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.**

Early identification of children with FASD has been limited by the difficulty of reliably evaluating facial dysmorphology and because investigators have not yet identified a set of neurobehavioral deficits specifically related to prenatal alcohol exposure. However, a new generation of neurodevelopmental measures that have been linked more specifically to neural processes and pathways has the potential to improve FASD diagnosis. We have recently completed a prospective study of 159 infants born to mothers from the Cape Coloured (mixed race) community in South Africa, which has confirmed the exceptionally high incidence of maternal alcohol abuse and dependence and FAS previously documented in this population. Arithmetic and executive function (EF) are among the most consistently affected domains in older children with FASD. In our Cape Town cohort, we were able to detect deficits in these domains in infancy using two innovative infant assessments: a numerosity test, which assesses magnitude representation, a precursor of arithmetic that has been linked to inferior parietal function, and the A-not-B test, an early precursor of EF. New data from our Detroit prenatal alcohol exposure cohort also provide evidence of a specific deficit in magnitude representation in older children and poorer conflict monitoring, an aspect of EF believed to be mediated by the anterior cingulate cortex.

This component of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) will follow up our Cape Town cohort at 4 and 6 years of age. The specific aims are: (1) to administer new narrow-band tests of arithmetic and EF to determine which elements of these domains appear to be core deficits of FASD; (2) to administer event-related potential (ERP) assessments of magnitude representation and recognition of facial emotional expression, two domains that are hypothesized to relate specifically to FASD; (3) to test the hypothesis that two moderator variables—maternal age and the absence of an ADH2*2 allele—can improve the identification of FASD in prenatally exposed children; (4) to assess the predictive validity of infant numerosity and A-not-B in relation to cognition and attention in early childhood; (5) to determine the degree to which measures of craniofacial variation derived from 3-D photography can improve FASD diagnosis; and (6) to administer additional neurobehavioral assessments in common with other CIFASD projects to provide data on the sequelae of FASD that can be pooled and compared across age, site and ethnic group. Improvement of diagnosis in infancy and early childhood and a better understanding of the specific domains of neurobehavioral function affected by fetal alcohol exposure are critically important for the development and implementation of targeted, effective interventions for FASD.

PERFORMANCE SITE(S) *(organization, city, state)*

Wayne State University, Detroit MI

University of Cape Town, Cape Town, South Africa

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
Jacobson, Sandra W.	Wayne State University	Principal Investigator
Carr, Lucinda G.	Indiana University	Geneticist
Jacobson, Joseph L.	Wayne State University	Co-Principal Investigator
Jordan, Nancy C.	University of Delaware	Collaborator
Khaole, Nathaniel	University of Cape Town	Pediatrician/Dysmorphologist
Molteno, Christopher D.	University of Cape Town	Co-Principal Investigator
Nelson, Charles A.	University of Minnesota	Collaborator
Viljoen, Denis L.	University of the Witwatersrand	Co-Principal Investigator

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

A. SPECIFIC AIMS

The diagnosis of fetal alcohol syndrome (FAS) is facilitated by its characteristic dysmorphic facial features, including short palpebral fissures, flat midface, thin upper lip, and a flat or smooth philtrum. By contrast, the diagnosis of alcohol-related neurodevelopmental disorder (ARND) depends on "evidence of...behavior or cognitive abnormalities that are inconsistent with developmental level" and a history of heavy maternal alcohol use during pregnancy (Stratton et al., 1996). This diagnosis is difficult because the neurobehavioral abnormalities known to be related to fetal alcohol exposure are relatively nonspecific and reliable retrospective information regarding pregnancy drinking is difficult to obtain. In addition, despite extensive clinical evidence pointing to the importance of socioemotional dysfunction in fetal alcohol spectrum disorders (FASD), there has been little research on the relation of fetal alcohol exposure to specific affective endpoints. This component of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) will focus on identification of the core deficits of FASD. A new generation of neurocognitive and affective measures that have been linked more specifically to neural processes and pathways has the potential to improve our understanding of the etiology of FASD and, thereby, to improve FASD diagnosis and treatment. Moreover, researchers have discovered two moderators of alcohol-related developmental deficit—maternal age and the presence of a particular ADH2 allele—that may further facilitate the identification of affected individuals. In addition, 3-dimensional digitizing technology to be implemented by the CIFASD Facial Imaging Core has the potential to provide a new quantitative assessment of facial form that could aid in the diagnosis of fetal alcohol disorders in children whose craniofacial deviation is subtler than that seen in full FAS.

We have recently completed a prospective study of 159 mothers and infants recruited during pregnancy from the Cape Coloured (mixed ancestry) community in Cape Town, South Africa, where recent studies have documented an exceptionally high incidence of FAS. Of the 134 infants who have been diagnosed to date, 21 met standard criteria for FAS; 49 were heavily exposed, non-FAS; 12 were exposed prenatally at low-to-moderate levels; and 52 were born to abstainers. Given the exceptionally high incidence of heavy drinking and alcohol dependence in women of childbearing age in this community, we were able to test hypotheses and detect effects more rapidly and efficiently than in most prospective fetal alcohol studies. In Cape Town, prenatal alcohol exposure predicted growth and neurobehavioral deficits that were similar to those observed in our prospective, longitudinal study of 480 inner city, African American Detroit children but stronger in magnitude. Prenatal alcohol exposure was also associated with poorer performance on two innovative assessments: infant numerosity, which assesses a rudimentary form of magnitude representation, an important component of arithmetic, and the A-not-B test, which is considered an early precursor of executive function. If these deficits are found to be predictive of alcohol-related dysfunction in childhood, they could be important for the early identification of FASD. Early diagnosis can, in turn, facilitate treatment since early interventions are often most effective.

Arithmetic and executive function have been identified as two of the principal domains affected by prenatal alcohol exposure and are, therefore, particularly promising neurobehavioral indicators from a diagnostic perspective. Because both these domains are complex and multifaceted, one important challenge is to determine which specific aspects or components of these domains are most relevant. We have new data from our Detroit cohort, in which we administered arithmetic tests developed in collaboration with Stanislas Dehaene that are designed to discriminate among three aspects of number processing proficiency—exact calculation, arithmetic approximation, and the child's intuitive conceptual understanding of magnitude representation. Although prenatal alcohol exposure was related to all three dimensions, the strongest associations were with magnitude representation, which is believed to be mediated by neural activity in the inferior parietal cortex.

This finding is consistent with our Cape Town finding of a relation of fetal alcohol exposure with infant numerosity. With regard to executive function, we have begun to administer new, more narrowly-focused tests designed to distinguish between two aspects of executive function described by Jonathan Cohen and associates—cognitive control, which is mediated by the dorsolateral prefrontal cortex, and conflict monitoring, which is mediated by the anterior cingulate cortex.

This component of the Collaborative Initiative will follow up our Cape Town cohort at 4 and 6 years of age. The principal goals are to improve diagnosis of FASD by advancing our understanding of core deficits and their neural substrates and of moderator variables that influence fetal vulnerability and to provide data on endpoints to be assessed at multiple CIFASD sites that will be evaluated collaboratively with other CIFASD investigators. The specific aims are

1. To administer new tests of arithmetic and executive function based on the contemporary models derived from event-related potential (ERP) and neuroimaging studies, in order to determine which elements of these neurobehavioral domains appear to be core deficits of FASD.

2. To better describe the affective components of FASD by examining the relation of prenatal alcohol exposure to specific aspects of affective function using current ERP, social judgment, and teacher reported behavior assessments.

3. To test the hypothesis that two moderator variables—maternal age and the absence of an ADH2*2 allele —can improve the identification of FASD in prenatally-exposed children.

4. To evaluate the usefulness of infant numerosity and A-not-B for early diagnosis of FASD by assessing their predictive validity in relation to the specific elements of arithmetic and executive function found to be associated with prenatal alcohol exposure during early childhood.

5. To determine the degree to which photographs taken using a new 3-dimensional camera, following the protocol developed by the Facial Imaging Core, may make it possible to detect differences among FAS, ARND, and controls and improve the validity of FASD diagnosis by detecting subtle craniofacial anomalies in children with ARND.

6. To administer cognitive and behavioral assessments recommended by the CIFASD Neurobehavioral Core, including the Leiter International Performance Scale and the Cambridge Neuropsychological Testing Automated Battery (CANTAB), to provide data on the neurobehavioral sequelae of FASD that can be pooled and compared across age, site, and ethnic group.

This component of the Collaborative Initiative provides a unique opportunity to improve our understanding of neural processes, underlying mechanisms, and modifying factors associated with fetal alcohol exposure. The data to be collected have the potential to help identify core deficits of FASD, thereby substantially improving diagnosis and permitting earlier identification of affected children and more targeted interventions. We have established a successful, multidisciplinary collaboration with Western-trained medical research collaborators in Cape Town and have assembled an excellent research team. A broad range of control variables has been and/or will be examined, including maternal education and IQ, smoking and illicit drug use during pregnancy, quality of intellectual stimulation provided by the parents, maternal depression and psychopathology, and current maternal drinking, smoking, and drug use, which will permit statistical control for potential

confounding influences in all evaluations of prenatal alcohol effects. The dysmorphology, growth, facial imaging, and neurobehavioral data to be collected in common with other CIFASD projects will benefit from standardized protocols and expertise to be provided by the CIFASD cores, and participation in the Collaborative Initiative will provide a valuable opportunity to evaluate the consistency of the FASD phenotype across a range of ages and racial/ethnic groups. Our cohort will be available to examine promising new diagnostic assessments and hypotheses that emerge in the course of the CIFASD. Where we find effects based on the new, more focused assessments we propose for this project, we will invite other investigators to use these assessments with their cohorts, as well.

B. BACKGROUND AND SIGNIFICANCE

FAS, which is characterized by a distinctive craniofacial dysmorphology (short palpebral fissures, flat midface, thin upper lip, flat or smooth philtrum), growth retardation, and cognitive and/or behavioral dysfunction (Stratton et al., 1996), is the most severe of the fetal alcohol spectrum disorders (FASD). The term fetal alcohol effects (FAE) has been applied to children whose mothers were known to have drunk heavily during pregnancy and exhibit some, but not all, of the alcohol-related dysmorphic features (Streissguth et al., 1991). Many FAS and FAE patients are mentally retarded (IQ < 70), but a substantial proportion perform in the low average to average range (Streissguth et al., 1991; Spohr et al., 1993). The term ARND is applied to children exposed prenatally to alcohol who lack the characteristic FAS dysmorphology but exhibit measurable, albeit generally subtler neurobehavioral deficits (Stratton et al., 1996). Although the impairment is less severe, children with ARND usually exhibit deficits in the domains that are most compromised in FAS. Whereas the unique facial dysmorphology provides specificity to the FAS diagnosis, FAE and ARND are difficult to diagnose because investigators have not yet identified a set of neurobehavioral deficits specifically related to prenatal alcohol exposure.

Neurobehavioral Manifestations of FASD

In contrast with Down syndrome patients, who exhibit impairment in virtually all aspects of intellectual function, FAS patients often perform relatively well on language tests (e.g., Kodituwakku et al. 1995), although some have difficulty with complex language. Two of the most consistent cognitive deficits are in arithmetic (Streissguth et al. 1991; Clarren et al. 1994; Goldschmidt et al., 1996) and executive function (Coles et al., 1997; Kodituwakku et al. 1995; Mattson et al., 1999). Although less attention has been devoted in the research literature to affective outcomes, there is consistent evidence of increased aggressive behavior (Brown et al., 1991; S. Jacobson et al., 1998b), poor social judgment (Carmichael Olson et al., 1992; Streissguth, 1997), and increased long-term psychopathology (Steinhausen et al., 1993). It is important to recognize, however, that each of these neurobehavioral domains is complex and multifaceted and that each has been implicated in children with other disorders, such as attention deficit hyperactivity disorder (ADHD), autism spectrum disorders, and childhood depression. During the past decade, aided by advances in our understanding of the neural substrates and pathways that mediate these behavioral endpoints, advances have been made in breaking down these complex behaviors into more elemental components, some of which appear to be more specifically linked to fetal alcohol exposure.

