3. What Caused This To Happen And Can This Be Prevented?

- **Is there something in my genetic or family history that poses a risk for ASD?**
- **What environmental exposures pose risks for the development of ASD?**
- **How might genetics and the environment interact to influence the occurrence of ASD?**

What do we know?

As with many complex disorders, causation is generally thought to involve some forms of genetic risk interacting with some forms of non-genetic environmental exposure. The balance of genetic risk and environmental exposure likely varies across the spectrum of ASD. The greatly increased concordance of strictly defined autism in monozygotic (identical) twins (70 - 90%) compared to dizygotic (fraternal) twins (0-10%) argues for the importance of genetic factors (Bailey et al., 1995; Steffenburg et al., 1989). Moreover, there are subpopulations of those diagnosed with ASD that have a known genetic mutation, often associated with a genetic disorder, such as Fragile X syndrome, Rett syndrome, or tuberous sclerosis, understanding of which has led to identification of possible pharmaceutical interventions. In many cases the same genetic variation does not result in an ASD phenotype, suggesting possible genetic or environmental modifiers that could be important intervention targets.

Using new technology that reveals gaps and extra copies in DNA sequences, researchers have found that some people with ASD have deletions and duplications of genetic material not found in their parents' DNA (Sebat et al., 2007). Recent genetics research has identified common genetic variations (e.g., Wang et al., 2009; Weiss et al., 2009), changes in chromosomal structure in specific genomic regions, (Marshall et al., 2008; Kumar et al., 2008; Weiss et al., 2008) and rare mutations in genes all associated with synaptic connectivity (Alarçon et al., 2008; Bakkaloglu et al., 2008; Durand et al., 2007; Jamain et al., 2003; Laumonnier et al., 2004.; Strauss et al., 2006). Some of these findings have contributed to new hypotheses about the inheritance of ASD. In families with just one affected member, spontaneous deletions and duplications may be causal factors of ASD. However, what causes these spontaneous deletions and duplications is not clear and could be due to environmental exposures.

Taken together, rare genetic mutations, chromosomal abnormalities and sub-microscopic deletions and duplications of genetic material are involved in at least 10% of ASD cases, yet individually each abnormality is found in no more than about 1-2% of cases (Abrahams & Geschwind, 2008). Since common genetic variations confer only modest increase in risk, this suggests that the genetic factors in ASD may involve many different genes and interactions between genes and environment. Possible models include: many additional rare genetic mutations to be discovered; multiple common genetic variations each conferring a small increased risk; and, many forms of ASD with different genetic contributions, both common and rare in the population. There is growing recognition that the same genetic contributions can lead to a wide variety of different phenotypes across individuals. As one good example, deletions and duplications in chromosomal region 16p11 have been associated with a broad

range of phenotypes, including disorders outside the autism spectrum. The factors responsible for this variability in disease phenotypes remain to be defined.

Researchers are working to better understand the interaction of genetic vulnerability with developmental experiences, such as a specific environmental exposure. While gene-environment interactions have been hypothesized to play a role in many medical disorders, these interactions have been difficult to prove or disprove beyond statistical tests showing that some genetic subgroups have a greater response to some environmental factor. Epigenetics is one mechanism by which it is thought that environmental factors may be influencing gene expression, and now molecular tools are allowing researchers to gain insight into epigenetic phenomena that may be contributing to a variety of disorders, including ASD (Baccarelli and Bollati, 2009; Nagarajan et al., 2008).

While genetics maps the sequence of DNA, epigenetics maps the modifications of the structure of DNA due to proteins or other factors that bind to the DNA helix. DNA is essentially linear text that gets "read" into RNA that in turn codes for proteins. Epigenetic modifications do not change the text but they highlight or redact large sections of text, changing how it is read. Epigenetic modifications consist of biochemical "tags" that attach to the DNA in different places, leading to the "silencing" or "activation" of genes. The pattern of epigenetic silencing or activation of genes can differ between genders, between species or between generations, and can change during specific time windows in development or in response to environmental cues. It is thought that the addition or removal of epigenetic tags from DNA is one mechanism by which developmental experience (i.e. exposure to physical or emotional stimuli) can cause long-term biological and behavioral effects. In the past year, the first maps of the human epigenome have provided the first comprehensive look at where and how nature and nurture may interact (Lister et al., 2009).

