DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

The Pilot Project Core provides one mechanism by which this consortium acts as a dynamic entity. It provides a flexible means for developing and exploring new research activities and directions for the Consortium and a mechanism by which new sites can be added or projects can evolve into independently funded research projects. During the 5 years of this Consortium we believe that we can fund approximately 14 pilot projects. We will start with 4 pilot projects in the 01-year, each running for two years. New projects will start as these end, some will be funded for a single year and others for two years. Of course other projects might be phased in as a funded pilot project spins off early into a regular research projects, or the progress on a pilot project is such that it is terminated early. The Consortium Coordinator will manage the Pilot Project Core, although again the Steering Committee will be intimately involved in this process. Following the initial round of pilot projects, additional projects will be solicited from Consortium members, affiliated investigators, other scientists interested in FASD, and investigators from others areas who might be interested in becoming part of the consortium. Announcements of the availability of these projects will be made through the Fetal Alcohol Study Group of the RSA, and other professional outlets. Each application for a pilot project will be screened by at least two members of the Scientific Advisory Board for scientific merit, innovation, and relationship to other projects in the Consortium. Applications that pass this first screen will be sent to the Steering Committee, which will assign primary and secondary reviewers of each proposal. These proposals will be discussed at one of the two annual meetings of the group. Pilot projects for the initial term were chosen with new ideas in mind. There was an emphasis on adding new sites that might contribute to the overall functioning of the Consortium over the long-term. These sites would have unique resources available to them, or unique attributes about the population under study. Thus, projects were chosen to be conducted in Buffalo, NY; the Ukraine; and Rome, Italy. The other consideration was that the pilot projects should utilize new and novel methods to assist in the diagnosis or treatment of FASD. In this regard, a project was chosen with a potential of diagnosing FASD early in life, based upon ultrasound imaging of the corpus callos

PERFORMANCE SITE(S) *(organization, city, state)*
San Diego State University, San Diego, CA
University of New Mexico, Albuquerque, NM
University of California, San Diego, San Diego, CA
University of Washington, Seattle, Washington
University of Buffalo, Buffalo, N.Y.

KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Principal Investigator. List all other key personnel in alphabetical order, last name first.

Name	Organization	Role on Project
Riley, Edward P.	San Diego State University	P.I.
May, Phil	University of New Mexico	Project PI
Jones, Kenneth Lyons	University of California, San Diego	Project PI
Streissguth, Ann	University of Washington	Project PI
Robinson, Luther	Buffalo	Project PI

Disclosure Permission Statement. Applicable to SBIR/STTR Only. See instructions. ☐ **Yes** ☒ **No**

MANAGEMENT OF THE PILOT PROJECT PROGRAMS.

Goals of the Pilot Project Core. One of the major concerns of investigators in the Consortium is that it be responsive to new findings in the field of FASD, as well as to findings in related areas of research. The Pilot Project Core is one mechanism by which new sites and ideas can be brought into the consortium. Furthermore, our goal is to integrate these pilot projects with existing projects ongoing in the consortium. For example, new sites can incorporate protocols already running at existing sites, or findings from the pilots can be incorporated into other sites existing in the consortium. We also plan to bring new investigators into the field, which the pilot projects will allow. We fully expect that successful pilot projects will gather sufficient information and preliminary data so that the investigator is able to attract funding through other mechanisms.

Administration and Management of the Pilot Project Core: Dr. Riley will take responsibility for managing the Pilot Project Core. His role will be to coordinate the evaluation of projects, oversee fiscal management, to communicate the findings of the pilot projects, and solicit and distribute proposals for new pilots to the Advisory Panel and the Steering Committee. Approximately 4-6 months prior to the end of one of the initial pilot projects, new pilot project proposals will be solicited. All consortium members will be informed of the availability of pilot funds and encouraged to relay this information to all interested parties. In addition, announcements will be made through the FAS Study Group of the RSA, as well as the general membership of the RSA. Announcements will also be posted on the Consortium web site. We will also post notices of the availability of pilot projects at professional meetings (e.g. INS). Applications will be available on the website. Following the receipt of a complete application, the application will be sent out to two members of the Science Advisory Panel. These individuals will provide short reviews on the scientific merit, the innovation of the project, and its relationship with other projects in the consortium and rank them according to a Likert scale for their inclusion in the Consortium. Based upon this initial recommendation, the Steering Committee will select projects for further review. The Steering Committee will function much as a study section, with a primary and secondary reviewer. Input of Core PIs will be solicited if a project utilizes Core resources. The review will be held at one of the two meetings of the Steering Committee during the year. Dr. Riley will chair this meeting. All applicants will be given written feedback on their proposals.

We envision that the majority of requests will be for two years of funding, but do expect to get some applications requesting only a single year of funding. This is because with the resources available to members of the consortium, there may be some questions that can be answered relatively quickly. We do not envision funding pilot projects for more than two years. At the meeting where new applications are discussed, progress of current pilot projects will also be reviewed. All pilots will be expected to submit annual progress reports to the Steering Committee. If projects are not proceeding according to the proposed time line, the Committee can discuss potential remedies and priorities for continued funding in collaboration with the Science Advisory Panel.

Pilot Projects Proposed in Years 1-2

	PI	Project Title-
1	Philip May	A FASD Epidemiology in Italy
2	Kenneth Lyons Jones	Prenatal Ultrasound Markers of FASD in Ukraine
3	Ann Streissguth	Detecting FASD from Neonatal Ultrasound
4	Luther Robinson	Comparison of Three Diagnostic Modalities in FASD and Related Disorders

Proposed Pilot Projects for Years 1-5

Year 01	Year 02	Year 03	Year 04	Year 05
Philip May	Philip May	Project 5	Project 5	Project 12
Kenneth Lyons Jones	Kenneth Lyons Jones	Project 6	Project 6	Project 13
Ann Streissguth	Ann Streissguth	Project 7	Project 7	Project 14
Luther Robinson	Luther Robinson	Project 8	Project 10	Project 10
		Project 9	Project 11	Project 11

BRIEF SUMMARY OF EACH CURRENT PILOT:

A FASD Epidemiology in Italy: Epidemiologic studies of FAS and other FASD using active case ascertainment have never been carried out in any European country to our knowledge. Virtually all such outreach studies have been undertaken in the United States and South Africa, mostly in very high risk, low SES, binge drinking populations. This study will be the first to examine the prevalence and characteristics of FASD in a European population where drinking is much more frequent among the female population, yet drinking patterns are normatively prescribed by culture and associated with meals and a general pattern of hospitality and familial sociability. Drinking in this context may have a very different effect on the fetus because of issues of lower gravidity and parity, lower blood alcohol concentrations (BAC) from limited quantity per occasion, better nutrition, and normative expectations of control, moderation, and appropriate contexts for drinking. In two consecutive years, a random sample of 25 schools in the Lazio region of Italy will be picked and all first graders in these schools assessed for FASD using the two-tier procedure outlined in the Dysmorphology Core. One hundred randomly selected controls will also be examined each year. All controls and those with some dysmorphological characteristics of FASD will advance to psychological testing. Extensive maternal risk factor interviews will be administered to all mothers to establish protective and risk factors for FASD. The results will provide a valid estimate of the true prevalence of FASD in this region of Italy. Furthermore, it will identify specific maternal risk and protective factors, specific characteristics of the FASD and normal children of this region, and insights into possible and feasible approaches to prevention and intervention where needed.

Prenatal Ultrasound Markers of FASD in Ukraine: This project will evaluate early markers of prenatal alcohol exposure. Standard 2-D prenatal ultrasounds will be performed at 4 specific time periods on 80 moderate-to-heavy drinking pregnant women and a similar number of minimal to non-drinking controls. The extent to which prenatal alcohol impacts somatic as well as brain growth will be determined. In addition, 3-D ultrasound equipment will be utilized to explore the capability of identifying abnormalities of brain and facial development in fetuses exposed to heavy quantities of alcohol. This project will interrelate with other sites in the consortium, as a postnatal ultrasound pilot project is proposed for a site in Seattle, Washington. Prenatally ascertained babies from the Ukraine pilot could be available for postnatal ultrasound evaluation using the Seattle pilot protocol. In addition, selected measures from the Ukraine pilot on somatic growth can be compared with similar data that will be collected from routine periodic prenatal ultrasound screenings in the Moscow Region consortium project and from another NIAAA-funded project in South Africa. Finally, children in this study will be available for neurobehavioral testing with advancing age. The choice of Ukraine as a site for this pilot project is based on the suspected high prevalence of both maternal drinking and FASD. In addition, the existing infrastructure that has been established in Ukraine over the last 5 years through the Ukrainian-American Birth Defects program makes Ukraine a site with exceptional potential for the development of future projects that could address development of a broader range of goals of the consortium.

Detecting FASD from Neonatal Ultrasound: In a series of papers, this group has shown that adults and adolescents with FAS or FASD can be detected with a high degree of accuracy by measuring shape variability of the corpus callosum (CC) from MRI. Furthermore, both diagnostic groups were equally deviant in CC shape, and 2 profiles of neuropsychological deficit were detected relative to CC shape variation: motor and executive function (EF). To extrapolate this work to newborn babies, these researchers have carried out a successful feasibility study developing a method of averaging ultrasound images of the CC which revealed a hypoplastic CC in 3 of 4 alcohol exposed infants and none of the controls. In this pilot investigation they expect to demonstrate significant differences in shape variation of the CC between 25 heavier alcohol-exposed babies and 25 unexposed babies. If this is successful, this group will apply for additional funding to carry out a full detection study on several hundred babies who could come easily from other consortium sites. A full detection study would flow easily from the expertise represented in the Consortium, which would confirm the diagnostic capability of the newborn ultrasound for detecting FASD.

Comparison of Three Diagnostic Modalities in FASD and Related Disorders: Epidemiological studies in the US suggest that FASD is more common among African Americans than persons of European background. A critical question, however, concerns whether facial features that are associated with FASD (e.g., depressed nasal bridge, epicanthal folds) also are common normal variations among African Americans and may contribute to over diagnosis of FASD in this group. In Buffalo, there is the opportunity to study this question by articulating the clinical efforts within the Consortium to assess the facial morphology of FAS and related disorders among African American subjects in comparison with similar subjects of European background. Individuals with Williams syndrome, an autosomal dominantly-inherited disorder with craniofacial dysmorphism, will serve as a disease control. Subjects will be recruited from the Ambulatory and Genetics Divisions in the Department of Pediatrics at SUNY-Buffalo. These investigators will

collaborate with researchers at Indiana University to compare and correlate three assessment modalities –dysmorphology, selected anthropometric measures, and 3-D digital imaging – to generate and analyze data (e.g., regression analysis, discriminant function analysis) that allow more reliable recognition of FASD in this population.