Arithmetic. Streissguth and associates have found arithmetic to be one of the most severely impaired subtests on the Wechsler IQ Scales among FAS/FAE patients (Streissguth et al., 1991) and ARND children at 7 and 14 years of age (Sampson et al., 1989; Streissguth et al., 1994b), and Goldschmidt et al. (1996) found deficits in arithmetic on an academic achievement test, even after controlling for IQ. Kopera-Frye et al. (1996) examined different types of number processing abilities

in 29 adolescent and adult patients with FAS/FAE matched for age, gender, and educational level with 29 controls. Whereas no group differences were found in the ability to read or write numbers, the FAS/FAE patients performed more poorly on exact addition, subtraction, and multiplication and approximate subtraction. The FAS/FAE patients also performed more poorly on a proximity judgment task ("Is 6 closer to 8 or to 5?") but did not differ on a number comparison task ("Which of these two numbers is larger?") In our Detroit study, however, prenatal alcohol exposure was associated with poorer performance on a somewhat more challenging number comparison task (i.e., where more pairs of numbers differed by only 1 or 2 points) (see "Preliminary Studies" below).

Dehaene (1997) distinguishes "number sense" or magnitude representation, which he considers a biologically-based ability to interpret quantities, from exact calculation, which depends heavily on working memory and rote learning. Dehaene and Cohen (1995) have hypothesized that magnitude representation, which is reflected most directly in number comparison and proximity judgment, is mediated by activation in the left and right inferior parietal cortex. This hypothesis is supported by data from both neuroimaging and lesion studies (Dehaene et al., 1996; Dehaene, 1997). A study of magnitude representation using event-related potentials (ERP) showed increased neural activity in the inferior parietal region with peak latencies and amplitudes that were very similar for comparisons of quantities, whether represented as dots on a domino or as Arabic numerals (Temple & Posner, 1998). Moreover, electrical activity during this task was very similar in 5-year-olds and adults, suggesting that magnitude representation may emerge very early in development. Wynn (1992) has designed a numerosity test, which appears to assess a rudimentary form of magnitude representation in infancy. We have recently found evidence of a specific deficit in magnitude representation in relation to prenatal alcohol exposure in our Detroit cohort on a number processing test adapted from Kopera-Frye et al. (1996) and in our Cape Town cohort on Wynn's (1992) infant numerosity test (see "Preliminary Studies" below).

Other developmental precursors of arithmetic to be assessed include principles of counting (Gelman & Gallistel, 1978; Gallistel & Gelman, 1992; Mix et al., 2002), nonverbal calculation, and number patterns. In counting, the "one-one principle" involves an understanding of one-to-one correspondence; in other words, that one and only one number word can be assigned to each object in a set. The "stable-order principle" states that number words are always assigned in the same order (e.g., 1-2-3, etc.). The "cardinality principle" refers to knowledge that, in counting, the final number word indicates the number of objects in the set. Nonverbal calculation emerges prior to verbal calculation and, by contrast to its verbal counterpart, is unrelated to social class (Jordan et al., 1992) or general cognitive ability (Jordan et al., 1995). The ability to recognize number patterns also emerges during the preschool period (Ginsburg et al., 1997). Patterns in number combinations and the relationships among them include the concept that, if 3 + 3 = 6, then 4 (which is 1 more than 3) + 2 (which is 1 less than 3) also = 6. An intuitive grasp of number patterns can enable the child to use answers from known combinations to solve unknown ones.

Executive function. Executive function, an important aspect of attention, is defined as the ability to coordinate, plan, and execute appropriate responses and to modify behavior flexibly in response to feedback. FASD has been linked to poorer cognitive flexibility on tests of verbal fluency, in which the child is asked to list as many words as possible from a given category (Kodituwakku et al., 1995; S. Jacobson et al., 1998a; Schonfeld et al., 2000). These tests assess the ability to monitor information retrieved from long-term memory for conformity with a prescribed rule. Reduced cognitive flexibility has also been found on a design fluency test (Schonfeld et al., 2000) and the California Trail Making Test (Mattson et al., 1999). Children with FASD also perform more poorly on the Wisconsin Card Sorting Test (Kodituwakku et al., 1995; Mattson et al., 1996; Coles et al., 1997; Carmichael Olson et al., 1998; S. Jacobson et al., 1998a), which assesses the

ability to utilize feedback to alter one's response when the criterion for the correct response changes and the ability to inhibit a previously learned but now inappropriate response. FASD children also perform more poorly on three variants of the Tower of Hanoi—the Progressive Planning Test (Kodituwakku et al., 1995), the Tower of London (S. Jacobson et al., 1998a), and the Tower of California (Mattson et al., 1999). The Tower of Hanoi assesses planning, including the ability to analyze a problem, devise a strategy, monitor one's performance, and modify one's strategy as performance proceeds.

In the Stroop Color-Word Test, the child is shown a page on which color names are printed in the "wrong" color (e.g., "red" printed in green ink) and asked to name the colors as quickly as possible. This "interference" test assesses the child's facility (speed) to name the colors that the words are printed in, which requires him/her to overcome an automatic response to read the printed word. The California Stroop Test adds a "switch" condition, in which some words on the page are surrounded by a box. The child is asked to read a word aloud if it is surrounded by a box but to name the ink color if it is not. Cohen and associates have used functional magnetic resonance imaging (fMRI) to distinguish between two aspects of executive function (MacDonald et al., 2000)—cognitive control and conflict monitoring. Cognitive control is defined as a resource-limited system that guides voluntary, complex actions, such as solving difficult tasks, overcoming habitual responses, and correcting errors. It uses a representation of the attentional demands of the task (a "rule"), which biases the child to respond in conformity with the rule and has been linked in several fMRI studies to activity of the dorsolateral prefrontal cortex. MacDonald et al. (2000) have identified a second component of executive function—conflict monitoring, which provides ongoing feedback indicating whether control is being allocated effectively and is typically activated in tasks which entail divided attention, such as the California Stroop "switch" condition. Conflict monitoring appears to be mediated by activity in the anterior cingulate cortex. Mattson et al. (1999) found that FAS and heavily exposed ARND children performed more poorly in both the interference and switch conditions, although for the ARND children the effect on switch fell below conventional levels of statistical significance.

Affective function. Compared with cognitive and attentional function, relatively little information is available regarding socioemotional impairment in FASD. Using the Achenbach Child Behavior Checklist-Teacher Report Form, Brown et al. (1991) found poorer social competence and more aggressive and destructive behavior in children whose mothers drank throughout pregnancy than in those whose mothers had abstained or stopped in mid-pregnancy, after controlling for current maternal drinking. In our Detroit cohort, we also found an association between prenatal alcohol exposure and teacher-reported aggression and social problems, even after controlling for current caregiver drinking and quality of child-rearing environment. These effects were largely independent of the attentional problems found in our laboratory assessments (S. Jacobson et al., 1998b), suggesting that they may be mediated by distinct neural processes and pathways. Children whose mothers drank at least 1 drink per day on average during pregnancy were more than twice as likely to exhibit aggressive behaviors in the clinical or borderline range; that is, to be rated by their teachers in the range in which clinic referrals are usually deemed appropriate.

Two studies that have evaluated FAS/FAE patients on the Vineland Adaptive Behavior Scale have found deficits in the socialization domain, which assesses interpersonal skill and the ability to conform to social conventions (Carmichael Olson et al., 1992; Thomas et al., 1996). The most salient problems are failure to consider the consequences of one's actions, lack of responsiveness to subtle social cues, and poor interpersonal relationships (Streissguth et al., 1991). Clinicians who have worked with FAS/FAE patients frequently describe them as being "unaware of the consequences of

[their] behavior, especially the social consequences," showing "poor judgment in whom to trust," and unable to "take a hint, [i.e., needing] strong clear commands" (Streissguth, 1997, p. 127).

Animal studies have implicated the amygdala in emotional and social behaviors, especially those related to fear and aggression (Rosvold et al., 1954; Kling et al., 1979, 1992), and recent studies in humans have focused on the role of the amygdala in social judgment. When asked to rate faces in terms of approachability and trustworthiness, patients with bilateral amygdala damage were more likely to assign positive ratings to faces that most observers would consider untrustworthy (Adolphs et al., 1998). Eight non-retarded adults who met DSM-IV criteria for autism showed normal ability to discriminate faces on the Benton Facial Recognition Test but assigned more positive ratings to the untrustworthy faces. This type of focused, experimental paradigm designed to assess a specific aspect of social judgment has not been used to date in fetal alcohol studies.

The ability to discriminate facial expression of emotion is an important aspect of social judgment. Nelson and Nugent (1990) studied ERPs in response to photos depicting happy and angry faces. ERPs are a class of evoked potentials that reflect changes in the brain's electrical activity in response to the presentation of a stimulus. The invoked activity propagates to the scalp surface, where it can be picked up by electrodes that have been fixed to the scalp. One important component, P300 (also known as P3 or P3b), is a positive voltage peak that occurs approximately 300-600 ms after stimulus presentation, which is believed to reflect the amount of attention allocated to the task (Donchin et al., 1986). Certain ERP components elicited prior to P300 reflect initial signal evaluation, early vigilance, and selective attention. For example, the N200 component is believed to reflect the focusing of attention and suppression of competing information from distracter stimuli (Luck & Hillyard, 1994). In 6-year-old children, a peak resembling P300 appears approximately 700 ms after stimulus presentation (Courchesne, 1978), and an earlier negative component at 400-500 ms ("N400") is believed to reflect initial vigilance.

Nelson and Nugent (1990) showed a sample of 4-6 year old children photos of a single model demonstrating two discrete emotions—happy and angry. The amplitude of the N400 component was larger in response to the angry face, even in trials when it was shown more frequently than the happy face, suggesting that this stimulus was a powerful elicitor of the child's initial attention. In a study of 6-12 year old children with a history of maltreatment, Pollak et al. (2001) found larger P300 amplitudes when angry faces appeared as targets in children with a history of maltreatment, suggesting that this task is sensitive to individual differences in affective responsiveness to social stimuli. By contrast, alcohol-exposed children with impaired social judgment might be expected to exhibit a reduced responsiveness to angry faces.

Tranel (1994) has described a disorder called "acquired sociopathy" found in patients with lesions in the ventromedial frontal cortex, who are unable to organize future activity and hold gainful employment. These patients exhibit a heightened sensitivity to immediate reward and diminished capacity to modify their behavior in response to punishment. They also exhibit a lack of sympathy and concern for others. Damasio's (1994, 1996) "somatic marker" hypothesis suggests that these patients have lost the ability to link knowledge about the potential long-term consequences of their actions to musculoskeletal and visceral responses (feelings) that constrain normal individuals from choosing short-term advantage in circumstances when this choice is outweighed by long-term risk. In a "gambling" task involving a series of decisions to choose from one of four decks of cards, Bechara et al. (1997) have shown that ventromedial frontal-lesioned patients continue to choose from the deck promising the greatest short-term gain even after they recognize intellectually that the gains from selecting from that deck are negated by even larger long-term losses. Moreover, these patients lack viscerally-mediated inhibitions, indicated by skin conductance response (SCR), that are activated

in control subjects once they recognize the risks associated with selecting the short-term gain deck. Alternative interpretations of this phenomenon have been suggested by Rolls (1999, 2000), Manes et al. (2002), and Monterosso et al. (2001).

Moderators of Effects of Prenatal Alcohol Exposure on Neurobehavioral Outcome

There are marked individual differences in vulnerability in children to the neurotoxic effects of prenatal alcohol exposure. One important moderator variable is maternal age. In case studies of Caucasian and Native American multiparous women, it has been noted that each successive child is almost always more severely impaired than the previous one if the mother continues to drink during pregnancy (Abel, 1988; May, 1991), a pattern that has been shown in controlled animal experiments to be due to maternal aging rather than parity (Abel & Dintcheff, 1984, 1985; Vorhees, 1988). Similarly, we have found that among the infants with ARND that we have studied in Detroit, alcohol-related deficits in cognitive function and physical growth are most severe and in many cases are seen only in infants born to women over 30 years of age (J. Jacobson et al., 1996). Possible explanatory mechanisms for this increased vulnerability include age-related changes in maternal alcohol metabolism, body fat-to-water ratio, and placental permeability. Analyses we have recently performed on our Cape Town cohort suggest that these effects may be due more to the number of years that the mother has been drinking rather than to maternal age per se (see "Preliminary Studies" below).

