exposure is related to subtle cognitive, behavioral and physiological differences, including working memory, attention, low birth weight, reduced brain volumes [4] and reduced caudate volumes [56]. In our current study, illicit drug exposure was significantly correlated with behavioral problems and reduced size of several brain regions, most notably the caudate. Of the illicit drugs reported in the current study, marijuana was most common, followed by cocaine. There was no reported prenatal exposure to opiates in this study population born between 1988 and 1996. Illicit drug exposure among the children with FASD was 10-fold higher (56%) than in the general population (6%).

Research shows that lower SES is associated with a wide array of adverse structural and functional brain outcomes in children across development [57]. Functional impairments include language, executive function and memory [8]. Structural abnormalities include smaller volumes of gray matter in hypocampi, middle temporal gyri and occipito-temporal gyri as well as lower diffusivity of the corpus callosum [58]. These findings are highly congruent with our observations. We observed associations between lower SES and impaired neuropsychological function across all domains of function (Figure 2). We also observed an association between lower SES and reduced volume of the corpus callosum. In a recent study focused on a group of children with FASD, Uban et al., [16], typically developing youth with no PAE exhibited increased subcortical brain volumes with increased SES, but surprisingly, the relationship was absent in adolescents with PAE (Figure 2) [16]. We replicated their univariate analysis to see if we too failed to observe a univariate correlation between SES and brain region volume between our Controls and subjects with FASD (Figure 4). It is important to note that 86% of our adolescents with FASD had high (Rank 4) PAE and spanned the full spectrum of FASD (FAS/PFAS, SE/AE and ND/AE). The subjects in the Uban et al., study had confirmed moderate to high PAE, but the severity of their cognitive outcomes or FASD diagnoses was not reported. We observed a somewhat weaker positive association between SES and frontal volume among our FASD group relative to our Control group (Figure 4A). But when we subdivided our FASD group into three groups from most severe to least severe (FAS/PFAS, SE/AE and ND/AE) the positive correlation between SES and frontal lobe volume was actually stronger among the FAS/PFAS than among the Controls (Figure 4B). The strength of the correlation decreased as the severity of FASD decreased from FASD/PFAS to SE/AE to ND/AE. When SES was regressed on WISC FSIQ, significant positive correlations were observed for both Control and FASD study groups (Figure 4C). Within the FASD study group, the strength of the correlation decreased once again as the severity of FASD decreased from FASD/PFAS to SE/AE to ND/AE (Figure 4D). These outcomes provide a potential explanation for why the Uban et al., study did not observe an SES-brain region correlation among the adolescents with PAE. The association could have been missed if the FASD study sample included too few individuals with severe FASD outcomes

