Are Low-to-Moderate Average Alcohol Consumption and Isolated Episodes of Binge Drinking in Early Pregnancy Associated with Facial Features Related to Fetal Alcohol Syndrome in 5-Year-Old Children?

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Background: Fetal alcohol syndrome (FAS) typically is observed among individuals with high prenatal alcohol exposures (PAE), but exposure histories obtained in clinical diagnostic settings are often inaccurate. The present analysis used the Lifestyle During Pregnancy Study (LDPS) to assess the potential effects of low-to-moderate average weekly alcohol consumption and binge drinking in early pregnancy on facial features associated with FAS among children 5 years of age.

Methods: The analysis is a prospective follow-up study of 670 women and their children sampled from the LDPS cohort based on maternal alcohol consumption during pregnancy. The 4-Digit Code FAS Facial Photographic Analysis Software was used to measure the magnitude of expression of the 3 diagnostic facial features of FAS from standardized digital photographs. Logistic regression was used to estimate the odds of presenting with the FAS/partial fetal alcohol syndrome (PFAS) facial phenotypes relative to different patterns of prenatal alcohol exposure.

Results: Ten children presented with the FAS/PFAS facial phenotypes. None of the children sampled met the central nervous system (CNS) criteria for FAS or PFAS at age 5 years. All remained at risk for PFAS since some types of CNS dysfunction associated with this diagnosis may only be assessed at older ages. The FAS/PFAS facial phenotypes were 8.5-fold more likely among children exposed to an average of 1 to 4 drinks/wk and 2.5-fold more likely among children with a single binge exposure in gestational weeks 3 to 4 compared to children with no such exposures. The magnitude of expression of the FAS facial phenotype was significantly correlated with all other diagnostic features of FAS: growth deficiency, microcephaly, and measures of CNS dysfunction.

Conclusions: These findings suggest that low-to-moderate levels of PAE or isolated binge exposures may place some fetuses at risk for FAS/PFAS. Thus, conservative advice is still for women to abstain from alcohol consumption during pregnancy.

Key Words: Alcohol, Alcohol Binge Drinking, Pregnancy, Fetal Alcohol Syndrome, Fetal Alcohol Spectrum Disorders.

FETAL ALCOHOL SYNDROME (FAS) is a permanent birth defect and developmental disability caused by in utero exposure to alcohol. FAS is characterized by growth deficiency, a unique constellation of minor facial anomalies, and structural, neurological, or functional central nervous system (CNS) abnormalities (Astley and Clarren, 2000; Bertrand et al., 2004; Stratton et al., 1996). Not all individuals exposed to and damaged by prenatal alcohol exposure have FAS, the most involved diagnosis under the umbrella of fetal alcohol spectrum disorders (FASDs). Prenatal exposure to alcohol can also result in more subtle adverse effects and diagnoses. The growth, facial, and CNS abnormalities can all present along separate continua from mild to severe (Stratton et al., 1996).

A number of FASD diagnostic schemes have been posed and applied worldwide (Astley, 2004; Bertrand et al., 2004; Bower and Elliott, 2016; Cook et al., 2016; American Psychiatric Association, 2013; Hoyme et al., 2016). All promote an interdisciplinary approach to diagnosis and broadly agree that FASDs are characterized by growth, facial, and CNS abnormalities. But, the specific criteria

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used to define each diagnosis under the umbrella of FASDs do differ across the diagnostic systems (Astley, 2011; Astley et al., 2017; Coles et al., 2016). It should be noted that all schemes assess facial features for an FAS diagnosis since these features reflect anomalies in prenatal brain development. The current analysis used criteria as outlined in Astley (2004), also known as the 4-Digit Code. Briefly, these criteria require all of the following:

1. Growth deficiency: prenatal and/or postnatal height and/or weight at or below the 10th percentile;
2. Facial dysmorphia: all 3 of the following: (i) short palpebral fissure lengths (PFLs; less than or equal to third percentile); (ii) smooth philtrum (Rank 4 or 5 on the University of Washington Lip-Philtrum Guide); and (iii) thin upper lip (Rank 4 or 5 on the University of Washington Lip-Philtrum Guide);
3. Evidence of severe CNS structural, neurological, and/or functional abnormalities;
4. Prenatal alcohol exposure: a confirmed or unknown history of exposure. FAS can be diagnosed in the absence of a confirmed prenatal alcohol exposure history if the 3 facial features (as defined by the Rank 4 facial phenotypes in the 4-Digit Code) are present. Empirical evidence confirms the Rank 4 facial phenotypes are so highly specific to (caused only by) prenatal alcohol exposure. Its presence can be used to confirm exposure when an exposure history is unavailable (Astley, 2013).

The FAS facial phenotype is not simply present or absent. It presents along a clinically meaningful continuum from mild to moderate to severe (Astley and Clarren, 2000). The magnitude of expression of the FAS facial phenotype not only increases with increasing prenatal alcohol exposure, but also correlates significantly with increasing severity of growth deficiency, microcephaly, and CNS dysfunction (Astley, 2013). These significant correlations serve to validate a causal association between prenatal alcohol exposure and the growth, facial, and CNS abnormalities currently used to define FAS (Astley, 2013; Astley and Clarren, 2001).

FAS is typically observed among individuals with reportedly high prenatal alcohol exposures (PAE; ≥6 drinks/d or 5 to 6 drinks within a short period of time) (O'Leary and Bower, 2012), but exposure histories obtained in clinical diagnostic settings often are inaccurate. For example, the average reported exposure among 154 individuals diagnosed with FAS or partial fetal alcohol syndrome (PFAS) at the University of Washington FAS Diagnostic & Prevention Network (FASDPN) using the 4-Digit Code was 8 to 12 drinks per drinking occasion, 5 to 6 days per week (Astley, 2010). This average exposure pattern, however, spanned a wide range. At the low end of the range, 1 of every 14 children with FAS or PFAS had a reported exposure of no more than 1 drink/d. Are these 1 in 14 cases especially vulnerable to the adverse effects of prenatal alcohol exposure, or were their lower exposures inaccurately reported? The Lifestyle During Pregnancy Study (LDPS) (Kesmodel et al., 2010, 2012) provided just such a dataset that addressed this issue by collecting prenatal alcohol exposure history during early pregnancy and using standardized measures of growth, face, and CNS.

The LDPS has previously provided data on the association between low-to-moderate alcohol intake and alcohol binge drinking and neuropsychological development, including intelligence, attention, psychomotor function, executive function, and behavior (Bay et al., 2012; Kesmodel et al., 2010, 2012, 2013). The objective of the present analysis was to use the LDPS to assess the potential effects of low-to-moderate average weekly alcohol consumption and binge drinking in early pregnancy on facial features associated with FAS among children 5 years of age. Specifically, we (i) document the occurrence of the individual FAS facial features and overall FAS facial phenotype in the study sample; (ii) assess the association between prenatal alcohol exposure and the magnitude of expression of the FAS facial features and phenotype; and (iii) assess the association between the magnitude of expression of the FAS facial phenotype and other diagnostic features of FAS, including cognitive impact, reduced head circumference, and growth deficiency.

MATERIALS AND METHODS

Study Sample

This study was part of the LDPS, which has been described in detail elsewhere (Kesmodel et al., 2010, 2012). Briefly, the study is a prospective follow-up study based on a subsample from the Danish National Birth Cohort (DNBC; Olsen et al., 2001).

A total of 1,628 mother–child pairs participated in the follow-up. Inclusion was based on a stratified sample with oversampling of women with low-to-moderate alcohol intake and binge drinking (Kesmodel et al., 2010, 2012). Exclusion criteria were inability to speak Danish, impaired hearing or vision causing inability to complete the cognitive tests, multiple pregnancies, and congenital diseases likely to cause mental retardation (Kesmodel et al., 2010). Data collection for the follow-up study took place from September 2003 to June 2008 (Kesmodel et al., 2010).