BRIEF DESCRIPTION OF THE INVESTIGATORS:

A FASD Epidemiology in Italy: PHILIP A. MAY, Ph.D. will oversee this project. He has worked with alcohol and prevention research and prevention topics for much of the past 32 years, and over the past 23 years he has directed many major research and prevention grants/contracts dealing with FAS and consulted on other FAS projects. He has served as the team leader and principal investigator of NIAAA community-based research projects on the epidemiology of FAS in South Africa, the Northern Plains, and Washington, D.C. Dysmorphology in the Italy project will be carried out by K.L. JONES, M.D., LUTHER ROBINSON, M.D., and H. EUGENE HOYME, M.D. The psychological assessment will be overseen by WENDY KALBERG, M.A., CED, and P.W. KODITUWAKKU, Ph.D., and carried out by Italian colleagues. Finally, medical and cultural translation in the project will be overseen by MARCELLO MAVIGLIA, M.D., an ASAM certified psychiatrist, who is a native of Italy, now a U.S. citizen, and has been a professional in the United States for the past 16 years. He is, however, new to the field of FAS, as will be approximately six other Italian colleagues who will be trained to work on this project.

Prenatal Ultrasonographic Markers of FASD in Ukraine: KENNETH LYONS JONES MD is the PI on this project. Drs. Jones and Smith in 1973 were the first to describe FAS. Since that time, Dr Jones's research has focused on the identification of a variety of human teratogens in addition to carrying out further studies on the effects of alcohol on fetal development. CHRISTINA D. CHAMBERS PhD, MPH is a Co-investigator. She is a birth defects epidemiologist who is relatively new to the FASD research field. However, she is presently involved in 4 studies relating to the prenatal effects of alcohol. ANDREW HULL MD is a perinatologist who has specialized expertise in prenatal 3-D ultrasonography. He is a leader in the development of this technology. Although he is new to the FASD research field, he is Director of the Perinatal HIV Clinic at UCSD. WLADIMIR WERTELECKI, MD is Chairman of the Department of Human Genetics at the University of South Alabama. In addition he is Program Director of the Ukrainian-American Birth Defects Program. In this role he has been instrumental in developing an infrastructure throughout Ukraine to identify at-risk pregnancies and children with birth defects in order to develop effective programs for the prevention of birth defects.

Detecting FASD from Neonatal Ultrasound: The PI of this project is University of Washington (UW) Professor ANN STREISSGUTH, Ph.D., who has been working with FASD since 1973. She is the PI of the Seattle Prospective Longitudinal Study on Alcohol and Pregnancy and the Neuroanatomic-Psychologic Analysis of FAS/FAE Deficits in Adults and Adolescents. UW colleagues, PAUL SAMPSON, Ph.D., Professor of Statistics, HELEN BARR, M.A., M.S., biostatistician, data base manager and PAUL CONNOR, Ph.D., have permitted testing the assumptions upon which the present Pilot Study rests. FRED BOOKSTEIN, Ph.D., Distinguished Research Scientist, Univ. of Michigan, Ann Arbor; and Honor Professor, University of Vienna, is a morphometrician, who, with Dr. Sampson, developed the method of geometric analysis of the corpus callosum (CC), which indicated that it is CC shape that is predictive of the impact of alcohol on prenatal brain development in individuals with FASD. To accomplish the aims of the Pilot Study, CHRISTINE GLEASON, M.D. UW Professor of Pediatrics and Head of the Division of Neonatalogy, and RAYMOND SZE, M.D., UW Assistant Professor and Ultrasound Radiologist will collaborate in obtaining the neonatal ultrasound images to be analyzed by Dr. Bookstein in the pilot study. Dr. Sze is new to the fetal alcohol work, but experienced in cranial ultrasound detection work.

Comparison of Three Diagnostic Modalities in FASD and Related Disorders: LUTHER K ROBINSON, MD is Associate Professor of Pediatrics at the State University of New York at Buffalo where he directs the Dysmorphology and Clinical Genetics program, of which the Fetal Alcohol Syndrome Evaluation is a part. Dr Robinson has worked in the field of dysmorphology for over twenty years, and brings expertise in the clinical evaluation of individuals with FASD and related disorders. He has been a member of the NIAAA-sponsored FASD study projects in South Africa and Russia and is senior dysmorphology consultant to the Fetal Alcohol Syndrome Epidemiology and Research project of the Great Plains.

THE PROJECTS

1. A FASD Epidemiology in Italy - May

A. Specific Aims

1. To determine the number, rate, and characteristics of children with FAS/FASD entering school each year in select towns in the Province of Rome, Italy by active case ascertainment and random sampling methods.

2. To screen suspects for FASD in schools with both normal and special needs children.
3. To determine if FAS and other FASD exist in Italy and if FASD is more prevalent among certain ethnic groups and immigrants, particularly those of low socioeconomic status.
4. To detail the specific physical, mental, and neurodevelopmental characteristics of Italian children with FAS and FASD.
5. To identify children on whom culturally appropriate neuropsychological tests can be applied to best diagnose and characterize Italian children with FAS and FASD.
6. To provide epidemiologic data on FASD in a select region of Italy that are uniquely applicable to targeted prevention programs. Such an approach has not been reported in European populations.

B. Background and Significance

The epidemiology of FAS is best established and understood in the United States (US) and The Republic of South Africa (SA) (Riley, et al., 2003). FAS occurs more frequently among lower socioeconomic status (SES) individuals in the US and SA, among African Americans in US inner city populations (Abel, 1995; May, et al., 2000a), and also among American Indians (AI) residing in impoverished reservations where binge drinking is common (May, et al., 1983; May, et al. 2002). In three recent clinical screenings for FAS in SA carried out under supplements to previous grants, the issues of SES, nutrition, body size, and specific biomarkers and genetics for FAS have been raised. Among women in SA who were the most impoverished, a substantially higher rate of children with FAS was found than among other women in the same region (May, et al., 2000; Viljoen, et al., 2002). This represents a significant over representation of FAS cases from poor rural areas ($p < .000$ OR = 4.41), women with lower education ($t = 3.22$, $p = 0.0019$), lower levels of religiosity ($t = 2.57$, $p = 0.012$), and more severe binge drinking patterns ($t = 4.93$, $p < 0.000$) (Viljoen, et al., 2002). These findings are similar to findings from a variety of studies on AI reservations (May, 1991; May et al., 2002) and elsewhere. Therefore, low SES, particularly living in poverty in rural or inner city areas among social networks characterized by severe binge drinking, appears to be a major risk factor for FAS (Abel, 1995).

European countries may well be another matter. Binge drinking is less common and SES is generally higher; but frequency of drinking is much greater. European norms treat alcohol as a culturally regulated food item, customs of moderate use are prevalent, a high proportion of the female population drinks, and SES levels are higher than among populations where FAS prevalence has previously been found to be high (Abel, 1998; Room, et al., 2002). In fact, Abel has written about the "The American Paradox." It holds that countries with the highest per capita consumption of alcohol are the least likely to report high rates of FAS. America, having a relatively low per capita consumption of alcohol, has a paradoxically high rate (Abel, 1998).

We have recently been invited by Italian officials to initiate this study. Proposed is an active case ascertainment study in a specific Italian population. It is unlike any performed in Europe previously. It will draw upon the experience gained in active case ascertainment studies in a number of US populations, particularly among AI, in Washington, D.C., as well as in SA. For a number of years, the proposed PI of this study has carried out active case ascertainment of FAS. Specifically, FAS studies among AI have been useful for determining the epidemiologic characteristics and prevalence of FAS (May, et al., 1983) and for designing relevant and targeted prevention programs (May and Hymbaugh, 1983). Currently, active case ascertainment studies are funded by NIAAA (U01AA11685) and underway in six communities of AI in the US (Montana, North Dakota, and South Dakota), Washington, D.C., Great Falls, MT, and SA. These studies and others have shown that FAS occurs frequently among mothers who are: less tied to their local culture, low status within their communities, of advanced maternal age (e.g. > 25 yrs. old), high gravidity and parity, and practice unstable, nomadic, binge drinking lifestyles. Binge drinkers especially have been found to give birth to multiple FAS and other FASD children. The prevalence of mothers bearing FAS children has been found to be low among AI tribes, one to thirty individuals per 1000 women of child bearing age produced all FAS cases (6.1 per 1000 or 0.6% in the early 1980's), and in recent studies this rate is in the lower part of the range (May et al., 2000b). Active case ascertainment studies by others in different AI communities have replicated these findings (Quaid, et al., 1993; Robinson, et al, 1987; Duimstra, et al, 1993). We do not know how these findings translate to European populations, as active case ascertainment methods have not been reported with any populations other than AI (IOM 1996), New Mexicans (May, et al., 2000b), and South Africans (May, et al., 2000).

Three Waves of NIAAA-funded research in SA have documented the highest rate of FAS in any major population in the world (46-75 per 1000). Children and mothers in SA exhibit characteristics of FAS and risk factors that are similar to other populations studied thus far. But because of a high rate of FAS in SA and special conditions there, researchers have raised new questions about susceptibility to FAS. Abel (1995,1998) and Abel and Hannigan (1995) have written that under nutrition and other biomarkers are major risk factors for FAS. Abel (1998) estimates that 4 to 5% of heavy drinking women have FAS children, and that not only are genetics and advancing age co-factors, but poor nutrition as well. Studies in SA indicate that the mothers with FAS children are not only prone to more binge drinking (Khaole and Li,

in progress), but they suffer from disadvantages of a low SES lifestyle including: relatively small body size (hgt, wgt, and OFC) and other effects of poor nutrition. Therefore, AI and SA studies are both useful for determining major FAS risk factors, and they lead us closer to understanding additional, more specific, risk factors. These studies have been carried out in entire populations (Kindig & Stoddart, 2003; Cohen, et al, 2003), and yield accurate estimates of epidemiologic characteristics that are not always possible in clinic or record-based studies (IOM, 1996; May, et al., 2002).

The proposed study in Italy will build on these previous studies to explore the six specific aims and enhance the collaborative initiative by providing another international population for cross-cultural examination of differences in FASD. We will focus on an epidemiologic study of a European population for the first time to explore some of these important variables linked to the specific etiology of FASD.

