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Research Article

High facial specificity and positive predictive value are required to diagnose fetal alcohol syndrome when prenatal alcohol exposure is unknown

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ABSTRACT

Background: Facial criteria with high specificity and positive predictive value (PPV) to prenatal alcohol exposure (PAE) are required to diagnose fetal alcohol syndrome (FAS) when documentation of PAE is unavailable. Not all fetal alcohol spectrum disorder (FASD) diagnostic guidelines appear to meet these criteria.

Methods: A dataset generated from a 10-year FAS screening of 1,602 children in fostercare conducted by the University of Washington FAS Diagnostic & Prevention Network was used to determine how well the FAS facial phenotype, microcephaly and growth deficiency (individually and in combination at varying levels of magnitude) predicted PAE.

Results: The 4-Digit-Code Rank 4 FAS facial phenotype was the only outcome that provided sufficient PPV and specificity to PAE (100%) to allow the facial phenotype to serve as confirmation of PAE in a diagnostic setting when PAE is unknown. Even minimal relaxation of the phenotype (e.g., Face Rank 3) resulted in PPV (35%) and specificity (88.7%) values too low to use as confirmation of PAE. Further relaxation of the facial criteria, as defined by the Hoyme et al., FASD guidelines, resulted in even lower PPV (17.9%) and specificity (76.6%); both too low to serve as confirmation of PAE in a diagnostic setting. The presence of all three physical features of FAS (Hoyme et al. FAS facial phenotype, growth and OFC ≤10th percentile) did not increase PPV beyond chance (52%).

Conclusion: FASD diagnostic guidelines that use relaxed criteria for the FAS facial phenotype risk misdiagnosing and over-diagnosing FAS and partial FAS when PAE is unknown.

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Introduction

Fetal alcohol syndrome (FAS) is a permanent birth defect syndrome caused by prenatal alcohol exposure (PAE). FAS is characterized by growth deficiency, a unique facial phenotype and structural and/or functional brain abnormalities [1,2]. To date, all fetal alcohol spectrum disorder (FASD) diagnostic guidelines permit a diagnosis of FAS and/or partial FAS (pFAS) to be rendered when a history of prenatal alcohol exposure is unknown [2-7]. Why? For most guidelines, it is because the diagnostic criteria for FAS require the presence of the FAS facial phenotype and the "FAS" facial phenotype is so unique to (caused only by) PAE, its presence serves as confirmation of PAE when written or verbal documentation of PAE is unavailable [2,8-10].

There are four screening metrics used in medicine to quantify how well the presence of a feature (e.g., the FAS facial phenotype) predicts the presence/absence of an exposure (e.g. PAE), and how well an exposure (e.g. PAE) predicts the presence/absence of an outcome (e.g., the FAS facial phenotype). These four metrics (positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity) are defined in Figure 1 [11,12].

If PAE is the only cause of the FAS facial phenotype [8,9,13], one would expect these two conditions to be true:

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		True Exposure		
		Prenatal Alcohol Exposure Documented	Prenatal Alcohol Exposure Not Documented	
Predicted Exposure based on the face	FAS Face Present (Face Rank 4)	True Positive (TP) = **20**	False Positive (FP) = **0**	**Positive Predictive Value (PPV) = 100%** = TP/ (TP + FP) = 20 / (20 + 0)
	FAS Face Absent (Face Ranks 1,2,3)	False Negative (FN) = **207**	True Negative (TN) = **1375**	**Negative Predictive Value (NPV) = 87.0%** = TN/ (FN + TN) = 1375 / (207 + 1375)
		Sensitivity 8.8% = TP / (TP + FN) = 20 / (20 + 207)	**Specificity 100.0%** = TN / (FP + TN) = 1375 / (0 + 1375)	

Figure 1. Demonstration: The ability of the Rank 4 FAS facial phenotype to correctly predict prenatal alcohol exposure.

Among 1,602 children in foster care, 14.2% had documented prenatal alcohol exposure (PAE). If the 4-Digit Code Rank 4 FAS facial phenotype is to be used to confirm PAE when a written or verbal history of exposure is not available, then the FAS facial phenotype must have a high PPV (all individuals with the facial phenotype have a documented PAE) and high specificity (the Rank 4 FAS face is never present in an individual with confirmed absence of PAE).

PPV: Probability that a subject with the FAS facial phenotype has PAE.

NPV: Probability that a subject without the FAS facial phenotype does not have PAE.

Sensitivity: Probability that a subject with PAE has the FAS facial phenotype.

Specificity: Probability that a subject without PAE does not have the FAS facial phenotype.

1. All individuals with the FAS facial phenotype will have PAE (PPV = 100%).
2. No individual with a confirmed absence of PAE will have the FAS facial phenotype (specificity = 100%).

And if, as research supports [14-17], the FAS facial phenotype is caused by prenatal alcohol exposure during a very narrow window of time (~weeks 2 and 3 of pregnancy, gastrulation stage of fetal development), one would expect these two conditions to be true:

1. Absence of the FAS facial phenotype would not confirm absence of PAE (NPV will be low).
2. Not all individuals with PAE would have the FAS facial phenotype (sensitivity will be very low).

While all FASD diagnostic systems allow a diagnosis of FAS to be rendered in the absence of a confirmed PAE, not all FASD diagnostic systems use the same clinical criteria for the FAS facial phenotype. The Australian 2016 [5] and Canadian 2015 [4] systems use the Rank 4 FAS facial phenotype as defined by the 4-Digit Code [3] (three facial features must be present (palpebral fissure lengths (PFL) at or below the 3rd percentile; a smooth philtrum (Rank 4 or 5 on the University of Washington (UW) Lip-Philtrum Guides) and a thin upper lip (Rank 4 or 5 on the UW Lip-Philtrum Guides)). The CDC 2004 guidelines use the 4-Digit Code criteria with one exception (the PFL criteria is relaxed to ≤10th percentile) [6]. The criteria for the FAS facial phenotype used by the Hoyme et al., 2016 [7] FASD diagnostic system is the most relaxed (only 2 of the 3 facial features must be present and 2 of the 3 facial features are relaxed in their magnitude relative to the 4-Digit Code (PFLs ≤ 10th percentile; a smooth philtrum (Rank 4 or 5 on the Hoyme et al., Lip/Philtrum Guides) and/or a thin upper lip (Rank 4 or 5 on the Hoyme et al., Lip/Philtrum Guides)). The Rank 4 thin upper lip on the Hoyme et al., North American Lip/Philtrum Guide is confirmed equivalent to the Rank 2 moderately thick upper lip on the UW Lip-Philtrum Guide [18,19].