Of the 1,628 participants' images available for measurement, 670 met the inclusion criteria for this study and had at least 1 of the 3 facial features measured (see details in Appendix).

Exposure Assessment

Information on alcohol intake during pregnancy was derived from the first prenatal DNBC interview. Among the subsample of women participating in the follow-up, the median week of gestation for completing the prenatal interview was 17 weeks (range: 7 to 39 weeks). During the interview, the women were asked about their average number of beers, glasses of wine, and glasses of spirits they currently consumed at the time of the interview over the course of a week, and based on this information, the total number of weekly drinks was calculated. These alcohol exposure questions have been shown to yield valid estimates of alcohol consumption throughout pregnancy (relative to other methods) and reliable information among pregnant Danish women (Kesmodel and Olsen, 2001). Information on binge drinking during pregnancy included data on the number of binge episodes (defined as intake of ≥5 drinks on a single occasion) and the timing (gestational week) of these episodes (Kesmodel, 2001) up until the time of the interview. A number of women

in the current sample reported 1 or more binge episodes during early weeks of pregnancy, although their average number of drinks per week at the time of interview was zero (Kesmodel et al., 2012). These women were classified accordingly as consuming zero average drinks per week during pregnancy, but with 1 or more previous binge episodes. The definition of a drink followed the definition from the Danish National Board of Health, with 1 standard drink being equal to 12 g of pure alcohol. The sampling stratification for average weekly consumption and binge consumption in the first trimester has been described previously (Kesmodel et al., 2010, 2012). This stratification resulted in 5 sampling categories used in this analysis.

Outcome Measures

Facial Features. The follow-up assessments were conducted at 4 sites located in Copenhagen, Aarhus, Odense, and Aalborg. The assessment comprised a comprehensive neuropsychological test battery which is described in detail elsewhere (Kesmodel et al., 2010, 2012).

Following the test session, standardized digital facial photographs were taken of each mother and child to allow subsequent measurement of (dysmorphic) facial features, including the philtrum, the upper lip, and PFL. Specific procedures for taking and coding photographs are described in Appendix. All testers were blind to the exposure status of the participants, and all tests were administered in Danish.

Briefly, the University of Washington FAS Facial Photographic Analysis Software (Astley, 2016) was used to measure the magnitude of expression of each of the 3 diagnostic facial features of FAS (short PFLs: 2 or more standard deviations (SD) below the mean; smooth philtrum (Rank 4 or 5 on the University of Washington Lip-Philtrum Guide) and thin upper lip (Rank 4 or 5 on the Lip-Philtrum Guide (Fig. 1), lip circularity ≥75.5) as defined by the University of Washington FASD 4-Digit Code (Astley, 2016). For the 366 children with photographs of sufficient quality to allow accurate measurement of all 3 facial features, the magnitude of expression of the overall FAS facial phenotype (Face Rank) was ranked on a 4-point Likert scale (Rank 1: normal phenotype; Rank 2: mild FAS phenotype; Rank 3: moderate FAS phenotype; and Rank 4: severe FAS phenotype) in accordance with the FASD 4-Digit Code (Astley, 2004). The Scandinavian PFL growth charts (Stromland et al., 1999) and University of Washington Lip-Philtrum Guide 1 were used for this Danish population.

Cognitive Function. Child intelligence was assessed using the Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) (Wechsler, 1990) covering the age span 3 to 7 years. The WPPSI-R includes 5 verbal and 5 performance subtests that are used to calculate an overall verbal intelligence quotient (VIQ), overall performance IQ (PIQ), and full-scale IQ (FSIQ). In this test battery, only 3 of the verbal (arithmetic, information, and vocabulary) and 3 of the performance (block design, geometric design, and object assembly) subtests were carried out to facilitate the child's cooperation throughout the testing. Standard procedures were used to prorate scores from the shortened test.

Child attention was assessed with the Test of Everyday Attention for Children at Five (TEACh-5; Underbjerg et al., 2012, 2013) covering the age span 5 years to 5 years and 3 months. For this study, 2 subtests assessing selective attention ("Great Balloon Hunt" and "Hide and Seek II") and 2 subtests assessing sustained attention ("Barking" and "Draw a line") were used. Each subtest score was standardized to a mean of 0 and a SD of 1. To calculate composite scores for overall, selective, and sustained attention, the means of the respective standardized subtest scores for each individual were calculated and restandardized to a mean of 0 and SD of 1.

Executive function was assessed using the Behavior Rating Inventory of Executive Function (BRIEF) questionnaire (Gioia et al., 2000) covering the age span 5 to 18 years. The questionnaire consists of 2 versions, 1 for parents and 1 for teachers. The parent version was used for these analyses because of higher participation. Each questionnaire evaluates 8 domains of executive functioning and forms the Global Executive Composite (GEC). Three of the 8 domains form the Behavioral Regulation Index (BRI), and 5 of the domains form the Metacognition Index (MI). Since the 8 domains do not follow a normal distribution, we performed a normalizing *t*-score transformation to standardize each domain to a mean of 50 and SD of 10. To compute the GEC, BRI, and MI, the means of the respective domains for each individual were calculated and restandardized to a mean of 50 and SD of 10. For all BRIEF scores, a higher score indicates more executive function difficulties.

Covariates

Factors demonstrated in previous research to influence child neurodevelopment were selected as covariates. The following covariates were obtained in the prenatal interview and subsequently coded as follows: parity (0, 1, ≥2); prenatal smoking (yes/no); and maternal prepregnancy body mass index (BMI) (weight in kg/[height in m]^2). At the time of the 5-year follow-up, the following variables were recorded: maternal marital status (single at either the prenatal interview or follow-up/with partner at both times) and parental education in years (total duration of attained education averaged for both parents or maternal only if information on the father was missing). Additional information on collection of covariate information is provided elsewhere (Kesmodel, 2012; Kesmodel et al., 2010).

Maternal age was obtained from the unique Danish personal identification number, as were sex and age of the child. Birthweight in grams, head circumference, and gestational age in days were obtained from the Danish Medical Birth Registry (Bliddal et al., 2018).

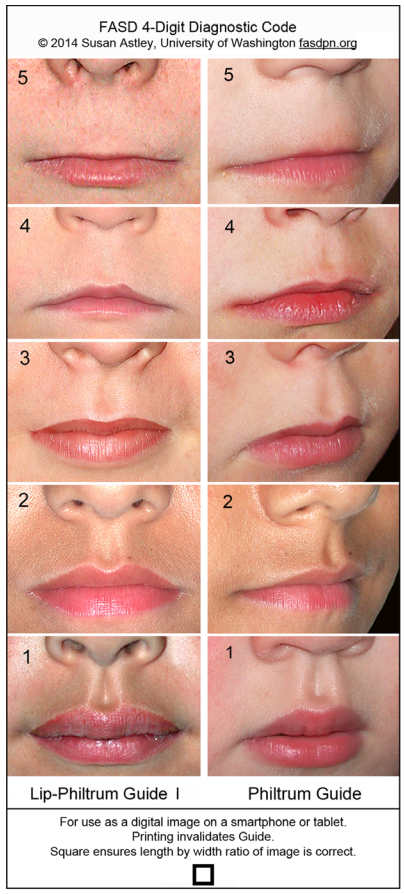
Data Analysis

Descriptive statistics (i.e., means, SDs, and proportions) were used to profile the sociodemographics of the study population, the maternal drinking patterns, and the magnitude of expression of the FAS facial features and phenotype. Logistic regression was used to document the odds of presenting with the FAS/PFAS facial phenotype (Face Rank 3 or 4), short PFLs (ABC-Score = C, 2 or more SDs below the mean), smooth philtrum (Rank 4 or 5), or thin upper lip (Rank 4 or 5) relative to 4 different patterns of prenatal alcohol exposure: (i) average number of drinks/week during pregnancy (0, 1 to 4, ≥5), (ii) binge drinking (yes/no), (iii) number of binge drinking episodes (0, 1, 2 ≥ 3), and (iv) gestational timing of the single binge drinking episode (no binge, weeks 1 to 2, weeks 3 to 4, weeks 5+; multiple episodes). Odds ratios were adjusted for predefined covariates (parity; prenatal smoking; maternal prepregnancy BMI; maternal marital status; parental education in years; maternal age at the birth of the index child; sex and age of the child).

