**Emory University Institutional Review Board**

**Sociobehavioral Protocol**

**Title**: Precursors of Social Engagement Before Birth

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**1.** **Background**

The goal of this study is to determine whether autism is already present at birth by measuring fetal behavioral responses to environmental stimulation *in utero*, and relating these responses to postnatal outcome.

The third trimester is the earliest known timepoint at which atypical behavior relevant to social development in autism may potentially be detected. Typically developing fetuses hear and respond to sound conducted both through ambient air and through the mother’s body by the thirty-fifth week of gestation. During the third trimester, fetuses develop preferences for specific sounds, including the maternal voice, that persist through birth and into postnatal life. Preference for voices learned *in utero*, and specifically the maternal voice, bootstrap the normal development of social engagement and bonding in the first months after birth. Social engagement and voice preferences in autism deviate from typical development by the first birthday, but it is not yet known whether the deficit in social interaction that characterizes autism is present at birth or develops later on.

The aim of this research is to determine the earliest point at which autism derails typical development of social engagement, by comparing fetal behavioral responses to voices and other sounds in typically developing fetuses with those of fetuses at risk of autism. We test the hypothesis that precursors of normal mechanisms of social engagement in infants who go on to develop autism are intact *in utero* against the hypothesis that the developmental derailment associated with the disorder has already begun before birth. By tracking a cohort of 130 infants who go on to develop autism (ASD), high-risk infants who do not develop autism (DD), and typically developing controls (TD) from 35 weeks gestation to diagnosis at 36 months of age, we will be able to relate prenatal behavior to postnatal outcome, and assess the potential for developing prenatal biomarkers for ASD.

**A. Specific Aims:**

*Aim 1: To characterize fetal behavioral responses to the maternal voice, other voices, and other sounds.*

Our first specific aim is to test whether fetuses respond differently to the maternal voice, other voices and other sounds, and to establish the mechanism behind these differences. Using 4D ultrasound to measure motor and cardiac responses to externally presented audio stimuli and audio stimuli that are spoken by the mother, we test the hypothesis that fetuses respond differently to sounds that are transmitted indirectly through the air and sounds that are also conducted directly through the body.

*Hypothesis H1*: Fetal preference for the maternal voice over other voices and sounds is driven by the selective contingent reinforcement that occurs because the mother’s body vibrates when she speaks at the same time that airborne sound radiates from her mouth and is transmitted to the womb.

*Aim 2: To determine differences in fetal behavioral responses to sound between infants who go on to develop autism, high-risk infants who do not develop autism, and typically developing controls.*

Our second specific aim is to determine whether fetuses who go on to develop autism respond differently to the maternal voice, other voices and other sounds relative to other diagnostic groups. By replicating existing findings in typically developing fetuses, and extending these to fetuses at risk of autism, we test the hypothesis that high-risk fetuses will differ from typically developing fetuses in showing no difference in response to different stimulus types, indicating prenatal onset of deficits in social engagement characteristic of autism.

*Hypothesis H2a*: Typically developing fetuses prefer the maternal voice to other voices. Fetuses who go on to develop autism show no preference for the voice of their mother.

*Hypothesis H2b*: Typically developing fetuses prefer voices to other sounds. Fetuses who go on to develop autism show no preference for voices.

*Aim 3: To relate differences in fetal behavioral responses to sound to postnatal outcome in infants who go on to develop autism, high-risk infants who do not develop autism, and typically developing controls.*

Our third specific aim is to determine whether differences in prenatal responsiveness to sound reflect postnatal differences in social communication. By comparing behavioral measures taken before birth with diagnostic assessments at 12, 24 and 36 months after birth, we test the hypothesis that fetal sensitivities to maternal voices relative to other voices, and voices relative to other sounds, can be used as: (a) categorical diagnostic markers; (b) predictors of autistic symptomatology; and (c) a means of defining endophenotypes within ASD.

*Hypothesis H3a*: Differential responses *in utero* to the maternal voice, other voices, and other sounds discriminate between TD/DD infants and infants diagnosed with ASD.

*Hypothesis H3b*: Dimensional outcome measures of social communication can be predicted from differential responses *in utero* to the maternal voice, other voices, and other sounds.

*Hypothesis H3c*: ASD subtypes derived from dimensional outcome measures correspond to subtypes derived from differential responses *in utero* to the maternal voice, other voices, and other sounds.

**B. Preliminary Studies:**

Autism Spectrum Disorders (ASD) are devastating neurodevelopmental disorders of early onset characterized by a triad of deficits in social interaction, communication, and repetitive and restricted behaviors (American Psychiatric Association, 2000) affecting 1 in 88 children in the United States (Centers for Disease Control and Prevention, 2012).

Genetic factors are known to influence the risk of autism (Folstein & Rutter, 1977), so the onset of the condition may well be present before birth, although symptoms usually appear during the first year of life and clinical diagnosis is often not confirmed until three years of age or after (Cox et al., 1999; Pinto-Martin & Levy, 2004; Stone et al., 1999).

Early intervention is known to be critical in ensuring optimal outcome for children affected with the disorder (Landa, 2007; Zwaigenbaum et al., 2007). However, clinical diagnosis is typically not reliable until at least two years of age, and it is unclear whether it may be possible to detect autism earlier in infancy. No studies of autism extend before two months of age, and none yet have probed for signs of autism before birth. The ability to intervene early is currently severely limited by late age of detection, and would be improved by pushing back the point of detection to the earliest point at which measurable symptoms of the condition begin to emerge.

Accordingly, motivated by previous studies of prenatal development, the aim of our research is to examine fetal behavioral responses to environmental stimuli in the third trimester to determine whether the necessary precursors of mechanisms of social engagement seen in typically developing children are in place at birth in children with autism, and to use these results to develop biomarkers of risk that will pick up any signs of autism by the time a child is born.

*Aim 1: To characterize fetal behavioral responses to the maternal voice, other voices, and other sounds.*

*Fetuses hear predominantly low-frequency components of sound by the 35th gestational week*. The auditory structures important for hearing (e.g. tympanic membrane, organ of Corti, cochlea, etc.) are developed by the eighth month of gestation, and the cochlea is developed and adultlike in its sensitivity by 35 weeks’ gestation (Pujol, Lavigne-Rebillard, & Uziel, 1990; Pujol & Uziel, 1988).

Infants are therefore able to hear in the womb, where they are exposed to both intra- and extra-uterine sound. Intra-uterine sound consists of low-frequency background noise produced by the maternal body (e.g., blood movement, borborygmi) (Querleu, Renard, Versyp, Paris-Delrue, & Crèpin, 1988; Querleu & Renard, 1981). Extra-uterine sound includes both ambient noise and human speech. Due to the severe attenuation of higher frequency sound waves by the abdominal wall (Benzaquen, Gagnon, Hunse, & Foreman, 1990; Querleu, Renard, Boutteville, & Crepin, 1989; Querleu & Renard, 1981), the information the fetus receives from speech is primarily limited to fundamental frequency contour and rhythmic components of intonation (Mehler et al., 1988; Nazzi, Bertoncini, & Mehler, 1998). Nevertheless, studies indicate that some consonant and vowel distinctions may be perceptible in the womb, and it may be possible for entire sentences to be identified (Griffiths, 1994; Smith, Gerhardt, Griffiths, Huang, & Abrams, 2003).

The maternal voice is a substantial component of the uterine sound environment. Although the abdominal wall attenuates higher frequency components of speech, intrauterine recordings made using a hydrophone demonstrate that low-frequency information in the maternal voice is carried to the fetus relatively unaltered, and is louder than either background noise or other extra-uterine sounds (Benzaquen et al., 1990; Querleu et al., 1989; Richards, Frentzen, Gerhardt, McCann, & Abrams, 1992; Vince, Billing, Baldwin, Toner, & Weller, 1985). Maternal speech is also distinct from other sounds in that it is transmitted to the fetus as intrauterine vibration as well as extrauterine sound. Since cutaneous sensation of vibration is functional by 11 weeks’ gestation (Humphrey, 1978), the third trimester fetus likely perceives co-occurring auditory and cutaneous stimulation during maternal speech. No study to date has established whether fetal perception of maternal body vibration facilitates fetal discrimination between socially relevant speech and irrelevant ambient noise. Further research is additionally needed to identify the specific acoustic qualities that may allow the fetus to distinguish between speech and nonspeech sound.

*Fetuses respond to sound with physiological changes that reflect the perceived salience of stimuli.* Fetal behavioral responses to instances of *ex utero* sounds indicate that the fetus both attends to and differentiates between different types of *ex utero* noise. These responses include fetal heart rate (FHR), eye blink movements, breathing movements, and gross motor movements (Nijhuis, Prechtl, Martin, & Bots, 1982). Changes in FHR typically provide the most statistically significant and reproducible reactions to external stimuli, although some studies report interactions suggestive of increasing response valence between FHR and a movement reflex (Lecanuet, Granier-Deferre, Cohen, Le Houezec, & Busnel, 1986; Zimmer et al., 1993). FHR deceleration occurs reliably in response to many classes of auditory stimuli (Fifer, 1994), and has been associated with recognition (Granier-Deferre, Ribeiro, Jacquet, & Bassereau, 2011) and the cardiac orienting response (Clarkson & Berg, 1983; Pomerleau-Malcuit & Clifton, 1973; Richards et al., 1992; Weisbard, Graham, & Graham, 1971) in older children. On this basis, many investigators use FHR deceleration as an indicator of perceived salience for the fetus when it occurs in response to stimuli played at less than 100 dB *ex utero* (Kisilevsky, Hains, Jacquet, Granier-Deferre, & Lecanuet, 2004; Lecanuet, Granier-Deferre, Jacquet, & Busnel, 1992). In accordance with the interpretation of FHR as an orienting response, fetuses only exhibit a statistically significant cardiac deceleration in response to some auditory stimuli classes (Kisilevsky et al., 2009). Differential amplitudes of cardiac deceleration to different stimuli classes have also been reported (Granier-Deferre et al., 2011). No studies have verified that these differential responses to voices and sound exist in autism, however.

