National CADDRE Study: Child Development and Autism
Sample Study Hypotheses and Data Collection Tools

1. Investigation of ASD Phenotype-related hypotheses

Children: General Hypotheses

Children with ASD are more likely than children in the neurodevelopmentally impaired comparison group or subcohort to have:

1. Abnormal physical findings/ dysmorphology: including greater head circumference, dysmorphic physical features, or a lower 2d: 4d digit (2^{nd} to 4^{th} digit) ratio

   Data collection tools: Physical exam (including dysmorphology exam)

2. Co-morbid medical conditions, including Tuberous sclerosis (TS), Neurofibromatosis, motor tics, seizure disorders; Thyroid disorders, autoimmune disorder, asthma and other allergic disorders; Fragile X (and other genetic syndromes such as Down Syndrome); gastrointestinal disturbances or disorders.

   Data collection tools: Caregiver Interview (primary or alternative), child medical record abstraction (neonatal and pediatric medical records), Autoimmune Survey, and Stool and Diet Diaries.

3. Developmental conditions, including cognitive delays and mental retardation; cognitive profile differences (visual-receptive skills that are greater than receptive and expressive language skills; exceptional cognitive skills of early reading) and attention/ hyperactivity problems (including decreased ability to shift attention); communication/speech delays; social deficits; specific language delays; sensory aberrancies; adaptive delays; heightened motor skills relative to other domains.

   Data collection tools: Neurodevelopmental disorders that have been diagnosed by a physician will be captured in the Caregiver Interview and through the child medical record abstraction (pediatric/specialty medical records). Other behavioral information will be captured through the CBCL, Vineland, ADOS, ADI-R, SRS, and Mullens.

4. Behavior difficulties, including lack of concern for physical dangers, self-injurious behaviors (such as head banging and biting), and increased activity level.

   Data collection tools: ADI-R, CBCL, and ADOS

Children: Regression Hypotheses

ASD children will be identified into 3 groups of progress - No regression (i.e., steady progress with or without delay), plateau of social and/or language skills (i.e. no loss of skills, but lack of addition of new skills), and regression of skills (i.e., actual loss of previously acquired language and/or social skills, but not motor or daily living skills). Children with regression of skills will be noted to lose skills by 12-30 months of age.

1. Children with plateau of skills or regression will use fewer words and phrases (with and without meaning) than children with no regression

   Data collection tools: Early Development Questionnaire and ADI-R

2. Children with regression will be more likely to have seizures than children in the other two groups

   Data collection tools: Early Development Questionnaire, ADI-R, Caregiver Interview, and child medical record abstraction (pediatric medical records).

3. Children with regression will be more likely to have more significant cognitive or adaptive delays than children in the other two groups

   Data collection tools: Early Development Questionnaire, Carey, Vineland, and Mullens.

4. Children with regression will be more likely to have more frequent gastrointestinal difficulties or disorders and/or growth impairment than children in the other two groups

   Data collection tools: Early Development Questionnaire, Caregiver Interview, child medical record abstraction (pediatric medical records), and Diet and Stool Diaries

5. Children with regression will be more likely to have a higher percentage of positive genetic testing results than children in the other two groups

   Data collection tools: Early Development Questionnaire, child medical record abstraction (pediatric/speciality medical records), and Biosampling

Parents

1. Parents of children with ASD are more likely to have medical and psychiatric conditions than parents of children in the neurodevelopmentally impaired comparison group or subcohort. Medical conditions include diabetes, thyroid disease, and other autoimmune disorders; seizures/epilepsy and other

specified neurological disorders (e.g., multiple sclerosis); tuberous sclerosis (TS); asthma; gastrointestinal disorders. Psychiatric conditions include depression, anxiety disorders, schizophrenia, OCD, withdrawn/antisocial personality disorder and OCD. Developmental disorders include developmental disabilities, ADHD, communication/speech delays, social deficits, learning and/or reading disabilities, and specific language delays.

Data collection tools: Autoimmune Survey, Maternal Medical History Form, Paternal Medical History Form and SRS. Medical history information of the mother can also be obtained from maternal medical record abstraction (prenatal medical records)

Siblings

1. Siblings of Children with ASD are more likely to have specific medical conditions than siblings of children in the neurodevelopmentally impaired comparison group or subcohort. Medical conditions include: diabetes, thyroid disease and other autoimmune disorders; seizures/epilepsy and other specified neurological disorders; fragile X, tuberous sclerosis (TS); asthma; gastrointestinal disorders, ADHD, developmental delay, communication/ speech delays, learning and/or reading disabilities, and specific language delays.