Not all photographs were of sufficient quality (e.g., facial expression, rotation, and focus) to generate accurate measures of all 3 facial features. As a result, participants were divided into 2 groups. Group A (N = 366) consisted of children whose photographs were of sufficient quality to measure all 3 facial features. Group B (N = 670) consisted of children whose photographs were of sufficient quality to measure 1 to all 3 of the facial features. Group A is a subset of Group B. Group B was used for analyses focused on the individual facial features. Group A was used for analyses focused on the overall facial phenotype.

All analyses were conducted in SAS and Stata 12 (StataCorp LP, College Station, TX, USA) and weighted by sampling probabilities. Statistical tests were 2-sided and deemed significant at the 5% level. Estimates are accompanied by 95% confidence intervals.

A



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B

Guide 1 Tables

Lip Rank	Lip Circularity	
	Pictured	Range
5	178	≥ 131.5
4	85	75.5 to 131.4
3	65	57.5 to 75.4
2	50	42.5 to 57.4
1	35	≤ 42.4

FACE TABLES

5-Point Rank for Philtrum or Lip	Z-scores for Palpebral Fissure Length	ABC-Scores for: Palpebral Fissure	Philtrum	Upper Lip
4 or 5	≤ - 2 SD	C	C	C
3	> -2 SD and ≤ -1 SD	B	B	B
1 or 2	> -1 SD	A	A	A

4-Digit Diagnostic Rank	Level of Expression of FAS Facial Features	Palpebral Fissure – Philtrum – Lip ABC-Score Combinations
4	Severe	CCC
3	Moderate	CCB, CBC, BCC
2	Mild	CCA, CAC, CBB, CBA, CAB, CAA BCB, BCA, BBC, BAC ACC, ACB, ACA, ABC, AAC
1	None	BBB, BBA, BAB, BAA ABB, ABA, AAB, AAA

GROWTH TABLES

Percentile Range	ABC-Scores for: Height	Weight
≤ 3rd	C	C
> 3rd and ≤ 10th	B	B
> 10th	A	A

4-Digit Diagnostic Rank	Growth Deficiency Category	Height-Weight ABC-Score Combinations
4	Severe	CC
3	Moderate	CB, BC, CA, AC
2	Mild	BA, BB, AB
1	None	AA

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Fig. 1. (**A**) University of Washington Lip-Philtrum Guide 1 used to rank lip thinness and philtrum smoothness on 5-point Likert scales. (**B**) The face tables on the backside of the Lip-Philtrum Guide outline how the magnitude of expression of the FAS facial phenotype is ranked on a 4-point scale (Rank 1: normal; Rank 2: mild; Rank 3: moderate; and Rank 4: severe) (Astley, 2004). Copyright Susan Astley Hemingway. Reprinted by permission.

RESULTS

Sample Characteristics

Although the 2 subsets, Group A and Group B, were not randomly selected from the 1,628, the sociodemographic profiles (Table 1) and maternal drinking patterns (Table 2) confirm that both subgroups were highly reflective of one another and highly representative of the 1,628 participants from which they were drawn. Of the 366 women in Group A, 308 (84%) reported, on average, low-to-moderate alcohol consumption with isolated episodes of binge drinking, and 58 reported no alcohol consumption during pregnancy. Of the 670 women in Group B, 561 (84%) reported, on average, low-to-moderate alcohol consumption with isolated episodes of binge drinking, and 109 reported no alcohol consumption during pregnancy (Table 2).

Occurrence of the FAS and PFAS Facial Phenotypes

Among the 366 participants with all 3 facial features measured, 308 had confirmed exposure to alcohol. Nine of the 308 (2.9%) met the 4-Digit Code criteria for the moderate

Table 1. Sample Characteristics of the Current Study Populations and the Original LDPS Population from Which They Were Drawn

	LDPS 2012 study participants Mother and child pairs from alcohol sampling categories 1 to 5	Current 2018 study participants Children from the LDPS with	
		Group A All 3 facial features measured	Group B 1 to 3 facial features measured
Sample characteristics			
n	1,628	366	670
Sampling fraction (median, 10th/90th percentile)	9.7 (1.5/49.6)	9.7 (1.5/49.6)	9.7 (1.2/49.6)
Timing of interview, gestational week (median, 10th/90th percentile)	17 (13/24)	17 (13/23)	17 (13/23)
Family characteristics			
Maternal age, years (mean ± SD)	30.9 ± 4.4	30.9 ± 4.3	30.9 ± 4.3
Parity			
0 (%)	50.1	48.9	49.1
1 (%)	32.2	31.7	32.1
≥2 (%)	17.8	19.4	18.8
Maternal BMI (before pregnancy), kg/m^2 (median, 10th/90th percentile)	22.6 (19.6/28.7)	22.5 (19.7/29.3)	22.6 (19.6/29.1)
Maternal marital status: single (%)	12.1	12.0	11.0
Parental education, years (median, 10th/90th percentile)	13.0 (11.0/16.0)	12.5 (11.0/16.0)	13.0 (11.0/16.0)
Family home index: suboptimal (%)	18.7	20.8	18.6
Maternal IQ (mean ± SD)	100.0 ± 15.0	100 ± 14.8	
Maternal smoking during pregnancy (%)	31.4	33.9	32.2
Parental postnatal smoking (%)	31.9	33.3	31.1
Maternal binge drinking in pregnancy (%)	69.6	70.8	67.6
Median number of drinks per week during pregnancy (median, 10th/90th percentile)	0.5 (0/5)	0.5 (0/5)	0,5 (0/5)
Child characteristics			
Male sex (%)	52.0	46.5	48.5
Age at testing, years (median, 10th/90th percentile)	5.2 (5.1/5.3)	5.2 (5.1/5.3)	5.2 (5.1/5.3)
Birthweight, grams (mean ± SD)	3601.9 ± 516.1	3,600 ± 507.3	3613.6 ± 521.4
Gestational age, days (median, 10th/90th percentile)	281 (267/293)	281 (267/293)	282 (269/293)
Medical condition or medication (%)	3.3	1.6	2.4
Impaired hearing abilities (%)	4.7	4.4	4.8
Impaired vision abilities (%)	2.9	2.5	2.7

expression of the FAS facial phenotype (Face Rank 3), and 1 (0.3%) met the criteria for the severe expression of the FAS facial phenotype (Face Rank 4) (Table 3). Measures of growth, CNS structure and function, and maternal drinking patterns are presented in Table 4 to document whether any of these 10 children met the diagnostic criteria for FAS or PFAS in accordance with the FASD 4-Digit Code. All children were alcohol-exposed. Their mothers reported an average intake of 0 to 7 drinks/wk before pregnancy, 0 to 2 drinks on average per week during pregnancy, and a maximum of 1 binge episode during the first 20 weeks of pregnancy. All children were born at term. Five of the 10 presented with growth, head circumference, and/or IQ measures between 1 and 2 SDs below the mean. None of the children presented with growth measures at or below the 10th percentile. One child presented with a head circumference at the 10th percentile. In the absence of microcephaly (head circumference less than or equal to third percentile), FAS and PFAS require evidence of brain dysfunction. The level of brain dysfunction required for FAS or PFAS (CNS Rank 3) is defined by the 4-Digit Code as 3 or more domains of brain function, 2 or more SDs below the mean based on a comprehensive assessment of language, memory, executive function, cognition, motor, attention, and adaptation, using validated instruments administered by clinical professionals (Astley, 2004). To confirm or rule out this level of brain dysfunction, these assessments must be administered when a child is old enough (typically >8 years) to engage in assessments of more complex, mature brain function (Astley, 2004). None of the 10 children met the above criteria for brain dysfunction based on the WPPSI-R IQ test. Thus, at 5 years of age, none of the 10 children met the 4-Digit Code CNS criteria for FAS or PFAS, but all remain at risk for PFAS because CNS dysfunction (CNS Rank 3) cannot be confirmed or ruled out at this young age.