When the 4-Digit-Code Rank 4 FAS facial phenotype was first identified and case-defined in a small, but rigorous split-half empirical study, PPV and specificity to FAS and PAE were 100% [20]. Subsequent studies in large clinical, foster care and general populations continued to document high PPV and specificity to PAE (> 95%) [8,9,13]. The specificity of the more relaxed Hoyme et al., FAS facial phenotype ranges from 71% to 75% as reported by the authors of the guidelines [21] and replicated in the FASDPN datasets [18,19]. A facial specificity of 71% to 75% is not sufficient to allow a valid diagnosis of FAS to be rendered when written or verbal documentation of PAE is unavailable. A specificity of 75% means 25% of individuals who present with the Hoyme et al., FAS facial phenotype do not have PAE. This was illustrated among twelve high functioning children (mean full scale IQ = 120) with confirmed absence of PAE enrolled as controls in a FASD MRI study [22]. Due to the relaxation of the facial criteria, 3 of the twelve children (25%) met the criteria for the Hoyme et al., FAS facial phenotype despite confirmed absence of PAE.

If a FAS facial phenotype with low specificity and PPV is used to confirm PAE, birth mothers are at risk of being wrongly accused of drinking during pregnancy and harming their unborn child; FAS will be misdiagnosed and over-diagnosed, and studies designed to generate population-based estimates of the prevalence of FAS will lead to inaccurate over-estimates [10].

If the criteria used to define the FAS facial phenotype do not have sufficiently high specificity and PPV to confirm PAE, can the specificity and PPV be increased by requiring additional FAS physical features be present? For example, what is the specificity and PPV to PAE when an individual presents with the FAS facial phenotype and microcephaly and growth deficiency? To answer this question, a dataset generated from a 10-yr population-based FAS screening of 1,602 children in foster care was used [9]. The screening activity collected height, weight, head circumference, computerized facial measures from facial photographs and presence/absence of documented PAE for each child.

The primary objective of this study was to determine how well the FAS facial phenotype, microcephaly and growth deficiency (individually and in combination at varying levels of magnitude) predict prenatal alcohol exposure. The following outcomes were postulated:

1. If the FAS facial phenotype is to be used to confirm PAE when a written or verbal history of exposure is not available, then the FAS facial phenotype must have 100% PPV (all people with the facial phenotype have PAE) and 100% specificity (the Rank 4 FAS face is never present in a person with confirmed absence of PAE).
2. Relaxation of the FAS facial phenotype criteria will cause a sharp drop in specificity and PPV to PAE.
3. The majority of individuals with PAE will not present with the FAS facial phenotype because there is a very narrow window of vulnerability in which PAE can cause the FAS facial features during fetal development (gestational weeks 2-3: the gastrulation stage) [16,17]. The sensitivity of the FAS

facial phenotype to predict PAE will be very low. If one were to use the FAS facial phenotype to screen a population for PAE, most of the individuals with PAE would be missed.

4. Since the majority of children in this foster care population are not expected to have the FAS facial phenotype and the majority will not have a documented PAE, the absence of the FAS facial phenotype will correctly predict the absence of PAE in the majority of the children in this foster care population by chance alone. NPV values will be uniformly higher under these circumstances as described more fully below, but this does not mean the absence of the FAS facial phenotype can be used to accurately rule-out PAE. NPV will not be 100%. It is well understood that the majority of individuals with PAE do not present with the FAS facial phenotype due to the narrow window of vulnerability in which PAE can cause the FAS facial phenotype.

5. Growth deficiency and microcephaly are caused by PAE but are not unique to (caused only by) PAE. PPV will be very low.

6. If the criteria for the FAS facial phenotype are relaxed, the combined presence of the relaxed facial phenotype, growth deficiency and microcephaly should increase specificity and PPV to PAE, but may not increase it sufficiently (> 95%) to serve as diagnostic confirmation of PAE when a verbal or written confirmation of PAE is not available.

Research Methodology

The dataset used for this study was generated from a 10-year FAS screening study of 1,602 children in foster care conducted by the University of Washington Fetal Alcohol Syndrome Diagnostic & Prevention Network (FASDPN) and the King County Washington Foster Care Passport Program (FCPP) between 1999 and 2009. Detailed methods and the outcomes of the first 600 children screened were reported in 2004 [9].

Enrollment into the foster care passport program

Briefly, all children, who were legally dependent with the State of Washington and enrolled in the Foster Care Passport Program (FCPP) in King County, Washington between 1999 and 2009 were eligible to participate in the FAS screening [9]. To be enrolled in the FCPP, a child had to be: a) legally supervised by the Department of Social and Family Services; b) 0-12 years of age at the time of enrollment, but may remain in the program after their 12th birthday; c) dependent and d) in out-of-home placement. Upon enrollment in the FCPP, each child's health and social service history from birth to present was abstracted from the child's medical records by a FCPP public health nurse and entered into a foster care Health & Education database. The database was used to produce a summary medical report for each child (a Health and Education "passport") along with health recommendations to be shared with the social worker, the foster parent and the child's health care provider(s). The "passport" included measures of height, weight and head circumference, medical conditions/concerns, and prenatal exposures.

Enrollment into the FAS screening

The FCPP identified all eligible children, obtained written consent from the child's legal guardian (Department of Child and Family Services social worker), sent the child's foster parents a letter that explained the purpose and process of the FAS screening, and sent the FASDPN clinic the list of all newly eligible, consented children, weekly. The FASDPN scheduler called each foster parent to schedule a photography appointment with the FASDPN photographer. The photographer measured the child's head size (occipital frontal circumference) and took 3 digital standardized facial photographs (frontal, oblique, and lateral) with a ¾ inch round paper sticker placed between the child's eyebrows to serve as an internal measure of scale (Figure 2A). The facial photographs were analyzed by the author using the FAS Facial Photographic Analysis Software, masked to the child's PAE status [23]. Over 95% of eligible children participated in the FAS screening program over a 10-year period [9].