All of these previous studies have been limited to the use of basic 2D Doppler ultrasound for descriptive assessments of fetal behavior, and have never attempted to explain the origin of prenatal sound preferences. Replicating these observations in our study using high spatial and temporal resolution 4D ultrasound in a series of tightly controlled hypothesis-driven experiments will not only enable us to finally achieve a fully quantified characterization of fetal behavioral responses to environmental stimulation, but will also allow us to identify the mechanism that may underlie preferential attention to the maternal voice and speech.

*Aim 2: To determine differences in fetal behavioral responses to sound between infants who go on to develop autism, high-risk infants who do not develop autism, and typically developing controls.*

*An infant responds to its own mother’s voice both before and after birth.* In typically developing children, differential prenatal exposure to the maternal voice precedes postnatal preferences for maternal speech, and thus bootstraps further development of language and social engagement. The low frequency components of the mother’s voice, specifically those carrying the mother’s intonation and speech prosody, are louder to the fetal ear than most other *in utero* sounds (see preliminary studies for Specific Aim 1). Subsequently, fetuses show a significant FHR response to the voice of the mother, but not to the voice of a father or a stranger female (Kisilevsky et al., 2009), although fetuses are capable of distinguishing between maternal and other voices (DeCasper, Sigafoos, & Prescott, 1984; Lecanuet & Granier-Deferre, 1993).

The elevated status of the maternal voice to the neonate continues after birth. Newborns orient towards their mother’s voice over other voices, including the father’s voice (DeCasper et al., 1984). An infant with little or no postnatal experience of its mother’s voice will show the most orienting movement towards the mother’s speech when compared with a female stranger’s voice (Fifer & Moon, 1994; Querleu et al., 1984). In a sucking paradigm, newborns activated a recording of the mother’s voice more often than a recording of a female stranger’s voice after only twelve hours of postnatal contact with the mother (DeCasper & Fifer, 1980). Preference for the mother’s voice is so strong that it can also act as a reinforcer for infants who must learn to control their suck rate precisely to elicit the mother’s voice (Moon, Bever, & Fifer, 1992) or to trigger the mother’s voice rather than that of a stranger female (DeCasper & Fifer, 1980).

Evidence exists that these preferences are formed from frequent prenatal exposure rather than an inherent preference for the birth mother’s voice. Newborns exhibit preferences for both adult-directed and infant-directed excerpts from maternal speech (Fifer & Moon, 1994), although their experience with adult-directed speech postnatally is limited. Infants also prefer low-pass filtered (i.e., prenatal approximation) to unaltered (i.e., postnatal approximation) maternal speech stimuli, although this preference is switched for non-maternal female speech samples (Spence & DeCasper, 1987; Spence & Freeman, 1996). It is possible that the mechanisms by which the maternal voice is made salient to the fetus, explored in Specific Aim 1, directly shape postnatal sound preferences.

*Individuals with ASD display lack of preference for maternal voice after age 3.* Preference for the maternal voice, and for intelligible speech in general, persists into late childhood for typically developing children (Klin, 1992). However, older children with autism do not display a preference for human speech over other sounds. Clinical observations note that autistic individuals display a lack of preference for speech (Clancy & McBride, 1969; Kanner, 1942). Studies show that autistic children over three years old do not display a preference for intelligible human voices (Klin, 1992), and may even favor complex nonspeech sounds over speech (Kuhl, Conboy, Padden, Nelson, & Pruitt, 2005; Lepistö et al., 2005; Vouloumanos & Werker, 2004). An EEG study found evidence that children with ASD orient to speech with more difficulty relative to nonspeech sounds when compared to a cohort of TD children (Lepistö et al., 2005). Older individuals with autism have a more difficult time perceiving human speech against a background of noise, which could be due in part to difficulty perceiving the human voice as salient (Alcántara, Weisblatt, Moore, & Bolton, 2004). Furthermore, this apparent indifference to human speech most likely does not emerge from a widespread hearing deficit characteristic of the condition (Rosenhall, Nordin, Sandström, Ahlsén, & Gillberg, 1999). Many autistic individuals, in fact, display heightened sensitivity to sounds (Iarocci & McDonald, 2006).

Evidence suggests that early preference for the maternal voice is crucial for normal development of social engagement. If individuals with autism fail to form an affinity for the maternal voice before birth, this may explain why social interaction and communication are impaired after birth. Exaggerated prosodic variation is the key feature of infant-directed speech, which caregivers use to draw infant attention in the first years of life. If difficulties with prosodic perception observed in older individuals with ASD are also present in the womb, when typically developing fetuses begin to respond to intonational cues, this may also explain the failure of infants with ASD to orient properly towards caregivers’ voices. For many possible reasons, fetal behavioral responses to environmental sound thus provide a critical scaffold for social engagement and spoken language that may be missing in autism.

To our knowledge, no studies have extended the investigation of responses to maternal voice in autism backwards to the fetal period, when we would expect a typically developing child to begin exhibiting a preference for the voice of its mother. There are also no studies that track the prosodic deficits in speech production and perception commonly observed in autism back towards birth. Our study will address both of these gaps in the literature. By using 4D ultrasound to characterize differences in fetal behavioral responses to environmental stimuli between typically developing fetuses and fetuses at risk of autism, we will be able to tell whether preferential attention to the intonation of the mother’s voice is intact in the womb. This will allow us to assess the potential for using prenatal ultrasound as the basis for developing biomarkers for risk of ASD.

*Aim 3: To relate prenatal behavioral responses to sound to postnatal outcome in infants who go on to develop autism, high-risk infants who do not develop autism, and typically developing controls.*

*Postnatal preferences for speech-relevant sounds reflect prenatal exposure to those sounds*. Infants will react preferentially to melodic features learned *in utero* (Granier-Deferre et al., 2011). Fetuses are capable of perceiving fundamental frequency modulation, for they respond to changes in fundamental frequencies in musical notes (Lecanuet, Granier-Deferre, Jacquet, & DeCasper, 2000) Neonates demonstrate a preference for (DeCasper & Spence, 1986), and fetuses an orienting response towards (DeCasper, 1994), a story that was read to them repeatedly during the last trimester of pregnancy. In those studies, a novel story was not reinforcing postnatally, nor did it elicit a prenatal orienting response.

In light of this prenatal learning of auditory preferences, prenatal exposure to speech may lead to the special status of speech for the infant postnatally. Children with typical language development reflexively orient towards speech sounds from birth (Vouloumanos & Werker, 2007), and will do so for longer than they orient environmental sounds or macaque calls (Shultz & Vouloumanos, 2010; Vouloumanos, Hauser, Werker, & Martin, 2010). These preferences extend to preferences for language; newborns and fetuses alike demonstrate the ability to discriminate between the mother’s native language and an unheard language, suggesting that experience with the mother’s speech *in utero* influences immediately postnatal preferences. By four days old, neonates both prefer the mother’s native language and discriminate between the maternal language and a foreign language (Mehler et al., 1988; Moon, Cooper, & Fifer, 1993). Within five days of delivery, newborns can distinguish between foreign languages using only rhythmic (Nazzi et al., 1998) and prosodic (Mehler et al., 1988) cues. Infant preference for the maternal native language exists even when the speech sample is low-pass filtered to remove almost all information save the fundamental frequency contour, suggesting that the acoustic information about voices learned *in utero* may rely on fundamental frequency or rhythm information (Mehler et al., 1988; Nazzi et al., 1998). If body vibration concurrent with sound allows the fetus to discriminate between maternal speech and other forms of speech, then body conduction of maternal speech may be the mechanism by which fetuses develop a preference for maternal voice in the womb.

*Learned preference for social vocal stimuli may underpin social visual development.* Learning a preference for voices may additionally scaffold a preference for faces and, therefore, prime an infant’s ability to interact socially. Growing evidence indicates that an association between the preferred maternal voice and the appearance of a talking face establishes the infant’s preference for specific faces early in life. A series of experiments demonstrated that an infant would not form a preference for the maternal face without seeing the speaking maternal face (Sai, 2005). In the absence of olfactory information, one-month-old infants only discriminated the mother’s face from a female stranger’s face in the presence of speech information (Burnham, 1993). By three months of age, infants demonstrated a preference for the maternal face without speech information. The infant might therefore learn to associate the synchronous movement of the maternal face with the preferred maternal voice until the face alone is sufficient to elicit interest and engagement from the infant. If humans use prenatal learning of the mother’s voice to scaffold later social interactions, they would not be the only species to do so; vocal learning during development prepares the bobwhite quail for important social communication after birth (Sleigh & Lickliter, 1998). Early preferences for voices, which may lead to early preferences for language and faces, may be responsible for social and communicative development in typically developing children.

All of these results suggest that prenatal experience impacts postnatal behavior, and that long-term postnatal outcome may well be predicted by behavioral responses that can be measured before birth. In the context of autism, it is possible that aberrant responses to voices *in utero* that we predict may be found in individuals with autism may later contribute to the core deficit in social communication associated with ASD that emerges in the first years of life. By correlating quantitative measures of fetal behavior with clinical measures of postnatal outcome assessed at 12, 24, and 36 months of age, we will be able to determine whether our biomarkers of risk for autism may serve as: (a) categorical diagnostic markers; (b) predictors of autistic symptomatology; and (c) a means of defining endophenotypes within ASD.

**C. Significance/Justification:**

Autism is a devastating neurodevelopmental disorder of early onset, characterized by a triad of deficits in social interaction, communication, and repetitive and restricted behaviors. Genetic factors are known to influence the risk of autism, so the onset of the condition may well be present before birth, although symptoms usually appear during the first year of life and clinical diagnosis is often not confirmed until three years of age or after. Early intervention is known to be critical in ensuring optimal outcome for children affected with the disorder, but at present there is no way to detect the onset of autism in infancy.

At present, autism can only be reliably diagnosed at around two years of age, through a battery of expensive and time-consuming clinical assessments that need to be conducted by experienced clinicians. Early diagnosis and intervention are critical in ensuring better outcome later in life, but we do not know enough about the nature or timing of the multiple developmental derailments implicated in ASD to be able to intervene effectively before the condition causes irreversible negative outcomes. Key goals of research in autism include lowering the age of diagnosis by seeking objective biomarkers of risk that can be measured automatically in infancy, and identifying the onset of specific developmental mechanisms affected by autism that can be targeted to develop interventions suited to each child.