Among the 304 children in which only 1 or 2 facial features could be measured, the full FAS facial phenotype (Face Rank 4) could effectively be ruled out in 96% and the moderate expression of FAS facial phenotype (Face Rank 3) could be ruled out in 77%. When combined with the facial outcomes of the 366 children with all 3 facial features measured, the FAS/PFAS facial phenotypes (Face Ranks 3 to 4) could be ruled out in 96.7% of the 670 children.

Table 2. Distribution of Maternal Drinking Patterns in the Original LDPS 2012 Study and the Current 2018 Study

Average standard drinks per week	Binge[a] drinking				LDPS 2012[b]	Current 2018 Facial Study	
	Gestational weeks				Participants	Group A All 3 facial features measured	Group B 1 to 3 facial features measured
During pregnancy	1 to 2	3 to 4	5 to 8	≥9	*N* = 1,622 *N*	*N* = 366 *N* (% of LDPS)	*N* = 670 *N* (% of LDPS)
0	No	No	No	No	257	58 (23)	109 (42)
0	Yes	No	No	No	113	28 (25)	47 (42)
0	No	Yes	No	No	104	25 (24)	42 (40)
0	No	No	Yes	No	109	24 (22)	55 (50)
0	No	No	No	Yes	94	21 (22)	38 (40)
					Total 677	Total 156 (23)	Total 291 (43)
1 to 4	No	No	No	No	155	30 (19)	72 (46)
1 to 4	Yes	No	No	No	113	30 (27)	43 (38)
1 to 4	No	Yes	No	No	120	23 (19)	43 (36)
1 to 4	No	No	Yes	No	93	28 (30)	45 (48)
1 to 4	No	No	No	Yes	114	21 (18)	39 (34)
					Total 595	Total 132 (22)	Total 242 (41)
0	Yes in at least 2				81	21 (26)	33 (41)
1 to 8	Yes in at least 2				82	15 (18)	28 (34)
	1 to 2	3 to 4	≥5		Total 163	Total 36 (22)	Total 61 (37)
5 to 8	No	No	No		79	17 (22)	35 (44)
5 to 8	Yes	No	No		11	1 (9)	1 (9)
5 to 8	No	Yes	No		37	7 (19)	15 (41)
5 to 8	No	No	Yes		40	12 (30)	18 (45)
≥9	No	No	No		15	4 (27)	5 (33)
≥9	Yes in at least 2				5	1 (20)	2 (40)
					Total 187	Total 42 (22)	Total 76 (41)
No. of binge drinking episodes during pregnancy							
				0	495	107 (22)	217 (44)
				1	783	182 (23)	312 (40)
				2	225	47 (21)	95 (42)
				3 to 12	114	30 (26)	46 (40)

[a]Defined as an intake of 5 or more standard drinks on one occasion.
[b]Lifestyle During Pregnancy Study 2012.

Occurrence of the Individual FAS Facial Features

Among the 670 participants with 1, 2, or all 3 of the facial features measured, 4% presented with PFLs 2 or more SDs below the mean (PFL ABC-Score = C), 11% presented with moderately-to-completely smooth philtrums (Philtrum Ranks 4 and 5; ABC-Score = C), and 41% presented with moderately-to-severely thin upper lips (Lip Ranks 4 and 5; ABC-Score = C; Table 3; Fig. 1). The prevalence of each FAS facial feature was nearly identical in the smaller subset of 366 participants that had all 3 facial features measured.

Association Between FAS/PFAS Facial Features and Prenatal Alcohol Exposure

Table 5 shows the odds of presenting with the FAS/PFAS facial phenotypes (Face Rank 3 or 4) across different patterns of quantity, frequency, and timing of prenatal alcohol exposure. Exposure to 1 to 4 drinks/wk on average during gestation was associated with a significant 8.5-fold increased odds for presenting with the FAS/PFAS facial phenotypes compared to participants with no average drinks per week. Exposure to a single binge drinking episode was associated with a significant 1.9-fold increased odds for the FAS/PFAS facial phenotypes. When the timing of the single binge exposure was in gestational weeks 3 to 4, participants were 2.5-fold more likely to present with the FAS/PFAS facial phenotypes than participants with no binge exposure. Single binge exposures occurring before or after gestational weeks 3 to 4 did not result in a significantly increased odds of the FAS/PFAS facial phenotypes.

Table 5 also presents the odds of presenting with each of the individual facial features of FAS (short PFL: ≤−2 SDs, ABC-Score = C), smooth philtrum (Rank 4 or 5, ABC-Score = C), and thin upper lip (Rank 4 or 5, ABC-Score = C) across the different patterns of prenatal alcohol exposure.

PFL. The odds of presenting with short PFLs (ABC-Score = C) increased significantly from 1.8-fold to 3.7-fold as the average number of drinks per week during pregnancy increased from 1 to 4 to ≥5. The odds of short PFLs increased significantly as the timing of binge exposure

Table 3. Distribution of the FAS Facial Features in the 2 Study Populations

	Group A All 3 facial features measured *N* (valid %)	Group B 1 to 3 facial features measured *N* (valid %)
Number of facial features measured[a]	Total *N* = 366	Total *N* = 670
Only 1	0 (0%)	102 (15%)
2 of the 3	0 (0%)	202 (30%)
All 3	366 (100%)	366 (55%)
FAS face rank		
Normal: Rank 1	172 (47%)	172
Mild: Rank 2	184 (50%)	184
Moderate: Rank 3	9 (2%)	9
Severe: Rank 4	1 (< 1%)	1
Among the 304 with only 1 or 2 features measured[b]		Total *N* = 304
Rank 1 ruled out	N/A	12 (4%)
Rank 4 ruled out	N/A	38 (12%)
Ranks 1 and 4 ruled out	N/A	20 (7%)
Ranks 3 and 4 ruled out	N/A	211 (69%)
Ranks 1, 3, and 4 ruled out	N/A	23 (8%)
PFL	Total *N* = 366	Total *N* = 491
ABC-Score[c]		
A (>−1 SD)	298 (81%)	411 (84%)
B (>−2 SDs & ≤−1 SD)	50 (14%)	58 (12%)
C (≤−2 SDs)	18 (5%)	22 (4%)
Philtrum smoothness		
ABC-Score[c]	Total *N* = 366	Total *N* = 648
A (Rank 1 or 2)	152 (41%)	261 (40%)
B (Rank 3)	168 (46%)	314 (49%)
C (Rank 4 or 5)	46 (13%)	73 (11%)
5-Point Rank[c]	Total *N* = 366	Total *N* = 648
1 (deeply grooved)	30 (8%)	54 (8%)
2 (moderately grooved)	122 (33%)	207 (32%)
3 (normal groove)	168 (46%)	314 (48%)
4 (moderately smooth)	42 (11%)	69 (11%)
5 (very smooth)	4 (1%)	4 (1%)
Upper lip thinness		
ABC-Score[c]	Total *N* = 366	Total *N* = 465
A (Rank 1 or 2)	75 (20%)	102 (22%)
B (Rank 3)	130 (36%)	174 (37%)
C (Rank 4 or 5)	161 (44%)	189 (41%)
5-Point Rank[c]	Total *N* = 366	Total *N* = 465
1 (very thick)	8 (2%)	11 (2%)
2 (moderately thick)	67 (18%)	91 (20%)
3 (normal thickness)	130 (36%)	174 (37%)
4 (moderately thin)	135 (37%)	161 (35%)
5 (very thin)	26 (7%)	28 (6%)