Screen-positive criteria

A child screened positive for FAS if they presented with the Rank 4 FAS facial phenotype as defined by the FASD 4-Digit Code. This facial phenotype is defined below and illustrated in Figure 2.

All children who screened positive were eligible to receive a comprehensive diagnostic evaluation and treatment plan at the FASDPN clinic by an interdisciplinary team (pediatrician, psychologist, speech language pathologist, occupational therapist, social worker and family advocate) using the 4-Digit Diagnostic Code. The screening activity was approved by the Human Research Review Boards of Washington State and the University of Washington. The outcomes of the screening program are reported separately [9].

Study dataset

The following fields of data from the 10-year FAS screening study were used in the current study.

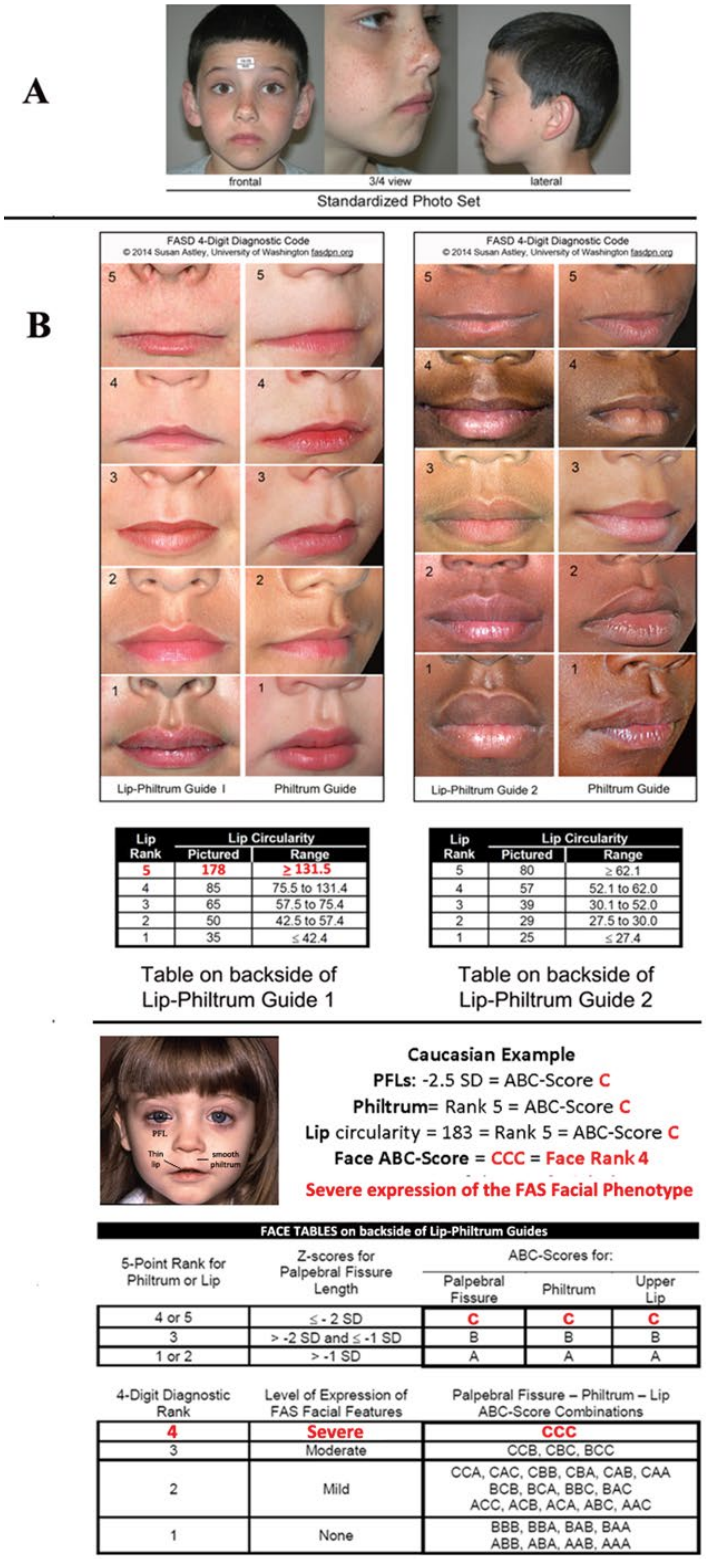
- The 3 individual facial features of FAS were measured from digital facial photographs using the FAS Facial Photographic Analysis Software [23] (Figure 2). A brief video demonstration of the software is provided at this weblink: http://depts.washington.edu/fasdpn/movie/software1024-768cd2.mp4

  1. Palpebral fissure lengths in mm.

  2. Philtrum smoothness: 5-point Likert Rank on the UW Lip-Philtrum Guides

  3. Lip thinness (circularity) was measured by outlining the perimeter of the upper lip using the FAS Facial Photographic Analysis Software [23]. Circularity equals perimeter2/area. The thinner the upper lip, the larger the circularity. The circularity tables printed on the backside of each Lip-Philtrum Guide (Figure 2B) were used to convert lip circularity into lip rank.

- Height (cm) and weight (kg) at enrollment into the screening study and at earlier time points when available in the medical records. The WHO [24] and CDC [25] growth charts were used to generate height and weight percentiles adjusted for age and gender. The FASD 4-Digit Code ranks growth deficiency on a 4-point Likert scale (Rank 1 normal; Rank 2, mild; Rank 3 moderate and Rank 4 severe) in



Lip Rank	Lip Circularity Pictured	Lip Circularity 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

Lip Rank	Lip Circularity Pictured	Lip Circularity Range
5	80	≥ 62.1
4	57	52.1 to 62.0
3	39	30.1 to 52.0
2	29	27.5 to 30.0
1	25	≤ 27.4