The research we propose is significant because we show that the deficit in social engagement in autism formally diagnosed at two years of age may actually be detectable in the womb. We also target a specific mechanism of derailment, by testing whether prenatal attention to the maternal voice is intact in autism. Attention to speech and voices, and specifically attention to patterns of intonation in the maternal voice, is known to be critical in orienting infant attention to talking faces immediately after birth. This in turn scaffolds social engagement and the development of spoken communication in the first years of life, both of which are derailed in autism. Demonstrating whether or not this mechanism is intact will either confirm or eliminate a possible pathway of derailment, and may lead to the development of better evidence-based methods for early diagnosis and treatment for children with ASD.

**2. Study Design**

**A. Study Sample:**

*Subject Population*

The total target sample for this study is 130 participant families, including healthy pregnant women older than 21 years of age and their newborn children after birth.

Recruitment will be limited to families who either have an older child already diagnosed with ASD or families who have no family history of developmental disorders. Study participants must be able to visit the Marcus Autism Center in person, so families will only be recruited within travelling distance of Atlanta. Male and female children will be enrolled. Enrollment will not be limited to specific ethnic or racial groups.

Based on information collected during an intake interview, participants will be assigned on enrollment to one of three groups. Participants from families who already have a child with ASD, whose newborn child will therefore be at increased risk of developing the disorder, will be assigned to a high-risk ASD group (“HR-ASD”). Participants from families with no history of developmental disorders, whose newborn child can be expected to follow typical development, will be assigned to a low-risk group (“LR-TDX”).

Clinical characterization procedures to determine diagnosis will be conducted on all participants at 12 and 24 months. All high-risk infants, as well as all low-risk infants who exhibit behaviors or symptoms consistent with developmental delays or ASD at 24 months, will be invited to return for a final diagnostic assessment at 36 months. All other cases without developmental problems or ASD features at 24 months will be screened at 36 months to detect false negatives.

Based on the results of the final diagnostic assessment at 36 months, participants will be assigned to one of four groups at outcome. High-risk infants who go on to develop autism will be assigned to an ASD group (“ASD”). Low-risk infants with no signs of developmental delays will be assigned to a typically developing group (“TD”). Infants from any group diagnosed with developmental delays other than autism will be assigned to a developmentally delayed group (“DD”). All other infants who are neither typically developing nor fully autistic will be excluded from the present analysis, but may be reanalyzed and considered as a separate group (“XX”) at a later date.

Power analyses conducted from previous studies indicate that a sample size of at least 15 will be needed at outcome for both the group of children who develop ASD and typically developing or developmentally delayed controls.

Since this is a prospective study, recruiting before birth, the sample size for recruitment must be estimated from prevalence rates in our target population. The current prevalence rate of ASD in the general population, as estimated by the CDC in metro Atlanta, is approximately 1%, compared to 10% for non-autistic developmental delays. Previous research has shown that the recurrence rate for younger siblings of children with ASD increases to 20%; another 23% of siblings can be expected to develop features of the broader autism phenotype (BAP) and a further 12% will exhibit developmental delays (DD) not specific to the autism phenotype. From past experience, an attrition rate of <10% can be expected for a study of this kind.

Based on these estimates of attrition and prevalence rates for ASD and developmental delays in the target population, the study aims to recruit 108 children at high risk of developing autism and 22 children at low risk with typical development expected, in order to obtain at least 15 children diagnosed with autism and 15 typically developing controls at outcome.

The full diagnostic breakdown at 36 months is predicted as follows:

20 children diagnosed with an autism spectrum disorder (HR-ASD ⇒ ASD)

23 children meeting criteria for broader autism phenotype (HR-ASD ⇒ XX)

12 high-risk siblings with developmental delays (HR-ASD ⇒ DD)

45 unaffected siblings who do not go on to develop ASD (HR-ASD ⇒ XX)

20 children with typical development (LR-TDX ⇒ TD)

10 children lost to attrition (LR-TDX, HR-ASD ⇒ XX)

By deliberately oversampling by a factor of about 1.5, we expect to guarantee that recruitment targets for this study will be met, even if the published statistics we have employed for prevalence of autism and developmental delays do not exactly match the population we sample from.

If these estimates are not achieved within the initial study period requested, an amendment will be submitted to extend recruitment and enrollment until the required targets for the ASD and TD groups are reached.

*Vulnerable Subjects*

This project is a prospective study of early markers of social engagement in autism, which involves measuring fetal behavioral responses to audio stimuli using ultrasound, followed by diagnostic assessments conducted after birth. Since we recruit from families with a history of ASD, some of our participants may receive treatment for autism and related developmental disorders at the Marcus Autism Center. The study therefore necessitates the use of pregnant women, fetuses, children and patients as study subjects, all of whom are potentially vulnerable populations. All of the procedures involved in this study carry minimal risk and safeguards will be put in place to ensure that the needs of these groups are met.

*Pregnant Women and Fetuses:*

The study design specifically involves measuring behavioral responses in fetuses, and therefore necessitates the inclusion of pregnant women and fetuses. The risk involved in this study is that those participants will be exposed to ultrasound.

The ultrasound procedures employed in this study are considered to be minimal risk, and are comparable to ultrasound assessment protocols routinely employed throughout pregnancy to monitor the health of the mother and her unborn child. Since we only recruit pregnant women who have already been receiving regular clinical ultrasound assessments as part of their existing program of prenatal care, we do not expect that participants will be exposed to any extra risk beyond the risk involved in completing a single additional ultrasound scan. Since the ultrasound examination in this study occurs late in pregnancy, around 35 weeks gestation, we expect that any potential problems with the pregnancy will already have been detected through previous examinations. Since ultrasound examinations are not usually conducted as late as 35 weeks, the additional scan completed during our study may actually be of benefit to the participant, since it will provide an additional opportunity to detect any remaining problems with the pregnancy that can be determined using ultrasound.

Risk to pregnant women and fetuses arising from the use of ultrasound will be minimized by strictly following medical guidelines on ultrasound safety published by the American Institute of Ultrasound in Medicine, and limiting the dosage and exposure to ultrasound during our experimental procedures. A trained clinical sonographer will carry out all procedures under the supervision of an attending obstetrician to ensure that safety measures are implemented and guidelines are followed.

As an additional safeguard, at the beginning of each session, the obstetrician and sonographer will carry out a biophysical profile (BPP) to assess the status of the fetus. Monitoring of vital signs will continue throughout the experiment to ensure that any signs of distress are immediately detected.

An emergency procedure has been established in collaboration with the Department of Obstetrics and Gynecology at Emory School of Medicine and the Maternal-Fetal Medicine Department at Emory Midtown Hospital to handle any adverse events that may arise, including detection of distress in the fetus during the BPP or at any time throughout the experimental protocol. An ambulance will be called using a direct phone line installed in the laboratory, and the obstetrician will attend to the mother until assistance arrives, then accompany the mother to Emory Midtown Hospital, where a 24/7 obstetric emergency team will provide appropriate medical care.

Mothers of children already diagnosed with autism may experience stress and anxiety during subsequent pregnancies, both before and after birth, due to knowledge of the increased risk of autism in their unborn child and uncertainty about the status of their new baby. Trained clinicians will be available at any time to discuss concerns with participant families. They will provide reassurance and professional support to minimize the risk of distress.

*Children:*

The study design requires clinical assessments to be carried out on each newborn child after birth, at 24 and 36 months, to establish diagnosis and assign group membership at outcome.

All of the clinical assessment procedures used in this study have no known hazards and are comparable to testing procedures that have been used in psychological research for decades. There are no known risks of physical or psychological damage associated with those procedures.

When child participants visit the Center to take part in an assessment, there is a risk of distress due to the unfamiliar environment and the length of time needed to complete assessments. To minimize distress to participants, a parent will remain with the child at all times. If a child expresses displeasure due to any cause, procedures will be suspended. With parental permission, procedures will continue when the child has calmed down and no longer expresses discomfort. Members of the research team are sensitive to concerns from both the child and the parent, and parents will always be able to request that a procedure be paused or ended. In addition, parents will be able to withdraw their child from the study at any time without penalty. During each study visit, families will be given time to rest between procedures, and all assessments and procedures will be scheduled around the times when children usually eat or sleep. If a child seems especially tired on a particular visit, parents will be asked whether they would like to come back on a different day. Our Center is baby/child-oriented and fully equipped with plenty of toys and activities to make children feel comfortable. We will strive to accommodate the needs of new mothers with baby-changing stations in the restroom, a rocking chair in the waiting area, and child-safe furniture throughout the Center. All of our clinical and research areas conform to necessary standards of safety and hygiene.

*Patients:*

The study design requires enrollment of children with older siblings already diagnosed with autism, and either those siblings or the participants themselves may receive treatment as patients at the Marcus Autism Center during the course of the study.

Enrollment in the high-risk group requires confirmation of the older sibling’s diagnosis. If the older child’s diagnosis was based on clinical evaluations carried out elsewhere (not at the Marcus Autism Center) and does not contain the standardized assessments used in this study, we will require confirmation of diagnosis using a parental screener without needing the older child to visit the clinic. The results of that confirmatory assessment will be communicated to parents if they wish. If the confirmatory diagnosis does not match the previous diagnosis, there is a risk that families may experience distress upon receiving the new diagnosis. Families may also experience distress when they receive a diagnosis for their newborn child.

To minimize the risk of distress, all diagnostic information will be communicated sensitively and professionally to families by experienced clinicians, and those clinicians will be available at any time during the study to answer any questions and address any concerns that families may have. If any child receives a diagnosis of autism or a related developmental disorder as a result of participating in this study, clinicians will be able to provide families with referrals to appropriate community service providers so that they can access early intervention services as quickly and easily as possible.

To avoid any risk of coercion, during the informed consent procedure, research staff will take care to explain to families that any decision they may make not to participate in the study, or to withdraw from the study, will in no way influence their access to care or the quality of the care they receive as patients either at the Marcus Autism Center or any other institution.

The risks to patients are otherwise the same as the risks to children, and will be minimized by adopting the same safeguards.

*Inclusionary and Exclusionary Criteria*

Determination of inclusionary and exclusionary criteria will involve review of each participant’s medical record, completion of a medical history checklist, and an intake interview.

*Inclusion Criteria:*

The study will recruit healthy pregnant women at least 21 years of age and their newborn children after birth.

