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ETHNIC DIFFERENCES IN DYSMORPHIC FEATURES AMONG CHILDREN WITH FETAL ALCOHOL SPECTRUM DISORDERS

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ABSTRACT

Children adversely affected by prenatal exposure to alcohol display a continuum of dysmorphic features, behavioral and neuropsychological deficits best termed fetal alcohol spectrum disorders (FASD). Alcohol exposure during critical stages of central nervous system and midfacial development leads to the observed pattern. Recent research on FASD focuses increasingly on quantifying physical traits (including head circumference, palpebral fissure length, philtrum length, morphology of the philtrum and vermilion border of the upper lip) and comparing them to specific neurocognitive findings. Various standards and cut-off points have been used by dysmorphologists to determine which of the structural features of children prenatally exposed to alcohol are significant. Many diagnosticians use a cut off point of <10th centile for determining such traits as microcephaly and palpebral fissure length; however, there is considerable debate as to what exactly should constitute these standards and others for the general U.S. population. Furthermore, there is little documentation of ethnic differences in morphology among children diagnosed with FASD. Both child and maternal data from three studies in the Northern Plains of the U.S., Italy, and South Africa were compared and revealed statistically significant differences in drinking patterns, drinks consumed on a typical drinking day during the index pregnancy, and percent bingeing (3 or more drinks) during pregnancy among the three populations. In this study we compare the impacts of these and other drinking measures within and across each of the three samples and examine the quantifiable dysmorphic features of the children (including head circumference, palpebral and philtral length, and morphology of the philtrum and

vermilion border of the upper lip). The analyses reveal statistically significant and meaningful differences in a variety of comparisons.

ETHNIC DIFFERENCES IN FETAL ALCOHOL SPECTRUM DISORDERS DYSMORPHOLOGY

The availability and regular use of a variety of alcoholic beverages by substantial segments of many populations make it one of the most prevalent teratogens. Indeed, it is estimated that fetal alcohol syndrome (FAS) has a prevalence ranging from 0.5 -3.0 per 1000 births in the United States (Stratton, et al., 1996; Abel, 1998; May and Gossage, 2001) and that fetal alcohol spectrum disorders (FASD) may affect 1 to 4% of many populations of developed countries (Sampson, 1998; May et al., 2006). While some people in particular populations may not believe their communities have cases of severe FASD, Abel's reviews (1995; 1998) have identified the presence of FAS in all racial and ethnic groups.

The term fetal alcohol syndrome was coined by Jones and Smith (1973) to encompass the features of children adversely affected by *in utero* exposure to alcohol. Even though others had described some of the clinical and epidemiological features of children exposed to substantial amounts of alcohol in the prenatal period (Sullivan, 1899; Lemoine, 1978; see review in Armstrong, 2002), the original work by Jones and Smith laid the foundation for identifying alcohol as the cause of this pattern of anomalies and catalogued the various features that combine to characterize the syndrome. The observations by Jones and Smith are now being refined as investigators further elucidate and clarify the major common features of prenatal alcohol exposure and its continuum of adverse effects. This vital for the efforts to strengthen screening and diagnostic methods for FAS and other fetal alcohol spectrum disorders (FASD).

Major morphological features of FAS include microcephaly, a hypoplastic midface which includes a smooth philtrum, a thin upper lip, and changes in the length of the philtrum. The size of the eye and the orbits are affected including the palpebral fissures which are shorter than normal. Unfortunately, there is considerable overlap in dysmorphology with other syndromes and teratogenic exposures. Therefore, a diagnosis must include additional physical, clinical findings. Additional findings currently in use in the refinement of a FAS diagnosis include: the shape and volume of the vermillion border features in the upper lip, neurobehavioral phenotype, and detailed explorations and documentation of the pattern and amount of maternal drinking and other associated risk factors (Wattendorf and Muenke, 2005; Kodituwakku et al., 2006; May et al., 2008; Sampson et al., 1997). By gathering data on multiple variables, investigators are better able to clarify the degree to which a child is affected with FAS.

In the past twenty years there has been significant progress in clarifying the syndrome. Although there is still debate about the degree of changes in normal morphology necessary to warrant a diagnosis of FAS (Astley, 2006), considerable efforts have elucidated maternal risk factors and clarified diagnostic criteria for making accurate diagnoses (Hoyme et al., 2005). In addition, recent work has elaborated on the array of features associated with the exposure of the fetus to alcohol (May et al., 2006; 2007) and the amounts of alcohol and patterns of consumption necessary to affect the fetus (Jacobson and Jacobson, 1994; Day et al. 2002). One of the difficulties of diagnosing FAS is the variability of the effects of alcohol and a broad spectrum of presentations. This diagnostic challenge led to the development of the terminology fetal alcohol spectrum disorders (FASD). This has helped in categorizing affected children.