C. Previous Studies

As far as we could determine, there have been no major FAS epidemiologic studies ever undertaken in Italy or in Europe using outreach or active case ascertainment. One clinic-based study of 535 mother-newborn pairs found significantly higher birth weights among children of drinkers (De Nigris, et al., 1981). Other studies in Milan, Italy have examined the relationship of alcohol consumption to spontaneous abortion/miscarriage. Parazzini, et al. (1990) found no association between either maternal or paternal drinking and miscarriage, and reported in another study that moderate drinking does not "increase markedly" the risk of miscarriage (Parazzini et al., 1994). Finally, in a study of coffee, alcohol and smoking, Parazzini et al. (1996) reported that the risk of multiple pregnancy is higher among women drinking $\geq$ 15 drinks per week compared to abstainers (OR = 2.3 to 2.6 for DZ and MZ twins). Similarly, women in Italy who drink 3 or more cups of coffee a day and are heavy smokers ($\geq$ 10 cigarettes per day) were also more likely to have multiple pregnancies.

Another clinic-based study in Italy (Primatesta, et al., 1993) examined 1516 women and infants in Milan and compared them to 996 pairs in Southampton, England. Although a high proportion of the women in both cities drank during pregnancy, no cases of FAS were found, and there was no association between birth weight and alcohol consumption. Lazzaroni and colleagues (1992, 1993a, 1993b) have published three articles on alcohol use in pregnancy among 2145 Italian women. A decrease in birth weight was associated with prenatal drinking and smoking, and an association was found between maternal drinking and early jaundice. Multivariate analyses found low birth weight to be more frequent in women who drank heavily regardless of smoking. Non-smokers who drank 10 grams (3.5 oz) of absolute alcohol or more a day were at highest risk for low birth weight (< 2500g.), and maternal alcohol consumption of 20 g. per day increased the risk of preterm delivery (OR = 2.35) (Lazzaroni, et al., 1993a). This study documents that 0.7 to 1.4 standard ethanol units (drinks) per day is associated with adverse pregnancy outcomes in Italy.

Bonati and Fellin (1991) studied 4966 Italian women delivering in Italian hospitals. Twenty-six percent of the mothers smoked pre-conception and 35% were daily drinkers. Preconceptual drinking was more prevalent among older women (39% among those 30-34 and 42.0% among 35-39) as was smoking and drinking (10-13% among 30-39 years old). Italian women were more likely to give up smoking than drinking during pregnancy, and the higher the education the more likely they were to quit either behavior. However, only one-third of the smokers gave it up during pregnancy and daily drinking declined even less, from 35% to 31%. In other words, drinking persisted (Bonati and Fellin, 1991). The authors were careful to distinguish between women who "drank between meals" and those who did not. Treating the former group as alcohol abusers, less than 1% (0.7) were classified in this category. Overall, smoking accounted for more "small for gestational age" (SGA) children and drinking was not associated with low birth weight. "There was no evidence of synergy between alcohol and cigarettes" (Bonati and Fellin, 1991). This study implies that in Italy drinking only during meals is protective and that the highest risk women (1%) are those who also drink between meals. The authors conclude that birth weight "is affected only by abuse."

We are proposing to use different methods of study than any previous Italian study. The various improvements that have been made to FAS epidemiology methods over the past 20+ years of study are reflected in current active case ascertainment methods. Active methods are comprehensive and utilize screening and information gathering in three major areas: 1) dysmorphology and physical development; 2) neuropsychological, intelligence, and social development testing; and 3) maternal risk factors. Not only are the dysmorphology methods well developed, the neuropsychological and developmental tests to be used our part of the study have been shown to be appropriate across cultures (Adnams, et al, 2001; Kodituwakku, et al., 2002). The maternal questionnaire to be translated and used has an extensive history. It was developed for a CDC-funded epidemiology project in New Mexico, it has been revised extensively three times in SA, and revised several more times in NIAAA work funded in the Plains. It utilizes questions based on content and findings from previous studies by Wilsnack et al. among women, and in studies of Day (Wilsnack and Beckman, 1984; Wilsnack, et al, 1984; 1991; Day, et al, 1991; 1993). By utilizing data from all three of these diagnosable domains, a complete study of FAS will be completed. With the methods of active case ascertainment, the clinical as well as the social, behavioral, and

environmental context of the problem is more fully understood for etiology, further research and prevention (May and Hymbaugh, 1983; 1989; Masis and May, 1991; May, 1995).

D. Methods

We will utilize active case ascertainment both in first grade classes of representative schools and in active outreach throughout the community in institutions for the severely retarded. Records of children in protective custody and, where possible, adoption agencies will also be consulted. The goal will be to use random selection of schools and capture/recapture methods (Egelund, et al., 1998) to produce a complete prevalence of FAS in a prescribed age cohort (approximately 5 to 7 years of age). Secondly, such activity will provide identification of a number of possible FAS and FASD cases, which can then be studied completely. Once some indicators of the FAS phenotype have been documented in a child by preliminary dysmorphology screening, maternal risk factor studies and neuropsychological studies will be completed and case conferences will determine the final diagnosis for each child (Appendix A, Figure 1). Then further neuropsychological testing by the consortium can follow.

Samples. The population of Italy is 57,680,000 and the targeted communities are in the Lazio region (pop. = 5,291,118). The targeted area is a wine producing area in the province of Rome. The region has 900,000 people under 18, and 3.6% are foreign immigrants compared to a national average of 2.8%. Single parent families make up 12.6% of all families in the region (11.6% nation wide). Perinatal deaths account for 41% of all deaths 0-14 years and 2.2% were listed from congenital malformations, the 2nd most common cause. The region of Rome has a relatively high number of requests for adoption (155 in 2001). The majority of children attend public schools with a minority attending Catholic and other private schools (Rapporto, 2002). The study will be carried out in 15 towns in the Lazio region that are listed in Appendix A, Tables 1 and 2. These towns had a total population of 239,672 in 2000.

Two samples will be selected from the 68 elementary schools within the 15 regional communities. They will be picked randomly. In two successive years (2004 and 2005), 25 schools selected for that year will be asked for permission to undertake complete screening of first grade classes. There are 2165 first grade students in these schools; therefore, we will screen a minimum of 800 children each year. The total sample size will be approximately 1600, representing 74% of all first graders in the schools.

A two tier screening system will be used as in SA, which implements the IOM FAS diagnostic system (See Appendix A, Table 3). In Tier I, first grade students are measured on height (hgt), weight (wgt), and head circumference (OFC) by school nurses or Italian university staff. Each child is then assessed for growth percentile. Growth charts of the National Center for Health Statistics will be used. In past studies, among a variety of ethnic groups, all of the classic FAS suspects were found to have hgt., wgt., and OFC measurements below the 25th centile. If below the 25th centile on OFC or on both hgt and wgt, a child will be referred for a complete (Tier II) examination by two of three dysmorphology teams consisting of one experienced dysmorphologist (Drs. Jones, Robinson, and Hoyme) and one Italian physician trainee in each team. Standardized IOM assessment criteria are used (IOM, 1996) (see Appendix A, Table 4). Exams occur in appropriately private rooms in the school. Assuming a normal growth distribution, approximately 200-250 suspect children will be seen each year in Tier II screening. At a pace similar to that in SA, 5-7 days are needed to screen. Six Italians (three physicians) have already attended and participated in training in SA. Trainees first observe, and then later take the lead in examining every child with the two physicians verifying one another's findings. Physicians are "blinded" from any prior knowledge of the child or mother. Once seen by one team, a child is directed to another "blinded" examination team who repeats the exam. The method provides training and a reliability check. Inter-rater reliability in SA studies (r) for this above diagnostic scheme (239 matched pairs) was .91 for ICD, .85 for IPD, .65 for PFL, and .84 for philtrum length (PL).

After the dysmorphology examinations, the child is assigned a preliminary diagnosis of FAS, Deferred, or not-FASD. Detailed FAS data forms are used. Children who receive the Deferred diagnosis are those who have some signs of FASD, but developmental tests and maternal interviews are required before settling on even a preliminary diagnosis of any level of FASD. Only those with the classic FAS phenotype and poor growth measurements receive a preliminary diagnosis of FAS. All children who receive FAS or Deferred diagnoses are advanced to the neuropsychological and development examination and for maternal risk factor studies (Appendix A, Figure 1). Final diagnosis is made by a case conference of professionals representing each domain: dysmorphology, psychological development, and maternal characteristics. Assessment in each area is done "blinded" from findings in other domains.

Special education classrooms and special schools for children with most developmental disabilities do not exist in Italy. Children with special needs attend the normal schools, and they stay in the same classroom with assignment to a special "holding teacher" to receive some special lessons from the holding teacher in specified hours of the day. They, therefore, can be examined at the same schools at the same time as all other children (personal communication, Dr. Fiorentino), but we also look for cases in institutions for the severely retarded.

Control Children. A number of controls for each child in Tier II screening will be picked randomly from the 25 schools under study each year and included in the screening at the same time as the FASD suspects. To be safe, a minimum of 100 controls will be picked each year prior to the Tier II exams. Identical exams and developmental testing are performed on all of the FAS, Deferred, and matched controls, and the physicians are blinded to their status.

Psychological and Neurodevelopmental examinations. Because this is an epidemiological study, only two different types of tests will be used to help assess the gross type or level of FASD for each child. The basic intelligence measure is the Italian translation of the WISC (Weschler, 1989). It will be used on all suspected FASD and control children. Additionally, a life skills test, the PBCL-36 (Streissguth, et al., 1998) will also be used, and teachers will be given the Parent/Teacher Disruptive Behavior Rating Scale (Pelham, 2002). From these two types of tests, sufficient information will be gathered for the epidemiology assessment of final diagnosis. Italian colleagues will administer all initial tests. In addition to the diagnosis of FASD in the epidemiology study, this pilot will identify cases and controls for further neuropsychologic examinations of those children with FASD for an even better understanding of true characteristics of FASD in this European population. Tests such as the Raven (Raven, 1995), the Wide Range Assessment of Memory and Learning (Adams and Sheslow, 1990), Reversal Learning (Rolls, et al., 1994), Matching Familiar Figure Test (Kagan, 1965), and others may well be used in follow-up studies.

Maternal Interviews and Maternal Controls. The mothers of the control children become the maternal controls. Structured maternal interviews contain 300 items which explore: childbearing pattern; drinking patterns (and estimation of peak BAC) before, during, and after the index pregnancy; marital and cohabitation pattern; SES; demographic factors such as self-esteem, locus of control, fatalism, and social environment, and nutrition. All questions will be translated into Italian and pilot tested. Italian research staff in the field will perform all interviews (English version of the instrument in Appendix B). Some mothers may be deceased or not locatable, and some of the children will have been adopted with records sealed. With such barriers, collateral sources and existing records will be used.