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

Figure 2. The FASD 4-Digit Code method for measuring the FAS facial phenotype using the FAS Facial Photographic Analysis Software [3,10,23]. The Rank 4 FAS facial phenotype is defined by 3 features: short PFLs ≤ -2 SDs, smooth philtrum Rank 4 or 5 and thin upper lip Rank 4 or 5 on the University of Washington Lip-Philtrum Guides. A) Three digital facial photographs are obtained with a ¾ inch adhesive sticker serving as an internal measure of scale. B) The PFL is measured in mm by clicking the mouse on the inner and outer corners of each eye. Using the Face Tables, the software converts the PFL in mm to a z-score and then to a PFL ABC-Score. The red perimeter of the upper lip is traced with the mouse to compute lip circularity (perimeter^2/area). The software converts lip circularity to lip rank and then to a lip ABC-Score. Finally, the software converts the philtrum rank to a philtrum ABC-Score. The 3 individual ABC-scores are combined in the order PFL-Philtrum-Lip to create the overall Facial ABC-Score. Highlighted in red font is an example of a child with PFL -2.5 SDs, philtrum Rank 5 and lip circularity 183. These three features produce a facial ABC-Score of CCC, representing the severe expression of the FAS facial phenotype (Face Rank 4). The FAS facial phenotype presents on a continuum: Rank 1: none of the 3 features present; Rank 2: 1 or 2 features present; Rank 3: 2.5 of the 3 features present; and Rank 4: all 3 features present. A video demonstration of the facial software is presented at this weblink: https://depts.washington.edu/fasdpn/movie/software1024-768cd2.mp4 Copyright Susan Astley Hemingway University of Washington

accordance with the Growth Tables printed on the backside of the Lip-Philtrum Guides (Figure 3). The Hoyme et al., FASD guidelines dichotomize growth deficiency as present (height and/or weight ≤ 10th percentile) or absent.

- Occipital frontal circumference (OFC) in cm at the time of enrollment in the screening and earlier time points when available in the medical records. The WHO [24] and CDC [25] growth charts were used to generate OFC percentiles adjusted for age and gender. The 4-Digit Code defines microcephaly as OFC ≤ 3rd percentile. The Hoyme et al., FASD guidelines define "microcephaly" as OFC ≤ 10th percentile.
- PAE (documented, not documented) from review of all medical and social service records from birth to present.
- Gender
- Age in years at screening
- Race/ethnicity

The 3 FAS facial features (PFL in mm, philtrum rank, and lip circularity) were used to generate the following FAS facial phenotypes in accordance with the 4-Digit Code [2,3] and Hoyme et al., 2016 [7] FASD diagnostic systems:

4-Digit Code FAS facial phenotype:

The magnitude of expression of the FAS facial phenotype is ranked on a 4-point Likert scale in accordance with the lip circularity and face tables printed on the backside of each Lip-Philtrum Guide (Figure 2B). The FAS facial phenotype (Face Rank 4) requires the presence of all three facial features: 1) palpebral fissure lengths ≤ 3rd percentile, 2) a smooth philtrum (Likert rank 4 or 5 on the UW Lip-Philtrum Guide) and 3) a thin upper lip (Likert rank 4 or 5 on the 5-point Lip-Philtrum Guide) (Figure 2B). The Iosub [26] PFL growth charts were used to generate PFL percentiles by gender and age for all full and mixed race African American children. The Stromland Scandinavian [27] PFL growth charts were used for all other races. The UW Lip-Philtrum Guide 1 was used to Rank lip thinness and philtrum smoothness for Caucasians and all races that indigenously present with thinner lips like Caucasians. The UW Lip-Philtrum Guide 2 was used to Rank lip thinness and philtrum smoothness for African Americans and all races (e.g., aboriginal Australians and some east Asian populations) that indigenously present with thicker lips like African Americans. Based on the racial makeup of the current study population, Lip-Philtrum Guide 2 was used on all full and mixed-race African Americans; Lip-Philtrum Guide 1 was used on all other races. The 27 Asian children in this study were not east Asian.

Hoyme et al., 2016 FAS Facial Phenotype:

The Hoyme et al., FAS facial phenotype is classified on a dichotomous scale (present/absent). The Hoyme et al., FAS facial phenotype requires 2 of the following 3 facial features (PFLs ≤ 10th percentile; smooth philtrum (Rank 4 or 5 on the Hoyme et al., Lip/Philtrum Guides); thin upper lip (Rank 4 or 5 on the Hoyme et al., Lip/Philtrum Guides)) [7]. Hoyme et al., provide two Lip/Philtrum Guides; one for South African Cape Coloured [28] and one for North Americans [7]. The Cape Coloured Lip/Philtrum Guide is not appropriate for use on African Americans, for it was

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

Example

Height: 8th percentile = ABC-Score B

Weight: 2nd percentile = ABC-Score C

Height-Weight ABC-Score = BC = Growth Rank 3

Moderate Growth Deficiency

Figure 3. FASD 4-Digit Code growth tables for converting height and weight percentiles into Growth Ranks.

The FASD 4-Digit Code documents growth deficiency on a 4-point Likert scale from Rank 1 normal growth to Rank 4 severe growth deficiency. Highlighted in red font is an example of an individual who presented with a height at the 8th percentile and weight at the 2nd percentile. Using the Growth Tables printed on the backside of the Lip-Philtrum Guides, these growth percentiles would translate into a Growth Rank 3, moderate growth deficiency.

developed by Hoyme et al. specifically for the Cape Coloured (mixed race) population in the Western Cape Province of South Africa. The investigators reported that neither of the University of Washington Caucasian or African American Lip-Philtrum Guides were an exact "fit" for the Cape Coloured population. Due to the absence of a Hoyme et al. Lip/Philtrum guide appropriate for use on African Americans, the Hoyme et al., FAS facial phenotype was not generated for subjects in this study that were full or mixed race African American. For all other races, the Hoyme et al., North American Lip/Philtrum Guide was used to Rank lip thinness and philtrum smoothness. Based on a previous study [18], the Rank 4 lip on the Hoyme et al., North American Lip/Philtrum Guide has a circularity of 52.5 (see video demonstration at this weblink: http://depts.washington.edu/fasdpn/movie/Fig2Cvideo.mp4). Thus, all lips with circularity ≥ 52.5 met the Hoyme et al., FAS facial phenotype criteria for a "thin upper lip". The Stromland [27] PFL normal growth charts were used to generate PFL percentiles adjusted for gender and age.