Another important moderator of vulnerability to the effects of prenatal alcohol exposure relates to the ADH2 allele, which plays an important role in alcohol metabolism. McCarver et al. (1997) found that drinking during pregnancy is associated with lower Bayley Mental Development Index scores in African American infants of mothers who lack an ADH2*3 allele but not in infants whose mothers have at least one ADH2*3 allele. Mothers with this allele metabolize alcohol more rapidly. The infant may be protected because the mother may drink less since she requires less alcohol intake to achieve intoxication and/or because her more rapid metabolism may reduce the amount of alcohol that reaches the fetus. We have replicated and extended the findings reported by McCarver et al. by documenting differences in fetal alcohol vulnerability on a large number of infant and child developmental outcomes (see "Preliminary Studies" below). Viljoen et al. (2001) found that, although the ADH2*3 allele is rare in the Cape Coloured population, there is an ADH2*2 allele in this population that appears to confer a similar protective effect against alcohol-related developmental deficit.

3-Dimensional Photography

It has been demonstrated that anthropometric data can be used to identify a subset of variables that can accurately distinguish among individuals with FAS, those who were alcohol exposed but do not manifest the full spectrum of clinical features (FAE/ARND), and those who were not alcohol exposed (Moore et al., 2002). Collection of these data, however, is time-consuming and requires extensive specialized training. The advent of new technology, such as three-dimensional (3-D) digitizing instruments, could make the collection of such data easier and more cost-effective. The utilization of images obtained from a 3-D laser camera can allow for new analytical approaches coupled with quantitative assessment of facial form. This new methodology, which will be developed by the CIFASD Facial Imaging Core, should make it possible to build on previous work to create a more efficient and broadly applicable approach to identifying children of various ages and ethnicities who have been affected by prenatal exposure to alcohol. Greater understanding of the phenotypic correlates of prenatal alcohol exposure will allow for better understanding of the pathophysiology of alcohol exposure, especially when these data are combined with information

collected on other systems affected by alcohol exposure, such as neural and cognitive development. The proposed integration of these research efforts in the Collaborative Initiative can help to clarify the degree to which craniofacial variation reflects underlying disruptions in brain form and function.

FASD in Cape Town

Recent studies have documented an exceptionally high incidence of FAS among the Cape Coloured population in the Western Cape Province of South Africa, which includes Cape Town (May et al., 2000; Riley et al., 2003). Rates of FAS in this community have been estimated to be 18 to 141 times greater than in the United States. The Cape Coloured population, composed mainly of descendants of white European, Malaysian, Khoi (Hottentot), and black African ancestors, has historically comprised the large majority of workers in the wine-producing and fruit-growing region of the Western Cape. The high prevalence of FAS in this community is a consequence of very heavy maternal drinking during pregnancy (Croxford & Viljoen, 1999), which is due to very poor psychosocial circumstances and the traditional *dop* system, in which farm laborers on grape and fruit farms were paid, in part, with wine. Although the *dop* system has been outlawed since the 1920s, regular and heavy alcohol consumption persists in both the urban and rural Cape Coloured communities. Inexpensive wine, beer, and liquor can be obtained in *shebeens* (illegal bars), and weekend binge drinking is a major source of recreation for many in the community. Given the large number of heavy drinking pregnant women and heavily exposed children in this population, research in this community offers a unique opportunity to advance our understanding of FASD, including its etiology, diagnosis, and treatment.

C. PRELIMINARY STUDIES

This component of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) is in response to Request for Applications No. AA-03-002. This project represents a collaboration among Sandra W. Jacobson, Ph.D., and Joseph L. Jacobson, Ph.D., Wayne State University; Christopher D. Molteno, M.D., University of Cape Town; and Denis L. Viljoen, M.D., University of the Witwatersrand. Drs. Sandra Jacobson and Joseph Jacobson have conducted extensive research on the developmental effects of prenatal alcohol exposure, including a 13-year prospective study of a socioeconomically disadvantaged cohort of 480 mothers and children in inner city Detroit, Michigan (R01-AA06966 and R01-AA09524). They have also conducted an 11-year prospective, longitudinal study of the effects of prenatal exposure to polychlorinated biphenyls (PCBs), an environmental contaminant, in Michigan (R01-ES03256 and R01-ES05843) and are currently conducting a prospective, longitudinal study on prenatal exposure to environmental contaminants in Inuit children in Arctic Quebec and Greenland (R01-ES07902). Their Cape Town study on FASD has been conducted in collaboration with Drs. Viljoen and Molteno. This study, which was initiated in 1998 and funded by grants from the National Institute on Alcohol Abuse and Alcoholism (NIAAA), NIH Office of Research on Minority Health (ORMH), and the Foundation for Alcohol Related Research (FARR), is described in detail below.

Dr. Viljoen, Head of the Department of Medical Genetics, University of the Witwatersrand School of Pathology, Johannesburg, is a physician and geneticist with extensive research experience studying heavy prenatal exposure to alcohol in South African populations and its effects on human health. He has spearheaded the very active fetal alcohol research programs currently being conducted in South Africa and is the founder and director of the Foundation for Alcohol Related Research (FARR), University of Cape Town. Dr. Molteno, a developmental pediatrician, who is affiliated with Red Cross Children's Hospital, the Psychiatry Department of the University of Cape Town, and

Groote Schuur Hospital, has been Co-Principal Investigator on our Cape Town infant alcohol study and a Co-Investigator on a study, funded by the Wellcome Trust, on maternal depression and infant outcome in Khayelitsha, a black South African township.

Other key personnel who will be involved in this study include Charles A. Nelson, Ph.D., of the University of Minnesota, a developmental psychologist who is internationally known for his ERP research with infants and children; Nancy Jordan, Ph.D., University of Delaware, a cognitive psychologist with expertise in assessing precursors of arithmetic in young children; Lucinda G. Carr, Ph.D., Indiana University School of Medicine, a geneticist with expertise in the genotyping of the ADH2 allele; and Nathaniel Khaole, M.B., Ch.B. (M.D.), University of Cape Town, a pediatrician and FAS dysmorphologist trained by Kenneth Lyons Jones, M.D., one of the first U.S. physicians to identify FAS (Jones & Smith, 1973). Stanislas Dehaene, Ph.D., Research Director of the Institut de la Santé et de la Recherche Médicale, Paris, an internationally respected cognitive neuroscientist with expertise on the neurobiology of number processing, will serve as a consultant on the ERP assessment of magnitude estimation and other arithmetic tasks.

This project will also draw heavily on the expertise provided by four of the CIFASD cores—the Facial Imaging Core, which will provide the protocol and initial analysis for the 3-dimensional digitized photos described above; the Dysmorphology Core, which will provide the protocol and supervision for the dysmorphology assessments; the Neurobehavioral Core, which will provide standardized administration procedures and equipment for the Leiter International Performance Scale, the Cambridge Neuropsychological Testing Automated Battery (CANTAB), and other tasks to be conducted in common across multiple projects; and the Informatics Core, which will facilitate pooling of data across projects and will provide expertise in statistical analysis. This project will also benefit from frequent interactions with our CIFASD collaborators who will be conducting related projects. The Moscow infant project and the Ukraine infant pilot will use the same timeline follow-back approach used in maternal alcohol assessment in our Detroit and Cape Town studies. They will also administer some of the narrow band, domain-specific infant assessments from the infant phase of our Cape Town study, whose predictive validity will be assessed in this project. The Collaborative Initiative will offer an important opportunity to determine the robustness of our infant findings in diverse ethnic and social contexts, and we welcome the opportunity to consult with these investigators in the analysis and interpretation of their data.

The recent collaboration among Drs. S. Jacobson, Molteno, Viljoen, and J. Jacobson has demonstrated our capacity to recruit and evaluate heavy drinking women and their offspring from the Cape Coloured community, where rates of alcohol abuse and dependence during pregnancy, FAS, and other forms of FASD are among the highest in the world. Because FAS is relatively rare in the U.S. (about 1/1000 births), this is the first study to provide data from a cohort with a large number of prospectively ascertained FAS children. This study has demonstrated our ability to conduct comprehensive pediatric and developmental follow-up in Cape Town and the value of data generated by research on FASD in this community.

Cape Town Infant Study

Participants. The mothers were recruited from the Hanover Park antenatal clinic, a Midwife Obstetric Unit (MOU) serving the Cape Coloured population, where most of the mothers also deliver their infants. This clinic was selected for its high prevalence of heavy alcohol use on the basis of data collected from pregnant women at six MOUs serving the Cape Town metropolitan region (Croxford & Viljoen, 1999). Each mother was interviewed regarding her alcohol consumption, both at the time of recruitment and retrospectively at conception, using a timeline follow-back approach to determine

incidence and amount of drinking on a day-by-day basis during a typical 2-week period (Sokol et al., 1983; S. Jacobson et al., 2002a). Volume was recorded for each type of beverage consumed each day, converted to absolute alcohol (AA), and averaged across the clinic visits to provide a contemporaneous report of "oz AA/day during pregnancy." Each oz of AA is equivalent to 2 standard drinks. Any woman reporting a minimum of 14 drinks per week (1.0 oz AA/day) or at least two incidents of binge drinking ($\geq$ 5 drinks) per month during the first trimester of pregnancy was invited to participate in the study. The next woman initiating antenatal care whose gestational age was within 2 weeks of the heavy drinking mother was also invited to participate in the study, provided that she drank fewer than seven drinks per week (0.5 oz of AA per day) and did not binge drink.

A staff driver and a research nurse transported the mothers and infants to our infant development laboratory at the University of Cape Town School of Medicine. Each mother was interviewed in the laboratory antenatally and at 1 month postpartum regarding her alcohol and drug use. The interviews were conducted in Afrikaans. At 6.5, 12, and 13 months, the infants were evaluated on a comprehensive battery of narrow band infant assessments that have been found (S. Jacobson et al., 1993) or were hypothesized to be sensitive to prenatal alcohol exposure. Assessments of preterm infants (born at < 38 weeks gestation) were scheduled to correspond to the date when they would have reached the appropriate age if they had been born at term. Infant examiners were blind with respect to maternal alcohol use. 135 infants were recruited and seen through 13 months. Near the end of the study, 24 additional infants were recruited from the same population for a maternal nutrition study, which followed the same research protocol through 6.5 months. Virtually all of the mothers (93.1%) seen antenatally were retained in the sample for the duration of the infant assessments. Six (3.8%) infants died, one (0.6%) abnormal pregnancy was terminated at 20 weeks gestation, three (1.9%) moved away from Cape Town, and one (0.6%) withdrew from the study.

Maternal alcohol abuse and dependence. By contrast to most prospective studies of pregnant drinking women, a substantial proportion (40.3%) of the heavy drinking mothers in the cohort met DSM-IV criteria for alcohol dependence or abuse, assessed on the alcohol-abuse module of the Diagnostic Interview Schedule (Robins et al., 1995). The women drank much more heavily than in the economically disadvantaged African American cohort that we have been studying in Detroit (S. Jacobson et al., 2002b; Riley et al., 2003), with 40% of the heavy drinkers consuming 5 to 10 drinks per occasion, as compared with only 15% in the Detroit cohort (Fig. 1). As in Detroit (S. Jacobson et al., 2000), the women in Cape Town concentrated their drinking primarily on the weekends, i.e., 1 to 3 days per week. Consistent with the high levels of alcohol abuse and dependence, we found a high rate of severe depression on the Beck Depression Inventory in relation to alcohol use during pregnancy among the heavy drinkers.