Recruitment will be limited to families who either have an older child already diagnosed with ASD (HR-ASD) or families who have no family history of developmental disorders (LR-TDX). Diagnosis of an older sibling will require confirmation. If the older child’s diagnosis was based on clinical evaluations carried out elsewhere (not at the Marcus Autism Center) and does not contain the standardized assessments used in this study, confirmation of diagnosis using a parental screener will be required.

Women interested in participating will only be recruited when pregnancy and an expected delivery date have been confirmed by a medical professional. Women expecting to get pregnant will not be considered as potential subjects until their pregnancy has been confirmed. Only pregnant women who have already received regular ultrasound scans as a part of their program of prenatal care will be eligible for participation. The physician who was primarily responsible for the mother’s obstetric care will need to provide written permission for her participation in this study before she is eligible to participate. Pregnant mothers may be recruited at any time before they give birth, although they must be enrolled early enough to allow enough time for experimental procedures to be carried out at 35 weeks gestation.

Study participants must be able to visit the Marcus Autism Center in person, so families will only be recruited within travelling distance of Atlanta. Male and female children will be enrolled. Enrollment will not be limited to specific ethnic or racial groups.

*Exclusion Criteria:*

Potential participants will be excluded from the study if they fail to meet inclusion criteria for one of the three intake groups. Participants will also be excluded if they or their newborn child displays signs of any of the following conditions, determined from the medical record:

* + Hearing loss or any other disorder influencing speech perception
  + Cleft palate or any other disorder influencing speech production
  + Non-febrile seizure disorders
  + Medical conditions associated with autism or any other identified genetic disorder

(e.g., Fragile X Syndrome, Tuberous Sclerosis)

* + Medical conditions requiring tube feeding or ventilation
  + Medical history indicating increased risk of complications during pregnancy

Participants will also be excluded at any time during the study if they exhibit signs of any medical condition that would prevent successful completion of the experimental and clinical assessment procedures needed as part of the study design, or would involve additional unforeseen risk.

Employees of Emory University Hospital, the Emory Clinic, Emory University, or Emory Healthcare will be excluded from the study.

**B. Setting**

All experimental procedures, clinical assessments and parent interviews will take place in the Spoken Communication Laboratory at the Marcus Autism Center (address below). The experimental procedures will be carried out in a dedicated ultrasound imaging facility by a licensed sonographer under the supervision of an obstetrician/gynecologist. The assessment procedures and parent interviews will be carried out in dedicated private clinical assessment rooms by trained clinicians. Parents will be able to complete any questionnaires required for this study at their own convenience in a location of their choice.

Addresses of study sites

Marcus Autism Center

1920 Briarcliff Road

Atlanta, GA 30329

**C. Recruitment**

*Recruitment for All Participants:*

Recruitment for the study will be carried out within the local community through flyers, brochures, newspaper advertisements, website posts, and notices sent to service providers. Advertising using flyers and newspaper advertisements will be limited to a reasonable radius of Atlanta to minimize travel burden on participant families.

Additionally, healthcare providers in the Atlanta area will be asked to refer families who are eligible for the study. Obstetricians, pediatricians and other providers will be asked to give families information about the study, and potential participants who express interest in enrolling will be invited to contact the study coordinator directly themselves by completing a prepaid response card provided to them and returning it by mail. Healthcare providers will only release contact information for families after a family has expressed interest, contacted the study coordinator, and given written permission for their information to be released.

Potential subjects will be identified when they express an interest in participating in the study by contacting our research staff themselves by phone, e-mail, or mail.

Once subjects identify themselves, research staff will contact them by phone to describe the study in detail, confirm that the subject is interested in participating, and conduct a 10-minute intake interview to determine whether they are eligible to take part. If the applicant cannot be accepted into the study, the study coordinator will convey this to them with due care and sensitivity, and will provide them with alternatives if any are known. No woman will be approached regarding participation in this study or accepted into this study at time of delivery or in any other compromising or stressful situation during her pregnancy. Women who are expecting to get pregnant will not be considered eligible until pregnancy is confirmed. Women who are already pregnant will not be considered eligible unless they provide confirmation of pregnancy by a medical professional with an expected delivery date.

Eligible subjects will be invited to visit the Marcus Autism Center, where the details of the study will be explained to them and discussed. After they have had a chance to ask questions, informed consent for participation in the study will be obtained in writing. Separate consent forms will be used to document consent for pregnant women and their partners. If participants wish to inspect the consent form beforehand, or if they wish to examine the consent form further before giving consent, a copy of the consent form will be delivered to them or given to them to take away. If participants are unable to sign the consent form in person, a copy will be mailed to them to sign and return. Should this be necessary, a member of the research team will then meet with the participant during their first subsequent visit to the Marcus Autism Center, confirm that the participant fully understood the form, answer any further questions, and verify that informed consent has been given before any study procedures take place.

With the mother’s consent, the research staff will contact the physician primarily responsible for her obstetric care during pregnancy to inform them that their patient intends to take part in the study, explain what the study involves, and obtain their consent for their patient to participate. A separate consent form will be used to document the consent of the physician. If consent is not given, the applicant will be excluded from participation in the study, and the reason for their exclusion will be explained to them.

Newborn children taking part in this study are too young to give informed consent, and consent cannot be obtained before they are born. Mothers will give permission for their child’s participation the first time the child is brought to the Marcus Autism Center for assessment. A separate form will be used to document permission for the child.

Equitable recruitment will be monitored by keeping records of gender, race, ethnicity, and demographic status for all potential research subjects who ask to participate in the study, as well as all research subjects who are actually enrolled in the study. All recruitment statistics will be de-identified to protect the identity of all applicants. Research staff will monitor recruitment statistics throughout the study to identify potential signs of inequitable recruitment, and will review recruitment procedures to address any issues as soon as they arise.

*Additional Recruitment Strategies for High-Risk ASD Sibling Group:*

The research in this study specifically requires recruitment of siblings of children with ASD, and additional recruitment strategies will be used to reach this group.

The Marcus Autism Center maintains mailing lists of families with children already diagnosed with autism who have previously been evaluated at the Marcus Autism Center and have given written consent to be contacted to participate in future research studies. Some of those families may be expecting another child, and may be eligible for recruitment to the present study. A recruitment letter along with a prepaid response card explaining the purpose of the study will be sent out to parents of children with autism who are likely to want to participate. Families will always be able to decline to participate in this study when contacted, and will be clearly told they may do so.

Families who have been affected by autism may also be reached through the Marcus Autism Center web site, Georgia Birth-To-Three service providers, parent support networks, as well as local offices of developmental pediatricians and obstetricians and other clinics in the area that specialize in ASD. These providers will be given brochures advertising the study, which will be made available to their clients. Parents of children with autism who are expecting another child will be invited to contact the study coordinator directly themselves, if they wish, by completing a prepaid response card and returning it by mail.

As part of the initial intake interview, families who already have a child with autism who did not receive a diagnosis of ASD from the Marcus Autism Center will be screened with a parent interview that will verify the diagnosis of the older sibling. If a reliable diagnosis of ASD cannot be ascertained for the older sibling, the family will not be enrolled in the study.

**D. Procedures**

*Experimental Procedures:*

Testing of fetal behavioral responses to sound will be carried out by making audio and electroglottographic recordings of mothers and their partners, using those recordings to generate audio stimuli, presenting those stimuli to fetuses using a loudspeaker, and recording fetal cardiac and motor responses using 4D ultrasound, electrocardiography, and cardiotocography.

*Audiovisual Recordings:*

Audio and video recordings of the mother and her partner will be made using a high-quality microphone and a high-resolution, high-speed video camera.

*Electroglottography:*

The intonation of the mother’s voice and her partner’s voice will be recorded using an electroglottograph (EGG). A tiny voltage (equivalent to a flashlight battery) is applied across the throat using two electrodes. By monitoring the change in electrical impedance between the electrodes caused by the opening and closing of the glottis during speech, it is possible to measure the vibration of the vocal folds and extract the time-varying fundamental frequency of the voice. The electrodes are held in place using a Velcro strap around the neck, which the participant can adjust. An alcohol swab is used to clean the surface of the skin beforehand, and hypoallergenic electroconductive gel is used to wet the electrodes and improve the electrical contact with the skin surface. Participants can easily remove the electrodes themselves at any time if they wish.

*Audio Stimulus Presentation:*

Audio stimuli will be presented to the fetus using a high-quality loudspeaker securely attached to an adjustable wall-mounted arm designed for use in clinical settings, which allows it to be positioned safely over the mother’s abdomen while she reclines on a hydraulically adjustable ultrasound bench. The loudspeaker will be calibrated before each recording session to ensure that loudness levels are identical across experimental sessions, and within safe limits to avoid causing fetal distress. The mother will wear sound-isolating headphones throughout the experiment to avoid any possible influence on fetal behavior of maternal response to stimuli.

*Ultrasound:*

Physiological responses to audio stimuli will be recorded using a 4D ultrasound scanner (General Electric Voluson E8 Pro), operated by a certified sonographer under the supervision of an attending obstetrician. The ultrasound scanner can be used in a variety of different modes to record fetal motion and fetal heart rate, and can also be used to carry out basic assessments of fetal health. At the beginning of each session, the ultrasound scanner will be used to complete a biophysical profile (BPP). The obstetrician will use the BPP to determine the health of the fetus. During the experimental procedures, ultrasound will be used to make 4D movies of fetal motion and/or cardiac response, under the direction of the investigators. Safety mechanisms are integrated into the hardware and software of the ultrasound scanner to ensure that ultrasound intensity is kept within safe limits. Overall exposure to ultrasound will be limited to 40 minutes over the entire session, in accordance with safety guidelines published by the American Association for Ultrasound in Medicine.

*Electrocardiography:*

Fetal cardiac responses to audio stimulus presentation may also be monitored using an electrocardiograph (ECG). The electrocardiography system is integrated with the ultrasound scanner. ECG electrodes are applied to the mother’s abdomen to measure electrical activity in the womb, and signal processing techniques are then used to extract the fetal heart rate. An alcohol swab is used to clean the surface of the skin beforehand, and hypoallergenic electroconductive gel is used to wet the electrodes and improve the electrical contact with the skin surface.

*Cardiotocography:*

Fetal cardiac responses to audio stimulus presentation may also be monitored using a cardiotocograph (CTG). CTG transducers are applied to the mother’s abdomen to detect fetal heart movements using Doppler ultrasound, and signal processing techniques are then used to extract the fetal heart rate. An alcohol swab is used to clean the surface of the skin beforehand, and hypoallergenic gel is used to wet the transducers and improve contact with the skin surface.