Specificity, sensitivity, PPV and NPV were calculated in accordance with Figure 1 to determine how accurately the FAS facial phenotype, microcephaly and growth deficiency (individually and in combination at varying levels of magnitude) predict PAE. Confidence intervals for sensitivity and specificity are "exact" Clopper-Pearson confidence intervals [29]. Confidence intervals for the predictive values are the standard logit confidence intervals given by Mercaldo et al. [30].

Sensitivity: (true positive rate) The proportion of people with documented PAE that have the FAS facial phenotype. We expect sensitivity to be very low; most people with PAE do not present with the FAS facial phenotype. If the FAS facial phenotype was used to screen for individuals with PAE, most individuals with PAE would be missed.

Specificity: (true negative rate) The proportion of people with no documented PAE that do not have the FAS facial phenotype. We expect specificity to be very high. If PAE is the only cause of the FAS facial phenotype, then a person cannot have the FAS facial phenotype if they were not exposed to alcohol.

PPV: The probability that a person with the FAS facial phenotype has a documented PAE. We expect this to be very high. If PAE is the only cause of the FAS facial phenotype, then a person with the FAS facial phenotype must have been exposed to alcohol in-utero.

NPV: The probability that a person without the FAS facial phenotype has no documented PAE. We expect this to be relatively high for the reasons outlined below.

Sensitivity and specificity are attributes of the diagnostic test. They are not influenced by the prevalence of PAE in the population. PPV and NPV are influenced by the sensitivity and specificity of the test and by the prevalence of PAE in the study population. As the prevalence of PAE decreases, the PPV (the ability of the FAS facial phenotype to correctly predict PAE) decreases because there will be more false positives for every true positive. This is because one is hunting for a "needle in a haystack" (e.g. only 14% of the children in this foster care have documented PAE). Since there are so many more children without documented PAE (86%) in this foster population, chance alone dictates one has a greater probability of selecting a child without documented PAE, than one with documented PAE. As the prevalence of PAE decreases, the NPV (the ability of the absence of the facial phenotype to correctly predict the absence of PAE) increases because there will be more true negatives for every false negative. Again, since there are so many more children without documented PAE (86%) in this foster care population, chance alone dictates one has a greater probability of selecting an individual without documented PAE, than one with PAE.

Results

Sociodemographic and FASD clinical profiles

The sociodemographic and FASD clinical profiles of the 1,602 children in foster care that participated in the FAS screening are presented in Table 1. The population was predominantly Caucasian (52%) and full or mixed race African American (31%); ranged in age from 3 months to 17 years old with 47% under 4 years of age. Roughly half (49%) were female.

Growth deficiency (height and/or weight ≤ 10th percentile) was observed in 14% of the population.

The 4-Digit Code Rank 4 FAS facial phenotype was observed in 20 (1.2%) of 1,602 children across all races (13 (2.6%) among the 502 African American; 7 (0.6%) among the remaining 1,100 children from all other races). The prevalence of PAE was highest (18.5%) among the 502 African American; 12.1% among all other races. Of the 20 with the Rank 4 FAS facial phenotype, 100% had documented PAE. The Hoyme et al., FAS facial phenotype was observed in 274 (25%) of the 1,093 children from all races except African Americans (exclusion of African Americans is explained above). Of the 274 with the Hoyme et al., FAS facial phenotype, 17.9% had documented PAE.

OFC ≤ 3rd percentile (microcephaly) was observed among 6.6% of the 1,602 children. OFC ≤ 10th percentile was observed among 11.7% of the children.

Prenatal alcohol exposure was documented in 227 (14.2%) of the 1,602 children.

Specificity, sensitivity, PPV and NPV

The PPV, NPV, specificity and sensitivity documenting how well the FAS facial phenotype, microcephaly and/or growth deficiency (individually and in combination at varying levels of magnitude) predicted prenatal alcohol exposure is presented in Table 2.

Table 1. Sociodemographic and clinical profile of the 1,602 children that participated in the foster care FAS screening.

Characteristic		Total N 1,602	
Race (n, %)	Caucasian	828	51.7
	African American (full or mixed-race)	502	31.3
	Native American	157	9.8
	Hispanic	26	1.6
	Asian	27	1.7
	All others including mixed race	62	3.9
Age years (n, %)	0-3.9	756	47.2
	4-5.9	210	13.1
	6-8.9	235	14.7
	9-12.9	328	20.5
	13-17.4	73	4.6
	mean (SD) range	5.5 (4.2)	0.3-17.4
Gender (n, %)	female	778	48.5
Growth Rank[A] (n, %)	normal (height & weight > 10th percentile): Rank 1	1383	86.3
	mild (height and/or weight 4-9th percentile): Rank 2	146	9.1
	moderate (height or weight ≤ 3rd percentile): Rank 3	32	2
	severe (height & weight ≤ 3rd percentile): Rank 4	41	2.6
	height and/or weight ≤ 10th percentile	219	13.7

FAS face Rank[A] (n, %)	normal (none of the 3 features): Rank 1	776	48.4
	mild (1 or 2 of the 3 features): Rank 2	685	42.8
	moderate (2.5 of the 3 features): Rank 3	121	7.6
	severe (all 3 features): Rank 4	20	1.2
Head Circumference (n, %)	microcephaly: OFC ≤ 3rd percentile	95	6.6
	microcephaly: OFC ≤ 3rd percentile & PAE	35	2.4
	OFC ≤ 10th percentile	167	11.7
	OFC ≤ 10th percentile & PAE	43	3
Prenatal alcohol exposure (n, %)	none documented	1,375	85.8
	documented	227	14.2

A. See Figures 2 and 3 for how growth and face are Ranked by the FASD 4-Digit Code.

Abbreviations: OFC: occipital frontal head circumference; PAE: prenatal alcohol exposure

Specificity and PPV of the FAS facial phenotype

The 4-Digit-Code Rank 4 FAS facial phenotype was 100% specific to PAE and 100% PPV for PAE. Twenty children presented with the Rank 4 FAS facial phenotype; all 20 had documented PAE (100% PPV). Of the 1,330 children with no documented PAE; none presented with the Rank 4 FAS facial phenotype (100% specificity).