There are several methods that are used to screen for FASD including: passive surveillance, clinic-based studies, and active case ascertainment studies (May and Gossage, 2001). Each method has its own strengths and limitations; however, ongoing research in schools and referral clinics using active case ascertainment methods with Plains Indians, communities in South Africa and in Italy have been successful in screening for and documenting FASD in these general populations. These studies identified FAS rates 1.0 – 8.97 per 1000 children (May et al., 2002), 51.3 - 67.2 per 1000 children (May et al., 2007; Viljoen et al., 2005) and 3.7 -7.4 per 1000 children (May et al., 2006) respectively. Rates of FAS and Partial FAS (PFAS) are also high, with two to four times as many cases of partial FAS (PFAS) as FAS and the percentages of FASD ranging from 2 to 4% in some studies (Quaid et al, 1993; Duimstra et al., 1995; May et al., 2002; 2006; 2007). These findings have revealed groups with high rates of FASD, but also have further clarified the prevalence of both FAS and other FASD in general populations. The overall conclusion of our studies thus far is that the prevalence of FAS and other FASD may be substantially higher than has been previously estimated from clinic-based referral studies.

Original comparisons of Plains Indian women and the women of a South African community provided interesting results revealing unusual variation in the effects of alcohol observed in the fetus (May et al, 2004). Alcohol seemed to have a differential effect on the children born in one group over the other, and the dysmorphology varied from one racial group to the next. The variables contributing to variation in risk for FASD included maternal age at pregnancy, gravidity, parity, body mass index, lifelong and current nutrition, and binge drinking. Comparing the above two diverse populations

demonstrated the importance and difficulty in interpreting environmental and genetic influences and impressed upon us the need to compare the dysmorphology of FASD between groups.

In this paper we attempt to use data from active case ascertainment studies of three distinct populations to better clarify the similarities and differences in dysmorphology features, growth, and maternal risk factors between subjects from vastly different ethnic groups. The data presented here help illuminate the importance of the different morphological features within and between three different populations. They may also be useful in identifying and defining differences in the clinical features of FASD found in general populations as compared to the more severe features identified in studies from referral clinics.

METHODS

Sampling Methods and Samples

The Northern Plains Indian data come from a National Institute on Alcohol Abuse and Alcoholism (NIAAA)-funded epidemiologic study, which used active case ascertainment through outreach in entire Plains Indian communities for identifying cases of FAS (Stratton et al, 1996; May and Gossage, 2001; 2002). Physicians, teachers, and other representatives are taught to recognize common traits of FASD and refer children to special clinics for assessing children with possible FASD. An interdisciplinary team examines each child for the morphological characteristics of FASD and other birth defects, IQ (verbal and non-verbal) and neuropsychologic traits of the children. While the children are being examined for the effects of exposure to alcohol in utero, their

mothers are interviewed and queried about prenatal experience including gravidity, parity, diet, medical history, social and demographic conditions, and consumption of alcohol before, during, and after pregnancy. In addition, a sample of unaffected children matched for age, sex, and community of residence was collected for comparison (controls). The mothers of the control children were interviewed and evaluated in the same manner and used as maternal controls. The total sample for the Plains group is 321 children, 98 of which are cases of FAS (n=49) or PFAS (n=49).

The South African and Italian data are derived from studies using active case ascertainment methods in a two-tiered, diagnostic process in the elementary schools (May et al., 2000; 2007; Viljoen, et al., 2005). Diagnostic criteria were established from components and criteria of the US Institute of Medicine (IOM) categories (Hoyme, et al., 2005). The two-tiered screening method in the elementary schools collected height, weight, and occipitofrontal (head) circumference (OFC) in the first tier. If the measurements were $\leq$ 10th centile on OFC and/or both height and weight, a child was referred to the second tier consisting of a complete physical exam including dysmorphology assessment by at least two pediatric dysmorphologists, each blinded to the background, history, and reason for the exam, random selection or referral for growth or behavior .

The South African study was funded by the NIAAA and National Institute on Minority Health Disparities (NIMHD). Following parental permission, all children with consent participated in a two tier, active case ascertainment screening process. The evaluation included assessing physical growth, dysmorphology, psychological and behavioral development, prenatal exposure to alcohol, and detailed assessment of

maternal risk factors and characteristics through interviews (May et al., 2006). Controls were established using randomly-selected children, confirmed to be without a FASD, from the same first grade classes in the exact schools attended by the children with a FASD. The total sample presented here (n=300) is from the third wave of screening in this South African town in 2002, and there are 73 children with FAS (n=55) or PFAS (n=18).

The Italian study was carried out through joint funding from the NIAAA and the Health Department of the Lazio regional government. In the Italian study, primary schools in two health districts of the Lazio region were randomly selected and recruited for the study. Two tier screening identical to that described above for the South African studies (May, et al., 2006) was carried out in Italian schools with the permission of Italian and US institutional review boards. The total sample from Italy is 300 children, and 48 of them are cases of FASD (8 FAS, 36 PFAS) and 256 are randomly selected controls

Intellectual and Behavioral Assessment

The diagnostic process on each child in Tier II of the screening at each site also included developmental tests that measured both intellectual development and behavioral problems often associated with FASD. Similar psychological and behavioral testing, although utilizing different tests and different languages with each of the three populations, were completed for each child, both subject and control.

Controls

The control children in the South African and Italian in-school studies were selected via random selection from a list of all children enrolled. For the referral study in the Plains, controls represent a convenience sample of children from the same reservation

community or the same town in the Northern Plains. Control children underwent the same testing as those children who were diagnosed with FAS. Mothers of the control children became the maternal controls.

Maternal Interviews

Similar, structured interviews were performed with the mothers of both control and affected children as part of Tier II of the assessment in each of the three populations. These interviews collected maternal risk data including childbearing, socioeconomic status, alcohol and other substance use via similar timeline follow back methods, demographics and social variables.