[a]The quality of a child's photoset did not always allow all 3 facial features to be measured.
[b]Even though only 1 or 2 facial features could be measured, the outcome of those features allowed 1 or more Face Ranks to be ruled out.
[c]See Fig. 1.

occurred earlier in gestation. The odds were highest when binge(s) occurred in weeks 1 to 2 and lowest when binge(s) occurred during or after gestational week 5, although not statistically significant. The odds of short PFLs was highest with a single binge exposure and significantly lower with 2 or more binge episodes.

Philtrum. Odds of a smooth philtrum (ABC-Score = C) appeared to be more dependent on the timing of binge exposure than the number of binge exposures. The odds were significant and highest (1.3-fold higher) when binge drinking occurred in weeks 1 to 2. Odds decreased linearly as binge drinking occurred later in gestation. Intake of 1 to 4 drinks/wk on average and 2 binge episodes in early pregnancy were associated with significantly lower odds of a smooth philtrum.

Lip. Odds of upper lip thinness (ABC-Score = C) also appeared to be more dependent on the timing of binge exposure rather than the number of binge exposures. Participants with 1 binge exposure were at significantly higher odds (Odds ratios [OR] 1.19) for thin upper lip than participants with no binge exposures. When binge exposure occurred in weeks 3 to 4, odds of a thin upper lip was greatest (OR 1.66). When binge exposure occurred in week 5 or later, children were significantly less likely to present with a thin upper lip (OR 0.83).

Associations Between the Magnitude of Expression of the FAS Facial Phenotype and Other Diagnostic Features of FASD

Individuals with short PFLs (≤−2 SDs) had significantly lower mean FSIQ and PIQ scores (5 to 7 points lower) than

Table 4. Characteristics of the 10 Children With Rank 3 or Rank 4 FAS Facial Phenotypes. Growth[a] and Neuropsychological[b] Outcomes Provided in Terms of Deviation From the Mean in Standard Deviations Using Standardized Norms

Child		Maternal					Child									
			Average alcohol intake drinks per week		Alcohol binge episodes		At birth				At 5 years of age					
Face Rank	Sex	Planned pregnancy	Before pregnancy	During pregnancy	Number	Timing: gestational weeks	Gestational age[c]	Birthweight	Length at birth	Head circumference at birth	Weight at age 5	Height at age 5	Head circumference at age 5	IQ at age 5	TEACh (overall attention, mean)	BRIEF (GEC mean)
3	Male	Yes	0.0	0.0	1	9+	39	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
3	Male	Yes	0.0	0.0	1	9+	40	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	−1.9 SD; −1 SD
3	Male	Yes	0.5	0.5	0		41	*N*	*N*	*N*	*N*	*N*	*N*	1 SD; 1.9 SD	*N*	*N*
3	Female	No	0.5	0.5	1	1 to 2	40	−1.9 SD; −1 SD	*N*	*N*	*N*	*N*	*N*	*N*	−1.9 SD; −1 SD	*N*
3	Male	Yes	3.0	0.0	1	3 to 4	40	*N*	*N*	*N*	*N*	*N*	−1.9 SD; −1 SD	*N*	1 SD; 1.9 SD	*N*
3	Female	Yes	3.0	2.0	0		41	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
3	Female	Yes	4.0	0.5	1	3 to 4	39	−1.9 SD; −1 SD	*N*	*N*	*N*	*N*	*N*	−1.9 SD; −1 SD	*N*	−1.9 SD; −1 SD
3	Male	No	6.0	0.0	1	5 to 8	39	*N*	*N*	*N*	1 SD; 1.9 SD	1 SD; 1.9 SD	*N*	*N*	*N*	*N*
3	Male	Yes	7.0	0.0	1	1 to 2	40	*N*	*N*	*N*	−1.9 SD; −1 SD	*N*	−1.9 SD; −1 SD	*N*	−1.9 SD; −1 SD	*N*
4	Female	Yes	1.0	0.5	1	3 to 4	39	−1.9 SD; −1 SD	*N*	*N*	1 SD; 1.9 SD	1 SD; 1.9 SD	*N*	*N*	*N*	*N*

[a]Anthropometric measures originally reported in grams (birthweight), kg (weight at age 5 years), or cm (length at birth, height at age 5 years, head circumference at birth and at age 5 years). Presented as deviations from the mean in SD (Danish standard growth curves) in order to avoid identification of individuals.
[b]FSIQ measured with WPPSI-R on standard IQ-scale (mean of 100, SD = 15). Attention measured with TEACh-5 (Test of Everyday Attention for Children at Five). Executive function measured with BRIEF (Behavior Rating Inventory of Executive Function). Presented as deviations from the mean: *N* = −1 SD to +1 SD from population means to avoid identification of individuals.
[c]Calculated in days and converted to completed weeks as presentation in days potentially allows for identification of individuals.

the reference group with normal PFLs (>−1 SD) (Table 6). Individuals with smooth philtrums (Rank 4 or 5) had significantly lower mean FSIQ and VIQ scores (3 to 4 points lower) than individuals with deep philtrums (Rank 1 or 2). Individuals with thin upper lip (Rank 4 or 5) had a significantly higher mean VIQ score (2.5 points higher) than individuals with thicker upper lips (Ranks 1 and 2). When the 3 facial features were assessed together, individuals with the Rank 3 or 4 FAS/PFAS facial phenotypes presented with mean FSIQ and PIQ scores that were 4 to 7 points lower than the individuals with normal facial phenotypes (Ranks 1 and 2). Although the magnitude and direction of association were equivalent to those observed for the individual facial features, the contrasts were not statistically significant. The smaller sample sizes resulted in insufficient power (<80%) to identify the 4 to 7 point contrasts as statistically significant.

We found no significant or clinically relevant differences between children with different facial phenotypes or different measures of individual facial features and executive function and attention (data not presented).

Mean birthweight, birth length, and birth head circumference decreased significantly with increasing magnitude of expression of the FAS facial phenotype (Face Ranks 1 to 4) among the 366 participants in Group A (Fig. 2).

DISCUSSION

Summary

There were 3 core findings in this study with a sample of 670 children in which 109 had no prenatal alcohol exposure and 561 had low-to-moderate exposure with isolated binge episodes. First, 10 children presented with the FAS/PFAS facial phenotypes (Face Rank 3 or 4). All 10 were alcohol-exposed. None met the diagnostic criteria for FAS or PFAS at 5 years of age. All 10, however, remain at risk for PFAS because they were too young at age 5 years to engage in the battery of neuropsychological assessments required to confirm or rule out brain dysfunction. Second, children exposed to 1 to 4 drinks/wk were 8.5-fold more likely to present with the FAS/PFAS facial phenotypes (Rank 3 or 4) than children with no prenatal alcohol exposure. Risk of the FAS/PFAS facial phenotypes was also significantly increased (2.5-fold) among children with a single binge exposure in gestational weeks 3 to 4 compared to children with no binge exposures. And third, the magnitude of expression of the FAS facial phenotype was significantly correlated with all other diagnostic features of FAS: growth deficiency, microcephaly, and measures of CNS dysfunction, even if measures of these features were within the normal range in this sample.