Training in the Overall Project. In this study, the training of the local professionals will focus on the constellation of birth defects and their unique presentation in Italian children. Each is dependent on both the physical features and the developmental assessment. The neurodevelopmental assessment is accomplished independent from the physical exam by a team overseen by Dr. Kodituwakku, Ms. Kalberg, and the PI. Maternal interviewers will be trained and overseen by the UNM team of Dr. Maviglia, Ms. Trujillo, Dr. Gossage, and the PI. Dr. Maviglia will oversee translation of the questionnaire to Italian.

Public schools were chosen as the sites for this initial study for four reasons: 1) both normal growth parameters in controls and FASD candidates can be accessed; 2) an estimate of FASD in schools will provide information to guide epidemiology, educational and public health interventions in Italy and possibly other European populations; 3) Italy emphasizes mainstreaming children with special needs; and 4) few children attend private school.

Italian colleagues will be working from governmental funding. They have secured $250,000 Euros from the Italian government for this project. This should fund the field activities (personnel, logistics, etc.). Their field staff will ensure follow-up in all domains, confidentiality, and provide appropriate information back to school personnel. They will also coordinate the use of facilities and the clinical evaluation phases with the advice from the US team. School nurses will aid in taking the current hgt, wgt, and OFC measurements of the children. The input from this pilot project, however, is important for the success of this program.

Data Gathering and Training in the Dysmorphology Phase. Before the first day of each screening week, three Italian physicians will take part in an educational seminar aimed at familiarizing them with information about clinical recognition of FAS. Diagnostic materials included in Appendix B will be given to each participant, and one-on-one training will supplement slide and lecture training. On each day, the three teams will travel to three to five of the selected elementary schools. As examinations are completed, the research assistants will assemble and review the completed forms for each child, checking for completeness and disparities in diagnosis. In the event of a disagreement concerning the preliminary diagnosis by the two examination teams, the child in question will be reexamined by all of the dysmorphologists simultaneously and a majority opinion reached. This methodology is intended to further diminish inter-observer variation and to provide the greatest possible diagnostic precision.

During this phase of the project, Drs. Maviglia, May, and Gossage will oversee the data gathering techniques, and will gather information about local culture affecting pregnancy and motherhood, demographics, socioeconomic status, norms of alcohol use, and other local conditions. They will train the local Italian field research assistants and professionals on data collection, management, and processing. Similarly, Dr. Kodituwakku and Ms. Kalberg will work with Drs. Deiana and Fiorentino and staff in protocols for the neurodevelopmental tests. These tests will be administered in accordance with procedures described in test manuals and Consortium criteria. Training sessions will be videotaped, and tapes independently rated by two observers to establish reliability. The neuropsychological protocols are carried out

after the dysmorphology exams are completed. US Staff will train and oversee the Italian Program Manager and the Data Coordinator in the maternal interview process. The questionnaire will be pilot tested during the site visit of the US researchers with UNM staff members present to ensure relevancy to local populations.

Follow-Up and Data Analysis. In the event a child is found to have any FASD, Italian colleagues will contact the parent(s) to inform them of the implications of this diagnosis, suggestions for meeting the educational needs of the child, and a recommended course of action. The biologic mother (when accessible) may also be referred to appropriate services. Ms. Kalberg and Dr. Maviglia will work with the Italian staff on follow-up protocols. The Italian Program Manager and the data coordinator will be responsible for disseminating the written results of the neurodevelopmental assessments and recommendations. Responsibility for initial data management, processing, and analysis will be the shared responsibility of both Italian and UNM researchers.

Final Estimates of Prevalence. Only a small number of children diagnosed with FASD is expected, but will represent complete ascertainment for the sub-population examined. Based upon the US literature, we might expect to find between 1 and 4 FAS and Partial FAS case per 1000 overall (n=2 to 6) in the children evaluated (Abel, 1995; May and Gossage, 2002). Based on the Italian literature, we may not find any children with FAS. One to 3 percent (n = 16 to 48) of the children may be found to have ARND or ARBD. Estimates of minimal prevalence will be calculated by comparing the number of confirmed FASD cases to the total age-specific population examined. Corrections will be made given contingencies in school attendance, and representativeness of identified cases. Additional analyses will project prevalence from the sampled population to the total geographic area, utilizing age, SES, sex, ethnic group, growth, or other variables.

Possibilities for Other Scientific Research and Prevention. This pilot epidemiology study will provide individual, "gold standard diagnosis" cases of FASD for further research. The ascertainment of children and mothers will identify a variety of possibilities for other studies to better understand FAS among a European population. Once the maternal risk factors and epidemiologic features of FAS have been determined in this Italian population, recommendations for prevention and intervention can be made. The results of this study will provide, at minimum, a prevalence estimate, which can be used to plan follow-on investigations, and will constitute a "proof-of-principle" regarding the use of these methods for epidemiologic study for FASD in Europe. The methodology and skills left in place after this study will facilitate future research in biology, neuropsychology, and intervention and prevention programs.

Probable Publication Topics. The proposed research will result in data collection and analysis that promise publications in several areas: 1) the prevalence and epidemiology of FASD, 2) clinical characteristics of FASD, 3) neurodevelopmental characteristics of Italians with FASD, 4) maternal risk factors for FASD in Italy and, 5) empirically-based recommendations for prevention of FASD in a European country.

Budget. The budget includes only travel expenses for the American professionals. At least two trips to Italy will be required for US researchers to provide technical assistance.

2. Prenatal Ultrasonographic Markers of FASD in Ukraine - Jones

A. Specific Aims. The **specific aim** of the study will be to use standard prenatal ultrasound techniques at 4 specified time points in the first, second and third trimesters, to compare patterns of somatic and brain growth in the fetuses of pregnant women with moderate to heavy alcohol consuming behaviors to the fetuses of pregnant women who drink low amounts or no alcohol at all. **A secondary aim** will be to utilize prenatal existing 3-D ultrasound equipment to explore the capability of identifying specific brain abnormalities as well as abnormalities in facial development in fetuses whose mothers report heavy alcohol use during pregnancy.

B. Background and Significance. Little has been done with imaging techniques to correlate timing of prenatal alcohol exposure with onset of specific alcohol-related effects, nor have imaging techniques been utilized to promote earlier diagnosis of FASD. Earlier identification fosters earlier access to services, reduced frequency of secondary disabilities, and provides earlier opportunities for prevention of recurrence through maternal interventions.

The most significant potential effect of prenatal alcohol exposure is on development of the central nervous system. A variety of structural defects have been described in autopsy studies, including defects in neuronal migration[1], partial to complete absence of the corpus callosum[2-4] and cerebellar abnormalities.[2,5,6,4] Magnetic resonance imaging (MRI) studies on living children exposed prenatally to alcohol have documented similar defects.[7-8]

Abnormalities of the brain, specifically reductions in the size of the frontal cortex, have also been demonstrated on prenatal ultrasound in fetuses exposed prenatally to alcohol.[9] Four brain measurements were taken at 1 to 6 time points during pregnancies ranging from 12 to 42 weeks gestation. Transcerebellar diameter, distance from the posterior margin of the thalamus to the inner calvarium, distance from the posterior margin of the cavum septum pellucidum to the inner surface of the calvarium (frontal lobe), and biparietal diameter were measured on 155 women including 29 heavy

drinkers. Whereas only 4% of fetuses in the control group had a frontal cortex measurement below the 10th percentile, 23% of heavily exposed fetuses fell below this cutoff point. With alcohol consumption level defined as absolute ounces of alcohol per day in weeks 1-4 postconception, heavy alcohol was significantly correlated in a linear regression model with frontal lobe size even after adjustment for transcerebellar diameter, distance from the posterior margin of the thalamus to the inner calvarium, maternal smoking, and the interaction between smoking and alcohol. Furthermore, the association was accentuated in women who were ≥30 years of age. Of particular importance, earlier ultrasound measurements appeared to be more sensitive to differences in size of the frontal lobe (Wass, personal communication). A limitation of this study was that not all women were scanned at multiple and consistent time points during pregnancy, comprehensive data on alcohol consumption was not collected, and more than 2/3 of the exposed women used substances other than alcohol. Since many women were recruited from treatment centers and might be expected to be mid- to late pregnancy abstainers, the relationship between timing of exposure and effects in frontal lobe or other brain structures could not be clarified.

To our knowledge, comprehensive data relative to prenatally assessed growth and major or minor structural abnormalities including facial dysmorphology in alcohol-exposed fetuses have not been published. Data collected on a sample of 60 heavily exposed fetuses and controls scanned in South Africa (Louise Matthews, personal communication) are currently being analyzed. Scans performed at intake, 22, 28 and 36 weeks gestation were performed and measurements of biparietal diameter, occipital frontal diameter, abdominal circumference, femur, humerus, and foot length, and orbital diameter were collected on all subjects. A subset of 20 subjects also had measurements of the frontal part of the brain. Preliminary analyses of these data show no significant growth falloff up to 28 weeks gestation; however, 15% of the exposed sample delivered preterm and therefore could not be scanned at 36 weeks. Prenatal orbital size measurements as a surrogate for palpebral fissure length have not previously been explored. Preliminary data from the South African sample suggest that measurement of the bony orbits can be quite easily accomplished by ultrasound prior to the last month of pregnancy and can be measured to within 1 mm reproducibility.