   Data collection tools: Caregiver Interview and Autoimmune Survey

2. Infection and Immune Function Hypotheses

Infection - Mother

1. Mothers of children with ASD are more likely to have more infections or infection-related exposures during pregnancy or through the end of breastfeeding than mothers of children in the neurodevelopmentally impaired comparison group or subcohort. Infections to consider are respiratory infections, oral infections, urinary/bladder/kidney infections, infections of the reproductive organs that include herpes, gonorrhea, syphilis, chlamydia, Trichomoniasis, PID, bacterial vaginosis, Candidiasis, genital warts, and endometriosis; major perinatal infections including measles, mumps, rubella, hepatitis A and B; mononucleosis, CMV, toxoplasmosis, parvovirus, shingles, chicken pox, Group B Strep, chorioamnionitis. Infection-related exposures include vaccinations, isoimmunization, vaginal douching, and fevers.

Data collection tools: Caregiver Interview (primary or alternative interviews) and maternal medical record abstraction (prenatal and labor and delivery medical records)

2. Mothers of children with ASD may have different treatment/intervention histories during pregnancy (including anti-infective treatments) or through the end of breastfeeding than mothers of children in the neurodevelopmentally impaired comparison group or subcohort. If mothers of children with ASD are more likely to experience infections or infection-related exposures during pregnancy or through the end of breastfeeding as compared to mothers of children in the neurodevelopmentally impaired comparison group or subcohort, they may be more likely to use antibiotics and other treatment regimes. Mothers of ASD children may be more likely than mothers of children in the neurodevelopmentally impaired comparison group or subcohort to have been given prescription medications.

Data collection tools: Caregiver Interview (primary or alternative interviews) and maternal medical record abstraction (prenatal and labor and delivery medical records).

Infection - Children

1. Children with ASD, from birth to 3rd birthday, are more likely to have experienced clinical illness from infections, including viral infections and ear infections that required medical attention including prescription medications, than children in the neurodevelopmentally impaired comparison group or subcohort. Infections to consider include: recurrent ear infections, viral and bacterial meningitis, encephalitis, pneumonia, Rheumatic fever, tonsillitis, tuberculosis, influenza, and TORCH infections.

Data collection tools: Caregiver Interview (primary or alternative), child medical record abstraction (neonatal and pediatric records).

2. Children with ASD, from birth to 3rd birthday, have different treatment histories for illness; and more reactions to treatment that led to a diagnosable condition, than children in the neurodevelopmentally impaired comparison group or subcohort.

Data collection tools: Caregiver Interview (primary or alternative interviews) and neonatal and pediatric medical record abstraction.

3. Children with ASD, from birth to 3rd birthday, are more likely to have febrile illness than children in the neurodevelopmentally impaired comparison group or subcohort.

Data collection tools: Neonatal and pediatric medical record abstraction (or alternative interview)

4. Children with ASD, from birth to 3rd birthday, have different vaccine histories; and reactions to vaccines within 6 weeks of vaccination, including reactions that requiring medical attention or treatment, than children in the neurodevelopmentally impaired comparison group or subcohort.

Data collection tools: Pediatric Medical Records. In the event pediatric records are not obtainable, the primary caregiver will be asked to complete an immunization card with the vaccinations the child received and dates of administration.

Immune function

3. Mothers of children with ASD are more likely to have autoimmune disorders as compared to mothers of children in the neurodevelopmentally impaired comparison group or subcohort. Among mothers with autoimmune disorders, the onset of autoimmune disorder among the mothers of children with ASD is more proximate to pregnancy than that of mothers of the children in the neurodevelopmentally impaired comparison group or subcohort.

Data collection tools: Autoimmune Survey and maternal medical record abstraction (prenatal medical records)

4. Children with ASD are more likely to have a family history of autoimmune disorders – overall, and by specific conditions than children in the neurodevelopmentally impaired comparison group or subcohort. Further, children in certain ASD subgroups may be more likely to have a family history of autoimmune disorders than other ASD subgroups.

Data collection tool: Autoimmune Survey (for entire nuclear family)

5. Children with ASD have altered immune function or dysfunction (including autoimmune disorders) compared to children in the neurodevelopmentally impaired comparison group or subcohort.

Data collection tools: Pediatric medical records, autoimmune survey, and biosampling techniques.

3. Hypotheses Related to Reproductive and Hormonal Features

Mother

1. Mothers of children with ASD have different menstrual and reproductive histories than mothers of children in the neurodevelopmentally impaired comparison group or subcohort. Menstrual and reproductive histories include: onset of menses, regularity of menses, history of infertility treatments, pregnancy history including number of pregnancies, duration and outcome of pregnancies, and birth/outcome interval.

Data collection tools: Caregiver interview (primary or alternative) and maternal medical record abstraction (prenatal medical records).