*Experimental Procedure:*

During the first visit, audio and EGG recordings will be made of mothers and their partners.

Each participant will be seated in a sound-isolated recording studio in front of a microphone. The investigator will place EGG electrodes on the participant’s throat. The participant will be prompted to produce a set of predefined utterances while watching a video recording of a baby. The resulting microphone and EGG recordings will be analyzed and post-processed on a computer to create a set of audio stimuli.

During the second visit, fetal behavioral responses to audio stimuli will be measured.

The mother will be seated comfortably on a hydraulically adjustable ultrasound bench in the Marcus Autism Center ultrasound laboratory. The experimental procedures will be explained to her and she will be able to ask any questions, and decide whether to continue with the experiment.

The obstetrician and sonographer will carry out a biophysical profile (BPP) to assess the status of the fetus. Monitoring of vital signs will continue throughout the experiment to ensure that any signs of distress are immediately detected. The experiment will be halted immediately by the investigators if any such signs are found. The mother will always be free to halt the experiment herself whenever she wishes. The experiment will only proceed further once the obstetrician has determined that it is safe to do so.

An emergency procedure has been established in collaboration with the Department of Obstetrics and Gynecology at Emory School of Medicine and the Maternal-Fetal Medicine Department at Emory Midtown Hospital to handle any adverse events that may arise, including detection of distress in the fetus during the BPP or at any time throughout the experimental protocol. An ambulance will be called using a direct phone line installed in the laboratory, and the obstetrician will attend to the mother until assistance arrives, then accompany her to Emory Midtown Hospital, where a 24/7 obstetric emergency team will provide appropriate medical care.

The mother will be asked to wear sound-isolating headphones during the experiment to ensure that there is no influence on fetal behavior of maternal response to stimulus presentation. A high-quality loudspeaker securely attached to an adjustable wall-mounted arm, designed for use in clinical settings, will be positioned safely over the mother’s abdomen to enable audio stimuli to be presented. A microphone integrated into the wall arm will enable her voice to be recorded. The investigator will apply EGG electrodes on the participant’s throat to enable the intonation of her voice to be measured at the same time. When the mother is ready, the sonographer will begin to acquire ultrasound, electrocardiograph, and cardiotocograph data so that the fetal heart rate and a full 3D view of fetal motion can be measured and recorded in real time. A real-time data acquisition system will be used to synchronize and record the microphone, EGG, ultrasound, ECG, and CTG channels simultaneously.

Once recording has started, the investigator will play audio stimuli through the loudspeaker. The stimuli will be presented in randomized blocks, with short pauses between blocks to enable the investigators to check that the mother is still comfortable.

One of the aims of the experiment is to determine whether fetuses are able to discriminate between voices that are conducted through the body and voices that are conducted through the air alone. Accordingly, at various points during the experiment, the mother will be asked to recite the same phrases that were used to generate the prerecorded audio stimuli.

When the experiment is over, the EGG, ECG, and CTG electrodes will be removed. All equipment is cleaned and sterilized before and after every experimental session.

In order not to exceed published safety guidelines for ultrasound exposure, the total time of the experiment involving ultrasound recordings will be limited to 30 minutes, regardless of whether or not all the experiment has been successfully completed.

Once the experiment has ended, the obstetrician and sonographer will review the ultrasound recordings with the mother and provide a clinical interpretation of the data where appropriate. They will answer any questions the mother may have. The mother will be referred to appropriate clinical services if there is anything in the ultrasound recordings that might require treatment.

The ultrasound recordings will be copied onto a DVD. This will be made available to the mother’s primary obstetric care physician if the mother and the physician have requested this. The primary obstetric care physician will decide whether to include any of the information provided in the participant’s medical record. The mother will receive a copy of the DVD if her primary obstetric care physician has consented to this.

All of the data recorded during the experiment will be de-identified and uploaded onto a secure data server for post-processing and analysis. Post-processed data may later be uploaded to other databases, including the National Database for Autism Research (NDAR), and made available to other researchers.

*Clinical Assessment Procedures:*

Characterization involves standardized screening and diagnostic procedures, assessment of developmental functioning, language, social communication, and adaptive behavior, pediatric medical and genetic examination, assignment to diagnostic group and screening for false negatives.

*Pediatric Medical Evaluations:*

All infants will have detailed family, medical, and genetic histories conducted over the course of 36 months using the recommended procedures set forth by the National Database for Autism Research, recorded on the *Medical History Questionnaire* and the *NIMH Physical Exam and Neurologic Evaluation Form*.

*Screening for Risk:*

All infants will receive a broadband screener, the *Infant-Toddler Checklist (ITC)* (Wetherby, Brosnan-Maddox, Peace, & Newton, 2008) for developmental concerns at the 12-month visit. HR-ASD infants and any controls that fail the initial screen will receive a secondary screen at 12 months that includes an autism-specific screener, the *Early Screening for Autism and Communication Disorders (ESAC)* (Wetherby, Woods, & Lord, 2006), an assessment of social communication (*Communication and Symbolic Behavior Scales, Developmental Profile (CSBS)* (Wetherby & Prizant, 2002a), and the *Systematic Observation of Red Flags of ASD (SORF)* (Wetherby, Woods, McCoy, & Stronach, 2011), coded with the CSBS.

*Parent Questionnaires:*

Family and demographic information will be collected at intake, 24 and 36 months using the *Family Demographic Information Form*, the *Family Information Form,* and the *Parent/Caregiver Questionnaires*. History of treatment and intervention practices will also be gathered at 24 and 36 months using the *Baby Sibling Research Consortium Intervention History Form*.

*Direct Assessment:*

All infants will receive a full diagnostic characterization at 24 months that includes the *Vineland* (Sparrow, Cicchetti, & Balla, 2005)*, Mullen* (Mullen, 1995)*, CSBS* (Wetherby & Prizant, 2002a), and *ADOS* (Lord, Luyster, Gotham, & Guthrie, 2012a, 2012b)*.* All HR infants, as well as LR infants classified as having ASD or a developmental disorder at the 24-month visit, will receive a confirmatory diagnostic evaluation at 36 months that includes all of the 24-month assessments, with advancement to *ADOS Modules 1 or 2* (Lord et al., 2012a) depending on language level, as well as the *SCQ* (Rutter, Bailey, & Lord, 2003), *CBCL* (Achenbach & Rescorla, 2000), and *SRS* (Constantino & Gruber, 2005). The 12- and 24-month assessments will be conducted and supervised by experienced and trained clinicians who will be blind to risk and/or treatment status. At the 36-month visit, an independent Clinician Best Estimate confirmatory diagnosis will be assigned by two senior-level clinicians, both of whom will be blind to treatment and diagnostic status.

*Confirmation of ASD Diagnosis in Older Sibling:*

In order to confirm a diagnosis of ASD in any older sibling, diagnostic and medical records for the older sibling will be requested from parents. In addition to record review, parents will be asked to complete a series of questionnaires regarding their older child’s symptoms and behavior. For children younger than 18 months, parents will complete the *Infant-Toddler Checklist (ITC)* (Wetherby & Prizant, 2002b)and the *Early Screening for Autism and Communication Disorders (ESAC)* (Wetherby et al., 2006). For children between 18 and 36 months old, parents will complete the *Modified Checklist for Autism in Toddlers* *(M-CHAT)* (Robins, Fein, Barton, & Green, 2001). For children over the age of 36 months, parents will complete the *SCQ*, *CBCL*, and *SRS*.

Participants in this study may already be enrolled in other research studies, or they may enroll in other research studies at a future date. It is possible that the results of this study may contain information that may be useful to those other studies, or that the results of those other studies may shed light on the results of this study. Accordingly, with the mother’s informed consent, we will make available the results of this study to other research studies, and we will reanalyze the results of this study at a later date whenever relevant data from other studies become available. Our intention to reanalyze the data we collect will be clearly communicated to participants as part of the informed consent process, and we will ensure that they understand what this entails.

**E. Measures**

*Experimental Measures:*

*Audio Recordings:* Microphone recordings of the mother’s voice and her partner’s voice will be stored as raw audio files.

*Electroglottograph Recordings:* Electroglottograph recordings will be stored as raw data files. The fundamental frequency contour will be extracted from each EGG signal and stored in a separate file.

*Electrocardiograph Recordings:* Electrocardiograph recordings will be stored as raw data files. The time-varying fetal heart rate will be extracted from each ECG signal and stored in a separate file.

*Cardiotocograph Recordings:* Cardiotocograph recordings will be stored as raw data files. The time-varying fetal heart rate will be extracted from each CTG signal and stored in a separate file.

*Ultrasound Recordings:* Ultrasound recordings will be stored as raw data files on the ultrasound scanner, which enables different views of the fetus to be created. Versions of each recording will be uploaded onto a server as DICOM files for postprocessing.

*Fetal Cardiac and Motor Response Measures:* The ultrasound recordings will be hand-coded according to the guidelines described by Kurjak et al. (2008) to extract measures that quantify changes in movement and heart rate during presentation of each audio stimulus. Inter-rater reliability will be calculated to ensure accurate coding. These measures will be stored in spreadsheets and data files for statistical analysis.

*Clinical Assessment Measures:*

*Autism Diagnostic Observation Schedule – Second Edition: Modules 1, 2 and Toddler Module* (Lord et al., 2012a, 2012b)*:* The ADOS is a comprehensive, investigator-basedprocedure that places the child in naturalistic social situations demanding specific social and communication reactions. Behaviors are coded in the areas of social communication, social relatedness, play and imagination, and restricted and/or repetitive behaviors. The ADOS provides a DSM-IV-based algorithm for the diagnosis of autism, ASD, and non-PDD. Either the ADOS, Toddler Module or ADOS, Modules 1-2 (depending on language level) will be administered to all infants at the 24-month visit, and to all infants determined to have developmental delays, including ASD, at the 36-month visit.