With respect to surrogate measures of palpebral fissure length, Braddock et al.[10] established the correlation between size of the eye globe and palpebral fissure length in a sample of normal newborns as well as a sample of adults and children with FAS using ultrasound measurements of globe size. In that the bony orbit is a reflection of the size of the ocular globe, it is reasonable to suggest that pre and postnatal bony orbital size will correlate with globe size and therefore also correlate with palpebral fissure length.[11]

3-D ultrasound offers several exciting possibilities in the prenatal detection of FASD. 1) Multiplanar imaging allows the observer to identify and assess specific anatomical structures in a standardized format, regardless of the plane of acquisition of the ultrasound dataset[12]. This technique is particularly applicable to the detailed evaluation of fetal intracranial anatomy, which may be difficult to assess using standard 2-D ultrasound.[13] It may also allow the systematic measurement of surface anatomical features such as philtrum length, ear size and profile contour. 2) Surface rendering allows a "photograph-like" depiction of the fetal face.[14] Such rendered images may be examined for dysmorphic features using both qualitative and quantitative parameters. 3) 3-D ultrasound may be used to accurately and precisely measure volumes of fetal structures, such as the orbit, potentially expanding the range of detectable FASD-related anomalies. 3-D datasets may be archived for later evaluation either locally or remotely and may be independently viewed, processed and assessed to evaluate intra- and interobserver variability in assessment.[15]

The choice of Ukraine as a site for this pilot is based in part on the suspected high prevalence of both maternal drinking and FASD in this country. Although there are limited epidemiological data on this issue, a preliminary and partially validated review of 526 children 0-3 years of age in 6 orphanages in Ukraine resulted in 25 children with a definite diagnosis of FAS and an additional 26 who were suspected, for a combined prevalence of 9.7%. In addition, there are a number of assets available in Ukraine that makes it an appealing site to conduct FASD research. 1) The Ukrainian-American Birth Defects Program was established in 1998. Through this program a sophisticated structure has been established involving neonatologists, obstetricians, medical geneticists and ultrasonographers in order to evaluate children with birth defects both prenatally and postnatally. 2) A Ukraine Alliance for the Prevention of Birth Defects has been created which provides experts in social policies who are interested in alcohol-related issues. FASD has been identified as a priority area for intervention and prevention. 3) A Birth Defects Monitoring System has been established based on international standards, and in the Rivne and Lutsk oblasts, a complete ascertainment newborn registry is in operation. 4) OMNI Resource Centers have been established at sites throughout the country to sustain telemedicine as well as to provide access to current medical information via the Internet.

D. Research Methods and Design.

Site: The Ukrainian-American Birth Defects Program, the Ukrainian Alliance for the Prevention of Birth Defects, as well as the US-Ukraine Foundation, all of whom are represented by Dr. Wertelecki have agreed to collaborate on this pilot study. Two oblasts in the Northwest area of Ukraine have been selected as sites due to the existing technical infrastructure, available equipment and expertise, and the centralized delivery of prenatal care in high-risk pregnancies drawn from the Rivne and Lutsk (Volney) regions. Approximately 25,000 births occur in these regions each year. Drs. Jones, Chambers and Hull will be responsible for the training of the Ukrainian collaborators in the study protocol as well as participating in the study implementation, analysis and reporting of results. Dr. Wertelecki will serve as the in-country coordinator for the project. Dr. Lyubov Yevtusok will serve as the Rivne team leader and Dr. Tetiana Vihovska will serve as the Volney team leader, each of whom will be responsible for insuring the quality and integrity of the data collection and recording, as well as participating in the preparation of results. Administrative and fiduciary matters will be coordinated through the U.S.-Ukraine Foundation headquartered in Kyiv and if necessary from headquarters in Washington, D.C. Memorandums of Understanding among all collaborators will be supplied upon approval of the project for funding and will include agreements as to data confidentiality, access to data (without identifiers) by investigators, data analysis, data reporting, publications and credits. Although limited funding is available in a pilot project to support frequent on-site involvement of the U.S. collaborators, the utilization of internet-based communication will allow for the accomplishment of the study objectives. The Ukrainian-American Birth Defects Program, as a collaborator on this project, over the last five years has already demonstrated the ability to utilize telecommunication including web-based technology to maintain quality assurance and meet international standards for projects being conducted over substantial geographic distances. In addition, the core staff of the Program located in Kyiv will be available to provide in-country assistance and to resolve matters of urgency.

Study Design: The study will be a prospective cohort design with subjects ascertained through early prenatal screening in several large obstetric services in two oblasts or regions of Northwestern Ukraine representing 1/3 or 8,000 of the area's annual births.

Sample Size and Power: Based on an estimated 12% of pregnant women in Ukraine who report drinking at least once a week, and 2% of women who report drinking 2-6 times per week (http://ibis-birthdefects.org/start/healths8.htm, 1996 survey data), and an estimated 0.3% of Ukrainian women who are registered alcoholics, a conservative estimate of 1% of the annual screened sample is expected to qualify as moderate to heavy drinkers (38% heavy and 62% moderate). Estimating approximately 20% attrition (10% spontaneous abortion or stillbirth after enrollment, and 10% lost to follow-up), recruitment will continue for 18 months. This will provide an exposed sample of approximately 80 subjects resulting in live births. An equal number of low or non-drinking subjects will be recruited over the same time frame using the same sources. With respect to the primary Specific Aim of this project, this sample size will be sufficient to detect an effect size for the frontal lobe (approximately 0.75 standard deviation) similar to that reported by Wass et al.,[9] when standardized measures of brain structure are averaged across time points for each subject and heavily exposed fetuses are compared to moderate or low.

Sample Selection and Exclusion Criteria: A short screening questionnaire will be administered at the site of the first prenatal visit to ascertain recent alcohol use. Women who meet the criteria for enrollment will be invited to participate and consented after referral to the Rivne Diagnostic Center or Pediatric Polyclinic in Lutsk. Women who report illicit drug use will be excluded (except for marijuana) and recruitment of controls will involve frequency matching on maternal smoking. Women who do not plan to continue the pregnancy will also be excluded from the study. All women who screen positive for alcohol, regardless of whether or not they consent to participation in the study, will be provided information regarding the risks of alcohol use in pregnancy and appropriate referrals will be offered.

Procedures: Structured Interviews: At intake at approximately 12 weeks gestation, and at 22, 28, and 34 weeks, a structured maternal interview focused on alcohol consumption around the time of conception and in the previous two week interval based on the instrument used by Jacobson[16-17] and set as a standard by the dysmorphology core, will be administered at the diagnostic center or pediatric hospital by a specially trained interviewer at the time of each ultrasound scan. Scans: Two specially trained ultrasonographers on staff at each site will perform standard prenatal 2-D scans at each of the specified time points without knowledge of the exposure group of the subject. A standard ultrasound report will be issued for each subject using Ukrainian growth norms to collect growth measures that are standardized for the source population. These measures include occipital frontal diameter, abdominal circumference, humerus, tibia, foot, and amniotic fluid index. Additional measures of brain structure will be included and specialized training provided by Dr. Hull. The 30 subjects who report heavy alcohol use will receive a second 3-D scan at the 28-week visit. Dr. Hull will also provide appropriate training for this. Images will be archived for future analysis using videotape or CD for selected

views on 2-D and 3-D scans. Physical Exam: Neonatologists at each of the four birthing hospitals will be trained by the dysmorphology core to provide the standard examination for structural features of FASD and to take standard digital photographs of the face and hands. The timeline for these procedures is as below.

Oct '03	Jan '04	Apr '04	July '04	Oct '04	Jan '05	Apr '05	July '05	Sept '05
Training	Enroll 40	Enroll 40	Enroll 40	Enroll 40	Enroll 40			Analysis
Start-up			Deliver 32	Deliver 32	Deliver 32	Deliver 32	Deliver 32	Write-Up

Data Handling and Analysis: All data and reports will be entered into a database specifically developed for the project and accessible for data entry at both the Rivne and Lutsk sites. A goal of the database development will be to achieve parsimony with the existing newborn registry software being utilized in these regions as it represents baseline information on all newborns in the oblasts selected for this study. This will facilitate comparison to the population from which the sample is drawn. Confidentiality of the data will be protected at every step of the project in accordance with human subjects requirements. The integrity and confidentiality of the stored data will be the responsibility of the Maternal Child Health authorities for each of the participating oblasts. Data analysis and interpretation will be performed in collaboration with a panel of Ukrainian scientific experts to be appointed by Drs. Wertelecki, Yevtushok and Vihovska.

The general approach of the data analysis will include the following. Prenatal ultrasound measurements will be standardized for gestational age and sex if appropriate at each time point and will be analyzed using alcohol exposure category groupings specific to timing of the ultrasound. Standard regression techniques will be used. Categorical and continuous measures of alcohol consumption over pregnancy will also be developed consistent with the standards set by the dysmorphology core, and repeated measures ANCOVA used to examine prenatal ultrasound data spanning all ultrasound time points. Drs. Hull and Jones will conduct descriptive, qualitative and quantitative analyses of the 3-D ultrasounds in collaboration with the Ukrainian experts. All prenatal and postnatal ultrasound data will be compared with the newborn physical examinations conducted by the neonatologists, and the correlation between abnormal findings on prenatal ultrasounds and physical features consistent with FASD will be explored.

Summary: This pilot project provides the opportunity to identify standard prenatal ultrasonographic markers of FASD as well as to explore the capability of identifying abnormalities in specific brain structures or facial development in fetuses whose mothers report heavy alcohol use utilizing 3-D prenatal ultrasonographic technology. Relative to this project's inter-relationship with other sites in the consortium, a postnatal ultrasound pilot study is proposed for a site in Seattle, Washington, and standard prenatal ultrasound data on growth will be a component of the prospective consortium project proposed in the Moscow Region of Russia. Similarly, these data can be compared to data previously obtained and to be collected at the South African site. In addition, the prenatally ascertained children in this study will be available for neurobehavioral testing with advancing age.

The successful completion of this pilot study will provide valuable information relative to earlier diagnosis of FASD. Furthermore, the training procedures and implementation of standard study protocols in Ukraine as part of this project will provide a basis for broader dissemination of these materials throughout the country, thereby improving the ability of Ukrainian physicians and other health care professionals to provide medical care with respect to FASD. Finally, the existing infrastructure that has been established in Ukraine over the last five years, as well as the suspected high prevalence of both maternal drinking and FASD in this country, make Ukraine a site with exceptional potential for development of future projects that could address a broader range of the goals of the consortium.

3. Neonatal Ultrasound Study to Detect Fetal Alcohol Brain Damage -Streissguth

The objective of this project is to develop methods and procedures for the earliest detection of babies born with prenatal brain damage from alcohol. This specific proposal is for a pilot study to demonstrate the effectiveness of an innovative new method for detecting not just those with the full FAS, but also those with the full spectrum of fetal alcohol damage. It is our hope that this pilot study, if successful, will allow us to obtain NIH funding for a full-scale study of several hundred babies to be carried out in several cross cultural sites simultaneously through the CIFASD consortium, including Seattle, South Africa, the Ukraine, and others. The project proposed here is a downward extension to infancy of our previous work on morphometric-neuropsychological deficits in adults and adolescents, which had over an 80% detection rate for patients with FAS/FAE vs. controls. Here, we propose to use neonatal cranial ultrasound to obtain the images, rather than Magnetic Resonance Imaging (MRI) as we did with older patients. Here the challenge is greater, as we must also make determinations about which newborns to scan for alcohol effects - in contrast to our MRI work, which involved diagnosed patients, all of whom were known to be alcohol affected vs. controls. We have demonstrated in a small

feasibility study that our procedures work, but we need a larger sample size to demonstrate quantifiable differences in the hypervariance signal between alcohol-exposed and unexposed infants.