Of the 274 children that presented with the Hoyme et al., FAS facial phenotype; only 49 had documented PAE (17.9% PPV). The Hoyme et al., FAS facial phenotype was not predictive of PAE. The vast majority (82.1%) with the Hoyme et al., FAS facial phenotype did not have documented PAE. Of the 960 children with no documented PAE; 735 did not have the FAS facial phenotype (76.6% specificity). In other words, 23.4% of the children with no documented PAE presented with the Hoyme et al., FAS facial phenotype. If the University of Washington Lip-Philtrum Guide 1 for Caucasians was used instead of the Hoyme et al., North American Lip-Philtrum Guide, 121 children presented with the Hoyme et al., FAS facial phenotype. Of these 121 children; only 36 had documented PAE (29.8% PPV). Relaxation of the PFL to the 10th percentile and requiring only two of the three facial features continued to produce a FAS facial phenotype that was not predictive of PAE.

Since the magnitude of expression of the 4-Digit Code FAS facial phenotype is ranked on a 4-point Likert scale from Rank 1 (absent) to Rank 4 (fully present), the specificity, sensitivity, PPV and NPV were calculated for each Rank to assess how incremental relaxation of the FAS facial phenotype impacts the phenotype's ability to predict PAE (PPV). When the mild Rank 2, moderate Rank 3 and severe Rank 4 expressions of the FAS facial phenotype were compared to Rank 1 (complete absence of the FAS facial phenotype), the ability of the FAS facial phenotype to predict PAE (PPV) dropped precipitously from 100% for the Rank 4 face to 26.5% for the Rank 3 face (Table 2). The PPV for the Rank 2 face dropped even further to 13.7%. Another clinically meaningful way to assess the predictive value was to compare each Face Rank to all Face Ranks below it. Again, as the magnitude of expression of the FAS facial phenotype was reduced, the PPV dropped precipitously. One additional clinically meaningful way to assess the predictive value of the FAS facial phenotype was to dichotomize the 4-point facial Likert scale into a present/absent scale, splitting it in half at different cut-points. For example, using the current definition of the FAS facial phenotype: present equals Rank 4 and absent equals Ranks 1, 2 and 3; the Rank 4 phenotype is 100% predictive of PAE (all children with the Rank 4 Face have a documented PAE). If the 4-Digit Code FAS facial phenotype was relaxed: redefined as present equals Ranks 4 and 3 and absent equals Ranks 2 and 1; the PPV drops to 36.9%. Only 36.9% of subjects with Rank 3 or 4 faces had documented PAE. If the facial phenotype was further relaxed; redefined as present equals Ranks 4, 3 and 2 and absent equals Rank 1; PPV drops to 17.7%. This PPV is essentially identical to the PPV of the Hoyme et al., facial phenotype (17.9%). In a previous study [18], the relaxation of the Hoyme et al., facial phenotype was confirmed to be equivalent to 4-Digit Facial Ranks 2, 3 and 4 combined.

Specificity and PPV for the combined presence of the FAS facial phenotype, growth deficiency and microcephaly

When all three physical features of FAS were present in accordance with the Hoyme et al., 2016 FASD guidelines (the Hoyme et al., FAS facial phenotype, growth deficiency ≤ 10th percentile and OFC ≤ 10th percentile), PPV only reached 52%. Twenty-one children presented with all three physical features (FAS facial phenotype, growth deficiency and small OFC); but only 11 of the 21 had a documented PAE (52% PPV). The presence of all three physical features failed to predict PAE above random chance (50%). Specificity to PAE increased to 99% (all 3 features were absent in 99% of children with no documented PAE).

Specificity and PPV of growth deficiency and microcephaly

The PPV for growth deficiency, even at the most severe level (height and weight ≤ 3rd percentile: Growth Rank 4), never rose above 48.5% (no better than random chance). Only 20 of 41 children with Rank 4 growth deficiency had documented PAE. The PPV for microcephaly (OFC ≤ 3rd percentile) was 36.8%; only 35 of the 95 children with microcephaly had documented PAE. These outcomes were anticipated, as it is well documented in the medical literature that PAE is not the only cause of growth deficiency and microcephaly.

Sensitivity and NPV

Since the majority of individuals with PAE do not present with the Rank 4 FAS facial phenotype, growth deficiency and/or microcephaly, as postulated, sensitivity was quite low. If one used these features to screen for PAE in a population, most individuals with PAE would be missed.

Table 2. How well the FAS facial phenotype, microcephaly and/or growth deficiency predict prenatal alcohol exposure.