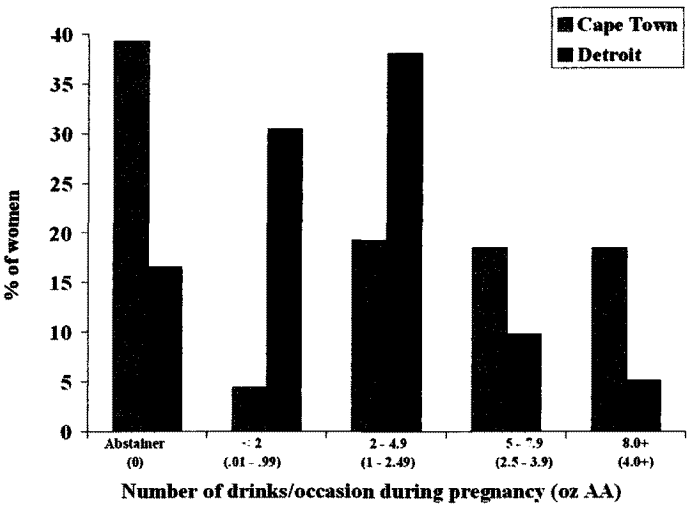

Fig 1. Comparison of drinks/occasion in the Cape Town and Detroit cohorts, $t = 2.74$, $p < .01$

FAS diagnosis. The infants were examined for presence of alcohol-related craniofacial dysmorphic features at the 1-, 12-, and 13-month visits by our colleague, Dr. Khaole, and/or Solly Zieff, M.D., dysmorphologists trained by Kenneth Lyons Jones, M.D., for the Wellington, South

Africa, FAS incidence study (May et al., 2000). Infants with the facial features characteristic of FAS (small palpebral fissures, flat midface, smooth philtrum), significant growth retardation (< 10th percentile for height and weight or < 3rd percentile for head circumference), and evidence of poor neurobehavioral function were diagnosed as having FAS. Dr. Jones subsequently confirmed the FAS diagnoses by examining frontal and side view facial photographs of the infants taken at 12 months of age. An extraordinarily large number of infants—21 of 68 whose mothers drank heavily during pregnancy (30.9%)—met criteria for full FAS.

Effects on infant growth and cognitive function. Five narrow-band, state-of-the-art assessments, which were related to prenatal alcohol exposure in our Detroit study (S. Jacobson et al., 1992, 1993, 1994; Jacobson, 1999), were administered to the infants. In the *Teller Visual Acuity Test,* which is based on infant preference for patterned stimuli, a visual target with vertical black and white stripes is displayed on the left or right side of a gray board. Over successive trials, progressively narrower stripes are displayed. Acuity is defined as the narrowest grid for which the child shows a visual preference. The *Fagan Test of Infant Intelligence (FTII)* consists of 10 problems, in which the infant is shown two identical target photographs of faces for a fixed period of time and is then shown the familiar target paired with a novel one. Novelty preference (the proportion of looking time to the novel stimulus) provides an index of visual recognition memory. The average duration of the infant's visual fixations to the stimuli provides a measure of information processing speed. In contrast to the traditional, global Bayley Scales of Infant Development, whose predictive validity for childhood cognitive function is generally poor prior to 2 years of age, novelty preference and processing speed, which focus specifically on memory and attention, are moderately predictive of childhood IQ (McCall & Carriger, 1993; Sigman et al., 1991; S. Jacobson et al., 1996). *Complexity of symbolic play*, which also has modest predictive validity to 7.5 years (S. Jacobson et al., 1996), was assessed at 12 months by using the Belsky et al. (1984) procedure. After 10 min of spontaneous free play, modeling is used to elicit higher levels of play than those spontaneously exhibited by the infant.

Two new assessments were added to the Cape Town infant battery that have not previously been examined in relation to prenatal alcohol exposure: the A-not-B test, an early precursor of EF, and a numerosity test (Wynn, 1992), which assesses magnitude representation, a precursor of arithmetic that has been linked to inferior parietal function. The *A-not-B Test*, which we adapted from Diamond (1985), is a version of the classic object permanence task, in which a toy is hidden for a specified period (3-11 sec). Young infants tend to search for the toy where it was previously found rather than where they saw it hidden most recently, particularly after longer delays. This task is believed to index the early development of executive function, an aspect of attention involving the ability to use new information to override a prepotent response—in this case, to use visual input to override the impulse to reach where the toy was previously found (Diamond, 1985). This interpretation has been questioned by Thelen and associates (Diedrich et al., 2000), however, who have argued that the A-not-B error is due to a lack of maturity of a specific aspect of motor function—visually-guided reaching—rather than a generalized deficit in attentional function. In the *Infant Numerosity Test*, one or two toy penguins are displayed and then covered by a screen. The infant sees one penguin added or taken away, and the screen is then lifted. "Number sense" or magnitude representation is inferred if the infant looks longer when the number of penguins appearing after the screen was lifted differs from what would be expected on the basis of the manipulation the infant had observed.

Prenatal alcohol exposure was associated with smaller infant size at birth and at 6.5 and 12 months (Table 1). At 6.5 months, maternal drinking was associated with poorer visual acuity (Carter et al., 2001 & submitted) and at 12 months with poorer FTII novelty preference and spontaneous and

elicited/imitative play. Poorer visual acuity and symbolic play were previously found at the lower levels of prenatal alcohol exposure in our Detroit cohort (S. Jacobson, 1999; S. Jacobson et al., 1993), but the more heavily exposed Cape Town infants are the first to show a deficit in recognition memory on the FTII. The alcohol-exposed infants also showed less ability to maintain attention during longer delays on the A-not-B Test and failed to attend longer when the display differed from what would be expected on the Infant Numerosity Test (see Fig. 2), indicating that alcohol-related deficits in precursors of executive function and magnitude representation can already be detected in infancy.

Table 1. Relation of Drinking during Pregnancy to Infant Growth and Neurobehavioral Outcome

	N	Average AA/day: At conception	Average AA/day: During pregnancy
Birth weight	159	-.33***	-.32***
Gestational Age	159	-.14†	-.09
6-month size			
Weight	133	-.17†	-.23**
Length	133	-.19†	-.23**
Head circumference	133	-.06	-.09
12-month size			
Weight	124	-.26**	-.33***
Length	124	-.25**	-.31***
Head circumference	124	-.16†	-.19*
Visual acuity	131	-.21*	-.22*
FTII			
Novelty preference			
6.5 months	114	-.03	-.05
12 months	90	-.23*	-.24*
Processing speed			
6.5 months	114	.04	.02
12 months	90	.10	.09
A-not-B	103	-.22*	-.21*
Symbolic play			
Spontaneous	114	-.18*	-.18*
Elicited	117	-.28**	-.34***

†$p < 0.10$ *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

Maternal age as a moderator of the effects of prenatal alcohol exposure. The hypothesis that children born to older mothers are more vulnerable to the effects of prenatal alcohol exposure was confirmed in these Cape Town infants. As can be seen in Table 2, the associations with visual acuity, FTII recognition memory, and A-not-B executive function were markedly stronger in infants born to mothers 30 years of age and older. The absence of increased vulnerability in spontaneous and elicited play suggests that these effects might be mediated by a different teratogenic process, from which the infants of the younger mothers are not protected. Not surprising, number of years of maternal drinking, which we assessed in this cohort for the first time, is highly correlated with maternal age ($r = 0.60$, $p < 0.001$). Years of drinking also moderated the effects on visual acuity and A-not-B, which were seen primarily in infants born to women who had been drinking regularly for at least 12 years. These findings indicate that the differential vulnerability associated with maternal age may be due, in part, to differences in the number of years of alcohol abuse, suggesting that heavy drinking may erode the viability of the mother's reproductive system progressively over time. A recent animal study found that long-term exposure of female mice to daily binge-like alcohol prior to conception resulted in lower offspring body weights, ovarian anomalies, and fewer follicles that reached maturity, even when the animals were not exposed to alcohol during gestation (Tucker Gutierrez et al., 2002).

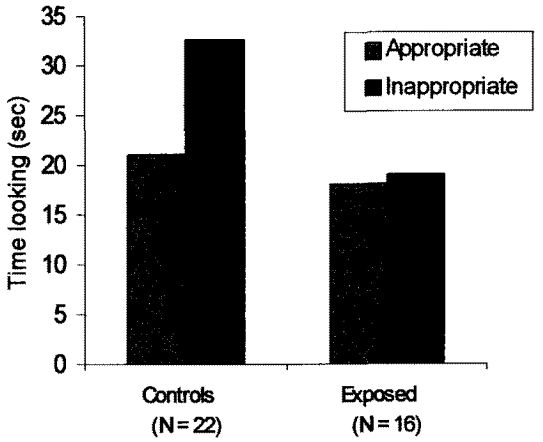

Fig. 3. Infant numerosity performance in alcohol-exposed infants and controls. As predicted, nonexposed infants look longer at inappropriate (1 + 1 = 1 or 2 – 1 = 2) than at appropriate (1 + 1 = 2 or 2 – 1 = 1) displays [$t(21) = 2.8$, $p < .01$]. Alcohol-exposed infants did not discriminate between appropriate and inappropriate displays [$t(17) = 0.3$, n.s.]

Home visitor intervention. Once the unusually high levels of alcoholism and high number of

Table 2. Relation of Pregnancy Drinking to Neurobehavioral Outcome by Mother's Age.

	Average AA/day			
	Mother's age < 30		Mother's age ≥ 30	
	N	r	N	r
Visual acuity	91	-.08	40	**-.40****
FTII				
Novelty preference				
6.5 months	77	-.09	37	.00
12 months	59	-.12	31	**-.41***
Processing speed				
6.5 months	77	-.06	37	.21
12 months	59	.08	31	.07
A-not-B	66	-.05	37	**-.38***
Symbolic play				
Spontaneous	77	-.18	37	-.21
Elicited	78	**-.31****	39	**-.35***

$^{\dagger}p < 0.10$ $^{*}p < 0.05$ $^{**}p < 0.01$ $^{***}p < 0.001$

FAS births became evident, a home visitor intervention was immediately implemented in collaboration with Mireille Landman, M.A., a clinical psychologist with extensive experience in training of home visitors; the Parent Centre, a community-based parenting program; and Stephen Rollnick, Ph.D., an expert on motivational interviewing. The intervention was designed to help the women stop drinking or decrease their alcohol use during pregnancy by encouraging them to speak more openly about their alcohol use and family and pregnancy problems. Arrangements were made to provide any woman seeking treatment for alcoholism with a referral to an alcohol treatment facility affiliated with the Department of Obstetrics, University of Cape Town School of Medicine. In connection with this study, we trained a social worker and two home visitors from the Parent Centre to use techniques from motivational interviewing to encourage open discussion of pregnancy drinking with the mothers.

Detroit Longitudinal Study

Participants. The sample consists of 480 African American infants whose mothers were recruited between September 1986 and April 1989 during their first prenatal visit to a large urban maternity hospital clinic (M = 23.4 weeks gestation, SD = 7.9). Each mother was interviewed regarding her alcohol consumption both currently and at conception using the timeline follow-back approach described above. All women reporting alcohol consumption at conception averaging at least 7 drinks/week and a 5% random sample of lower level drinkers and abstainers were invited to participate. To reduce the risk that alcohol would be confounded with cocaine exposure, 78 heavy cocaine (≥ 2 days/week) light alcohol (< 7 drinks/week) users were also included in the sample. The sample was predominantly lower class; 83.5% were receiving welfare, and only 10.0% were married.

The children were evaluated at 6.5, 12, and 13 months and again at 7.5 and 13 years. Each child was also assessed on a 46-item fetal alcohol dysmorphology checklist (Golden et al., 1982: Ernhart et al., 1987) by a project research psychologist trained and supervised by our collaborator, Erawati V. Bawle, M.D., a pediatric geneticist and Director of Genetics Department, Children's Hospital of Michigan. When a child was identified as potentially FAS, Dr. Bawle examined him/her to confirm the diagnosis. Front and profile photographs were also taken of the child's face. Based on these examinations and a review of the photographs and checklists, three children were identified as having the characteristic FAS pattern of facial anomalies. All three also met the criteria for growth retardation and CNS impairment required for an FAS diagnosis. The photographs of these three children, others who were suspect for FAS dysmorphology, and a random sample of children lacking FAS dysmorphic features were reviewed by Kenneth Lyons Jones, M.D., and Sterling Clarren, M.D., two distinguished FAS dysmorphologists. Both independently confirmed the diagnosis of the three FAS children, and neither identified any other children as meeting criteria for FAS. If we extrapolate back to the larger population from which our sample overrepresenting moderate-to-heavy drinking mothers was drawn, the rate of FAS is 3/2562 or approximately 1 per thousand, which is consistent with estimates of the incidence of FAS reported elsewhere (Abel, 1995; Sampson et al., 1997).