Here, we propose to enroll 50 mother/child pairs: 25 recently postpartum mothers who are heavier drinkers and at risk of having alcohol affected offspring according to BARC, our binge-alcohol rating criteria and 25 non-drinking or lightly drinking mothers. An HDL Ultrasound Scanner using a C8-5 Pediatric Cephalic Transducer with cine memory will be used to obtain multiple images of each baby's corpus callosum (CC) in the midsagittal plane. These will be sent electronically to our consultant Fred Bookstein for data preparation and analysis. We predict that the exposed children will exhibit corpus callosum that are hypervariable in shape compared to controls.

The public health goal of this project is to facilitate early intervention with alcohol affected babies and their mothers. The consortium goal of this pilot study is to develop a brain-based protocol that can be used cross-culturally for the neonatal detection of babies who have the whole spectrum of fetal alcohol disorders. Together, we will be testing a model that can be built into any modern neonatal unit in almost any country of the world, and will be suitable even for babies of those highest risk mothers who fail to receive prenatal care.

Specific Aims. This pilot project aims to demonstrate that our methodology for averaging cranial ultrasound images of the corpus callosum (CC) can differentiate between alcohol-exposed and unexposed newborns.

Background and Rationale. It is well known that alcohol is teratogenic and that the brain is the most vulnerable organ to the effects of prenatal alcohol exposure (1). It is also well known that neurobehavioral deficits have been associated with prenatal alcohol exposure in both laboratory studies (2) and in epidemiological studies of humans beginning with day 1 of life (3) and continuing into adolescence (4,5) and adulthood (6,7). The corpus callosum (CC) is one of the areas of brain most visibly affected by prenatal alcohol, ranging from complete agenesis, to dysgenesis, and to mild hypoplasia (8-13). During early embryonic development, glial cells in the developing CNS are crucial in providing a framework for the forming nervous system, and assisting neuronal migration (14-16). In culture, the astroglial cells have a greater sensitivity to alcohol than neurons, and even moderate levels of alcohol delays growth and maturation of these cells and induces alterations in astrocyte development. The CC appears to be an important focus for fetal alcohol damage.

Agenesis of the CC (ACC) has been found in 2.3%of a developmentally disabled population based on chart review (17). Mental retardation/developmental disability was noted in 73% of patients with ACC in a literature survey (18). In 1991 Jeret and Serur (19) predicted that as FAS becomes more widely recognized, it will become the most common syndrome associated with ACC and that MRI may assist in the diagnosis. The next year, Mattson, Riley, and colleagues published the first report of a child with FAS who had ACC detected with MRI in a case report that also described neuropsychological deficits (20), beginning their decade of work in this arena (11-13).

Thirty years have now elapsed since Jones and Smith identified Fetal Alcohol Syndrome (FAS) in infants (21). It is 24 years since Hanson (22) in our Seattle Prospective Longitudinal Study (SPLS) demonstrated that a skilled dysmorphologist could identify, on blind clinical examination of newborn babies of moderate- to heavy-drinking mothers, those (with both FAS and FAE) who were significantly different from those of light drinking or abstaining mothers. Surprisingly, there are <u>still</u> no methods developed for the easy clinical detection of newborn babies who are fetal alcohol affected, and no clear protocols for diagnosing the full spectrum of fetal alcohol affected babies in clinical settings. We address those problems here.

Preliminary studies from our Unit. In recent work (through AA-1086) we have utilized a brain-based methodology for measuring both landmarks and curves from MR images using a protocol developed by Fred Bookstein. In a sample of 180 subjects, those with both FAS and FAE could be detected at better than 80% sensitivity and specificity with respect to age and sex matched controls (23-24). Detection was by hypervariability in CC shape, which we found to be a stronger signal for alcohol than size or volume. It is this finding that we hope to replicate with neonatal ultrasound.

When these MRI data were combined with scores from a neurobehavioral battery, they produced a striking quadratic discrimination of FAS/FAE from controls, with nearly 90% sensitivity and 93% specificity. Subjects with the thickest callosa had deficits in executive function (EF); those with the thinnest callosa had deficits in motor function (25). (See Appendix these 3 papers: (23-25)

Using the database from our SPLS (AA-01455), Helen Barr developed a binge-alcohol rating criterion (BARC) based on self-reported drinking patterns during and prior to pregnancy (26). BARC+ scores derive from a weighted sum of monthly frequency of 3-4 drinks per occasion plus monthly frequency of 5 or more drinks per occasion. BARC+ predicted an elevated risk of having offspring diagnosed ARND, FAS, and FAE (26) and an elevated risk of compromised neurocognitive function in adulthood (27). BARC can easily be calculated from our Hospital Screening Questionnaire

(HSQ) (28), a one-page self-report screening form that we have used to screen over 9000 women the day after delivery at hospitals in Seattle and Tacoma [first for a big cocaine study (29) and then for our Birth to 3 Parent-Child Assistance Program (30,31) and now for our current CSAP study, headed by Therese Grant].

In a feasibility study for the present proposal, valid ultrasound scans were obtained on 4 heavily exposed (BARC+) neonates and 4 unexposed. Between 8 and 29 CC images were collected per baby and these were averaged using a method developed by Bookstein (32). One exposed infant demonstrated partial agenesis of the splenium of the CC, a finding observed in 3 of the 120 adult/adolescents with FAS/FAE in our MRI study (24). That this anomaly can be visualized using averaged ultrasound images at 13 weeks of age in the baby of a heavily drinking mother is very important for the potential of this work for early diagnosis and intervention. Of the other 3 exposed babies, one had an abnormally thin CC and another had abnormal thinning of the anterior arch. We note from this feasibility study, not only the unusually large proportion of CC abnormalities in BARC+ neonates, but also that the ability to visualize these anomalies was enhanced by the averaging of the images with the Bookstein procedures. At the end of the Pilot Study data collection, when a larger sample size is available, the data will be quantified in a manner similar to that presented in our Brain/Behavior Study of MR images (23,24), but specifically designed for ultrasound images.

Rationale. The early embryonic development of the CC coincides with a time when women use more alcohol, as they may not yet know that they are pregnant. This is congruent with our SPLS finding that alcohol use prior to pregnancy is more strongly related to neurobehavioral outcomes, than are alcohol use scores obtained at mid-pregnancy. Our use of the HSQ and the BARC as a first screen for selecting mothers whose babies may be at risk of fetal alcohol brain damage should be an effective strategy that could be used for identifying mothers drinking heavily during this early embryonic period (or throughout pregnancy) in any delivery or postpartum unit. Our feasibility study demonstrates the unique opportunity we have during these first few months of postnatal life before the fontanelle closes, to image the CC, using neonatal cranial ultrasound, the CC, the same brain region which we found to be so fruitful for discriminating heavily exposed adolescents and adults from controls. The innovative work of Bookstein in averaging many incomplete ultrasound images to produce a single "averaged" image for each baby has permitted enhanced visualization of the CC. Our feasibility study findings that three of four BARC+ babies (and zero of four unexposed babies) had some CC anomalies on visual inspection of the averaged scans shows the potential of the proposed methodology. Detecting newborn babies with FASD is the first step in developing appropriate interventions for mother and child. At the present time, help is available for mothers in our P-CAP program and others, to prevent future alcohol-affected births, but no one is detecting babies who themselves are in need of help.

The purpose of the Pilot Study is to demonstrate that the signal we glean from neonatal cranial ultrasound images is strong enough to show a significant shape variance difference in the anticipated direction between alcohol-exposed and unexposed babies, utilizing just 2 data collection points: maternal alcohol use and neonatal ultrasound. The pilot is not a "detection" study by itself, but merely a demonstration that the ultrasound CC signal will, with sufficient sample size and two additional data collection points (dysmorphology and neurobehavior), be strong enough to support funding a full-fledged Detection Study of Fetal Alcohol Spectrum Disorders (FASD) in Neonates. A successful Pilot Study should make the full detection study possible through the consortium.

METHODS

Subjects. Recruitment of appropriate subjects will be through a modified screening/referral process at University Hospital in Seattle, Tacoma General Hospital in Tacoma, (which collectively have 2300 births/year) and other community venues. An ongoing CSAP study indicates that 3% of the delivering mothers are BARC + and that about 6 BARC + mothers a month are delivered, from whom at least 2 exposed babies and at least 2 controls will be enrolled each month. Eligibility for the Pilot Study includes the following criteria: Mother consents to participate, is BARC+ or BARC- (controls), is not deemed medically unsuitable for study, and does not live too far away. Baby is not under 37 weeks gestation, is a singleton birth, has no serious medical complications or brain anomalies not known to be caused by alcohol (i.e., perinatal strokes), does not have a previous sibling enrolled in the study, is between birth and 3 1/2 months old, and can be successfully scanned. Control babies will be stratified for nicotine exposure. All data collection will occur in Year 1.

Additional Procedures include the Pregnancy Drinking Calendar we developed for our Feasibility Study in order to obtain a consecutive view of alcohol exposure throughout gestation, and the UWASI (University of Washington Addiction Severity Index, a well standardized interview that we modified for use with high risk pregnant women). The UWASI reveals a clinical picture of the duration and intensity of alcohol use and abuse, and of other drugs.

All data will be obtained and analyzed in accordance with Human Subjects without knowledge of a baby's exposure history. Dr. Bookstein will train the technicians in our procedures for "freezing" multiple ultrasound scans of the CC per baby and transmitting the data electronically. Mothers suspected of having significant alcohol problems will be given

appropriate community referrals if not already in P-CAP, our state-funded program for high-risk mothers. The Ultrasound Radiologist, Dr. Sze, will report any clinically significant ultrasounds to our neonatologist, Dr. Gleason, for appropriate action.

Ultrasound images. The same ultrasound protocol used in our Feasibility Study will be followed in this Pilot Study. We use a HDL 5000 Ultrasound Scanner using a C8-5 Pediatric Cephalic Transducer with cine memory. These images can be acquired on any machine that includes a cine freeze frame capacity along with the ability to export these frames on some electronic medium. The machine operator is instructed to image the midsagittal plane, as indicated by characteristic midline structures such as the aqueduct or the falx. Such frames are selected "on the fly" in a redundant fashion (perhaps 24 in total, of which 10 or so are ultimately used for processing). Usable ultrasound data, collected in this fashion on 50 babies, will be transmitted electronically to Bookstein, who has previously developed and published the basic idea of image averaging (32).