*Communication and Symbolic Behavior Scales* (Wetherby & Prizant, 2002a)*:* The CSBS is a standardized assessment tool designed to evaluate verbal and nonverbal communication, social-affective, and symbolic abilities of children whose chronological ages range from 6 to 24 months, and with preschool children with delayed development up to the chronological age of 6 years. The sampling procedures used in this measure consist of a set of probes designed to elicit communication and social interaction, as well as functional and symbolic play. Quantitative data on subject responses to these sampling procedures are collected and summarized in a set of cluster scores, which include: 1) emotion and gaze sharing; 2) rate of communication; 3) functions of communication; 4) means of communication: gestures/sounds/words/word combinations; 4) understanding of words; and 5) symbolic and functional object use. The CSBS will be administered to all infants at the 24-month visit, and to all infants determined to have developmental delays, including ASD, at the 36-month visit.

*Mullen Scales of Early Learning* (Mullen, 1995)*:*TheMullen is an individually administered comprehensive measure of cognitive functioning for infants and preschool children, from birth through 68 months. The Mullen assesses the child’s abilities and provides standardized scores in five domains: Visual Reception (non-verbal problem-solving skills), Receptive Language (ability to understand language), Expressive Language (ability to use language to communicate), Fine Motor Skills, and Gross Motor Skills. Additionally, the Mullen provides an Early Learning Composite. The Mullen will be administered to all infants at the 24-month visit, and to all infants determined to have developmental delays, including ASD, at the 36-month visit.

*SORF: Systematic Observation of Red Flags of Autism Spectrum Disorder* (Wetherby et al., 2011)*:*The SORF is a direct observational measure that allows observers to assess symptoms of ASD that are directly keyed into the diagnostic criteria for autism. The SORF will be conducted during observations of the CSBS-DP at 12 months.

*Parent Interviews and Inventories*

*Parent/Caregiver Questionnaires:* These measures cover the prenatal, perinatal, and general health history of the baby, as well as the intervention history (if any) while the baby is developing. Different sections of the questionnaires appropriate to the age and background of the child will be chosen and administered by a trained researcher at each visit to our clinic according to the needs of the study.

*Social Communication Questionnaire* (Berument, Rutter, Lord, Pickles, & Bailey, 1999)*:* The SCQ is a 10-minute instrument for parents that evaluates communication skills and social functioning in children who may have autism or autism spectrum disorders. Parents will be asked about their child’s development over the entire 36 months of life, with greater emphasis on the most recent 3-month period, as well as for use in confirming diagnosis of an older sibling with ASD.

*Baby Sibling Research Consortium Intervention History Form:* The BSRC Intervention History Form is used to ask parents about their child’s intervention history, and will be administered at 24 and 36 months.

*Child Behavior Checklist* (Achenbach & Rescorla, 2000)*:* The CBCL is a parent rating scale containing 99 items that describe behavioral/emotional problems. This scale also contains an open-ended item for additional problems. The purpose of this assessment is to obtain a parental rating of children’s behavioral and emotional problems evident at the age of 36 months, as well as for confirming diagnosis of an older sibling with ASD.

*Communication and Symbolic Behavior Scales – Developmental Profile, Infant-Toddler Checklist* (Wetherby & Prizant, 2002b)*:* The CSBS-DP, Infant-Toddler Checklist is a broadband screener that is useful for detecting at-risk behaviors for general developmental delays in children between the ages of 6 and 24 months. It is a parent-report measure that is very efficient to administer and can be extremely useful in detecting developmental and communication delays in children who are at risk for ASD. The ITC will be completed by parents for the older sibling with ASD to confirm diagnosis if the older sibling is under the age of 18 months.

*Early Screening for Autism and Communication Disorders* (Wetherby et al., 2006)*:* The ESAC is a parent-report screener for autism spectrum disorders and communication disorders. It consists of 47 items that are either ratings or checklists of behavior, with 7 additional open-ended questions to allow parents to elaborate on their child’s behavior. The ESAC will be completed by parents for the older sibling with ASD to confirm diagnosis if the older sibling is under the age of 18 months.

*Modified Checklist for Autism in Toddlers* (Robins et al., 2001)*:* The M-CHAT is a 23-item parent-report checklist designed to detect behaviors that indicate risk for ASD in toddlers between the ages of 16 and 30 months. For children who fail key items on the M-CHAT, there is a follow-up interview that can be conducted with the parent that reduces the false positive rate for ASD. The M-CHAT will be used to confirm ASD diagnosis in older siblings if the sibling is between 18 and 36 months old.

*Social Responsiveness Scale* (Constantino & Gruber, 2005)*:* The SRS is a 65-item rating scale that measures the severity of autistic symptoms as they occur in natural social settings. The scale can be completed by a parent in just 15 minutes, and it provides a clear picture of a child’s social impairments, assessing social awareness, anxiety/avoidance, and autistic preoccupations and traits. The toddler version is appropriate for use with children who are 3 years old, and will be used only at the 36-month visit, as well as for confirming diagnosis of siblings with ASD older than 36 months.

*Vineland Adaptive Behavior Scales – II* (Sparrow et al., 2005)*:* The Vineland-II retains the original framework of the earlier Vineland tests: four domains (Communication, Daily Living Skills, Socialization, and Motor Skills), and the Maladaptive Behavior Domain. It is the most widespread instrument for assessing adaptive behavior. In addition to providing measures of adaptive skills, it has been thoroughly studied as an instrument for research on social functioning in autism and related conditions. The Vineland-II Survey Form will be administered by a trained research assistant to parents of all infants at the 24-month visit, and to parents of all infants determined to have developmental delays, including ASD, at the 36-month visit.

**F. Risks**

All members of the research team will strive to ensure that participants are subjected to the least possible amount of risk necessary to achieve the objectives of the research.

The risks involved in this study arise from the use of ultrasound, electrocardiography and cardiotocography on pregnant women and their fetuses, the use of electroglottography and microphone recordings on pregnant women and their partners, and the potential distress that may result from the administration of clinical assessments to children, or the communication of the results of those assessments to families. Loss of confidentiality is also a potential risk.

*Ultrasound:*

Ultrasound is needed to measure cardiac and motor responses to audio stimuli in the fetus.

The ultrasound procedures employed in this study are believed to involve minimal risk to participants and their unborn children, based on evidence from a large number of similar previous studies. The IRB classifies ultrasound as a minimal risk procedure. No adverse outcomes in humans resulting from ultrasound use have ever been reported, despite the widespread use of the procedure for both healthy and complicated pregnancies (Medical Ultrasound Safety, Second Edition, American Institute of Ultrasound in Medicine). Although there is no known instance of human injury as a result of exposure to diagnostic ultrasound, there is a potential for tissue damage whenever ultrasound travels through human tissue that needs to be carefully considered (Ang, Gluncic, Duque, Schafer, & Rakic, 2006; Kieler, Axelsson, Haglund, Nilsson, & Salvesen, 1998). From animal studies, we know that heating of the tissue may occur with ultrasound, and we also know that an elevated temperature inside the womb, regardless of its cause, may lead to birth defects in fetuses. To safeguard against possible damage, the sonographer will carefully control both the output level and the total exposure during our experiment, according to guidelines for medical ultrasound safety published by the American Institute of Ultrasound in Medicine. Research staff will provide our participants with a copy of those guidelines upon request, and will be happy to discuss any concerns participants may have about the use of ultrasound in our research. Safety checks are incorporated in both the hardware and the software used to control the ultrasound scanner used in our laboratory that prevent the ultrasound beam intensity from exceeding a user-programmed threshold. This threshold will be set to minimize the risk of adverse ultrasound exposure levels to the mother and the fetus. In designing our study, we have been careful to follow those guidelines, and to weigh up the risk our research potentially poses to participant health against the future benefits that our research may provide in helping to improve diagnosis and treatment for children at risk of autism.

There is a risk that the fetus may be in a state of distress before or during the experiment, either due to external circumstances or as a result of the stimuli presented as part of our experimental procedure. It is known that excessively loud sounds or body vibration may cause fetal distress, and although our stimuli and laboratory procedures are designed to avoid this, there is a small risk that this may occur. To safeguard against this possibility, at the beginning of each session the obstetrician and sonographer will carry out a biophysical profile (BPP) to assess the status of the fetus. Monitoring of vital signs will continue throughout the experiment to ensure that any signs of distress are immediately detected. The experiment will be halted immediately by the investigators if any such signs are found. The mother will always be free to halt the experiment herself whenever she wishes.

An emergency procedure has been established in collaboration with the Department of Obstetrics and Gynecology at Emory School of Medicine and the Maternal-Fetal Medicine Department at Emory Midtown Hospital to handle any adverse events that may arise, including detection of distress in the fetus during the BPP or at any time throughout the experimental protocol. An ambulance will be called using a direct phone line installed in the laboratory, and the obstetrician will attend to the mother until assistance arrives, then accompany the mother to Emory Midtown Hospital, where a 24/7 obstetric emergency team will provide appropriate medical care.

In the interest of delivering efficient and timely emergency care, we will be unable to accommodate requests for treatment at hospitals other than Emory Midtown. If a mother participating in our study wishes to pursue an alternate course of emergency action, she will be free to do so; however, she must sign a waiver we provide releasing our research group and its affiliated institutions from liability for the consequences of her decision.

*Electrocardiography:*

Electrocardiography is needed to monitor fetal heart rate. According to the National Heart, Lung, and Blood Institute of the U.S. Department of Health and Human Services, electrocardiography (ECG) is painless and harmless to both pregnant women and fetuses. Standard electroconductive gel approved for use with ECG electrodes is applied to the skin to ensure good electrical contact. Occasionally, electrode gel may cause a mild skin rash. In the unlikely event that this should occur, the participant may experience mild discomfort, but any rash will usually disappear within days without medical treatment.

*Cardiotocography:*

Cardiotocography is needed to monitor fetal heart rate. According to the National Heart, Lung, and Blood Institute of the U.S. Department of Health and Human Services, cardiotocography (CTG) is painless and harmless to both pregnant women and fetuses. Standard hypoallergenic gel approved for use with CTG electrodes is applied to the skin to ensure good electrical contact. Occasionally, electrode gel may cause a mild skin rash. In the unlikely event that this should occur, the participant may experience mild discomfort, but any rash will usually disappear within days without medical treatment.