Case Conferences for Final Diagnoses

All of the individual examinations, assessments, tests, and interviews were performed by investigators from various disciplines, each blinded to history, background, findings of other researchers, and reason for examination. Once all of the data were collected, interdisciplinary case conferences were held to review each child's case and provide a final diagnosis. In the case conference, all data from each of the domains are presented by one of the examiners from each domain (physical growth and development, behavioral, and maternal risks), pictures of each child are reviewed, discussion is pursued by all clinicians involved with each case, and final diagnosis is made by the group.

Institute of Medicine (IOM) Categories and Criteria for Fetal Alcohol Spectrum Disorders

Diagnostic components of the revised U.S. Institute of Medicine (IOM) categories (Hoyme, et al., 2005) were used to assess specific FASD in the children. The diagnostic components of the revised U.S. IOM categories (Stratton, et al., 1996; Hoyme, et al.,

2005) describe the full continuum of FASD, from severe to mild. The four specific diagnoses within this delineation, from most dysmorphic and severely affected in intelligence and behavior, to the less obvious and severe, are: fetal alcohol syndrome (FAS), partial FAS (PFAS), alcohol-related neurobehavioral disorders (ARND) and alcohol-related birth defects (ARBD).

The two most severe diagnoses of a FASD, FAS and PFAS, are currently most readily recognized and are most dependent on dysmorphology. Furthermore, for most in-school studies carried out with our methods, these are the two most common manifestations diagnosed (May et al., 2000; 2005; 2006; 2007). For the diagnosis of FAS a child must have: 1.) evidence of a characteristic pattern of minor facial anomalies including at least 2 or more of the key facial features of FAS (palpebral fissures $\leq 10^{th}$ centile, thin vermilion border, or smooth philtrum), 2.) evidence of prenatal and/or postnatal growth retardation (height or weight $\leq 10^{th}$ centile), 3.) evidence of deficient brain growth (structural brain anomalies or occiptofrontal head circumference (OFC) $\leq 10^{th}$ centile), and if possible, 4.) confirmation of maternal alcohol consumption directly from the mother or a knowledgeable collateral source. For a diagnosis of Partial FAS (PFAS), a child must have: 1.) evidence of a characteristic pattern of facial anomalies including 2 or more of the three mentioned above, 2.) one or more other characteristics, such as prenatal or postnatal growth retardation ($\leq 10^{th}$ centile) in height or weight), 3.) small OFC ($\leq 10^{th}$ centile), and/or evidence of a complex pattern of behavioral or cognitive abnormalities inconsistent with developmental level and unexplainable by genetic composition, family background, or environment alone, and if possible, 4.)

confirmation of maternal alcohol consumption directly from the mother or a collateral source.

All physical growth and dysmorphology features observed and measured in a child's examination are recorded on a standardized and weighted checklist (Hoyme et al, 2005). Possible dysmorphology scores range from 0 to 35. This checklist is a later generation instrument which has been developed by dysmorphologists over the past 25 years. A high dysmorphology score and specific, key features of FASD must be present for a positive diagnosis of one of the more severe FASD. Furthermore, for any of the FASD diagnoses to be made by the clinical team, other known birth defects and patterns of other disabilities must be excluded. For example, some other recognizable patterns of malformations and behavioral disorders share a number of the symptoms that are also characteristic of FASD, e.g., Williams syndrome, Down syndrome, de Lange and fragile X syndromes.

The diagnoses of alcohol-related neurodevelopmental disorder (ARND) and alcohol-related birth defects (ARBD), are not utilized in this paper, even though we have provided these diagnoses in clinical diagnostic studies and occasionally in the in-school studies in Italy. They are not nearly as dependent on dysmorphology for the diagnosis, and because our methods begin with indicators of deficient growth and development for inclusion, we may not detect ARND and ARBD cases in the unscreened school populations.

Data Analysis

Analysis of the data from the three studies was carried out by first comparing cases to controls and significance evaluated with a Bonferoni-adjusted alpha level of

≤.0015 with t-tests and one way analysis of variance. Descriptive data analysis was first performed using EPI Info software of the US Centers for Disease Control and Prevention (Dean et al., 1996). All comparative analyses and regression measures were performed using the Statistical Package for the Social Sciences (SPSS). The alpha level in the logistic regression tables was also set at .0015 for significance of individual traits to adjust for multiple comparisons.

Regression Analyses – Specific Considerations

The preliminary analyses consisted of two sets of three separate sequential regressions for data from Plains Indians, Italy, and the third wave from the community in South Africa. In each, 18 physical characteristics were sequentially entered into a regression equation to predict dysmorphology. Predictors, in order of entry into the equation, were height, weight, head circumference, PFL, narrow vermillion, smooth philtrum, long philtrum, strabismus, interpupillary distance, ptosis, intercanthal distance, nasal bridge, anteverted nostrils, aberrant palm crease, fifth finger clinodactyly, compodactyly, heart murmur, and railroad ears. The first set of analyses used the entire sample of children from each site. The second set of analyses omitted children diagnosed as "partial FAS".