Data Preparation and Analysis, to be carried out by Fred Bookstein in Year 2:

Image Averaging: The unwarped images representing multiple frames of a session are first inspected to locate a subset that appear to be sufficiently coplanar to share image structures such as the margin of the medial thalamus. Working in the Edgewarp program package (33), fiducial curves are selected on all the images and averaged within the subject. The individual ultrasound images are then unwarped to the average, and the unwarped images averaged. In this composite average, most of the outline of the CC is visible (which is not true of the individual images before averaging).

Measurement. Still working in Edgewarp, the outline of the callosum is fitted by a smooth curve connecting a sequence of points that are individually midway up the gradient of the composite (averaged) outline shape. From the fitted curve, we extract size measures (overall length and area, for instance), measures of arch form (for instance, height), and a series of measures of arch thickness along the full length of the structure.

Data Analysis involves examining all the statistics of the curve using the same methods that Bookstein and our team developed for our MRI Study (23). We will examine the distribution of all the size and shape measures simultaneously by alcohol exposure data. We expect the data for the unexposed to cluster in the center of this distribution, surrounded by a wide variety of anomalous size/shape profiles representing mostly exposed infants. Figure 1, below, depicts the hypervariability of CC shape that is the key finding in our MRI Study in terms of differentiating alcohol-exposed from unexposed subjects. We expect to show a similar figure from the analysis of our 50 neonates. Figures 2 and 3, below, depict two averaged ultrasound images from our Feasibility Study. When this Pilot Study is concluded, we can analyze 50 sets of ultrasound data like this, to hopefully produce a hypervariability finding similar to that depicted in the figure from our MRI study (Figure 1 below). Examination of these 3 figures reveals the necessity for a 2-step process in the data analysis. While the averaged images might permit a clinical interpretation in the most severely affected babies, quantification is necessary for analysis of the spectrum of alcohol effects.

Power calculations. Based on the findings of our CC studies of adolescents and adults with FAS and FAE, we expect three times as much variance in the affected group as in the unexposed. Assuming half of a sample of 25 exposed are affected, the chance of finding a hypervariance in the exposed subsample is about 97%. Such a replication of the adolescent-adult finding in this age range would certainly justify initiating a true detection study with several hundred babies and funding in unison with other collaborating sites (Ukraine, South Africa, and perhaps Finland, all areas of high alcohol use among women). A statistically significant difference in CC shape variance between controls and highly alcohol-exposed infants in a small study like this suggests that differences are large enough to make actual detection possible when a larger sample is accrued. (A nominally significant difference in a large sample would not necessarily suggest that effective detection was likely).

Time Line

April/May 2003: Obtain Human Subjects approval.
May 19-23, 2003: Fred Bookstein in town and trains sonographers.
Oct. 1, 2003: Data collection for this Pilot Study will occur in Year 1, at the rate of at least one ultrasound per week. Data preparation, analysis and grant writing for the full detection study will be in Year 2.

Significance. This study addresses what we believe to be two of the main public health problems in the field of clinical alcohol teratogenesis: [1] failure to detect clinically affected babies early in life and [2] failure to detect brain anomalies in babies that could foreshadow later neurobehavioral problems caused by prenatal alcohol. Both of these problems prevent the initiation of early interventions, and even handicap research on what appropriate early interventions might be. We know, however, from our own research on secondary disabilities (34) that an early diagnosis relates to a significant reduction in such adverse outcomes as school dropout and expulsion, trouble with the law, and alcohol and drug problems.

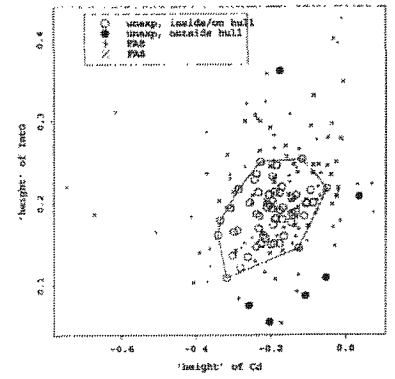
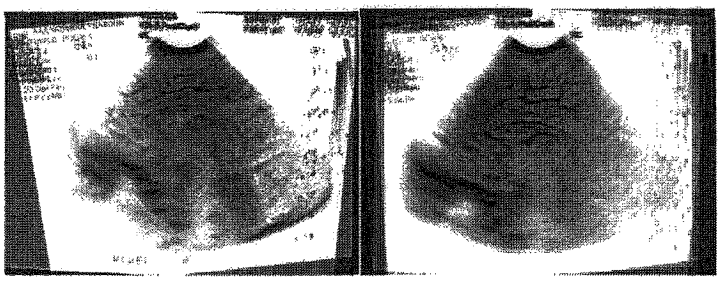


Figure 1



Baby 8, 3 wk, control

Baby 14, 13 wk, exposed

Figure 2 Figure 3

We hope that the successful completion of this Pilot Study will demonstrate both the power of this new postnatal ultrasound methodology and the ease and reliability of these procedures in the neonatal care milieu. We are hopeful that this Pilot Study will permit other sites to utilize these methods and to join with us in a full-fledged detection study of several hundred children, ultimately possible through R01 funding.

Relationship of this pilot to the consortium. The full-fledged detection study for FASD in newborn babies will require two more data points (neonatal dysmorphology and 12 month infant neurobehavioral assessment). Assuming that our Pilot Study is successful, we will immediately seek R01 funding for the full study, which will require several hundred babies.

We will collaborate with Ken Jones of the Dysmorphology Core to train our Pediatric Fellow because we want to assure that the diagnoses we use in this FASD detection study are congruent with the diagnostic traditions of our previous studies with David Smith and his fellows, and with the rest of the work in the CIFASD. We will collaborate with Sandy Jacobson of the Infant Neurobehavioral Core of the CIFASD to train our Developmental Psychology Fellow to carry out the procedures for our 12-month neurobehavioral assessment to be congruent with her South Africa Study. These include infant measures of Executive function and Numerosity in addition to the Bayley Scales of Mental and Motor Development, (and perhaps A not B). Fred Bookstein, consultant to our Unit, will train the Ultrasound Radiologists in Ukraine and South Africa so that our data across all sites will be comparable. Electronic transmission of data from all sites will not only enable Dr. Bookstein to average and analyze the Neonatal Ultrasound data collectively but also to transmit Ultrasound "diagnostic" data back to collaborators. Other consortium sites can also be involved and Finland has also expressed interest.

This Neonatal Ultrasound component will be strengthened in two important ways by the mission of this consortium/collaboration. Most importantly, it would be impossible for us in Seattle to obtain enough "pure" alcohol exposed babies in a reasonable time to conduct a full fledged detection study of FASD without the collaboration of at least Ukraine and South Africa. Inclusion of some drug exposed babies will not preclude a successful pilot study of our Neonatal Ultrasound to detect CC differences, but to test the ultimate goal of detecting FASD the study will require several hundred babies who are alcohol but not drug exposed (and controls) who all have neonatal ultrasound, neonatal dysmorphology and 12 month neurobehavioral data. Additionally, our Neonatal Ultrasound "diagnoses" will be available for collaborators to enhance their infant studies.

In addition to our Neonatal Ultrasound, our team at the Fetal Alcohol and Drug Unit is ready to help in any way we can to facilitate the important work of this cross cultural, multidisciplinary endeavor.

Key Personnel on project. Ann Streissguth Ph.D., Christine Gleason, M.D., Raymond Sze, M.D., Fred Bookstein, Ph.D., Therese Grant, Ph.D. Additional personnel contributing from out unit. Paul Sampson, Ph.D., Helen Barr, MA, MS, Paul Connor, Ph.D., Janet Huggins, Ph.D., and Cara Ernst, M.S.

4. Comparison of Three Diagnostic Modalities in FASD and Related Disorders

A. Specific Aims. This pilot project will be a collaborative effort involving and articulating our local efforts with those of the Facial Imaging Core. The goal of the project is to test the feasibility of applying and integrating new computerized imaging technology into the clinical evaluation of individuals with FASD and related disorders. It will analyze and otherwise compare clinical (i.e., morphological and neuropsychological) and selected anthropometric measures with 3-D digitized image data of individuals with FASD and related conditions. We will generate and analyze data from a group of individuals with FAS, an age and gender matched unexposed control group, and a disease control group consisting of patients with Williams syndrome, an autosomal dominantly inherited disorder of elastin biosynthesis. We will evaluate in particular the discriminating power of 3-D digital imaging relative to these disorders and will assess the feasibility of translating these data to the screening of large populations of individuals at risk of FAS. Comparability of these data may allow for widespread screening and diagnosis of FASD.

The specific aims of this project are:

1) To evaluate the sensitivity and specificity of selected clinical and anthropometric craniofacial measurements among a group of racially diverse American patients with FAS and phenotypically similar disorders.
2) To compare the above measurements with those derived from 3-D digital imaging.
3) To analyze and otherwise reflect on the relationships between the two assessment measures and to analyze which of these measures best predict a diagnosis of FAS or its milder physical manifestations

B. Background and significance of research

Fetal alcohol spectrum disorder (FASD) refers to the breadth of growth, structural, developmental, and social disabilities experienced among individuals with prenatal exposures to the human teratogen, ethyl alcohol. Initial reports (Lemoine et al, 1968, Jones et al, 1973) called attention to the growth deficits, developmental disabilities, and physical differences of moderate to severely affected individuals and this recognizable pattern of malformation was termed fetal alcohol syndrome (FAS) by Jones and Smith in 1973. Subsequent reports (Hanson et al, 1978) called attention to the physical and developmental disabilities of more mildly affected individuals, and more recent data have disclosed that cognitive and functional impairments are likely the most consistent disabilities among individuals with FAS (Streissguth et al, 1985, Streissguth, 1991). Moreover, the scientific studies of Mattson and others illuminate the anecdotal experience of providers and families, namely that these cognitive and functional impairments frequently occur in the absence of physical features of FAS (Mattson et al, 1995). These cognitive, functional and social impairments have been referred to as alcohol-related neurodevelopmental disorder (ARND) (Stratton and Howe, 1996). Recognition of the breadth of morbidities associated with prenatal alcohol exposure prompted consideration of the term fetal alcohol spectrum disorder (FASD) in 2002.