*Electroglottography:*

Electroglottography is needed to measure the intonation of the participant’s voice. A tiny voltage (equivalent to a flashlight battery) is applied across the throat using two electrodes. By monitoring the change in electrical impedance between the electrodes caused by the opening and closing of the glottis during speech, it is possible to measure the vibration of the vocal folds and extract the time-varying fundamental frequency of the voice. The electrodes are held in place using a Velcro strap around the neck, which the participant can adjust. An alcohol swab is used to clean the surface of the skin beforehand, and hypoallergenic electroconductive gel is used to wet the electrodes and improve the electrical contact with the skin surface. Participants can easily remove the electrodes themselves at any time if they wish. Occasionally, electrode gel may cause a mild skin rash. In the unlikely event that this should occur, the participant may experience mild discomfort, but any rash will usually disappear within days without medical treatment.

*Microphone Recordings:*

Microphone recordings are needed to record the voice of the mother and her partner. There are no known risks from conducting microphone recordings on human subjects, other than a risk of loss of confidentiality. All recordings will be deidentified to safeguard against this possibility.

*Clinical Assessments:*

Clinical assessments need to be carried out at 12 months, 24 months and 36 months after birth to determine a diagnosis for each child, in order to assign group membership at outcome.

All of the clinical assessment procedures used in this study have no known hazards and are comparable to testing procedures that have been used in psychological research for decades. There are no known risks of physical or psychological damage associated with those procedures.

When child participants visit the Center to take part in an assessment, there is a risk of distress due to the unfamiliar environment and the length of time needed to complete assessments. To minimize distress to participants, a parent will remain with the child at all times. If a child expresses displeasure due to any cause, procedures will be suspended. With parental permission, procedures will continue when the child has calmed down and no longer expresses discomfort. Members of the research team are sensitive to concerns from both the child and the parent, and parents will always be able to request that a procedure be paused or ended. In addition, parents will be able to withdraw their child from the study at any time without penalty. During each study visit, families will be given time to rest between procedures, and all assessments and procedures will be scheduled around the times when children usually eat or sleep. If a child seems especially tired on a particular visit, parents will be asked whether they would like to come back on a different day. Our Center is baby/child-oriented and fully equipped with plenty of toys and activities to make children feel comfortable. We will strive to accommodate the needs of new mothers with baby-changing stations in the restroom, a rocking chair in the waiting area, and child-safe furniture throughout the Center. All of our clinical and research areas conform to necessary standards of safety and hygiene.

All newborn children enrolled in the study will be required to participate in clinical assessments at 12, 24 and 36 months. If any of the clinical assessments we conduct indicate a developmental delay or disability in the participant’s child, there is a risk that this will cause distress when the results of the assessment are communicated to the family.

Enrollment in the high-risk group also requires confirmation of the older sibling’s diagnosis. If the older child’s diagnosis was based on clinical evaluations carried out elsewhere (not at the Marcus Autism Center) and does not contain the standardized assessments used in this study, we will require confirmation of diagnosis using a parental screener without needing the older child to visit the clinic. The results of that confirmatory assessment will be communicated to parents if they wish. If the confirmatory diagnosis does not match the previous diagnosis, there is a risk that families may experience distress upon receiving the new diagnosis.

To minimize the risk of distress, all diagnostic information will be communicated sensitively and professionally to families by experienced clinicians, and those clinicians will be available at any time during the study to answer any questions and address any concerns that families may have. Families will always be able to choose not to receive the results of any assessments if they wish. If any child receives a diagnosis of autism or a related developmental disorder as a result of participating in this study, clinicians will be able to provide families with referrals to appropriate community service providers so that they can access early intervention services as quickly and easily as possible. If any problems are detected during pregnancy, families will be referred to a counselor for guidance. Clinical information will always be communicated sensitively to families in a private setting.

To avoid any risk of coercion, during the informed consent procedure, research staff will take care to explain to families that any decision they may make not to participate in the study, or to withdraw from the study, will in no way influence their access to care or the quality of the care they receive as patients either at the Marcus Autism Center or any other institution.

*Travel:*

Participants must visit the Marcus Autism Center several times in order to participate in the study. Although we will only recruit within a reasonable distance of Atlanta, there is a risk that stress and anxiety will be increased due to the inconvenience and fatigue involved in travelling to our clinic, and it may be difficult for participants to travel during pregnancy or after their child is born. To minimize this risk, parents will be given a tentative schedule of all study visits at the beginning of their participation to allow them to plan for visits well in advance. In addition, parents will be able to reschedule the day and time of a visit if they need to. Each visit will be carefully organized and will not last longer than 4 hours in total. If a visit requires more than 4 hours of time – for instance, if a visit includes a confirmation of an autism diagnosis via assessments – the procedure will include frequent breaks to ease the stress on the child. If participants have any concerns about the inconvenience involved in coming to the Marcus Autism Center for their visits, they will be able to discuss this with our research staff.

*Loss of Confidentiality:*

The information our participants provide will be kept strictly confidential. Although every measure is taken to maintain confidentiality, there is a potential risk for loss of confidentiality. We will make every effort to minimize this risk. A full discussion of the steps that will be taken to minimize risks associated with loss of confidentiality is given below.

*Cost of Participation:*

There are no costs to participants for taking part in this research.

No physical, emotional, psychological, social stigmatization, economic or legal risk to participants is anticipated other than those listed above. Because we are employing new research procedures, participation in this study may involve additional risks that are currently unknown. Any unexpected risks are believed to be minimal. The Principal Investigator will evaluate any unanticipated adverse events that occur determine whether the adverse event affects the risk/benefit ratio of the study and whether modifications to the protocol or consent form are required. A summary of any adverse events will be reported to the IRB when annual re-approval of the protocol is sought.

**G. Benefits**

Information gained as a result of this research may indirectly benefit participants or other individuals with ASDs, by leading to increased understanding of the underlying pathophysiology of these disorders, and thereby facilitating the development of new treatments.

*Ultrasound Examination:*

Pregnant women participating in this study will receive an ultrasound evaluation carried out by a trained sonographer and obstetrician. This evaluation will take place in the third trimester, later in pregnancy than ultrasound evaluations are usually routinely administered. The evaluation will include a biophysical profile of the fetus and will allow the fetus to be monitored for signs of distress. A potential benefit of participating in the study is that the additional ultrasound evaluation will provide an opportunity to detect any potential late-occurring problems with the pregnancy.

*Clinical Assessment:*

Children participating in the study will receive clinical assessments for autism and related disorders at 12, 24 and 36 months, administered by experienced clinicians. This will facilitate early diagnosis of developmental disabilities, and will ensure that any affected children and their families can be placed in touch with appropriate community service providers so that they can access early intervention services as quickly and easily as possible.

*Risk-Benefit Ratio:*

The study has a low risk-benefit ratio. The benefit to families for participating in this study, namely the potential for early diagnosis of developmental disabilities, outweighs the minimal risk associated with the experimental and clinical assessment procedures involved in taking part in the study. The minimal level of risk to the subject population entailed by the study procedures is counterbalanced by the potential importance to the broader population of the new knowledge that will be gained about the pathophysiology of autism. Anticipated risks to subjects are therefore reasonable in relation to expected benefits.

*Compensation for Participation:*

Participants will receive compensation for participating in the study. Mothers will receive $25 for completing the first study visit (audio recordings), $50 for the second study visit (ultrasound recording), and $25 for the final three visits (clinical assessments). Partners will receive $25 for completing the first study visit (audio recordings). The total compensation that can be received by the mother, her partner, and her child for participating in this study is $150 over a period of 3 years.

All payments will be made using a personal payment card, which is issued to participants for free. The payment card is a prepaid debit card, which can be used exactly like a Mastercard. Money is loaded onto the card electronically every time a participant needs to be paid. The card scheme is run by Greenphire, an independent company specializing in payments for research studies and clinical trials. To issue a card, Greenphire needs to be given personal information about a participant. Banks and other financial institutions can access this information if they need to verify identity when participants use their card. Additionally, Emory University is required by law to report any participant payments to the IRS. To do this, Emory University Department of Finance needs to keep each participant’s Social Security Number on file. Participants will therefore be asked to allow their name, address, date of birth, research study name and Social Security Number to be communicated to Greenphire and Emory University Department of Finance. If participants want to receive e-mail or text alerts when payments are made to them, they will also be asked to provide their e-mail or phone number as well. All of this information will be stored on computers owned by Greenphire. Greenphire will not have access to any other information collected during this study.

Subjects will be provided with full instructions about using their payment card when it is issued, and they will have the opportunity to ask questions or voice concerns about the card scheme or the use of their personal information as part of the informed consent procedure.

**H. Data Analysis**

The research design is based on testing for significant differences in fetal behavioral responses to different categories of audio stimulus. Time-varying fetal cardiac and motor responses are measured from ECG, CTG, and ultrasound recordings relative to baseline responses and aligned relative to the start of each audio stimulus. Functional Data Analysis (Leng & Müller, 2006; H. Müller, Stadtmuller, & Yao, 2006; H.-G. Müller & Stadtmuller, 2005; H.-G. Müller, 2009; Ramsay & Silverman, 2002, 2006; Tang & Muller, 2008; Yao, Müller, & Wang, 2005, 2006) is used to model the statistical ensemble of response curves, and a 2-way Functional Analysis of Variance is used to test for significant differences in cardiac response and motor response curves with factors for diagnostic group (3 levels: TD, DD, ASD) and stimulus type (multiple levels depending on experiment).

*Power Analysis:*

Calculations to determine the required sample size have been based on previous studies of fetal responses to sound *in utero*, e.g. d=1.33 for Zimmer et al. (1993); d=2.71 for DeCasper (1994); d=2.975 for Granier-Deferre et al. (2011). The proposed resulting sample of 130 subjects (22 at low risk for autism, 108 at high risk, of which 20 are expected to develop an ASD) will provide the power (>.90) required to detect statistically significant differences (p < .05) in fetal cardiac responses to sound, which can be expected to be medium to large based on data from previous studies. Power and effect size calculations follow general recommendations (Cohen, 1988).

Our expected cohort of 20 TD infants and 20 infants with ASD at final outcome will allow us to meet this requirement despite uncertainties in the actual prevalence statistics for autism and developmental delays in the population we sample.

**3. Training**

All persons involved in the design and/or conduct of research involving human subjects as part of this study have completed HIPAA and confidentiality training. All research staff involved in the study are required to complete CITI training through Emory University before being allowed to conduct research involving human subjects at the Marcus Autism Center. Additionally, all study personnel have experience working with children and families and are trained in accordance with their specific role on the research project. No member of the study staff will be allowed to participate in the study unless the PI or another appointed member of the study team has approved their ability to do so.