FAS Physical Features	N Group1 vs. 2	PPV	95% C.I.	NPV	95% C.I.	Specificity	95% C.I.	Sensitivity	95% CI
4-Digit Face Ranks 4, 3, 2 vs. Rank 1 [A]									
Face 4 vs. 1	20 vs. 776	100		89.6	88.6-90.4	100	99.5-100.0	19.8	12.5-28.9
Face 3 vs. 1	121 vs. 776	26.5	20.2-33.8	89.6	88.4-90.6	88.7	86.2-90.8	28.3	20.2-37.6
Face 2 vs. 1	685 vs. 776	13.7	12.0-15.6	89.6	87.9-91.0	54	51.3-56.8	53.7	46.0-61.3
Each face rank vs. all ranks below[A]									
Face 4 vs. 3,2,1[B]	20 vs. 1582	100		87	86.5-87.4	100	99.7-100.0	8.8	5.5-13.3
Face 3 vs. 2,1	121 vs. 1461	26.5	19.8-34.4	88	87.4-88.6	93.5	92.1-94.8	15.5	10.8-21.2
Face 2 vs. 1	685 vs. 776	13.7	12.0-15.6	89.6	87.9-91.0	54	51.3-56.8	53.7	46.0-61.3
Face Rank Scale Dichotomized[A]									
Face Rank 4 vs. 3,2,1	20 vs. 1582	100		86.9	86.5-87.4	100	99.7-100.0	8.8	5.5-13.3
Face Ranks 3,4 vs. 2,1	141 vs. 1461	36.9	30.0-44.4	88	87.2-88.8	93.5	92.1-94.8	23	17.6-28.1
Face Ranks 2,3,4 vs. 1	826 vs. 776	17.7	16.1-19.3	89.6	87.7-91.2	50.7	47.9-53.2	64.3	57.7-70.6
Face Rank 4 vs. 3,2,1 African Americans excluded	7 vs. 1093	100		88.4	88.0-88.8	100	99.6-100.0	5.2	2.1-10.5
Hoyme 2016 FASD Guidelines[C]									
FAS Face vs. no FAS Face	274 vs. 819	17.9	14.5-21.9	89.7	88.4-90.9	76.6	73.8-79.2	36.8	28.7-45.6
FAS Face + growth ≤ 10%[D]	48 vs. 1045	39.6	27.4-53.2	89.1	84.8-89.8	97	95.7-98.0	14.3	8.8-21.4
FAS Face + OFC ≤ 10%[D]	48 vs. 1029	37.5	25.6-51.1	88.9	88.2-89.6	96.8	95.5-97.90	13.6	8.3-20.7
FAS Face + growth ≤ 10% + OFC ≤ 10% [E]	21 vs. 1075	52.4	32.3-71.8	88.6	88.0-89.1	98.9	98.1-99.5	8.2	4.2-14.2
Hoyme et al., FAS face using UW Lip-Philtrum Guide 1	121 vs. 979	29.8	23.1-37.4	89.9	89.0-90.9	91.2	89.2-92.9	26.9	19.6-35.2
Growth and Microcephaly									
Growth Rank 4 vs. 1	41 vs. 1383	48.8	34.5-63.3	87.9	87.4-88.5	98.3	97.4-99.0	10.7	6.7-16.0
Growth Rank 3 vs. 1	32 vs. 1383	31.3	18.0-48.6	87.9	87.5-88.3	98.2	97.3-99.0	5.7	2.7-10.1
Growth Rank 2 vs. 1	146 vs. 1383	20.6	15.1-27.3	87.9	87.3-88.6	91.3	86.7-92.3	15.2	10.5-21.0
Growth ≤ 3% (Ranks 3,4 vs. 2,1)	73 vs. 1529	41.1	30.9-52.1	87.1	86.5-87.7	96.9	95.8-97.7	13.2	9.1-18.3
Growth ≤ 10% (Growth Ranks 2,3,4 vs. 1)	219 vs. 1383	27.4	22.5-32.9	87.9	87.1-88.8	88.4	86.6-90.1	26.4	20.8-32.7
Growth ≤ 10% African Americans excluded	145 vs. 955	24.1	18.5-30.8	89.6	88.6-90.6	88.6	86.4-90.6	26.1	18.9-34.4
OFC ≤ 3%	95 vs. 1338	36.8	28.3-46.3	83.5	81.4-85.4	95.1	93.7-96.2	16.5	11.8-22.2
OFC ≤ 10%	167 vs. 1264	25.8	20.2-32.2	86.8	86.0-87.6	89.8	88.0-91.5	20.5	15.2-26.6
OFC ≤ 10% African Americans excluded	127 vs. 894	22.8	17.0-30.0	89	88.1-90.0	89	86.8-91.0	22.8	15.9-31.2

A. See Figures 2 and 3 for how growth and face are ranked by the FASD 4-Digit Code.

B. This outcome is portrayed in Figure 1.

C. The study population used to assess the Hoyme et al., FASD criteria does not include African Americans as detailed in the Methods section.

D. Hoyme et al., 2016 FASD guidelines [7] require 2 physical features be present to diagnose PFAS when PAE is unknown (the FAS facial phenotype and growth ≤ 10th percentile or the FAS facial phenotype and OFC ≤ 10th percentile).

E. Hoyme et al., 2016 FASD guidelines require 3 physical features be present to diagnose FAS when PAE is unknown (the FAS facial phenotype and growth ≤ 10th percentile and OFC ≤ 10th percentile).

Abbreviations: CI: confidence interval; NPV: negative predictive value; OFC: occipital frontal circumference (head circumference); PPV: positive predictive value; UW: University of Washington.; vs: versus

As anticipated, NPV was uniformly high (83.5% to 89.6%) across each of the physical features (the FAS facial phenotype, growth deficiency ≤ 10th percentile and OFC ≤ 10th percentile). The absence of one or more features correctly predicted the absence of PAE 83% to 89% of the time. But it is important to remember that NPV is influenced by the prevalence of PAE in the study population. As the prevalence of PAE decreases, the probability of correctly predicting the absence of PAE increases.

Discussion

The observed outcomes in this study were concordant with the postulated outcomes. The Rank 4 FAS facial phenotype, as defined by the FASD 4-Digit Code [3], was the only physical outcome that provided sufficient PPV and specificity to PAE (100%) to allow the facial phenotype to be used as confirmation of PAE in a diagnostic setting when a written or verbal documentation of PAE is not available. Even minimal relaxation of the facial phenotype criteria (e.g., Face Rank 3) resulted in a precipitous decrease in PPV (35%) and specificity (88.7%), rendering the relaxed phenotypes incapable of serving as confirmation of PAE. It is for this reason that the FASD 4-Digit Code requires confirmed PAE for all diagnoses with the exception of FAS. The high PPV and

specificity metrics generated from the current study are consistent with the PPV and specificity metrics generated from our previous studies dating back to 1995 [8,9,20,31].

The FAS facial phenotype as defined by the Hoyme et al., 2016 FASD guidelines resulted in a very low PPV (17.9%) and low specificity (76.6%), both far too low to serve as valid confirmation of PAE in a diagnostic setting when written or verbal confirmation of PAE is unavailable. Requiring the presence of all three physical features of FAS (the Hoyme et al., FAS facial phenotype and growth ≤ 10th percentile and OFC ≤ 10th percentile) did not increase PPV sufficiently to allow the cluster of physical features to serve as diagnostic confirmation of PAE. PPV increased only to 52%. Of the 21 children that presented with all three of these physical features, only 52% had a documented PAE. The presence of all three features predicted PAE no better than chance (50%). It is important not to misinterpret this finding. The low PPV associated with growth deficiency and OFC ≤ 10th percentile does not mean PAE does not cause growth deficiency and microcephaly. The low PPV simply reflects the fact that there are many risk factors (not just PAE) that cause growth deficiency and reduced head circumference, especially in this high-risk foster population. Growth deficiency and microcephaly are strongly correlated with PAE, highly predictive of cognitive/behavioral dysfunction and essential to the diagnosis of FASD [32].