A primary objective of this study was to determine whether adverse outcomes typically observed among populations with high PAE could be found in a population with much lower exposure. Since the facial features that define FAS/PFAS were measured using the same software (Astley, 2016), personnel, and FASD diagnostic system (Astley,

Table 5. Odds Ratios[a] for Dysmorphic Facial Features Among the 366 Children from GROUP A With all 3 Facial Features Measured and the 670 Children From Group B With 1 to 3 Facial Features Measured in Relation to Pattern of Maternal Alcohol Consumption During Pregnancy

Alcohol pattern	Face ranks 3 to 4 versus 1 to 2 *N* = 10 versus *n* = 356 From Group A: *N* = 366 OR (95% CI)	PFL ABC-Scores[b] C versus AB *N* = 22 versus *N* = 469 From Group B: *N* = 670 OR (95% CI)	Philtrum ABC-Scores[b] C versus AB *N* = 73 versus *N* = 575 From Group B: *N* = 670 OR (95% CI)	Upper lip ABC-Scores[b] C versus AB *N* = 189 versus *N* = 276 From Group B: *N* = 670 OR (95% CI)
Average number of drinks per week during pregnancy				
0	Reference	Reference	Reference	Reference
1 to 4	8.50 (6.03 to 12.0)	1.76 (1.42 to 2.16)	0.87 (0.79 to 0.96)	0.97 (0.90 to 1.05)
≥5	*No rank 3 to 4 faces in this category*	3.71 (2.15 to 6.40)	0.76 (0.47 to 1.24)	1.16 (0.85 to 1.58)
p-Value[c]	<0.001	<0.001	0.01	0.46
Binge drinking in pregnancy				
No	Reference[d]	Reference	Reference	Reference
Yes	1.36 (1.03 to 1.78)	0.85 (0.67 to 1.08)	0.92 (0.82 to 1.03)	1.08 (0.99 to 1.18)
p-Value	0.03	0.19	0.15	0.07
Number of binge drinking episodes in pregnancy				
0	Reference	Reference[c]	Reference	Reference
1	1.94 (1.48 to 2.54)	1.20 (0.94 to 1.54)	1.00 (0.87 to 1.14)	1.19 (1.08 to 1.31)
2	*No rank 3 to 4 faces in this category*	0.11 (0.03 to 0.35)	0.71 (0.57 to 0.89)	0.86 (0.74 to 0.99)
≥3	*No rank 3 to 4 faces in this category*	0.44 (0.21 to 0.96)	0.96 (0.70 to 1.31)	1.08 (0.87 to 1.35)
p-Value	<0.001	<0.001	0.02	0.93
Timing of binge drinking episodes in pregnancy (gestational week)				
No binge	Reference	Reference	Reference	Reference
Weeks 1 to 2 only	1.46 (0.90 to 2.35)	1.13 (0.76 to 1.69)	1.32 (1.08 to 1.62)	1.12 (0.95 to 1.31)
Weeks 3 to 4 only	2.47 (1.79 to 3.41)	1.10 (0.78 to 1.56)	0.91 (0.76 to 1.08)	1.66 (1.46 to 1.90)
Week ≥5 only	0.80 (0.43 to 1.49)	0.99 (0.66 to 1.48)	0.87 (0.69 to 1.08)	0.83 (0.71 to 0.98)
Multiple episodes	*No rank 3 to 4 faces in this category*	*No rank C PFLs in this category*	0.79 (0.62 to 1.01)	1.05 (0.89 to 1.24)
p-Value	<0.001	<0.001	0.007	<0.001

[a]OR adjusted for sociodemographic and sampling factors. 95% confidence intervals that do not span 1.0 are statistically significant at $p < 0.05$.
[b]Facial ABC-Scores from the FASD 4-Digit Code are defined in Fig. 1.
[c]*p*-Value for the hypothesis of no difference in facial fetaures across levels of alcohol intake.
[d]The reference groups have zero exposure only for the type of exposure pattern being assessed, but have the full range of exposure based on the other patterns. For example, this reference group of 109 participants has zero binge episodes of exposure, but 30 (28%) were exposed to an average of 1 to 4 drinks/wk during pregnancy and 21 (19%) were exposed to an average of 5 or more drinks/wk during pregnancy.

Table 6. Association Between Facial Features and Child IQ[a]

Intelligence	Full-scale IQ (standard score)				Performance IQ (standard score)				Verbal IQ (standard score)			
	Mean	Mean difference	95% CI	*p*-Value	Mean	Mean difference	95% CI	*p*-Value	Mean	Mean difference	95% CI	*p*-Value
Facial features measure[b]												
Facial phenotype rank												
Ranks 1 and 2 (N = 355)	106.7	Reference			107.4	Reference			104.8	Reference		
Ranks 3 and 4 (N = 10)	102.5	−4.2	−11.7, 3.3	0.269	100.6	−6.8	−16.6, 3.0	0.175	103.6	−1.2	−7.6, 5.3	0.724
PFL ABC-Score												
A (>−1 SD) (N = 410)	107.2	Reference			107.7	Reference			105.2	Reference		
B (>−2 SD & ≤−1 SD) (N = 57)	103.9	−3.3	−6.6, 0.06	0.055	102.4	−5.3	−9.6, −1.0	0.015	104.4	−0.9	−3.7, 2.0	0.553
C (≤−2 SD) (N = 22)	101.3	−5.9	−11.0, −0.7	0.025	100.4	−7.3	−13.8, −0.8	0.027	102.0	−3.2	−7.7, 1.3	0.160
Philtrum ABC-Score												
A (Rank 1 or 2) (N = 260)	107.8	Reference			107.9	Reference			106.1	Reference		
B (Rank 3) (N = 314)	105.5	−2.3	−4.4, −0.2	0.034	105.6	−2.2	−5.0, 0.5	0.115	104.3	−1.8	−3.6, −0.07	0.041
C (Rank 4 or 5) (N = 73)	104.1	−3.5	−6.9, −0.1	0.042	104.3	−3.4	−7.6, 0.8	0.116	103	−2.9	−5.8, −0.03	0.048
Upper Lip ABC-Score												
A (Rank 1 or 2) (N = 102)	105.9	Reference			107.3	Reference			103.4	Reference		
B (Rank 3) (N = 174)	106.0	0.1	−3.2, 3.4	0.950	106.7	−0.7	−4.9, 3.5	0.757	104.1	0.7	−2.0, 3.4	0.613
C (Rank 4 or 5) (N = 188)	107.2	1.3	−1.7, 4.3	0.388	106.9	−0.5	−4.4, 3.4	0.810	106.0	2.5	0.06, 5.0	0.045

PFL, palpebral fissure length.
[a]IQ measured with WPPSI-R.
[b]Face Ranks and ABC-Scores described in Fig. 1.

2004) used to measure facial features in the University of Washington FASDPN clinical population, relevant comparisons can be made between the 2 populations. The FASDPN dataset includes over 3,000 individuals with prenatal alcohol exposure who received an interdisciplinary FASD diagnostic evaluation using the FASD 4-Digit Diagnostic Code (Astley, 2010). The alcohol exposures reported in the current study population (83% reported no more than 1 to 8 drinks/wk and/or isolated binge episode [drinking categories 1a to 4c; Table 1]) were considerably lower than the alcohol exposures reported in the FASDPN clinical population (76% report greater than 1 to 8 drinks/wk; average exposure is 7 to 9 drinks per occasion, 4 to 5 d/wk) (Astley, 2010).