Prevalence rates of FAS range from 1 - 2 per 1000 births, and it is estimated that as many as six to ten times as many prenatally exposed individuals suffer from ARND. Presently, FASD is identified by recognizing the pattern of morphological (e.g., the craniofacial) features associated with FAS; there is no agreement on the diagnostic criteria for ARND. Diagnosis of FASD is labor intensive, requiring a comprehensive, transdisciplinary evaluation that includes the ascertaining of maternal drinking histories, reviewing or generating developmental or neurocognitive data, and assessing the morphological characteristics of at-risk individuals. Clinical resources (e.g., trained physicians, comprehensive evaluation centers) for recognition of FASD are limited, and the number of centers in which clinical research on FAS is conducted is even smaller. Efforts to train pediatricians with little or no previous experience in FAS diagnosis have been successful (Robinson, et al, 2001), but the number of such expert clinicians still remains small. Thus, many affected individuals, particularly those with milder physical manifestations of FAS, often go undiagnosed, and opportunities for early, effective interventions are missed.

The inadequacy of resources for diagnosis of FASD has prompted the use of other modalities for screening and diagnosis. Screening instruments such as so-called "dysmorphia checklists" using weighted morphological scales have shown promise in individual studies (Burd et al, 1999), but none have been validated. Similarly, photographic analytical studies such as those of Astley and Clarren (1996, 2000) have met with success in the hands of the investigators but the technique has not been validated in other centers. Simple statistical analyses of morphological data from our clinical sample suggest that short palperal fissures or smooth philtral columns are sensitive indicators of FAS in exposed individuals. Recent anthropometric studies from Indiana University demonstrate that full-blown FAS and even milder cases (e.g., partial FAS) can be identified with sensitivity and specificity by adding simple craniofacial measurements (e.g., midfacial depth [distance from tragus to nasal base] and maxillary arc [distance from tragus to tragus via nasal base]) to the morphological

examination. Preliminary data from Indiana University also suggest that craniofacial measurements derived from three-dimensional computerized imaging compare favorably with clinical and anthropometric measures.

A limitation of the above studies is that few have had sufficient subjects from diverse racial phenotypes or have assessed the reliability of discriminating FAS from other disorders associated with craniofacial and developmental disabilities that are similar to those of FAS (*i.e.*, FAS phenocopies). In Buffalo, where 30% of our FAS clinical sample are of African American background, we have an opportunity to evaluate the discriminating power of 3-D digital imaging in the diagnosis of FAS and similar disorders across American populations. The sensitivity and discriminating power of such studies coupled with narrow band neuropsychological studies of executive functioning, memory and learning, rather than global tests of cognition (*e.g.*, IQ tests) may provide a more complete perspective of FASD.

We will test the following hypotheses:

1) Statistical analyses of morphological and anthropometric craniofacial data among patients with FASD, Williams syndrome, and controls will yield significant differences relative to each diagnosis.

2) Digital imaging techniques will confirm these differences at a statistically significant level and discriminate FAS from disorders with similar facial features.

Methods and pilot data:

This will be an observational study that compares and correlates the clinical, neuropsychological, and selected anthropometric characteristics of individuals with FAS, Williams syndrome, and unexposed controls with 3-D imaging techniques. The FAS Evaluation program in the Division of Genetics, Department of Pediatrics at SUNY Buffalo School of Medicine and Biomedical Sciences provides clinical diagnostic services to individuals with known or suspected FASD. Patients are referred by their primary care providers, human service workers, or are self-referred, largely as a result of our active community outreach efforts. Under a contract from the NIAAA, we demonstrated our ability to implement a project of "academic detailing", engaging area physicians to enhance their awareness of FASD in Buffalo, NY and refer patients in need of screening or diagnosis of FASD. We demonstrated that engagement and periodic, on site contact with physicians increased knowledge and skills concerning FAS screening and increased the appropriate referral of individuals suspected of having FASD. We will build on this record of physician engagement and referral to conduct this study.

Data from our FAS clinical program suggest that four variables (growth deficiency, short palpebral fissure, altered philtral columns and thin vermilion border) are highly sensitive markers of FAS in our population, predicting an FAS diagnosis with 93% accuracy. Our data are consistent with those of other studies that demonstrate that patients with full-blown FAS and its milder morphological manifestations (*i.e.,* partial FAS, PFAS) can be identified with 96% to 100% accuracy using head circumference, palpebral fissure measurements and lip and philtral assessments (Astley and Clarren, 1996) or head circumference in conjunction with other anthropometric measurements such as bigonial breadth, facial depth and maxillary arc (Moore et al, 2001).

Individuals with FAS, partial FAS (PFAS) (n=40), Williams syndrome (n=20) will be selected from the FAS Evaluation and the Williams syndrome programs in the Division of Genetics, Department of Pediatrics at the State University of New York (SUNY) at Buffalo. A group of age, gender, and race-matched comparison subjects (n= 50 - 60) will be recruited from primary care (e.g., pediatrics, family medicine) practices in the Buffalo area.

Diagnoses of FAS and partial FAS (PFAS) will have been made by the PI in accordance with IOM criteria (Stratton and Howe, 1996) and will have been assigned to subjects who display the following:

I. FAS with confirmed maternal alcohol exposure

A. Confirmed maternal alcohol exposure

B. Evidence of a characteristic pattern of minor facial anomalies, including:
   - short palpebral fissures (less than or equal to the 10th centile)
   - midfacial hypoplasia
   - thin vermilion border of the upper lip (score 4 or 5 on the Clarren lip/philtrum guide)
   - smooth philtrum (score 4 or 5 on the Clarren lip/philtrum guide)

C. Evidence of prenatal and/or postnatal growth retardation
   - height or weight less than or equal to the 10th centile, corrected for racial norms, if possible

D. Evidence of CNS neurodevelopmental abnormalities, including one or more of the following:

head circumference less than or equal to the 10th centile
structural brain abnormalities
neurological hard or soft signs (as age appropriate)

II. FAS without confirmed maternal alcohol exposure

1B, 1C, and 1D as above

III. Partial FAS with confirmed maternal alcohol exposure

A. Confirmed maternal alcohol exposure

B. Evidence of some components of the pattern of characteristic facial anomalies, including at least *two* of the following:
   a. short palpebral fissures (less than or equal to the 10th centile)
   b. thin vermilion border of the upper lip (score 4 or 5 on the Clarren lip/philtrum guide)
   c. smooth philtrum (score 4 or 5 on the Clarren lip/philtrum guide)

C. One of the following:
   a. Evidence of prenatal and/or postnatal growth retardation (*e.g.*, height or weight less than or equal to the 10th centile, corrected for racial norms, if possible
   b. Evidence of CNS neurodevelopmental abnormalities, including one or more of the following:
      1. head circumference less than or equal to the 10th centile
      2. structural brain anomalies
      3. neurological hard or soft signs (as age appropriate)

D. Evidence of a complex pattern of behavior or cognitive abnormalities that are inconsistent with developmental level and *cannot be explained by genetic predisposition, family background or environment alone*. This pattern may include learning difficulties; deficits in school performance; poor impulse control; problems in social perception; deficits in higher-level receptive and expressive language; poor capacity for abstraction or metacognition; relative deficits in mathematical skills; or problems in memory, attention, or judgment

Diagnosis of Williams syndrome will have been made by clinical examination by the PI with documentation of abnormal fluorescence *in situ* hybridization ("FISH") analysis of the characteristic submicroscopic deletion in the elastin gene region on the long or "q" arm of chromosome 7 (7q). Subjects with other malformation syndromes or with prenatal exposures to known human teratogens such as phenytoin will be excluded from this pilot project.

The PI, who is a member of the Dysmorphology Core of the CIFAS, will perform morphological examinations on all subjects. The examinations will be standardized and articulate with those of the Dysmorphology Core Examination (Appendix) and will include measurements of height, weight, head circumference, palpebral fissure length, inner canthal distance, ear length, and philtral length. Weight, length or height, and head circumference data will be compared with centiles for infants and children of the same age and sex using the National Center for Health Statistics growth curves (CDC; www.cdc.gov/nchs/). Growth data for premature infants will be adjusted for gestation until age 24 months and calculated using the most appropriate growth curves for the ethnic distribution of the sample. Palpebral fissure length, inner canthal distance, and philtral length also will be compared with distributions for infants of the same age. Assessment of the philtral columns and upper lip will be quantitated with the lip-philtrum guide of Astley and Clarren (1996). For the purposes of this pilot project the measurements of midfacial depth (distance from tragus to midpoint of nasal base) and maxillary arc (distance from tragus to contralateral tragus) in accordance with the studies at Indiana University (Moore et al) will be added to the morphological examination.

After morphological examinations and assignment of diagnoses, subjects will undergo digitized imaging of the face in accordance with the protocol of the Imaging Core. The local imaging professional is a member of the medical photography group of the Women and Children's Hospital of Buffalo. He has over thirty years' experience in photography and digital imaging technology, and will have attended one or more sessions on 3-D imaging at Indiana University before the local imaging protocol is implemented. The PI and his staff will coordinate clinical evaluations and imaging, and he and the imaging professional will work closely to ensure local quality assurance of the imaging protocol and resultant images.

Data on morphology will be analyzed with SPSS statistical software program and will articulate with data of the Dysmorphology and Facial Imaging Cores.

Informed consent (see Appendices)

Budget justification. Project funds will be used to compensate the professional time of the principal investigator, project program coordinator, project patient care coordinator, and the imaging professional. Funds also will be used for honoraria to project subjects.

Timetable

Year one
Months 1 – 6
- Attend training to develop expertise in the use of 3-D digital imaging hardware and software
- Contact potential subjects for imaging studies
- Conduct examinations and begin imaging protocol (one week on four occasions)

Months 6 - 12
- Continue subject examinations and imaging (one week of imaging)
- Begin preliminary data analysis
- Begin project evaluation

Month 10 – 12
- Project-to-date evaluation and planning for Year 2

Year 2
- Continue examinations and imaging of subjects
- Continue data analysis
- Continue project evaluation
- Prepare written evaluation report on pilot project
- Seek additional funding (e.g. R01, etc)

Relationship to the Consortium. This pilot project articulates the goals of the Consortium by presenting opportunities to share data derived from our ethnically diverse population. Moreover, by adhering to consistent protocols across the Consortium, our local data will be comparable with those of other sites.

THE PILOT PROJECT CORE

E. **Human Subjects**. Each of these projects has applied for human subject approval at their own institution. The PI of the Pilot Project Core will insure that all human subjects issues have been dealt with and approvals are up to date. Some consent forms from specific projects are provided in the Appendix.

F. **Vertebrate Animals. NA**

G. **Consortium/Contractual Arrangements**

I. **Letters of Support**

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