The obstetrician and sonographer who will be responsible for supervising and administering the ultrasound examinations needed during this study maintain professional certification in their respective fields, and have been credentialed through Children’s Healthcare of Atlanta.

**4. Data Management and Monitoring**

*A. Data Management*

Research data collected during this study will be derived from the following sources:

* + Demographic information
  + Medical records
  + Microphone and electroglottograph recordings
  + Ultrasound, electrocardiograph, and cardiotocograph recordings
  + Results of experimental procedures
  + Results of clinical assessments

All research data collected by the study will be entered and checked for errors and internal consistency using a standardized data flow procedure. All data will be deidentified and labeled with a numerical identifier linking the data to the study and the participant. Deidentified data will be stored on a central data server, which will be password-protected and located in a secure locked room. Data will be archived daily onto local network storage, and backed up weekly, monthly, and quarterly onto magnetic tape media archived on-site and off-site in fireproof and waterproof data vaults.

*B. Data Safety and Monitoring Plan*

The Principal Investigator will be responsible for monitoring data and conducting performance safety reviews every six months. Both the Principal Investigator and the IRB have the authority to stop or modify the study. Excepting the risks noted in the “Risk” section of this protocol, no physical, emotional, psychological, social stigmatization, economic, or legal risk is anticipated. The Principal Investigator will evaluate any unanticipated adverse event that may occur and determine whether the adverse event affects the risk/benefit ratio of the study and whether modifications to the protocol or consent form are required. A summary of any adverse events will be reported to the IRB.

**5. Confidentiality**

**A. Plans to Protect Privacy of Subjects and Confidentiality of Data**

Private identifiable information about individual participants will be collected and used in the course of this study. Data will be collected by investigators, clinicians, and research assistants. All study personnel are CITI-trained and HIPAA-certified. The data will consist of Personal Health Information (PHI), raw data collected during experimental procedures, and processed data derived from analysis of raw data. PHI will be stored separately from all other data. Raw and processed data will be deidentified and labeled with a numerical identifier linking the data to the study and the participant. All data will be stored on secure data servers, which will be password-protected and physically located in secure locked rooms. Data will be stored as individual computer files and may also be entered into HIPAA-compliant research databases that protect PHI. Where data are collected on paper, hard copies will be scanned electronically and converted into computer files, and the originals then shredded. Data will be maintained indefinitely, with the informed consent of all participants. The quality, integrity and security of all stored data will be monitored regularly by the PI.

All information that is collected in connection with this study will remain confidential and will only be disclosed when required by U.S. or State Law. Examples of information that we are legally required to disclose include abuse of a child or elderly person, or certain reportable diseases.

Only investigators and research staff collaborating on this study will be allowed to access the data. However, funding agencies may require that data from this study be shared with other researchers. Specifically, NIH requires that deidentified data from this project be submitted to the National Database for Autism Research (NDAR), a HIPAA-compliant biomedical informatics system and data repository created by the U.S. Department of Health and Human Services to encourage and facilitate sharing of data between researchers working in the field of autism. In addition, the Emory IRB may need to review study records and conduct regular audits to ensure that federal regulations regarding research involving human subjects are being followed.

Permission to access data collected during this study will in all circumstances only be given after careful consideration of the need to maintain confidentiality and protect subject privacy.

Every reasonable precaution will be taken to maintain confidentiality and protect subject privacy, but there is always a potential risk of loss of privacy and confidentiality due to failure of data safety and monitoring procedures or breach of computer security. To guard against failures of data safety and monitoring procedures, the PI will conduct performance safety reviews every six months, and there will be annual reviews by the IRB. To protect against any breach of computer security, all computers are password-protected and equipped with firewalls and anti-spy/anti-virus software, in accordance with the general guidelines of Emory University and Children’s Healthcare of Atlanta Information Technology and HIPAA offices.

**B. Plans to Link Data to Identifiers**

All data collected as part of this research study will be identified by a numerical study identifier linking it to the research study and a numerical subject identifier linking it to the participant from whom it was collected. Numerical study identifiers will be assigned to each study using an internal coding system specific to the Marcus Autism Center. Numerical subject identifiers will be assigned to each participant using the Global Unique Identifier (GUID) derived from the National Database for Autism Research (NDAR). The GUID is a nationally adopted coding system, based on the participant name, gender, and date and place of birth, which allows for easy identification of overlap with other databases and studies involved in research on autism. Neither study identifier nor subject identifier can be used to retrieve participant identity.

**C. Plans to Protect Linkage**

The linkage list associating numerical study identifiers and numerical subject identifiers with individual participants will be stored on a password-protected computer in a secure location, separate from all deidentified data. Only the PI and authorized study personnel who have a specific need to associate the identities of individual participants with the data collected from them will be permitted to access the linkage list. Nobody outside the study will be allowed to access the linkage list unless this is mandated by law, or as part of external auditing procedures imposed by funding agencies or internal auditing procedures imposed by Emory University and Children’s Healthcare of Atlanta. All decisions about access to the linkage list will be made by the PI.

**6. Informed Consent**

**A. Consent Personnel**

Principal Investigator, Co-Investigators, Study Coordinator and Research Associates.

**B. Assessment of Capacity to Consent**

Adults will be allowed to participate in the study only if they are deemed capable of giving informed consent and only after voluntary, informed consent has been obtained from them in writing. Children are not capable of giving informed consent, so permission for children to participate in the study will be given on their behalf by a parent, in writing, if the parent agrees for them to take part.

Assessment of capacity to consent will be determined on the basis of cognitive ability, which will be screened by the study coordinator during the initial intake interview, before the informed consent process takes place. Individuals with an estimated mental age of less than 7 years will not be considered capable of providing informed consent. In cases where cognitive ability is in doubt, a trained clinician will be consulted. If there is any doubt about ability to provide informed consent, the individual will not be enrolled in the study.

**C. Process of Obtaining Consent/Permission**

Potential subjects will be identified when they express an interest in participating in the study by contacting our research staff themselves by phone, e-mail, or mail.

Once subjects identify themselves, research staff will contact them by phone to describe the study in detail, confirm that they are interested in participating, and conduct a 10-minute intake interview to determine whether they are eligible to take part. If the applicant cannot be accepted into the study, the study coordinator will convey this to them with due care and sensitivity, and will provide them with alternatives if any are known. No woman will be approached regarding participation in this study or accepted into this study at time of delivery or in any other compromising or stressful situation during her pregnancy. Women who are expecting to get pregnant will not be considered eligible until pregnancy is confirmed. Women who are already pregnant will not be considered eligible unless they provide confirmation of pregnancy by a medical professional with an expected delivery date.

Eligible subjects will be invited to visit the Marcus Autism Center, where the details of the study will be explained to them and discussed. The person obtaining consent will describe the study protocol, risks, benefits, and tentative appointment schedule based on the expected date of birth. They will answer any questions regarding the study and will ensure that applicants feel they have had adequate time to make a decision. In addition, applicants will be reminded that participation in the study is voluntary and may be terminated at any time without penalty, and that refusal to participate or withdrawal from the study will in no way influence their relationship with the Marcus Autism Center or any other institution.

After they have had a chance to ask questions and are satisfied that they still wish to take part, informed consent for participation in the study will be obtained in writing. Separate consent forms will be used to document consent for pregnant women and their partners. If participants wish to inspect the consent form beforehand, or if they wish to examine the consent form further before giving consent, a copy of the consent form will be delivered to them or given to them to take away. If participants are unable to sign the consent form in person, a copy will be mailed to them to sign and return. Should this be necessary, a member of the research team will then meet with the participant during their first subsequent visit to the Marcus Autism Center, confirm that the participant fully understood the form, answer any further questions, and verify that informed consent has been given before any study procedures take place.

We will ask pregnant women who are interested in participating in the study to provide their consent at least one month prior to giving birth, and they will be asked to sign a release of information form at that time to enable study personnel to verify their medical history. Families who already have an older child with autism will be asked to provide confirmation of

With the mother’s consent, the research staff will contact the physician primarily responsible for her obstetric care during pregnancy to inform them that their patient intends to take part in the study, explain what the study involves, and obtain their consent for their patient to participate. A separate consent form will be used to document the consent of the physician. If consent is not given, the applicant will be excluded from participation in the study, and the reason for their exclusion will be explained to them.

Newborn children taking part in this study are too young to give informed consent, and consent cannot be obtained before they are born. Mothers will give permission for their child’s participation the first time the child is brought to the Marcus Autism Center for assessment, before assessment takes place. A separate form will be used to document permission for the child.

The older sibling’s diagnostic information will be collected only through the mother. Since the older sibling will not be required to visit the Center or engage in any direct testing, the mother will not need to issue signed consent for the older sibling’s participation in the study. However, the mother will be asked to sign a release of the older sibling’s health information before the diagnostic parent interview takes place.

**D. Documentation of Consent/Permission**

Consent Form (Mother of Infant Sibling): to document consent for mothers of infants at risk of ASD.

Consent Form (Mother of Infant Control): to document consent for mothers of other infants.

Consent Form (Partner of Mother): to document consent for partners of mothers.

Permission Form (Child): to document permission for child participants.

Permission Form (Primary Care Physician): to document permission from primary care physician.

Release of Information Form (Adult): to obtain medical record files from a Hospital/Physician’s Office.

Release of Information Form (Child): to obtain medical record files from a Hospital/Physician’s Office.

HIPAA Authorization: to use or disclose healthcare information.

Recording Permission Form (Adult): to make audio/video/photo recordings of assessments.

Recording Permission Form (Child): to make audio/video/photo recordings of assessments.

Compensation Signature Form (Adult): to acknowledge payment for study participation.

Compensation Signature Form (Child): to acknowledge payment for study participation.

**7. New Information**

This is a minimal risk study, and new information discovered during this study is not expected to have any negative implications for the health or wellbeing of study participants or their families. Any new information or study findings that emerge during the course of this study that could potentially influence the health or wellbeing of study participants, or affect families’ decisions to remain in the study, will be immediately shared with families and reported to the Emory IRB. Furthermore, families will never be pressured to remain in the study, and they will be able to withdraw from the study without penalty at any time they wish.

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