Prevalence of FAS Facial Features and Correlation with Prenatal Alcohol Exposure

In the current study population with low-to-moderate prenatal alcohol exposure, 3.2% (10/308) presented with the FAS/PFAS facial phenotypes (Rank 3 or 4). All were exposed to no more than 7 drinks/wk and no more than a single episode of binge drinking. In contrast, a much higher proportion of individuals (19%) present with the FAS/PFAS facial phenotypes in the FASDPN patient population (Astley, 2010). Although individuals in the FASDPN patient population are, on average, highly exposed, 1 of every 14 diagnosed with FAS/PFAS has a reported exposure of no more than 7 drinks/wk. This is similar to the 10 children with the FAS/PFAS facial phenotypes in the current study. Although prenatal alcohol exposure may have been underreported for these 1 in 14 cases, it is also possible that these children are particularly vulnerable to lower levels of exposure. Future research may want to examine this possibility. The outcomes in the current study suggest that lower exposures may, in fact, be sufficient to produce the FAS/PFAS facial phenotypes in a small proportion of children. Timing of exposure also appears to be important. Perhaps one of the most compelling findings in the current study was a significant 2.5-fold increased odds of the FAS/PFAS facial phenotypes among children with a single binge exposure in gestational weeks 3 to 4. Gestational weeks 3 and 4 reflect the primitive streak and gastrulation stage of embryogenesis—a critical period of induction of alcohol-induced craniofacial alterations (Astley, 2013; Astley et al., 1999; Sulik, 1984).

FAS and PFAS require more than just the Rank 3 or 4 facial phenotype. Although 10 children in the current study presented with the Rank 3 or 4 FAS/PFAS facial phenotypes, none met the diagnostic criteria for FAS or PFAS (in accordance with the 4-Digit Code) at the young age of 5 years. FAS is defined by growth ≤10th percentile, a Rank 4 facial phenotype, and microcephaly (less than or equal to third percentile) and/or brain dysfunction (3 or more domains of brain function 2 or more SDs below the mean) (Astley, 2004). PFAS is defined by normal growth,

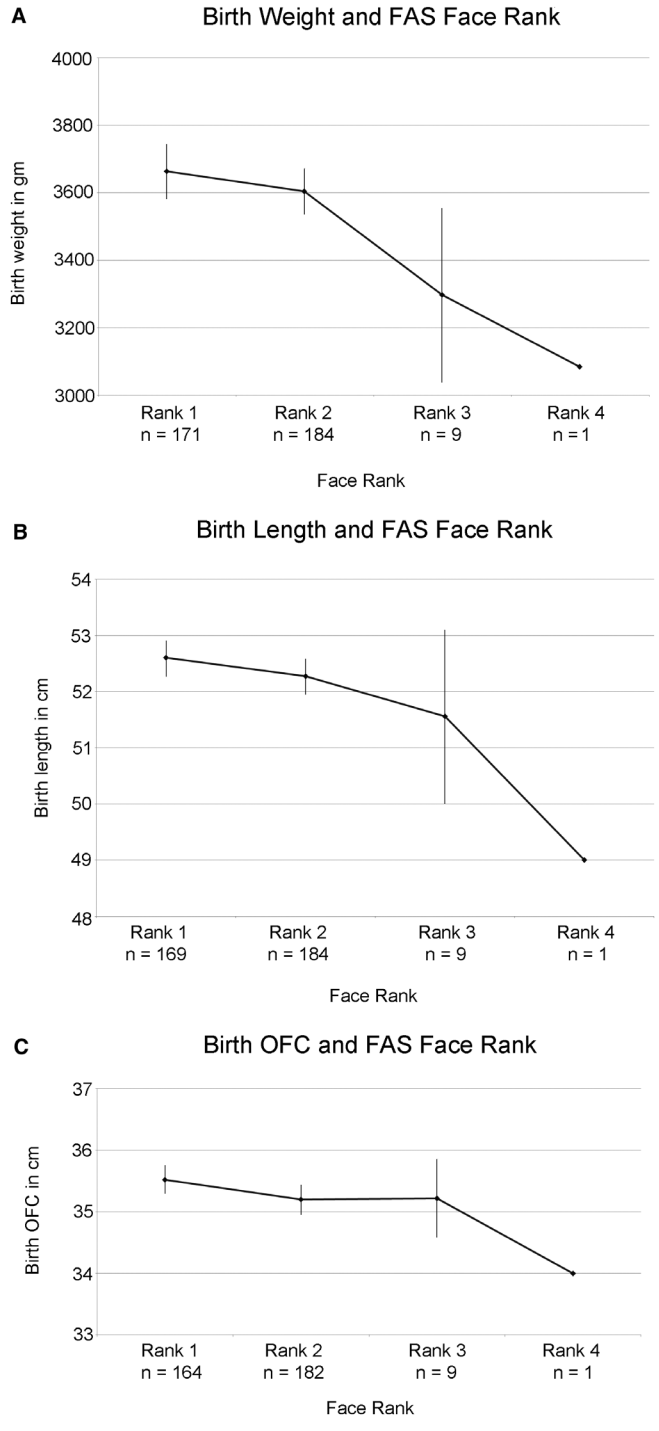

Fig. 2. Mean birthweight, birth length, and birth head circumference decreased significantly with increasing magnitude of expression of the FAS facial phenotype (Face Ranks: 1, normal; 2, mild; 3, moderate; and 4, severe) among the 366 participants in Group A. Error bars reflect 95% CIs. One-way ANOVA test for linear trend *p*-values: birth length 0.04, and birthweight and head circumference 0.001.

a Rank 3 or 4 facial phenotype, and microcephaly and/or brain dysfunction (3 or more domains of brain function 2 or more SDs below the mean). Since no child presented with growth ≤10th percentile, no child met the criteria for FAS. In contrast, all 10 children met the growth and facial criteria for PFAS. None of them presented with microcephaly; therefore, CNS dysfunction would be required to meet the CNS criteria for PFAS. Nevertheless, at 5 years of age, all were too young to participate in the battery of assessments required to confirm or rule out CNS dysfunction. As documented in the FASDPN clinical population, most children with FAS or PFAS do not present with severe brain dysfunction until later in childhood. For example, among 87 children ≤5 years of age at the time of their FAS/PFAS diagnosis at the FASDPN, only 24% met the criteria for severe CNS dysfunction (3 or more domains of function 2 or more SDs below the mean). Among 152 children >5 years of age at the time of their FAS/PFAS diagnosis, 84% met the criteria for severe CNS dysfunction. In addition, recent research (Astley et al., 2016) documents that 67 and 70% of young children with prenatal alcohol exposure that present with the Rank 3 or 4 FAS facial phenotypes, respectively, will present with severe CNS dysfunction (3 or more domains of brain function 2 or more SDs below the mean) when they are old enough (>8 years of age) to engage in more sophisticated assessments of brain function. Thus, if any of the 10 children with the Rank 3 or 4 FAS/PFAS facial phenotypes present with brain dysfunction (3 or more domains of brain function 2 or more SDs below the mean) later in childhood, they would meet the diagnostic criteria for PFAS.