A two-level hierarchical model then assessed the effects of the predictors over the three locations. First level units were children and second level units were locations. Hierarchical models are those in which data collected at different levels of analyses (e.g., children and locations) may be studied without violating assumptions of independence in linear regression. For example, the fact that different physicians test children in different environments means that responses from children within each location are not

independent of one another. Multilevel modeling takes account of these dependencies by estimating variance associated with group (e.g., location) differences in average response (intercepts) and group differences in associations (slopes) between predictors and outcomes (e.g., location differences in the relationship between narrow vermillion and square root of dysmorphology). This is accomplished by declaring intercepts and slopes to be random effects. Thus, fixed effects are average slopes over all locations and random effects are group difference in average dysmorphology and in relationships between dysmorphology and each predictor.

The dependent variable, dysmorphology, showed substantial departure from normality in all three data sets. Therefore, a square root transformation was applied in all three analyses. Several of the predictors were converted to centiles: height, weight, head circumference, interpupillary distance, and intercanthal distance.

The three data sets varied in the amount of missing data. The South African data set had no missing values. Two cases were omitted from the Italy data set due to missing values for one or more predictors. Because most of the Italian children did not have measurements for interpupillary distance, that variable was omitted from the analyses of Italy data. For the Plains Indians data set, 21% of the cases were missing values for interpupillary distance, with lesser amounts of missing values for four other predictors. SPSS MVA was used to impute a complete data set using the EM algorithm, after omitting the two cases with missing values on dysmorphology.

Multivariate outliers ($p < .001$) were numerous in all of the data sets. However, these outliers were caused by extremely uneven splits in some of the dichotomous predictors, which were not amenable to transformation. Therefore, the decision was

made to set a criterion of $p < .0015$ for tests of individual predictors to compensate for underestimation of standard errors as well as inflated familywise Type I error rate.

RESULTS

Table 1 introduces the data of interest. First, the data are grouped by study population: Plains Indians, Italians, and South Africans. Then within each study sample, the data are grouped by final diagnosis for the children: FASD (either FAS or PFAS), or not FASD (Hoyme, et al., 2005). Forty-four sets of analyses are reported in Table 1, of those 29 are statistically significant at the standard alpha of $\leq .05$, but 24 are significant when the adjusted multiple comparison level of $\leq .0015$ is applied.

Overall, sixty-four percent of the mothers consumed alcohol during the pregnancy; more mothers of children with FASD reported dranking than did control mothers (84% vs. 44%). Plains Indian mothers consumed an average of 6 drinks on a typical drinking day which was substantially more on average than reported by Italian or South African mothers. Binge drinking (3 or more drinks per occasion) was more prevalent among Plains Indian and South African mothers. The experience of our interviewers indicated that there may have been some significant underreporting of alcohol use in the Italian sample.

As observed in all three waves of research in South Africa, the majority of South African children from wave III in Table 1 are very small when plotted on growth charts, with very low centile scores for height, weight, head circumference, and palbebral fissure length. And as shown in the rest of Table 1, other features consistent with a diagnosis of FASD are observed more often in children with FASD in all three populations. More

detail in descriptive data on both the children and the mothers in these populations is found in other publications (May et al. 2000; 2004; 2006; 2007; Viljoen et al., 2002; 2005).

Sequential Regression Analyses of Full Sample

Among Plains Indians, the set of 18 variables significantly predicts square root of dysmorphology, $F(18, 302) = 99.54$, $p < .001$, adjusted $R^2 = .85$ with 95% CI from .81 to .87. The last column of Table 2 shows that 12 of the variables predict square root of dysmorphology at the point at which they enter the sequential model. These include centiles for the common size measurements (height, weight, and head circumference), PFL, vermillion and philtrum measurements, nasal bridge, anteverted nostrils, aberrant palm crease, compodactyly, and railroad ears. Looking at each predictor in the context of the entire set (column 2), however, the centiles of common size measurements no longer significantly predict dysmorphology after adjustment for other predictors, because of their correlations among all predictors.

In Italy, measurements of interpupillary distance were made on only a small portion of the sample (79 of 302 cases). Therefore, that predictor was omitted from the analysis. The remaining set of 17 variables significantly predicts square root of dysmorphology, $F(17, 282) = 60.01$, $p < .001$, adjusted $R^2 = .77$ with 95% CI from .70 to .80. The last column of Table 3 shows that 10 of the variables predict square root of dysmorphology at the point at which they enter the sequential model. These include centiles for the common size measurements (height, weight, and head circumference), PFL, vermillion and philtrum measurements, anteverted nostrils, aberrant palm crease,

and fifth finger clinodactyly. Again, the common size measurements no longer predict square root of dysmorphology once adjusted for the entire set of predictors. In this analysis, PFL also fails to predict dysmorphology after adjusting for other predictors.

For the third wave of the South African data, the set of 18 variables also significantly predicts square root of dysmorphology, $F(18, 226) = 87.79$, $p < .001$, adjusted $R^2 = .87$ with 95% CI from .82 to .89. The last column of Table 4 shows that 11 of the variables predict square root of dysmorphology at the point at which they enter the sequential model. These include centiles for the common size measurements (height, weight, and head circumference), PFL, vermillion and philtrum measurements, ptosis, aberrant palm crease, and fifth finger clinodactyly. All of these except centile of height continue to predict square root of dysmorphology even after adjustment for all other variables.