2. Mothers of children with ASD have different index pregnancy histories than mothers of children in the neurodevelopmentally impaired comparison group or subcohort. Index pregnancy history includes use of fertility treatments, morning sickness, prenatal care, maternal serum alpha-fetoprotein (MSAFP) and/or multiple marker screening (i.e., human chorionic gonadotropin and either MSAFP or unconjugated estriol or both), infection of the reproductive organs, major perinatal infections, obstetric complications, labor and delivery complications, placental features.

Data collection tools: Caregiver Interview (primary or alternative) and maternal medical record abstraction (prenatal and labor and delivery medical records).

3. Mothers of children with ASD have different patterns of exogenous hormone exposures (hormonal medications including oral contraceptives, infertility treatments, treatments for conditions, and medications administered during the labor and delivery and perinatal period such as oxytocin and pitocin) during pregnancy and through the end of breastfeeding than mothers of children in the neurodevelopmentally impaired comparison group or subcohort.

Data collection tools: Caregiver interview (primary or alternative), and maternal medical record abstraction (prenatal and labor and delivery records).

4. Mothers of children with ASD have different patterns of endogenous hormone levels during pregnancy (i.e., history of hypothyroidism, testosterone level as indicated by proband's 2D:4D ratio) than mothers of children in the neurodevelopmentally impaired comparison group or subcohort.

Data collection tools: Autoimmune Survey (thyroid disease), maternal record abstraction (prenatal medical records), physical exam of proband (anthropometrics and dysmorphology examination).

Children

1. Children with ASD, compared to children in the neurodevelopmentally impaired comparison group or subcohort, have different levels of serotonin, melatonin, oxytocin, and vasopressin.

Data collection tools: Biosampling techniques

4. Hypotheses Related to Gastrointestinal (GI) Features

1. Children with autism and non-specific developmental delay will have more gastrointestinal symptoms than children in the subcohort (most of whom have typical development).

   Data collection tools: Caregiver Interview (primary or alternative), Diet and Stool Diaries

2. Children with autism will have more loose or watery stools than children in the neurodevelopmentally impaired comparison group or subcohort.

   Data collection tools: Caregiver Interview (primary or alternative), Diet and Stool Diaries

3. ASD children with gastrointestinal symptoms are more likely to have a history of regression, greater cognitive delay, and family history of gastrointestinal and/or autoimmune disorders than ASD children without gastrointestinal symptoms or children in the neurodevelopmentally impaired comparison group.

   Data collection tools: Caregiver Interview (primary or alternative), Diet and Stool Diaries, Early Development Questionnaire, ADI-R, Mullens, Autoimmune Survey, Maternal Medical History Form and Paternal Medical History Form.

4. Gastrointestinal symptoms will be associated with dietary fiber intake, carbohydrate to fat ratio, and fluid intake.

   Data collection tools: Caregiver Interview (primary or alternative), Diet and Stool Diaries.

5. Children with autism will be more likely than children in the neurodevelopmentally impaired comparison group or subcohort to have very restricted diets/less than 15 foods eaten.

   Data collection tools: Caregiver Interview (primary or alternative) and Diet Diary

6. ASD children with restricted diets will be more likely to have higher overall scores on the Carey Temperament Scales than ASD children without restricted diets or children in the neurodevelopmentally impaired comparison group or subcohort.

   Data collection tools: Caregiver Interview (primary or alternative) and Carey.

7. Nutritional intake will be deficient in children with extremely restricted diets.

Data collection tools: Caregiver Interview (primary or alternative) and Diet Diary

8. ASD children with gastrointestinal symptoms will have differences in levels of platelet serotonin, vasoactive intestinal peptide, immune mediators, other biomarkers and candidate genes relative to ASD children without gastrointestinal symptoms or children in the neurodevelopmentally impaired comparison group or subcohort.

   Data collection tools: Caregiver Interview (primary or alternative) and biosampling techniques

5. Genetic Hypotheses

Autism spectrum disorders are associated with genotypes related to:

1) immune function, including autoimmunity;
2) response to infection, especially viral infection;
3) response to other pre- and peri-natal stressors;
4) hormone function;
5) metabolism of xenobiotics;
6) neuronal development and function.

For each hypothesis above:

1) Are these genetic effects offspring- or parentally-mediated?
2) Are there gene-gene interactions among the candidate genotypes?
3) Do the candidate genotypes modify the effects of related prenatal environmental exposures?
4) Do the candidate genotypes modify the effects of related postnatal environmental exposures?

Data collection tools: Biosampling techniques.

6. Sociodemographic Factors Hypotheses

Children with ASD and their families have different sociodemographic characteristics than children/families in the neurodevelopmentally impaired comparison group or subcohort. Sociodemographic characteristics include maternal and paternal age, birthplace, race, ethnicity, education level, and income.