Arithmetic. Eighty-two children were assessed at 13 years on a 7-part, computer-administered test, which we expanded and adapted from Kopera-Frye et al. (1996) in collaboration with Stanislas Dehaene. Each of the seven subtests had 32 items. On exact addition, subtraction, and multiplication, the child entered the solution on the computer keypad. On approximate addition and subtraction, s/he selected which of the two choices shown on the monitor provided the best approximation; in number comparison, which of two numbers displayed was larger. In proximity judgment, a number was displayed on the left side of the screen, and the child chose which of two numbers on the right side was closest to it. The child's performance on each of these subtests was examined in relation to maternal drinking during pregnancy (average oz AA/day), after controlling for potential confounding variables. Twelve control variables were considered for potential confounding in these analyses: maternal age, marital status, socioeconomic status, years of education, Peabody Picture Vocabulary Test score; smoking, cocaine, and marijuana use during pregnancy; and child's gender, parity, age when tested, and examiner. Any control variable that was weakly related to the outcome being examined (at $p < 0.10$) was controlled statistically by multiple regression analysis.

Prenatal alcohol exposure was associated with poorer performance on exact addition and multiplication, with effects on approximate addition and subtraction that fell short of conventional levels of statistical significance (Table 3). However, the strongest associations were with number comparison and proximity judgment. A factor analysis of the seven subtests yielded two factors—one for exact and approximate calculation ("calculation"); the other, for comparison and proximity ("magnitude representation"). Multiple regression analysis showed that the relation of prenatal alcohol exposure to calculation was mediated completely by its relation to magnitude representation since the entry of magnitude representation into the regression reduced the effect of alcohol on calculation to $\beta = 0.03$. These data suggest that the poorer numerical processing associated with prenatal alcohol exposure is due to a specific deficit in magnitude representation, which Dehaene et al. (in press) have linked to bilateral activation of the horizontal segment of the intraparietal sulcus. When full scale IQ scores were added at the final step of the Dehaene subtest regressions, the effect of alcohol on the exact and approximate calculation subtests was reduced, indicating that some of the association with calculation is attributable to a prenatal alcohol effect on general intellectual ability (Table 3). By contrast, the entry of IQ did not alter the effect of alcohol on number comparison or proximity judgment, suggesting that alcohol has a direct effect on magnitude representation that is not mediated by its impact on other aspects of the child's intellectual function.

Table 3. Relation of Prenatal Alcohol Exposure to Performance on the Dehaene Arithmetic Subtests (N=82).

	Average AA/day		
	r	β^1	β^2
Individual subtests			
Exact addition	**-.37*****	**-.33****	**-.25***
Exact subtraction	**-.19***	-.14	-.05
Exact multiplication	**-.27****	**-.23***	-.15
Approximate addition	**-.19***	**-.19**†	-.12
Approximate subtraction	**-.22***	**-.21**†	-.13
Number comparison	**-.61*****	**-.55*****	**-.53*****
Proximity judgment	**-.46*****	**-.42*****	**-.42*****
Composite measures			
Calculation	**-.28****	**-.21**†	-.14
Magnitude representation	**-.61*****	**-.58*****	**-.56*****

[1] After control for confounders
[2] After control for confounders and 13-year IQ score
$^{\dagger}p < 0.10$ $^{*}p < 0.05$ $^{**}p < 0.01$ $^{***}p < 0.001$

Executive function. The relation of prenatal alcohol exposure to performance on two tests of executive function administered in the 7.5-year follow-up is shown in Table 4. The same control variables examined for the Dehaene subtest regressions were considered as potential confounders in these analyses. Maternal drinking during pregnancy was associated with poorer verbal fluency and

poorer planning on the Tower of London. In the 13-year follow-up, we administered the California Stroop Test, which is described under "Background and Significance" above. The California Stroop makes it possible to distinguish between "cognitive control," which is mediated by activity of the dorsolateral prefrontal cortex, and "conflict monitoring," which is mediated by activity in the anterior cingulate cortex (MacDonald et al., 2000). Cognitive control is assessed in the standard Stroop interference condition; conflict monitoring, in the California Stroop's "switch" condition. By contrast to the findings for FAS and heavily exposed ARND children (Mattson et al., 1999), at the moderate-to-heavy levels of exposure in our Detroit cohort, the relation between prenatal alcohol exposure and performance in the Stroop interference condition was not significant (Table 4). Instead, prenatal alcohol was associated primarily with poorer performance on the speed and accuracy measures for the switch condition. These findings suggest that the fetal alcohol deficit in executive function may relate more specifically to conflict monitoring, that is, ongoing feedback regarding whether control is being allocated effectively, than to cognitive control per se.

Table 4. Relation of Prenatal Alcohol Exposure to Executive Function Measures in the Detroit Cohort.

		At Conception		During Pregnancy	
	N	r	β[1]	r	β[1]
7.5 years					
Verbal fluency	333	-.12*	-.12*	-.16**	-.17***
Tower of London	333	-.12*	-.10†	-.17***	-.14**
13 year Stroop					
Interference-accuracy	95	.14†	.12†	.16†	.14
Interference-speed	95	.16†	.18†	.08	.13
Switch-accuracy	95	.28**	.24*	.31***	.28**
Switch-speed	95	.25**	.21*	.19*	.16†

[1] After control for confounders.

Table 5. Relation of Prenatal Alcohol to Developmental Outcome by Presence of an ADH2*3 Allele

	Allele absent (N=99)	Allele present (N = 60)
Infancy		
Birth weight	-.24*	-.20
Head circumference	-.32**	-.01
Bayley Scales		
Mental Development Index	.30**	.11
Psychomotor Development Index	-.08	.02
Cross Modal Transfer	.39**	-.05
Fagan Test of Infant Intelligence [a]		
Length of look	.05	.34*
Visual Expectancy Paradigm [b]		
Median reaction time	.44*	.05
% Fast response	-.45*	-.06
% Quick response	-.54*	-.12
Elicited Play		
7.5 Year		
Digit cancellation (omission errors)	.23*	-.03
Mental Rotation	.26**	-.02
Forward reaction time	.29**	.14
Backward reaction time	.22*	.11
Sternberg [c]		
Yes reaction time	.21†	.11
No reaction time	.19	.12
Magnitude estimation		
Reaction time (numbers)	.29**	.06
Slope (numbers)	.32**	.11
Reaction time (arrows)	.20†	.33*
Slope (arrows)	.30**	.01
Verbal fluency	-.17†	.08

Values are Pearson rs.
[a] Average of 6- and 12- month visits. N = 60 for allele absent;
N = 42 for allele present.
[b] N = 20 for allele absent; N = 16 for allele present.
[c] N = 69 for allele absent; N = 43 for allele present.
†p < 0.10 *p < 0.05 **p < 0.01

The ADH2*3 allele as a moderator of the effects of prenatal alcohol exposure. The performance of 99 children from the Detroit cohort whose mothers lacked the ADH2*3 allele was compared with that of 60 whose mothers had at least one ADH2*3 allele using data from the infant and 7.5-year phases of the study. These analyses indicated lower birth weight, smaller head circumference, poorer Bayley Mental Development scores, slower Cross Modal Transfer and Visual Expectancy reaction times, and poorer symbolic play in relation to prenatal alcohol exposure among infants whose mothers lacked the ADH2*3 allele but not among those whose mothers had at least once ADH2*3 allele (Table 5). Similarly, at 7.5 years adverse effects were seen in the offspring of drinking women without the allele, whereas virtually none were seen in children of mothers with this allele. Performance of 116 children who themselves lacked the allele was compared with that of 71 who had at least one ADH2*3 allele (Croxford et al., in press). The presence of at least one ADH2*3 allele in the

child also conferred protection from the adverse effects of prenatal alcohol exposure seen in children lacking the allele. These data lend additional support to the hypothesis that the more rapid metabolism of alcohol conferred by the ADH2*3 allele may provide some protection to the fetus from alcohol exposure *in utero*. As noted, although the ADH2*3 allele is rare in the Cape Coloured population, an ADH2*2 allele in this population appears to confer a similar protective effect against alcohol-related developmental deficits (Viljoen et al., 2001).

D. RESEARCH DESIGN AND METHODS

Participants

The sample will consist of 133 children, who will be assessed at 4 and 6 years of age. Given the extremely low attrition rate (6.9%) during the infant phase of the study, we expect an additional attrition of no more than 10%. Therefore, of the 148 children retained over the course of the infant phase of the study, we expect to assess 133 at 4 and 6 years. Given this sample size, we will have sufficient power to detect a Pearson $r = 0.24$ with an alpha < 0.05, two-tail, and a power of 0.80.

Neurodevelopmental Assessment

The children will be assessed within 6 weeks of their fourth and sixth birthdays. The child and the mother will be transported early in the morning to the FARR research laboratory at the University of Cape Town School of Medicine by our staff driver and research nurse. Because we found in the infant phase of this study that the mothers often arrive at the laboratory hungry, a breakfast will initially be served. The study procedures will then be explained in detail to the mother, who will be asked to sign an informed consent at each visit. The 4- and 6-year visits will each entail a 3-hr child assessment. The mother will stay with the child initially and, once the child adapts to the testing environment, the mother will be interviewed in a separate room by the research nurse to provide control variable information. The Junior South African Intelligence Scale is available in Afrikaans, the language of the Cape Coloured community. The instructions for the other assessments, which are mostly nonverbal, will be translated into Afrikaans and then back translated into English to ensure accuracy. At the end of each visit, the mother will be given $10 and a photograph of her child, and the child will receive a small gift for his/her participation.

This project will incorporate several assessment procedures to be administered concurrently by other CIFASD projects in conjunction with three of the CIFASD Cores. The Facial Imaging (FI) Core will provide a 3-dimensional camera and detailed protocol for photographing the children's faces. The FI Core will analyze these data to identify key craniofacial parameters, which we will then examine in relation to prenatal alcohol exposure and child neurobehavioral outcome. Nathaniel Khaole, M.D., our pediatrician/dysmorphologist, will perform the dysmorphology assessments according to the protocol to be provided by the Dysmorphology Core. Dr. Khaole was trained in dysmorphology by Kenneth Lyons Jones, M.D., Director of the Dysmorphology Core, who reviewed and confirmed all of our FAS diagnoses during infancy. The neurodevelopmental assessment battery will include several tests recommended by the Neurobehavioral Core, including the *Leiter International Performance Scale-Revised*, the *Cambridge Neuropsychological Testing Automated Battery (CANTAB)*, the *Edinburgh Handedness Inventory*, the Achenbach *Child Behavior Checklist and Teacher Report Form*, the Pelham *Parent/Teacher Disruptive Behavior Rating Scale*, and the *Vineland Adaptive Behavior Scales*. These tests will be administered according to a standardized protocol to be developed by the Neurobehavioral Core. Administration across multiple projects will provide a valuable opportunity to determine the degree to which the neurobehavioral deficits that characterize FASD are consistent across a range of ages and racial/ethnic groups.

4-year Child Assessment

General Intellectual Ability. The child will be assessed on the *Junior South African Intelligence Scale*, which is based on the Stanford-Binet IQ Test and is valid for children ages 3-7 years, 11 months.

Arithmetic. Five tasks designed to assess specific precursors of arithmetic will be administered. The first three tasks, which were developed by Geary et al. (1992), focus on *counting*. In each of these tasks, the child is presented with a row of eight alternating red and blue chips. A puppet who is learning to count counts the chips, correctly in some trials; making an error in others. The child's task is to determine if the puppet's count is correct. The tasks are designed to assess the child's grasp of the one-one correspondence, stable order, and cardinality principles. For example, in one trial of the stable order task, the puppet reverses the number sequence (4, 6, 5); in another, he skips a number (4, 6, 7).