Sequential Regression Analyses Sample Excluding Partial FAS

Table 5 shows the results of the statistically significant prediction of square root of dysmorphology for the Plains Indians sample without partial FAS by the set of 18 variables, $F(18, 253) = 84.51$, $p < .001$, adjusted $R^2 = .85$ with 95% CI from .80 to .87. Ten of the variables predict square root of dysmorphology at the point they enter the sequential model. These 10 also were significant predictors for the entire sample, however, compodactyly and railroad ears no longer add to prediction after adjusting for higher priority variables. Eight of these 10 predictors also contribute to prediction after adjustment for all other variables; height and head circumference centiles do not contribute to prediction after adjustment for all other variables.

For the Italy sample, excluding partial FAS children, the 17 variables (exclusive of interpupillary distance) significantly predicts square root of dysmorphology, $F(17, 246) = 43.47$, $p < .001$, adjusted $R^2 = .73$ with 95% CI from .65 to .77. Eleven of the variables were significant predictors at the point at which they enter the sequential model, as seen in the last column of Table 6. These are the same as for the entire sample of children, with the addition of compodactyly when partial FAS children are excluded. Ten of these 11 predictors remain significant after adjustment for all other variables. Height and weight are significant predictors when entering the model early, but no longer contribute once all other variables are taken into account.

For the third-wave South African sample without partial FAS, $F(18, 208) = 77.17$, $p < .001$, adjusted $R^2 = .86$ with 95% CI from .81 to .88. Twelve of the variables significantly predict square root of dysmorphology at the point at which they enter the sequential model. One of these, compodactyly was not a significant predictor when the sample included partial FAS children. All of these predictors except height continued to contribute to the model prediction even after adjustment for all of the other variables, as seen in Table 7.

DISCUSSION

While each of the included studies has been examined independently, this is one of the first attempts to compare diagnostic findings across populations. As is commonly acknowledged, when all of the features of severe FASD are considered, the summation of positive and significant findings are predictive of FASD. This work shows that this

approach holds true among different ethnicities, in the face of variations in prevalence of significant dysmorphic findings among controls.

While the sample study populations may be considered small, other interesting findings are revealed when comparing other FASD traits in addition to the common physical size variables (e.g., height and weight). The South African controls have higher percentages of: narrow vermillion border, smooth philtrum, short interpupillary distance, flat nasal bridge, and anteverted nostrils. Meanwhile, Plains Indian controls have higher percentages of "railroad track" ears, likely a normal genetic variant. Italian controls, however, fall between the two groups in most categories with the exception of philtrum length and strabismus. These findings suggest inherent differences in morphology that make it necessary for clinicians to be cautious when suggesting a diagnosis of FASD with few dysmorphologic traits, to be aware of normal genetic traits in a population, and for researchers to utilize controls for the same population.

The sequential regression analyses for the multiple variables with each ethnic group reveal variation in the utility and importance of the common size measurements. Plains Indian children showed that height, weight, and head circumference have less value in predicting FASD diagnoses. Similarly, in Italian children height, weight, and head circumference have less importance in predicting FASD; however, PFL, a very commonly cited finding in FASD, also held less value. This suggests that the diminished value of the common size measurements is related to their syndromal relationship with the individual dysmorphologic variables.

In the South African children, weight, head circumference, and PFL remain important predictors of FASD. It is unclear why these measurements retain their

importance in South African children, while being less important in the other populations. However, this finding is important in the clinical setting. Many clinicians use height, weight, and head circumference centiles to quickly assess whether children may be afflicted with a developmental defect. In Plains Indian and Italian children, these findings, although suggestive of FASD, appear to have less value for diagnosing children with FAS and PFAS and indicate the need to place more emphasis on the other findings. Meanwhile, in South African children, focusing on common size measurements may hold more importance for FASD diagnosis, but abnormal findings are also more common in South Africa. The growth data in South Africa may make the case for growth charts specific to the South African Coloured population.

Although the predictive value of height, weight, and head circumference varies between groups, all of the children with FASD show substantial deficits in these traits. The extreme difference in both controls and FASD children between the South African children and the other groups is clear. It is possible the substantial difference in these characteristics is related to nutrition (Viljoen et al., 2005; May, et al., 2007). Previous analysis of the South African study revealed that a mother's nutritional status was important in predicting FASD. These findings, coupled with previous analysis and the extremely high rate of FASD in the South African community (May et al., 2007) suggest that nutritional status may play a large role in development of FASD or the severity of the dysmorphology.

Previous work indicated that FASD rates were highest in the South African study community (May et al, 2004) compared with the Plains Indians. Interestingly, Plains Indian mothers show a predilection to drinking more alcohol overall and higher rates of

drinking than South African and Italian mothers. These findings suggest that there are other factors (e.g., nutritional, environmental or genetic) that facilitate South African Coloured women who consume alcohol to produce more children with FASD in spite of consuming lower amounts of alcohol.

This study supports the use of multiple abnormal syndromic findings together to make a diagnosis of severe FASD. Furthermore, it shows that although there are variations between ethnicities in morphological features, that using the syndromic features together, an accurate FASD diagnosis may still be made. In addition, it shows that there are additional underlying genetic, nutritional, and/or environmental factors which affect the development of an FASD. Some, but not all, of these additional factors await further study.