Prenatal alcohol exposure was significantly correlated with the FAS facial phenotype and the 3 individual features that comprise the FAS facial phenotype. The strongest correlations with alcohol (ORs of 1.9 to 8.5) were observed when the 3 features appeared together to produce the Rank 3 or 4 FAS/PFAS facial phenotypes (Table 5). Since the Rank 4 FAS facial phenotype is confirmed to be highly specific to prenatal alcohol exposure (Astley, 2013; Astley and Clarren, 1996), it is highly likely that the FAS/PFAS facial phenotypes observed in these 10 children were caused by their prenatal alcohol exposure. Weaker, but statistically significant, correlations (ORs of 1.2 to 3.7) were observed between prenatal alcohol exposure and each individual FAS facial feature. This would be expected since alcohol is not the only factor influencing the length of a palpebral fissure, the depth of a philtrum, or the thickness of an upper lip. Perhaps one of the strongest factors other than alcohol influencing the physical presentation of these 3 facial features is familial genetics. A unique strength of the current study was the opportunity to measure the birth mothers' facial features. Among the 10 children who presented with the Rank 3 or 4 FAS facial phenotypes, all of their birth mothers presented with normal facial phenotypes (Face Ranks 1 and 2).

Correlations Between the FAS Facial Phenotype and Growth Deficiency, Microcephaly, and CNS Dysfunction

The correlations between face, growth, and CNS abnormalities observed in the current study (Fig. 2) are nearly

identical to those documented in the FASDPN clinical population (figures 8 and 9 in Astley, 2013). This study extends understanding of these correlations to a population of children with low-to-moderate prenatal alcohol exposure.

Strengths

The sample of women and children used for this study form part of a well-described, prospective cohort (Kesmodel et al., 2010; Olsen et al., 2001). While information bias is always a potential problem in observational studies (Kesmodel, 2018), the risk of information bias was minimized. Information on alcohol drinking patterns was collected directly from the birth mothers during pregnancy using validated instruments (Kesmodel, 2001; Kesmodel and Olsen, 2001), and all facial measures were performed by the inventor of the software system (Astley, 2016) used in this paper, thereby eliminating any interobserver variability and reducing the likelihood of measurement error. Further, facial measurements were taken blind to the child's exposure history. Because of the detailed information available on all participants, confounding could be addressed by adjusting for a priori selected potential confounders (Howards, 2018), following the same criteria as previous papers based on this cohort (Kesmodel et al., 2012). Also, it has previously been shown that despite selection problems in the DNBC, the external validity of measures of association seems to be good (Nohr and Liew, 2018).

Weaknesses

The DNBC represents only approximately 30% of all Danish pregnant women and hence is not a representative sample (Olsen et al., 2001). Further, the LDPS sample is a stratified sample within the DNBC (Kesmodel et al., 2010), making the current sample even less representative of the background population. While such selection may make the sample less suitable for firm statements about the overall prevalence of specific traits, inferences based on measures of association have been shown to be valid within the cohort (Nohr and Liew, 2018). Finally, since only 10 children presented with the Rank 3 to 4 facial phenotypes, the representativeness of this small group may be limited, but the statistical power was sufficient to identify significant associations with level and timing of prenatal alcohol exposure.

CONCLUSION

In conclusion, we found that approximately 3% (10/308) of the children whose mothers reported low-to-moderate alcohol intake, not usually associated with the full FAS, met the criteria for moderate-to-severe expression of the FAS facial phenotypes, Face Ranks 3 to 4. None met the diagnostic criteria for FAS or PFAS at 5 years of age. However, all 10 remain at risk for PFAS because they were too young at age 5 years to engage in the battery of neuropsychological assessments required to confirm or rule out severe brain dysfunction. The risk of FAS/PFAS facial phenotypes (Ranks 3 to 4) was significantly increased among both women with average alcohol intake of 1 to 4 drinks/wk and women with isolated episodes of binge drinking, particularly during gestational weeks 3 to 4. These findings suggest that low-to-moderate levels of prenatal alcohol exposure or isolated binge exposures may place some fetuses at risk for FAS, PFAS, or other FASDs. Thus, conservative advice is still for women to abstain from alcohol consumption during pregnancy.

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CONFLICT OF INTEREST

None.

ETHICS

The LDPS was approved by the DNBC Board of Directors, the DNBC Steering Committee, the Regional Ethics Committee, the Danish Data Protection Agency, and the Institutional Review Board at the Centers for Disease Control and Prevention. Signed informed consent was obtained for the LDPS. The current analyses were approved by the DNBC Steering Committee and the Danish Data Protection Agency.

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APPENDIX • DETAILED PROCEDURES FOR TAKING AND SELECTING PHOTOGRAPHS FOR FACIAL CODING:

To examine the association between facial features used to diagnose FAS/PFAS and low-to-moderate prenatal alcohol exposure, digital photographs were obtained, selected, and categorized for FAS/PFAS criteria according to the FASD 4-Digit Code (Astley, 2004). Details of these procedures are described in this appendix.

Taking Digital Photographs

Each participant had a standardized frontal, oblique, and lateral digital facial photograph taken in accordance with the FAS Facial Photographic Analysis Software instructions (Astley, 2016). Briefly, the child had a relaxed facial expression (no smile, lips gently closed, eyes fully open with no eyeglasses), and the digital images had proper rotation, exposure, and focus. A 19.05-mm-diameter round paper sticker was placed between the participant's eyebrows as an internal measure of scale. Photographs were taken according to the protocol outlined in Astley (2016), and lead psychologists received in-person training on how to take the photographs by SA.

Selection of Photographs for Facial Coding

Resources and photograph quality did not permit the complete analysis of all 1,628 participants' photographs. Thus, a stepwise approach was used to identify those children with clear or suggestive indication of facial dysmorphia for further measurement. The goal was to identify all individuals that presented with 1, 2, or all 3 of the FAS facial features as defined above. The photographs were measured by authors AG and SA in a 2-step process, masked to the participant's alcohol exposure.

- Step 1: AG measured the PFLs and lip circularities of all 1,628 participants regardless of the quality of the feature in the photograph (e.g., the eyes were not fully open, the child was smiling, or the sticker curled). If the eyes are not fully open, the child is smiling, or the sticker is slightly curled, the direction of error will always be in 1 direction; the PFLs will be shorter, the lip thinner, and the philtrum smoother than they truly are. SA reviewed the subset from Step 1 that appeared to have short PFLs ≤ -1.5 SDs and/or thin upper lips (lip circularities ≥ 70) and identified the subset that had sufficient image quality to ensure the PFL and lip circularity measures could be accurately measured. SA then remeasured the PFLs and lip circularities of this subset to ensure the highest level of consistency and accuracy across all facial measures.
- Step 2: SA also reviewed the philtrum of all 1,628 participants and ranked only the subset with philtrum images of sufficient image quality and met criteria for Rank 4 or 5.

Final FAS/PFAS Determination

For all viable photographs, whenever a participant was identified as having at least 1 facial feature in the FAS range, the other 2 facial features were also measured if the quality of the image was sufficient. Once measurement of the 3 facial features was complete, the software generated a 4-Digit Code Facial ABC-Score and Face Rank (Fig. 1). For example, if a child presented with PFLs 2.6 SDs below the mean, a Rank 3 philtrum, and a Rank 2 upper lip, they would receive a Facial ABC-Score of CBA and a Face Rank of 2 (mild). If 1 or 2 of the 3 facial features could not be measured, an "X" was placed in the ABC-Score to signify its absence (e.g., the ABC-Score XCA signifies that the PFL could not be measured, but the philtrum was a "C" and the lip was an "A" [see Fig. 1*B*]). A Face Rank could not be generated if 1 or 2 of the 3 features could not be measured, but Facial ABC-Scores with 1 or 2 missing features could be used to accurately rule out 1 or more of Face Ranks 1 to 4. For example, if a Facial ABC-Score was XXA, Face Ranks 3 and 4 can be accurately ruled out despite not knowing the outcome of the PFL or philtrum, because neither can include a feature with a Rank A.