The *nonverbal calculation* task (Levine et al., 1992) uses two 28 x 15 cm cardboard mats (one placed in front of the experimenter; the other in front of the child), a set of 20 black disks (2 cm in diameter), a box for the disks, and a cover for the disks. The nonverbal calculation is preceded by a matching task. The experimenter takes a disk from the box and places it on her mat in full view of the child. The disk is then hidden under a cover. The experimenter puts a disk on the child's mat and lifts the cover from his/her own mat so that the child sees that the two mats have the same number of disks on them. The demonstration item is presented again, except that this time the child is asked to place the appropriate number of disks on his/her mat after the experimenter's disks are hidden. The task is repeated with disks varying in number from 2 to 7. The nonverbal calculation trials are then presented. For addition, the experimenter places the set of disks comprising the augend in a horizontal line on her mat and then covers it. S/he then puts the set of disks comprising the addend in a horizontal line in full view of the child and slides them under the cover one at a time. The two terms of the problem are never simultaneously in view. The child indicates how many disks were hidden under the cover by placing the appropriate number of disks on his/her mat. A comparable procedure is used for subtraction, but the disks comprising the subtrahend are removed from under the cover one at a time. No verbal labels are provided on any of the problems, nor is the child asked to generate them.

The *number pattern* task, which was adapted from Klein and Starkey (2003) by our collaborator Nancy Jordan, uses a set of colored blocks. In the pattern duplication trials, the experimenter places a preconstructed pattern of blocks on the table and asks the child to make a pattern that "looks just like mine" with his/her blocks. The patterns include repeated (ABAB or ABCABC) and growing (e.g., ABAABAAAB). In the pattern extension trials, the child is asked to add his/her blocks to the pattern presented by the investigator.

Executive function. Two executive function tasks designed for preschool-age children will be administered. Diamond and colleagues' (Diamond et al., 2002; Gerstadt et al., 1994) Stroop-like *day-night* task examines the young child's ability to inhibit a prepotent or learned response. In the original Stroop (MacLeod, 1991), color names are printed in the "wrong" color. The participant is asked to name the color the word is printed in and to inhibit the automatic tendency to read the word as printed. Because most young children cannot read words, the Stroop test is not difficult for them. The *day-night* task requires the child to say "night" in response to a picture of the sun and "day" in

response to a picture of the moon, responses that involve the inhibition of a prepotent response that is difficult for children who are 3.5-4.5 years of age (Diamond et al., 2002).

Zelazo et al. (1996) have developed a simplified version of the Wisconsin Card Sorting Test that can be used with preschool-age children. In this *preschool card sorting* task the child is asked to sort a deck of cards first by color or shape. S/he is then asked to sort the cards by the other dimension, which requires inhibition of the previously learned response. The authors report that very young children will often continue to sort the cards according to the initial dimension even though they can correctly tell the examiner that the rule has changed and are reminded of the new dimension before they respond in each consecutive trial (Kirkham et al., 2000; Zelazo & Muller, 2002). The authors have also incorporated a "switch" condition, which makes this task analogous to the California Stroop (described under "Background and Significance" above). Children who pass at least 7 of 8 initial trials are then told to sort by color but, if the card has a black border, to switch and sort by shape. This "switch" condition provides a measure of conflict monitoring, an aspect of executive function that MacDonald et al. (2000) have linked to activation of the anterior cingulate cortex.

Affective function. The children will also be administered the *Children's Gambling Task* (Kerr & Zelazo, in press), which is based on Bechara et al. (1994, 1997) but modified for young children. In this task, the child is presented with two decks of cards (presorted in a fixed order) that, when turned over, display happy faces on the top half of the card and sad faces on the bottom half. Happy faces indicate the number of candies won; sad faces indicate candies lost. The sad faces are covered with a Post-It sticky note when first turned over. The examiner then takes candies (M&Ms) from an opaque plastic container and places them on top of each happy face. These candies, which correspond to the number of happy faces on the card, are then put into a transparent glass cylinder in full view of the child. The Post-It note is removed, revealing the number of sad faces on the card. Candies are removed from the glass cylinder and placed on each sad face on the card and then returned to the plastic container. The child is told to try to win as much candy as possible before the end of the "game" (i.e., 50 card selections) but is not told that one deck is advantageous and the other is not. Cards in the advantageous deck always provide a gain of one reward (one happy face) together with zero or one loss (with a net average of 5 candies gained per block of 10 cards). Cards in the disadvantageous deck always provide a gain of two rewards together with losses of 0, 4, 5, or 6 candies (with a net average of 5 candies lost per 10 cards). The key dependent measure is the proportion of choices made from the disadvantageous deck.

6-year Child Assessment

General Intellectual Ability. The child will be assessed on the *Leiter International Performance Scale-Revised* (Stoelting, 2001), a nonverbal measure of intelligence that has been recommended by the CIFASD Neurobehavioral Core and will be administered in several of the CIFASD projects. Both the Visualization and Reasoning and the Attention and Memory batteries will be administered.

Arithmetic. The Temple and Posner (1998) *magnitude representation ERP paradigm* will be administered. EEG will be recorded using a electrode cap with silver-silver-chloride electrodes. Placement of the electrodes will be based on an adaptation of the international 10-20 system (Jasper, 1958) with nine additional electrodes added to provide more complete coverage. Recordings will be made from Fz, Pz, Cz, POz, F3/4, F7/8, AF3/4, FC5/6, FC1/2, C4/3, T3/4, T5/6, CP5/6, P3/4, PO3/4, O1/2, initially referenced to Cz and then re-referenced to linked mastoids off line. The electrooculogram (EOG) will be recorded from bipolar, miniature electrodes placed vertically above

and below the right eye. Pre- and posttest impedances will be considered acceptable if they are below 5 Kohms. Grass/Astromed amplifiers will be used, with a gain of 50 for scalp electrodes and 5 for EOG electrodes. The amplifier bandpass will be 0.1-30 Hz, and a 60 Hz notch filer will be engaged. Data will be sampled at 200 Hz beginning 100 ms before stimulus onset and continuing for 1700 ms. EOG artifacts that occur during stimulus presentation will result in deleting that trial; those that occur after stimulus offset will be corrected using a standard blink correction algorithm (see Gratton et al., 1983). All elements of the experiment, including stimulus presentation, data collection, and data reduction, will be under the control of ERPw software developed by our collaborator Charles Nelson.

Before the ERP arithmetic assessment, a number knowledge test adapted from Griffin et al. (1995) is administered to the child. In each ERP trial, a stimulus is presented consisting of one of the digits 1, 4, 6, or 9 or an array of dots corresponding to those digits. Digits are presented in Helvetica font 5 cm high; the dots vary in size such that all arrays fit into a 5-cm square. The child is told to press one computer key if the stimulus is larger than 5; another, if it is smaller than 5. The stimulus remains on the screen until the button is pressed (up to 7 s). If the child's response is correct, a "smiley face" is presented. A session consists of a practice set with 24 trials, followed by four blocks of 40 recorded trials.

Executive function. The Cambridge Neuropsychological Testing Automated Battery (CANTAB; Robbins et al., 1998; Robbins, 1996; Hughes et al., 1994) consists of a set of nonverbal tasks, administered on a touch-screen computer, which are derived from experimental paradigms with longstanding traditions in brain-behavior studies that have been adapted for administration to children. The CIFASD Neurobehavioral Core will provide Training and equipment for administration of the CANTAB.

Three executive function tests from the CANTAB will be administered at 6 years. The *Motor Screening Test* is administered first to provide a baseline measure of psychomotor speed and accuracy, the dependent variables in each of the CANTAB tests. *Big-Little Circle* assesses the child's ability to follow an explicit rule and then reverse the rule. The child is presented with two boxes on the screen, one containing a big circle and the other a little circle, and is told to touch the little circle. After 20 trials, the rule is changed, and the child is told to touch the big circle. *Intradimensional/Extradimensional Set-Shift* is a modification of the Wisconsin Card Sorting Test, designed to provide more specific information regarding the child's ability to use computer-provided feedback to learn response contingencies. Nine stages of set shifting are administered, ranging from a simple discrimination to complex between-category response set shifting. A lesion study in marmosets showed that one type of set shift, a within-category reversal shift (e.g., when the correct response shifts from one lined figure to another previously non-reinforced lined figure) is mediated by the orbitofrontal cortex, whereas between category set-shifting (e.g., when the correct response shifts from lined figures to colored shapes) is mediated by the dorsolateral prefrontal cortex (Dias et al., 1996). *Stockings of Cambridge* is a variant of the Tower of London planning task, in which the child solves problems by moving colored disks among three locations in a prescribed number of moves.

Two fluency tasks from the Delis-Kaplan Executive Function battery will be administered. In the *Design Fluency Test*, the child is asked to create as many unique line designs as possible. In the first condition, s/he is presented with a 5 x10 dot array and is allowed to use only four straight lines to connect the dots. In the second condition, there are five filled and five empty dots, and the child is told to connect only the empty dots while ignoring the filled dots. In the third condition, the child must alternate between the empty and filled dots (set-shifting). Two conditions of the *Verbal*

Fluency Test will be administered—category fluency (list as many words as possible from the categories "animals" and "boys' names") and set-shift (alternate between two semantic categories, e.g., "fruit" and "furniture").

Affective function. In the *discrimination of facial expressions of emotion ERP* task (Pollak et al., 2001), the child is shown three photos of the same women—one with a happy, one with an angry, and one with a fearful emotional expression (Ekman, 1976, slide numbers 48, 53, and 50). Each trial consists of two consecutive blocks of 160 randomized presentations. In each trial, one emotion is designated as the target, and the child is asked to press a button whenever it appears. The target emotion appears in 25% of the trials, an infrequent non-target in 25% of the trials, and a frequent non-target in 50% of the trials. Each of the two non-target emotions serves as the frequent non-target in one of the blocks and the infrequent non-target in the other. Each child is presented with one trial for each target emotion, with the order of the target emotions counterbalanced across children.

Social judgment of trustworthiness will be assessed by adapting from the procedure developed by Adolphs et al. (2001) for use with children. The child will first be shown 39 facial expressions of basic emotions—six instances each of happiness, surprise, fear, anger, disgust, and sadness, as well as three neutral emotions (Ekman, 1976)—and asked to identify the emotion in each face ("How does this person feel?") and to rate its intensity ("How happy is s/he?") by selecting one of four thermometers drawn as being 25% full, 50% full, 75% full, and 100% full, respectively. The child will then be shown a series of 50 faces, half of which have been rated highly trustworthy and approachable and half of which have been rated highly untrustworthy and unapproachable by normal controls in several studies. This test will be adapted for use with children at this age through pilot testing that we will first conduct in Detroit and subsequently in Cape Town. For example, the child may be told to imagine that the person in the photo has asked to borrow one of his/her favorite toys or his/her pet and will then be asked whether s/he trusts that that person will return the toy/pet. The child will again be shown the drawing of the four thermometers and will be asked to indicate for each photo how sure s/he feels about whether s/he is likely or not likely to get the toy/pet back.

At the 6-year visit, the Achenbach (1991, 2001) *Child Behavior Checklist* (CBCL), the *Disruptive Behavior Disorders Rating Scale* (DBD; Pelham et al., 1992), and the *Vineland Adaptive Behavior Scales-Revised* (VABS-R; Sparrow et al., 1984) will be administered to the mother. All three of these instruments have been recommended by the Neurobehavioral Core and will be administered in other CIFASD projects, as well. The CBCL interview assesses social competence and behavior problems. The 1991 version, which has been translated into Afrikaans, contains 112 behavior problem rating scales that are summarized in terms of eight problem syndromes—withdrawn, somatic complaints, anxious/depressed, social problems, thought problems, attention problems, delinquent behavior, and aggressive behavior—and three summary scales—internalizing, externalizing, and total. The new items introduced in the 2001 version will be translated into Afrikaans. In addition to the problem syndromes, the new version also provides six DSM-oriented summary scales—affective problems, anxiety problems, somatic problems, attention deficit hyperactivity problems, oppositional defiant problems, and conduct problems. The DBD is a 45-item checklist assessing attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder, and conduct disorder based on DSM-IV criteria. The VABS-R, a semi-structured interview, assesses adaptive behavior in three domains: communication, socialization, and daily living. The DBD and the VABS-R will be translated into Afrikaans. The mother will also be asked the name and school address of the child's classroom teacher and will be asked to sign a consent form requesting that the teacher provide information about the child's classroom behavior. The teacher will be sent the Achenbach *Child Behavior Checklist-Teacher Report Form* and the DBD.