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Table 1: Comparison of Measures Across Three Studies, FASD[e] vs. Controls

	Plains		Italy		South Africa		
Variable	**Controls**	**FASD**	**Controls**	**FASD**	**Controls**	**FASD**	**Significance *p***
Mother's Variables Reported drinking during index pregnancy, %	**(n=152)** 62.5	**(n=52)** 92.3	**(n=179)** 42.5	**(n=39)** 53.8	**(n=157)** 28.0	**(n=66)** 95.5	0.000[a] ** 0.000[b] **
Drinks consumed on typical drinking day, Mean (SD)	4.3 (5.09)	7.6 (6.27)	0.3 (0.52)	0.4 (0.60)	0.6 (1.82)	4.2 (4.12)	0.000[c] ** 0.000[d] **
Binged (3+) during Index pregnancy, %	51.4	83.7	0.8	5.4	13.3	74.1	0.000[a] ** 0.000[b] **
Child's Variables Age (months), Mean (SD)	**(n=221)** 85.4 (52.66)	**(n=98)** 92.8 (52.08)	**(n=256)** 80.2 (4.56)	**(n=44)** 79.7 (4.14)	**(n=172)** 87.7 ((6.49)	**(n=73)** 93.2 (9.51)	0.000[c] ** 0.009[d] *
Height, centile, Mean (SD)	57.7 (28.94)	27.9 (25.00)	53.3 (29.20)	37.5 (29.31)	25.9 (24.20)	5.6 (6.65)	0.000[c] ** 0.000[d] **
Weight, centile, Mean (SD)	66.3 (28.59)	32.1 (26.0)	58.7 (30.14)	40.3 (30.52)	27.1 (25.75)	4.6 (5.79)	0.000[c] ** 0.000[d] **
Head circumference (OFC),	60.3 (28.22)	24.5 (25.40)	47.4 (29.20)	25.6 (28.47)	28.3 (26.40)	4.2 (6.62)	0.000[c] ** 0.000[d] **

centile Mean (SD)							
Palbebral fissure length (PFL), centile, Mean (SD)	41.8 (21.05)	12.5 (15.57)	27.6 (14.35)	19.4 (18.94)	29.0 (16.93)	6.8 (8.75)	0.000[c] ** 0.000[d] **
Narrow vermillion border, %	11.7	93.9	24.8	95.5	42.4	84.9	0.000[a] ** 0.067[b]
Smooth philtrum, %	10.3	82.7	18.2	90.9	63.4	86.3	0.000[a] ** 0.422[b]
Long philtrum, %	34.5	41.8	40.9	54.5	36.0	45.2	0.328[a] 0.371[b]
Strabismus, %	1.3	6.1	3.1	6.8	1.7	4.1	0.380[a] 0.786[b]
Short interpupillary distance, %	4.9	30.6	0.0	21.7	32.6	54.8	0.000[a] ** 0.001[b] **
Ptosis, %	1.3	9.2	3.1	6.8	4.7	15.1	0.149[a] 0.303[b]
Short intercanthal distance, %	3.1	13.3	11.3	20.5	19.2	23.3	0.000[a] ** 0.220[b]
Flat nasal bridge, %	4.5	17.3	0.4	0.0	30.2	53.4	0.000[a] ** 0.000[b] **
Anteverted nostrils, %	4.5	19.4	10.9	22.7	13.4	26.0	0.006[a] * 0.586[b]
Palmer crease, %	21.5	32.7	22.9	36.4	27.3	42.5	0.380[a] 0.420[b]
Clinodactyly, %	6.3	7.1	32.6	54.5	44.2	53.4	0.000[a] ** 0.000[b] **

Camptodactyly, %	6.3	20.4	8.1	11.4	9.3	34.2	0.523[a] 0.012[b] *
Heart murmur, %	1.8	5.1	0.8	2.3	4.1	13.7	0.055[a] 0.036[b] *
"Railroad track" ears, %	11.7	19.4	6.6	13.6	3.5	15.1	0.007[a] * 0.624[b]

a = X^2 of affirmative responses across three samples of controls
b = X^2 of affirmative responses across three samples of FASD
c = ANOVA of data for controls across three samples
d = ANOVA of data for FASD across three samples
e = FASD is a category of anomalies under which there are four diagnoses: FAS, Patial FAS, alcohol-related neurodevelopmental deficits (ARND), and alcohol related birth defects 9ARBD). All of the above cases are the two most severe forms of FASD, FAS and Partial FAS.

*** Significant at the .05 level**

**** Significant at the adjusted alpha level of .0015**

Table 2. Sequential Regression for (Square Root of) Dysmorphology Scores, Plains Indians (N = 321)

Variables	B	SE B	β	sr^2 *(incremental)*
Height (centile)	<-0.01	<0.01	-.12	.22*
Weight (centile)	<-0.01	<0.01	-.10	.08*
Head circumference (centile)	<-0.01	<0.01	-.02	.06*
PFL (centile)	-0.01*	<0.01	-.19	.09*
Narrow vermillion	0.70*	0.09	.26	.19.*
Smooth philtrum	0.75*	0.08	.27	.07*
Long philtrum	0.77*	0.06	.29	.09*
Strabismus	-0.11	0.18	-.01	<.01
Interpupillary distance (centile)	-0.01	<0.01	-.09	<.01
Ptosis	0.29	0.16	.04	<.01
Intercanthal distance (centile)	<0.01	<0.01	<.01	<.01
Nasal bridge	0.43*	0.11	.09	.01*
Anteverted nostrils	0.47*	0.11	.10	.01*
Aberrant palm crease	0.37*	0.07	.13	.02*
Fifth finger clinodactyly	0.20	0.12	.04	<.01
Campodactyly	0.34*	0.10	.08	.01*
Heart murmur	0.41	0.18	.05	<.01
Railroad ears	0.29*	0.09	.08	.01*
Intercept	1.94	0.12		
			R^2 =	.86
		Adjusted	R^2 =	.85
			R =	.93