It is important to clarify that high PPV and specificity are required to confirm PAE in a FASD diagnostic setting. Diagnostic evaluations provide definitive information about the presence or absence of a condition or exposure. In contrast, lower levels (~80%) of PPV and specificity may be deemed acceptable in a FASD screening setting, when the goal is to identify individuals at risk for adverse outcomes caused by PAE. In a screening activity, one may be more willing to accept PAE false-positives so as not to miss PAE true-positives. One would expect the false positives to be corrected in the diagnostic phase of a screening. A recent study by Goh et al., [33] serves as a good example of a screening tool developed to determine which children with neurodevelopmental problems were likely to be affected by PAE and require clinical follow-up. The tool screens for different combinations of outcomes (e.g., IQ, measures from the Child Behavior Checklist [34] and Vineland Adaptive Behavior Scales [35], 1 or more of the 3 Hoyme et al., FAS facial features, ptosis, incomplete extension of one or more digits, and growth or head circumference ≤ 10th percentile) in hierarchical fashion to predict PAE. When the screening tool was administered to a group of 454 children, in which 145 (32%) had PAE, the tool performed with the following metrics: PPV = 70.7%, NPV 86.7, specificity 80.6%, and sensitivity 79.2%. The PPV of 70.7% means 29.3% of the children predicted to have PAE, did not have PAE (e.g. 29.3% were false positives, their birth mothers did not drink during pregnancy). A false-positive rate of this magnitude could be deemed acceptable in a screening activity; but would be unacceptable in a diagnostic clinic.

FASD diagnostic guidelines that use relaxed criteria for the FAS facial phenotype risk misdiagnosing and over-diagnosing FAS and partial FAS when PAE is unknown. These misdiagnoses can lead to over-estimates of the prevalence of FAS and PFAS in FASD screening/surveillance activities that target populations where confirmation of PAE is difficult to obtain. For example, May et al., [36] conducted a FASD screening among first grade students in a Midwestern U.S. community. Screen positives received FASD diagnostic evaluations in accordance with the Hoyme et al., [37] criteria that permit diagnosis of FAS and PFAS when PAE is unknown. The relaxed facial, growth and head circumference criteria for FAS and PFAS are identical between the Hoyme et al., 2005 [37] and Hoyme et al., 2016 [7] FASD diagnostic systems. The authors reported alcohol use during the index pregnancy was confirmed in only 33% of the cases diagnosed with FAS and 61% of the cases diagnosed with PFAS. It is unclear what feature(s) were present among two thirds of the FAS cases and one third of the PFAS cases that allowed these diagnoses to be rendered with unknown PAE. The authors concluded *"The prevalence of FAS cases in this study of first grade children in this general population is likely 6 to 9 per 1000. It is significantly higher than older, previously accepted estimates of FAS (0.2 to 3 per 1000) that were generated from less representative samples that did not use active case ascertainment. But these findings are similar to recent rates published for the United States, Italy, and Croatia, 2 to 7 per 1000, which used similar, active methods of case identification and ascertainment. For FAS and PFAS combined, the likely maximum range of rates is 17 to 26 per 1000, and for total FASD, the rates range from 24 to 48 per 1000. Therefore, rates from this study are all well above the old estimate of 1% for total FASD* [38]."

The outcomes of the current study continue to support that the Rank 4 FAS facial phenotype is unique to (caused only by) PAE. Nevertheless, updates in genetic testing technologies such as chromosomal microarray analysis suggest some of the physical and neurobehavioral abnormalities observed in FASD overlap with those observed among individuals with chromosomal disorders. Chromosomal microarray abnormalities occur among 15-20% of individuals with unexplained developmental disability/intellectual disability, autism spectrum disorder and multiple congenital anomalies [39]. Chromosomal abnormalities (often chromosomal micro-deletions or micro-duplications) have been reported in 8-14% of individuals with FASD [39-44]. But to date, these studies have been descriptive, not empirical in design. To empirically confirm chromosomal abnormalities serve as an alternate etiology for the Rank 4 FAS facial phenotype, one would have to identify a sufficiently large group of individuals who present the Rank 4 FAS facial phenotype, have the chromosomal abnormality and have a confirmed absence of PAE. As proffered by Kahila et al., [41], are chromosomal abnormalities an alternate etiology for the physical and neurobehavioral abnormalities observed among individuals with PAE or are the chromosomal abnormalities the consequence of PAE? Halsted et al. [45] suggested that alcohol consumption decreases the amount of folate, which is needed for cells' methionine cycle. Thus, alcohol could reduce the production of methyl groups for DNA and histone methylation, cause hypomethylation, and consequently decrease the stability of the chromosomes [41]. To date, there have been no reports of the Rank 4 FAS facial phenotype present in an individual with chromosomal abnormalities and confirmed absence of PAE.

Study strengths and limitations

Documentation of PAE was both a strength and limitation in this study. PAE is inherently difficult to accurately confirm or rule-out in any population. Due to the stigma associated with drinking

during pregnancy, birth mothers are typically reluctant to report their alcohol use during pregnancy. The most frequent source of confirmation of PAE in the FASDPN clinic is past birth, medical and social service records [46]. It takes time and effort to obtain these records, but in so doing, one is likely obtaining the most accurate record of PAE available. If there is error in the "true exposure" status of a study population, the classification/prediction parameters derived from that "true exposure" classification can be impacted. The strength of this study was the thoroughness with which all records were obtained and reviewed by the foster care program to identify, as accurately as possible, the "true PAE" status of each child.

Conclusion

FASD diagnostic guidelines that use relaxed criteria for the FAS facial phenotype risk misdiagnosing and over-diagnosing FAS and partial FAS when PAE is unknown. These misdiagnoses can lead to over-estimates of the prevalence of FAS and PFAS in FASD screening activities that target populations where confirmation of PAE is difficult to obtain.

Ethical Approval

FASD diagnostic guidelines that use relaxed criteria for the FAS facial phenotype risk misdiagnosing and over-diagnosing FAS and partial FAS when PAE is unknown. These misdiagnoses can lead to over-estimates of the prevalence of FAS and PFAS in FASD screening activities that target populations where confirmation of PAE is difficult to obtain.

Author Contributions

The author was the principal investigator for the original study and conducted all analyses and manuscript preparation for the current study.

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