Physical Growth and FAS Diagnosis

Weight, length, head circumference, and skinfold thickness will be measured at the end of both visits, following the protocol provided by the CIFASD Dysmorphology Core. As with all measures requiring judgment by the examiner, each examiner will be trained to a 90% reliability criterion, and interobserver reliability will be checked periodically throughout the study. The Edinburgh Handedness Inventory (Oldfield, 2000) will be administered at 4 years. At both visits, the child will be examined for presence of alcohol-related craniofacial dysmorphic features by Dr. Khaole according to the protocol provided by the Dysmorphology Core. Children with the facial features characteristic of FAS (small palpebral fissures, flat midface, smooth philtrum), significant growth retardation (< 10th percentile for height and weight or < 3rd percentile for head circumference), and evidence of poor neurobehavioral function will be diagnosed as having FAS. Frontal and profile 3-D photos will be taken of the child's face under Dr. Khaole's supervision using the protocol provided by the CIFASD Facial Imaging Core. The mother will also be asked about her child's health history at each visit, including major illnesses, accidents, and hospitalization.

Control Variables

Maternal report. In order to assess current maternal drinking, the mother will be interviewed regarding her alcohol and drug use using a timeline follow-back approach to determine incidence and amount of drinking on a day-by-day basis in a typical 1-week period during the past year (Sokol et al., 1983; S. Jacobson et al., 2002a). Volume will be recorded for each type of beverage consumed each day and converted to absolute alcohol (AA). Average oz AA/day, average number of drinks per drinking day (dose/occasion), and number of days (frequency) of drinking will be tabulated. The mother will also be asked about the number of years she has been drinking on a regular basis and frequency of her current binge drinking (≥ 5 drinks per occasion), smoking, and drug use. The Diagnostic Interview Schedule, Version IV, Section R (Robins et al., 1995), will be administered to determine whether the mother meets DSM-IV criteria for a diagnosis of alcohol abuse or alcohol dependence.

Three demographic measures that may have changed since the infant phase of this study—socioeconomic status (SES), martial status, and residential crowding—will be reassessed. SES is assessed on the Hollingshead (1975) Scale, which is based on parental education and occupational status. Residential crowding is defined as a ratio of more than one inhabitant per room in the child's home (Bradley et al., 1994). The mothers will also be asked questions regarding the type of housing (shanty, project, etc.) they live in; whether their home has running water, electricity, a telephone, stove, or refrigerator, which will be summarized in terms of number of residential amenities; and food security. The food security interview, which we have used in a nutritional study of a subgroup from our Cape Town cohort, is comprised of 18 questions regarding how often during the last year members of the family, including the child, have been hungry or had to miss or cut down the size of their meals because the family did not have the money to buy food.

The mother will be interviewed on the preschool version of the Home Observation of Measurement of the Environment (HOME Inventory, Caldwell & Bradley, 1978), a semi-structured interview that assesses quality of intellectual stimulation and emotional responsiveness provided by the caregiver. We have successfully administered the HOME Inventory to these mothers when their children were infants and to mothers in a cohort of Inuit infants that we are studying in Arctic

Quebec. The cross-cultural validity of the HOME was demonstrated in both settings in relation to SES, maternal intellectual competence on the Raven et al. (1966) Standard Progressive Matrices, maternal education, and infant/child performance on the FTII and symbolic play. The mother's verbal competence will be assessed on the Peabody Picture Vocabulary Test, which has been translated into Afrikaans. Nonverbal intellectual competence was previously assessed on the Raven. Maternal psychopathology will be assessed on the Beck Depression Inventory (Beck & Steer, 1987); the Brief Symptom Inventory (Derogatis, 1993), which assesses psychological symptoms (e.g., anxiety, hostility, paranoid ideation); and the Personality Diagnostic Questionnaire-4 (Hyler, 1994). The Depression module from the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) will be administered to determine whether the mother meets DSM-IV criteria for a depressive disorder. The Revised Conflict Tactics Scale (CTS2; Straus et al., 1996), a measure of intrafamilial conflict, will be used to assess the incidence and severity of verbal and physical conflict between the mother and her husband/boy friend in terms of five scales—negotiation, physical aggression, physical assault, sexual coercion, and physical injury. Using the Life Events Scale (Holmes & Rahe, 1967), the mother will be asked to rate any of 43 listed major life events she has experienced in the past in terms of how stressful she found each experience.

Biological measures. After the neurobehavioral assessment, the mother and child will be transported to Red Cross Hospital. A 6-cc (about 1 tsp) venous blood sample will be obtained from the child and analyzed for lead using atomic absorption spectrophotometry with a graphite rod. The child's blood samples will also be analyzed for iron deficiency anemia, based on levels of hemoglobin, mean corpuscular volume, red cell distribution width, ferritin, and transferrin. Five drops of maternal and child blood will be placed on diagnostic filter paper for genotyping of the ADH2 and ALDH2 loci, which will be performed at the University of Indiana School of Medicine under the direction of our collaborator, Lucinda Carr.

Data Verification and Transformation

We have developed detailed procedures for insuring the integrity of the data based on recommendations by the U.S. Environmental Protection Agency's Expert Committee on Pediatric Neurobehavioral Evaluations (Jones et al., 1983). Interobserver reliability is checked periodically at random intervals for all assessments requiring discretionary judgments by the examiner. Tests that entail manual scoring are scored twice, and any discrepancies are resolved by a senior investigator. Data are recorded on scannable forms for use with a Teleform program, which permits scanning of numeric data directly into the computer without requiring manual entry. This program verifies the data entry by identifying questionable values (e.g., a numeral that poorly matches the computer's template for that numeral) so that a research assistant can verify those entries by hand. Data base files will be constructed to be consistent with the format recommended by the Informatics Core, in order to facilitate the pooling of data for cross-project analyses. To reduce the influence of extreme outliers, highly skewed variables (skew > 3.0) will be subjected to log X + 1 transformation, and values greater than 3 standard deviations (SD) beyond the mean will be recoded to one greater than the highest observed value, as recommended by Winer (1971).

Data Analysis

In addition to the data analysis plan described below, we will confer with investigators from the Informatics Core and the other CIFASD projects, and additional collaborative analyses will be conducted, where deemed appropriate.

Multiple comparisons. The administration of an extensive neurobehavioral test battery raises the concern that a certain proportion of associations between exposure and outcome might be significant by chance (see Jacobson & Jacobson, in press). Although a large number of neurobehavioral endpoints will be assessed in this study, a large proportion of these endpoints have been selected to reflect three neurobehavioral domains that have been linked to prenatal alcohol exposure in previous studies. Eight of the endpoints represent elements of arithmetic, executive function, or social behavior that have previously been found to relate to fetal alcohol exposure—magnitude representation (to be assessed on the ERP arithmetic task); executive function (to be assessed on the Interdimensional/Extradimensional shift, Stockings of Cambridge, and verbal fluency tasks); attention, social problems, and externalizing behavior on the TRF; and ADHD diagnosis on the DBD. Other neurobehavioral endpoints represent elements of these same domains that have not previously been examined in relation to prenatal alcohol exposure. Where the data replicate previous findings, there is a low probability of chance findings because the odds of obtaining the same significant effect twice by chance are very low. Effects on endpoints that have not previously been examined will be considered more tentative and will be interpreted only where they are consistent with a pattern of effects on other related endpoints. Evidence of confirmation or disconfirmation will be sought in data from other CIFASD projects and future studies.

Selection of potential confounders. Potential confounding influences will be controlled statistically by multivariate analysis (see Jacobson & Jacobson, 1996). Since a control variable cannot be the true cause of an observed deficit unless it is related to both exposure and outcome (Schlesselman, 1982), association with either exposure or outcome can be used as a criterion for identification of potential confounders. Selection in relation to outcome has the additional advantage of including covariates unrelated to exposure, which can increase precision (Kleinbaum et al., 1988). Control variables that are related even weakly (at $p < 0.10$) to a given developmental outcome will be controlled statistically as potential confounders in the analyses of effects on that outcome. Six sets of control variables will be considered for inclusion in these analyses: (1) demographic background—maternal age, education, marital status, parity, SES, residential crowding, food security, number of residential amenities, type of housing, and child gender; (2) maternal smoking and drug use during pregnancy; (3) current maternal alcohol, smoking, and drug use; (4) quality of the social environment, which will be assessed in terms of interview information from the mother on the HOME Inventory, the Raven Standard Progressive Matrices, the Peabody Picture Vocabulary Test, the Beck Depression Inventory, the Brief Symptom Inventory, the Personality Diagnostic Questionnaire-4, the Revised Conflict Tactics Scale, and the Life Events Scale; (5) two biological measures—child's postnatal lead exposure and iron deficiency anemia; and (6) two situational variables—examiner and child's age when tested.

Aims 1, 2, and 6: Relation of prenatal alcohol exposure to neurodevelopmental outcome. Each of the dependent measures will be examined in separate hierarchical multiple regression analyses in relation to two measures of prenatal alcohol exposure—absolute alcohol per day averaged across pregnancy and frequency of binge drinking during pregnancy. The potential confounders will be selected as indicated above and entered in the first step of each regression; the alcohol exposure measure will be entered at the second step. A prenatal alcohol effect will be inferred only if its relation to the developmental endpoint is significant (at $p < 0.05$), after adjustment for the effects of the potential confounders. Because the mother's drinking during pregnancy may be highly confounded with her current drinking, it may be difficult to determine how the variance should be apportioned between these two variables (Jacobson, 1998). Current maternal drinking will, therefore, not be entered until the third step of the regression so that the relation between prenatal alcohol exposure and the endpoint can be considered with and without the inclusion of current drinking in the analysis. Where prenatal alcohol exposure is found to relate to a developmental endpoint, the child's

overall IQ score will be added at the final step of the regression to examine the degree to which the observed alcohol effect is related to the effects of alcohol on other aspects of general intellectual ability or whether the alcohol effect is specific to that endpoint; i.e., independent of its effects on IQ (see our discussion of the role of IQ in Table 3 above).

Where prenatal alcohol exposure is found to relate to a developmental endpoint, clinical or functional significance will be assessed as follows. Where available, established normative criteria will be used; for example, IQ < 70 is generally considered indicative of mental retardation. For outcomes lacking established criteria, scores more than 1 SD below the mean will be considered indicative of functional impairment. The odds that prenatal alcohol exposure is associated with an increased incidence in functional impairment will be assessed by examining the dichotomized endpoint in a logistic regression in relation to prenatal alcohol and the relevant potential confounders. The degree to which the adverse effects seen in FAS are also found in children with ARND will be examined in analyses of covariance comparing the scores of FAS, ARND, and unaffected children, after adjustment for potential confounders.

Aim 3: Moderator variables. Two moderator variables—maternal age and the absence of at least one ADH2*2 allele—have been hypothesized to increase fetal vulnerability to the adverse effects of prenatal alcohol exposure. The maternal age hypothesis will be tested initially by adding a prenatal alcohol by maternal age interaction term to each regression where prenatal alcohol effects on particular endpoints have been identified. McClelland and Judd (1993) have shown, however, that the interaction term has very low power in a longitudinal cohort unless the sample has been selected to overrepresent extreme values on both the independent and moderator variables. To overcome this limitation, the sample will also be dichotomized at a maternal age of 30 years, and regressions will be run separately for the two subgroups. The hypothesis that maternal age influences the expression of fetal alcohol exposure on neurodevelopment will be supported to the degree that the alcohol effects are markedly stronger and/or seen only among the offspring of the older mothers (as in Table 2). In a second set of analyses, an interaction term constructed by multiplying prenatal alcohol by the presence/absence of a maternal ADH2*2 allele will be added to the regressions indicating an association between prenatal alcohol exposure and developmental outcome. Separate regressions will also be run to compare the effects of prenatal alcohol in the children of mothers who do or do not have at least one ADH2*2 allele.