*p < .0015

Table 3. Sequential Regression for (Square Root of) Dysmorphology Scores, Italy Data (N = 301)

Variables	*B*	SE *B*	β	sr^2 *(incremental)*
Height (centile)	<-0.01	<0.01	-.07	.11*
Weight (centile)	<-0.01	<0.01	-.12	.05*
Head circumference (centile)	-0.01	<0.01	-.17	.06*
PFL (centile)	-0.01	<0.01	-.15	.04*
Narrow vermillion	0.60*	0.08	.27	.27*
Smooth philtrum	0.60*	0.09	.25	.09*
Long philtrum	0.61*	0.06	.28	.09*
Strabismus	-0.12	0.18	-.02	<.01
Interpupillary distance (centile)	N.A.	N.A.	N.A.	N.A.
Ptosis	0.52	0.17	.09	.01
Intercanthal distance (centile)	<0.01	<0.01	-.01	<.01
Nasal bridge	0.30	0.53	.02	<.01
Anteverted nostrils	0.40*	0.09	.12	.02*
Aberrant palm crease	0.42*	0.07	.17.	.03*
Fifth finger clinodactyly	0.29*	0.06	13	.02*
Campodactyly	0.33	0.11	.09	.01
Heart murmur	0.26	0.30	.02	<.01
Railroad ears	0.29	0.12	.07	.01
Intercept	2.01	0.09		
			R^2 =	.78
		Adjusted	R^2 =	.77
			R =	.89

*p < .0015

Table 4. Sequential Regression for (Square Root of) Dysmorphology Scores, South African Sample ($N = 246$)

Variables	*B*	SE *B*	β	sr^2 *(incremental)*
Height (centile)	<-0.01	<0.01	-.10	.28*
Weight (centile)	-0.01*	<0.01	-.18	.12*
Head circumference (centile)	-0.01*	<0.01	-.20	.06*
PFL (centile)	-0.01*	<0.01	-.15	.06*
Narrow vermillion	0.48*	0.05	.24	.11*
Smooth philtrum	0.59*	0.06	.28	.12*
Long philtrum	0.34*	0.05	.17	.04*
Strabismus	0.06	0.15	.01	<.01
Interpupillary distance (centile)	<-0.01	<0.01	.02	<.01
Ptosis	0.39*	0.09	.11	.02*
Intercanthal distance (centile)	<-0.01	<0.01	-.02	<.01
Nasal bridge	0.30*	0.6	.15	.03*
Anteverted nostrils	0.17	0.07	.07	.01
Aberrant palm crease	0.20*	0.05	.10	.01*
Fifth finger clinodactyly	0.28*	0.05	.15	.02*
Campodactyly	0.19	0.07	.07	.01
Heart murmur	0.17	0.09	.04	<.01
Railroad ears	0.10	0.09	.03	<.01
Intercept	2.65	0.09		
			R^2 =	.88
		Adjusted	R^2 =	.87
			R =	.94

*$p < .0015$

Table 5. Sequential Regression for (Square Root of) Dysmorphology Scores, Plains Indians (N = 273, partial FAS omitted).

Variables	*B*	SE *B*	β	sr^2 *(incremental)*
Height (centile)	<0.01	<0.01	.01	.23*
Weight (centile)	-0.01*	<0.01	-.15	.09*
Head circumference (centile)	<0.01	<0.01	.01	.04*
PFL (centile)	-0.01*	<0.01	-.15	.07*
Narrow vermillion	0.64*	010	.22	.18.*
Smooth philtrum	0.80*	0.10	.27	.07*
Long philtrum	0.89*	0.07	.33	.10*
Strabismus	0.02	0.21	<.01	<.01
Interpupillary distance (centile)	-0.01	<0.01	-.09	.01
Ptosis	0.28	0.18	.04	<.01
Intercanthal distance (centile)	<0.01	<0.01	.03	<.01
Nasal bridge	0.44*	0.12	.10	.01*
Anteverted nostrils	0.63*	0.13	.13	.02*
Aberrant palm crease	0.40*	0.08	.13	.02*
Fifth finger clinodactyly	0.14	0.13	.03	<.01
Campodactyly	0.31	0.11	.07	.01
Heart murmur	0.39	0.18	.05	<.01
Railroad ears	0.26	0.09	.07	<.01
Intercept	1.89	0.13		
			R^2 = .86	
		Adjusted	R^2 = .85	
			R = .93	

*p < .0015

Table 6. Sequential Regression for (Square Root of) Dysmorphology Scores, Italy Data (N = 265, partial FAS omitted).