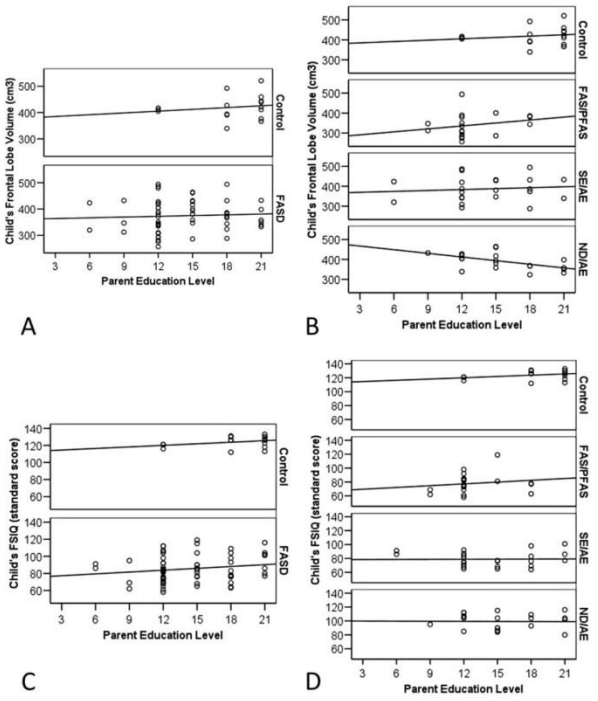

Figure 4. Scatterplots illustrating SES-brain associations for frontal lobe volume and the full scale intelligence quotient. The educational level of the current caregiver is codified in accordance with Hollingshead [24] with 3 reflecting<7th grade education and 21 reflecting a postgraduate education. A) A positive correlation between SES and frontal lobe volume is observed in the Control group, but not the FASD group, consistent with the findings of Uban et al., [16]. B) When the FASD group was subdivided into its three diagnostic categories, it became clear that a strong positive SES-frontal lobe correlation existed in the subgroup with the most severe FASD (the FAS/PFAS subgroup). Weaker correlations were observed among the less severe subgroups (SE/AE and ND/AE groups). The strong positive SES-frontal lobe correlation among children with FAS/PFAS may be masked if combined with children with less severe forms of FASD. C & D) Similar patterns of correlation were observed between SES and Full Scale IQ (FSIQ) with the strongest positive correlations observed among the FAS/PFAS group

Strengths and limitations

The observations in the current study are based on a small, but rigorous and comprehensive set of data. Replication in other datasets will be important to support/validate the outcomes observed in this exploratory study. To that end, we ran a few internal validation analyses on this current dataset to see if we could replicate findings observed in our larger clinical dataset. For example, as described in the Discussion section, we documented that prenatal tobacco exposure (not PAE) was the single FASD from the University of Washington FASDPN clinic [2]. When we replicated that analysis in the current dataset, once again, prenatal tobacco exposure was the single strongest factor influencing birth weight (adjusted R^2 = 0.17, F 10.2, p =.003). Another correlation that is well documented in our large clinical dataset [23] as well as our large population-based foster care FAS screening dataset [59] is the specificity of the Rank 4 FAS facial phenotype to PAE. When we regressed the same array of prenatal risk factors on a continuous measure of the FAS facial phenotype severity (the FAS facial d-score [27], as hypothesized, only PAE (days/week of drinking during pregnancy) explained a significant proportion of the variance in the FAS facial phenotype (adjusted R^2 = .16, F 10.1, p = .003). Finally, when we conducted a regression analysis in our larger clinical dataset using the same 14 prenatal and postnatal risk factors and regression model parameters to predict FSIQ, maternal drinking through the 3rd trimester explained the largest proportion of variance, with birth mother's years of education entering second and child neglect entering third explaining a total of 23.6% of the variance in FSIQ. This outcome was near identical to the outcome observed in the current study despite the fact that different metrics were used to document the prenatal and postnatal risk factors and the FSIQ was derived from various versions of the WISC (as is typical for a clinical dataset). Although 14 prenatal and postnatal risk factors were assessed in the current study, many other risks exist (e.g. pregnancy complications, prematurity, family genetics, parental verbal abuse, witnessing domestic violence, neglect, etc.). Understanding the interplay between risk factors and outcomes is complex. Teicher and Samson [9] present a series of questions that help convey this complexity and serve as a guide for future studies. 1) Does childhood abuse affect brain structure and function? 2) Does the type of maltreatment matter or are they all stressors? 3) Does age at the time of abuse matter? 4) What is the temporal association between exposure and brain changes? 5) Are boys and girls affected in the same way? 6) Do the observed structural and functional consequences make more sense as adaptive responses or as nonspecific damage? 7) Are the neurobiological consequences of childhood maltreatment reversible? 8) What is the relationship between childhood abuse, brain changes and psychiatric illness?

Conclusion

In conclusion, individuals with PAE present with a multitude of other prenatal and postnatal risk factors. The prevalence of these risk factors is often 3 to 7-fold higher than in the general population. PAE was the dominant risk factor explaining the largest proportion of variance in brain structural and functional outcomes in this study. Individually, each of the other risk factors explained a statistically significant, but smaller proportion of variance in brain outcome compared to PAE. In combination, however, the proportion of variance explained by the presence of multiple prenatal and postnatal risks rivaled that of PAE. A better understanding of the impact other prenatal and postnatal risk factors have on the neurodevelopmental outcomes of individuals with FASD can inform more effective primary prevention and intervention strategies.

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