Data collection tools: Caregiver Interview (primary or alternative)

7. Hypotheses in Other Etiologic Areas

Substance Use Hypothesis

Certain lifestyle practices (i.e., use of tobacco, alcohol and illegal substance use) during pregnancy are more common among mothers of ASD children compared to mothers of children in the neurodevelopmentally impaired comparison group or subcohort.

Data collection tools: Caregiver Interview (primary or alternative), maternal medical record abstraction (prenatal medical records).

Hospitalizations and Injuries Hypotheses

Children with ASD are more likely to have physical injuries/hospitalizations (overall, by specific injuries/hospitalizations) than children in the neurodevelopmentally impaired comparison group or subcohort.

Data collection tools: Caregiver Interview, child medical record abstraction (pediatric medical record).

Children: Sleep Hypotheses

1. The frequency of sleep disorders is greater in children with autism than in children in the neurodevelopmentally impaired comparison group or subcohort.

   Data collection tools: Sleep Habits Questionnaire, Caregiver Interview, child medical record abstraction (pediatric medical record), and CBCL.

2. The types of sleep disorders are different in children with autism than in children in the neurodevelopmentally impaired comparison group or subcohort.

   Data collection tools: Sleep Habits Questionnaire

3. Children with autism and sleep disorders have more cognitive impairments than children with autism who do not have sleep disorders.

   Data collection tools: Sleep Habits Questionnaire, Caregiver Interview, child medical record abstraction (pediatric records), CBCL, Vineland, and Mullens.

4. Children with autism and sleep disorders have more social impairments than children with autism who do not have sleep disorders.

Data collection tools: Sleep Habits Questionnaire, Caregiver Interview, child medical record abstraction (pediatric records), CBCL, Vineland, and ADI-R

5. Children with autism and sleep disorders have more regulatory dysfunction than children with autism who do not have sleep disorders.

   Data collection tools: Sleep Habits Questionnaire, Caregiver Interview, child medical record abstraction (pediatric medical records), CBCL, Diet and Stool Diaries

6. Children with autism and dysomnias are more likely to have abnormalities in "clock genes," serotonin, melatonin and other biomarkers than children with autism who do not have dysomnias or children in the neurodevelopmentally impaired comparison group or subcohort.

   Data collection tools: Sleep Habits Questionnaire, Caregiver Interview, child medical record abstraction (pediatric medical records), CBCL, biosampling techniques.

7. Children with autism and parasomnias are more likely to have elevated levels of anxiety and/or a family history of anxiety disorders and sleep disorders than children with autism and no parasomnias.

   Data collection tools: Sleep Habits Questionnaire, Caregiver Interview, child medical record abstraction (pediatric medical records), CBCL, Maternal Medical History Form, Paternal Medical History Form

Pre- and Postnatal mercury exposures hypothesis:

1. Children with ASD have higher exposure levels of mercury than children in the neurodevelopmentally impaired comparison group or subcohort from:

   a. prenatal exposure to i) RhoGAM; ii) maternal influenza vaccines
   b. postnatal vaccine exposure

Data collection tools: Prenatal exposure of mercury will be captured via Caregiver Interview (primary or alternative). Postnatal exposure of mercury will be captured via the child's medical record abstraction (neonatal and pediatric medical records). In the event, access to child's medical records is not obtained, an immunization card will be used to obtain information on vaccines that may have contained the mercury containing preservative, thimerosal.

2. Children with ASD have higher levels of mercury measured in hair samples than children in the neurodevelopmentally impaired comparison group or subcohort.

Data collection tools: Biosampling techniques.

Parental Occupation Hypotheses:

1. Parents of children with ASD are more likely to work or be trained in highly technical occupations that reflect systemizing and analytic skills, such as engineers or scientists, than parents of children in the comparison group.

Data collection tools: Maternal occupation will be captured via the Caregiver Interview. Paternal occupation will be ascertained in a self-administered questionnaire. If the father is not available, the mother may be asked to supply some basic information about his occupation if she can. Coding of occupation will be necessary and could be done using Bureau of Labor Statistics or census codes.

2. Around the time of pregnancy, parents of children with ASD are more likely to have worked in occupations that involve exposure to hazardous substances that may be neurotoxins or hormone disruptors, such as metals, solvents, and pesticides, than parents of children in the comparison group or subcohort.

Data collection tools: Maternal occupation and exposures will be captured via the Caregiver Interview. Paternal occupation and exposures will be ascertained in a self-administered questionnaire. The information about job duties may be coded by an industrial hygienist to provide an alternate source of information on likely exposures.