Aim 4: Predictive validity of infant assessments as indicators of FASD. The usefulness of infant numerosity and A-not-B as early indicators of fetal alcohol deficit will be assessed by evaluating the predictive validity of these measures for alcohol-related cognitive deficits in childhood. Each of the executive function measures found to be related to prenatal alcohol exposure will be examined in a multiple regression analysis in relation to pregnancy drinking and A-not-B performance. If the entry of A-not-B in the analysis reduces the association with pregnancy drinking, we can infer that the infancy measure provides an early indication of the prenatal alcohol effect on that endpoint. Similar analyses will be performed to determine the degree to which infant numerosity performance is predictive of alcohol effects on the arithmetic endpoints to be assessed at follow-up. Where an infant measure appears to provide an early indication of a specific deficit, receiver operating characteristics (R-O-C) curves will be constructed to evaluate the efficiency (i.e., sensitivity and specificity) of particular cutpoints on the infant measure in predicting the alcohol-related deficit in childhood.

Aim 5: 3-D photography. The utility of the key parameters generated by the quantitative assessment of the 3-dimensional digitized photos will be evaluated by examining these parameters in relation to the mother's report of her drinking during pregnancy (including timing and pattern of

drinking), the FAS diagnoses performed in infancy and at follow up, and neurobehavioral endpoints found to relate to prenatal alcohol exposure. The most promising parameters to emerge from these analyses will be selected to determine if, individually or in combination, they can be used to predict which of the alcohol-exposed children are impaired in terms of their cognitive and behavioral development. As in the predictive validity analyses described in the previous paragraph, multiple regression will be used to determine if the association of the neurobehavioral endpoint with prenatal alcohol exposure is reduced by the entry of these craniofacial parameters into the analysis.

Timetable

February 1 – May 31, 2004. Data collection protocols will be prepared for the 4-year follow-up and translated into Afrikaans, where necessary. After pilot testing the assessment procedures in Detroit, Dr. S. Jacobson will travel to Cape Town to adapt the protocols for this cohort and train Andrea Hay, M.A., a clinical psychologist who administered the assessments during the infant phase, to administer the tests to the children at 4 years. The Cape Town research staff will complete the pilot testing under Dr. Molteno's supervision after Dr. Jacobson returns to Detroit.

June 1, 2004 – December 31, 2005. The 4-year assessments will be performed, including data collection, protocol scoring, and data entry. Dr. S. Jacobson will return to Cape Town periodically to monitor the reliability of the assessments, trouble shoot, and respond to questions regarding the research protocol as they arise. Analysis of the 4-year data will begin toward the end of this period.

January 1 – May 31, 2006. Data collection protocols will be prepared for the 6-year follow-up and translated into Afrikaans, where necessary. After pilot testing in Detroit, Dr. S. Jacobson will travel to Cape Town to adapt the 6-year protocols for this cohort and to train Ms. Hay to administer the tests. Dr. Sivan will join Dr. Jacobson in Cape Town to set up the ERP equipment and train the research staff to administer the ERP assessments. After the Cape Town staff has practiced for 1 month with several children, Dr. Sivan will return to complete the training. The Cape Town research staff will complete the pilot testing of the 6-year protocols under Dr. Molteno's supervision.

June 1, 2006 – December 31, 2007. The 6-year assessments will be performed, including data collection, protocol scoring, and data entry. Drs. S. Jacobson and Sivan will return to Cape Town periodically to monitor the data collection. Analysis of the 4-year data will be completed, and analysis of the 6-year data will begin toward the end of this period.

January 1 – April 30, 2008. Analysis of the 6-year data will be completed, and the results of the study will be written up and submitted for publication.

E. HUMAN SUBJECTS

1. RISKS TO THE SUBJECTS

Human Subjects Involvement and Characteristics. An estimated 133 of the 159 children and mothers who participated in our original Cape Town Mother-Infant Collaborative study will be interviewed and evaluated when the children are 4 and 6 years old. Each child and his/her mother will be transported by the staff driver and research nurse to our research laboratory at the University of Cape Town Medical School for a 3-hour developmental assessment at 4 and 6 years. The mothers were recruited from the Hanover Park antenatal clinic, a Midwife Obstetric Unit (MOU) serving the Cape Coloured (mixed ancestry) population. The sample was selected to overrepresent women who drank at least 14 drinks per week (1.0 oz AA/day) or engaged in at least two incidents of binge

drinking (≥ 5 drinks) per month during the first trimester of pregnancy. The next woman initiating antenatal care whose gestational age was within 2 weeks of the heavy drinking mother was also invited to participate in the study, provided that she drank fewer than 7 drinks per week (0.5 oz of AA per day) and did not binge drink. Only Cape Coloured mothers and infants were included in the cohort because theirs is the only ethnic group in South Africa with an exceptionally high incidence of heavy alcohol use during pregnancy and fetal alcohol syndrome (FAS).

Sources of Materials. A neurobehavioral assessment battery (described under "Experimental Design and Methods" above) will be administered to the children at 4 and 6 years. Behavior rating scales will be completed by the child's mother and classroom teacher. The mother has been administered the Raven Progressive Matrices and will be administered the Peabody Picture Vocabulary Test. Standard instruments selected to assess quality of the child-rearing environment will be administered, including the HOME Inventory, the Conflict Tactics Scale, degree of life stress, and measures of maternal depression and psychopathology. The mother will also be interviewed regarding her socioeconomic, marital, and welfare status and current alcohol, smoking, and drug use. All interviews will be conducted in Afrikaans. Data on prenatal exposure to alcohol and illicit drugs were obtained by maternal interview during the infant phase of this research, and infant growth and neurodevelopmental data are also available. Alcohol-related craniofacial dysmorphology will be assessed at the 4- and 6-year visits by a physician trained to assess FAS. Frontal and profile 3-dimensional photos will be taken of the child, under the supervision of the pediatrician, who will examine the child. The child will also be weighed and measured. A 6-cc (about 1 tsp) venous blood sample will be obtained from the child and analyzed for lead levels and iron deficiency anemia at Red Cross Hospital. Five drops of maternal and child blood will also be placed on diagnostic filter paper for genotyping of the ADH2 and ALDH2 loci, which will be performed at the University of Indiana School of Medicine.

Potential Risks. The neurobehavioral assessment procedures administered in this study have been adapted for use with young children and should not entail any significant physical or psychological stress. The blood draw is the only invasive procedure. Risks are minimal because this procedure will be performed by an experienced phlebotomist at the Red Cross Hospital in Cape Town. Apart from some possible minor bruising of the child's arm from the blood draw, the only risks to the mother relate to the possibility of her experiencing some stress when reporting sensitive material regarding her alcohol and drug use, violence in the home, and psychological distress and the possibility that suspected child abuse or neglect might be reported by the investigators (see below). Confidentiality will be carefully maintained as described below. Any adverse events will be reported immediately in person or by telephone to the Co-Principal Investigator, Christopher D. Molteno, MD, who will promptly report them to the University of Cape Town ethics committee and to the Principal Investigator, Sandra W. Jacobson, PhD, who will then report them to the Wayne State University Human Investigation Committee.

Given the risk of child abuse and neglect and the legal requirement that suspected abuse or neglect be reported, there is some risk that the mother may expose herself to legal action by participating in this research. To inform her of this risk, the following language will be included in the consent form: "I understand that evidence of child abuse or neglect will be reported to the appropriate authorities." As with all elements of the informed consent, this information will also be communicated orally in Afrikaans before the mother is asked to sign the consent form.

2. ADEQUACY OF PROTECTION AGAINST RISKS

Recruitment and Informed Consent. The mother or guardian of each eligible child will be contacted by telephone and invited to participate in the follow-up study. If neither she nor the friends and relatives whose phone numbers she gave us during the earlier phases of the study can be reached by phone, our research nurse will try to locate her by visiting her at her last known address and those of the friends and relatives whose addresses she has given us. When the mother and child arrive at the laboratory, the examiner will review the study procedures in detail with the mother, emphasizing that participation is voluntary and that consent can be withdrawn at any time. The mother will also be told that any evidence of child abuse or neglect will be reported to the appropriate authorities. All questions raised regarding the study will be answered. A consent form in (Afrikaans) outlining the procedure to be followed will then be presented to the mother for her signature, and she will be given a copy to take home.

Protection Against Risk. To insure confidentiality, only subject identification numbers will be recorded on assessment forms and laboratory reports. Access to names will be limited to project staff members who need to contact participants by telephone or in person. Published reports will not use any names or information that could identify study participants unless specific informed consent is obtained.

3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

Benefits to participating families include small cash payments, photos, and gifts as well as feedback on the child's current capabilities and performance deficits. With the consent of the mother, referral for remedial or medical help or social services will be made in the event that evidence of clinically significant cognitive, medical, or emotional problems are found or if, in the course of reporting on substance abuse, violence in the home, or psychological distress, a referral is requested by the mother. Assessment information will be forwarded to school authorities or social services **only** if requested by the mother. If the child meets the criteria for iron deficiency anemia or for treatment of lead poisoning, we will contact the mother and recommend that the child be evaluated by a physician or nurse at Red Cross Hospital, for possible treatment.

4. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

While risks involved are minimal and the breach of confidentiality is extremely unlikely, the potential benefits to society are significant. These include the opportunity (a) to refine scientific understanding of the specific pattern of deficits associated with heavy prenatal alcohol exposure and examine the consistency between effects seen in children with FAS and ARND; (b) to obtain more specific information regarding alcohol-related deficits in number processing, executive function, and socioemotional information processing; (c) to evaluate the significance of these deficits for the ongoing, day-to-day function of the individual child; (d) to evaluate the extent to which two specific moderator variables—maternal age and the absence of an ADH2*2 allele—can improve the identification of FASD in prenatally-exposed children; and (e) to assess the utility of a new 3-dimensional photography procedure to improve the validity of FASD diagnosis by detecting subtle craniofacial anomalies in children with ARND. The major aim of this research is to improve the diagnosis of FASD, thereby resulting in earlier identification and treatment of affected children. Increased understanding of the core deficits associated with FASD will also facilitate development of more targeted interventions.

Collaborating Sites

The OHRP assurance number for the University of Cape Town and the affiliated hospitals (Groote Schuur, Red Cross, and Mowbray Maternity Hospitals) is FWA00001637.

Inclusion of Women, Minorities, and Children

Because effects on child development are the focus of this research, children and their mothers will be studied. The sample is limited to the Cape Coloured population because of the unusually heavy alcohol use during pregnancy and the extremely high incidence of FAS in this community. This population provides an unusual opportunity to study the role of prenatal alcohol exposure on neurobehavioral development and to better understand the specific deficits related to heavy alcohol use during pregnancy. Generalizability to other ethnic and racial groups will be assessed by comparing our data and findings with the other ethnic/racial groups represented in the other CIFASD cohorts.

Name of Applicant *(Last, first, middle):* Jacobson, Sandra W.

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: Identification of FASD in South African Children (U01 Research Project)

Total Enrollment: 159 **Protocol Number:** _____

Grant Number: _____

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Sex/Gender			
	Females	Males	Unknown or Not Reported	Total
Hispanic or Latino				**
Not Hispanic or Latino	76	83		159
Unknown (Individuals not reporting ethnicity)				
Ethnic Category: Total of All Subjects*	76	83		159 *
Racial Categories				
American Indian/Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American	76	83		159
White				
More than one race				
Unknown or not reported				
Racial Categories: Total of All Subjects*	76	83		159 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Unknown or Not Reported	Total
American Indian or Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White				
More Than One Race				
Unknown or not reported				
Racial Categories: Total of Hispanics or Latinos**	0	0	0	0 **

* These totals must agree.
** These totals must agree.

Targeted/Planned Enrollment Table
This report format should NOT be used for data collection from study participants.

Study Title: Identification of FASD in South African Children (U01 Research Project)

Total Planned Enrollment: 133

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino			
Not Hispanic or Latino	64	69	133
Ethnic Category: Total of All Subjects*	64	69	133
Racial Categories			
American Indian/Alaska Native			
Asian			
Native Hawaiian or Other Pacific Islander			
Black or African American	64	69	133
White			
Racial Categories: Total of All Subjects *	64	69	133

*The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: Total of All Subjects."

F. VERTEBRATE ANIMALS

None.

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