Variables	*B*	SE *B*	β	sr^2 *(incremental)*
Height (centile)	<-0.01	<0.01	-.08	.10*
Weight (centile)	<-0.01	<0.01	-.13	.04*
Head circumference (centile)	-0.01*	<0.01	-.16	.05*
PFL (centile)	-0.01*	<0.01	-.14	.04*
Narrow vermillion	0.58*	0.09	.25	.21*
Smooth philtrum	0.64*	0.10	.25	.09*
Long philtrum	0.65*	0.07	.31	.10*
Strabismus	-0.14	0.19	-.02	<.01
Interpupillary distance (centile)	N.A.	N.A.	N.A.	N.A.
Ptosis	0.69*	0.21	.11	.01
Intercanthal distance (centile)	<-0.01	<0.01	-.03	<.01
Nasal bridge	0.19	0.55	.01	<.01
Anteverted nostrils	0.48*	0.11	.15	.02*
Aberrant palm crease	0.45*	0.08	.19.	.04*
Fifth finger clinodactyly	0.30*	0.07	14	.02*
Campodactyly	0.47*	0.13	.13	.01*
Heart murmur	0.24	0.31	.03	<.01
Railroad ears	0.32	0.13	.08	.01
Intercept	1.98	0.10		
			R^2 =	.75
		Adjusted	R^2 =	.73
			R =	.87

*p < .0015

Table 7. Sequential Regression for (Square Root of) Dysmorphology Scores, South Africa Sample (N = 228, partial FAS omitted)

Variables	*B*	SE *B*	β	sr^2 *(incremental)*
Height (centile)	<-0.01	<0.01	-.10	.26*
Weight (centile)	-0.01*	<0.01	-.18	.13*
Head circumference (centile)	-0.01*	<0.01	-.19	.07*
PFL (centile)	-0.01*	<0.01	-.15	.05*
Narrow vermillion	0.48*	0.06	.25	.10*
Smooth philtrum	0.60*	0.06	.29	.13*
Long philtrum	0.35*	0.05	.18	.04*
Strabismus	0.08	0.16	.01	<.01
Interpupillary distance (centile)	<-0.01	<0.01	.02	<.01
Ptosis	0.43*	0.10	.12	.02*
Intercanthal distance (centile)	<-0.01	<0.01	-.04	<.01
Nasal bridge	0.30*	0.06	.15	.03*
Anteverted nostrils	0.21	0.08	.08	.01
Aberrant palm crease	0.20*	0.06	.10	.01*
Fifth finger clinodactyly	0.30*	0.05	.15	.02*
Campodactyly	0.23*	0.07	.09	.01*
Heart murmur	0.16	0.11	.04	.01
Railroad ears	0.11	0.10	.03	<.01
Intercept	2.62	0.09		
			R^2 =	.87
		Adjusted	R^2 =	.86
			R =	.93

*p < .0015

Table 8. Results of Two-Level Model of Square Root of Dysmorphology Score as Predicted from 17 Physical Variables (N = 868)

(a) Fixed effects (Averaged over Children and Locations)

Effect	Parameter Estimate	Standard Error	*t*-ratio df = 2	*p* (2-sided)
Height (centile)	-0.003	0.001	-2.30	.103
Weight (centile)	-0.005	0.001	-4.48	.090
Head circumference (centile)	-0.005	0.002	-3.06	.133
PFL (centile)	-0.010	0.001	-6.72	<.001
Narrow vermillion	0.595	0.682	8.73	<.001
Smooth philtrum	0.655	0.058	11.28	<.001
Long philtrum	0.561	0.101	5.54	.006
Strabismus	-0.070	0.110	-0.63	.592
Ptosis	0.397	0.090	4.39	.104
Intercanthal distance (centile)	-0.001	0.001	-1.51	.226
Nasal bridge	0.387	0.063	6.09	.001
Anteverted nostrils	0.333	0.083	4.02	.163
Aberrant palm crease	0.336	0.064	5.25	.015
Fifth finger clinodactyly	0.264	0.040	6.54	<.001
Campodactyly	0.286	0.064	4.47	.091
Heart murmur	0.297	0.107	2.76	.093
Railroad ears	0.224	0.075	3.00	.125
Intercept	2.197	0.19	11.44	<.001

(b) Random effects

Effect	Variance Component	Standard Deviation	χ^2 df = 1	p (1-sided)
Height (centile)	<0.001	0.001	2.30	.125
Weight (centile)	<0.001	0.001	1.20	.272
Head circumference (centile)	<0.001	0.003	10.32	.002
PFL (centile)	<0.001	0.002	2.75	.093
Narrow vermillion	0.009	0.096	5.43	.019
Smooth philtrum	0.005	0.070	2.59	.103
Long philtrum	0.027	0.165	21.96	<.001
Strabismus	0.009	0.096	0.86	>.500
Ptosis	0.007	0.083	0.59	>.500
Intercanthal distance (centile)	<0.001	0.001	0.79	>.500
Nasal bridge	0.004	0.065	2.07	.146
Anteverted nostrils	0.013	0.114	3.99	.043
Aberrant palm crease	0.008	0.091	4.44	.033
Fifth finger clinodactyly	0.009	0.029	0.20	>.500
Campodactyly	0.004	0.066	1.63	.198
Heart murmur	0.011	0.105	1.86	.170
Railroad ears	0.008	0.090	1.92	.162
Intercept	0.102	0.319	23.69	<.001
Residual	0.212	0.460		