Progress in identifying environmental factors which increase autism risk has been made recently (Eskenazi et al., 2007; Palmer et al., 2006; Palmer, Blanchard, & Wood, 2009; Rauh et al., 2006; Roberts et al., 2007; Windham et al., 2006), although this area of research has received less scientific attention and far fewer research dollars than genetic risk factors. Environmental factors may be pertinent not only to brain development but also to chronic systemic features of at least some subgroups of ASD. An Institute of Medicine (IOM) workshop held in 2007 summarized what is known and what is needed in this field (Forum on Neuroscience and Nervous System Disorders, Institute of Medicine, 2008). Numerous epidemiological studies have found no relationship between ASD and vaccines containing the mercury based preservative, thimerosal (Immunization Safety Review Committee, 2004). These data, as well as subsequent research, indicate that the link between autism and vaccines is unsupported by the epidemiological research literature. However, the IOM report acknowledged that the existing population-based studies were limited in their ability to detect small susceptible subpopulations that could be more genetically vulnerable to environmental exposures.

Of note, the Committee receives many public comments that reflect concerns about vaccines as a potential environmental factor in autism. Some members of the public are convinced that the current data are sufficient to demonstrate that vaccines do not play a causal role in autism and argue against

using limited autism research funds to do additional vaccine studies when many other scientific avenues remain to be explored. At the same time, those who believe that prior studies of the possible role of vaccines in ASD have been insufficient argue that investigation of a possible vaccine/ASD link should be a high priority for research (e.g., a large-scale study comparing vaccinated and unvaccinated groups). A third view urges shifting focus away from vaccines and onto much-needed attention toward the development of effective treatments, services and supports for those with ASD.

In addition, a number of other environmental factors are being explored through research because they are known or suspected to influence early development of the brain and nervous system. Recent studies suggest factors such as parental age, exposure to infections, toxins, and other biological agents may confer environmental risk. These findings require further investigation and testing, some of which is ongoing through the CADDRE Program, the Norwegian cohort study, the CHARGE study, the EARLI study, and the Children's Centers for Environmental Health and Disease Prevention supported by NIEHS and the Environmental Protection Agency (EPA).

What do we need?

Although most scientists believe that risk factors for ASD are both genetic and environmental, there is considerable debate about whether potential environmental causes, genetic precursors, or interactions between genes and environmental factors should be the highest priority for research aimed at identifying the causes of ASD. To date, few studies have ruled in or ruled out specific environmental factors. There are reports of associations of ASD with exposure to medications, maternal antibodies, toxicants, and infections prenatally or postnatally, however these observations need to be the subject of additional study. It is still not known whether any specific factor is necessary or sufficient to cause ASD. Similar to other disease areas, advancing research on the potential role of environmental factors requires resources and the attraction of scientific expertise. Bringing this to bear on autism will help define the environmental factors to study, as well as the best approach for staging studies to examine environmental factors, interaction between factors, and between individual susceptibility and various environmental factors.

For example, some researchers believe that it is important to study a large number of exposures, or classes of exposure, that are known to affect brain development. Others support more tightly focused studies of one exposure or a limited number of exposures, with greatest biologic plausibility for interacting with known or suspected biologic or genetic ASD risk factors. In addition, it is also important to design studies that assess environmental exposure during the most relevant exposure windows: pregnancy and early development. In doing this research, it will be important for the field to develop sound standards for identifying and claiming that environmental factors contribute to ASD, as it is for genetics.

Research studies on risk factors can be pursued through several means. Smaller, focused studies are needed for hypothesis testing and to provide insight for replication studies. Similar to other health outcomes research for relatively rare conditions, case-control studies can be an effective first line of inquiry. The CHARGE and CADDRE (SEED) studies are good examples of this approach where

environmental exposures and biological pathways, along with genetics, are being examined. Other existing cohorts could also be identified and used for epigenomic as well as traditional genomic and environmental studies.

To address public concerns regarding a possible vaccine/ASD link, it will be important for the IACC to continue to coordinate with the National Vaccine Advisory Committee (NVAC), a Federal advisory committee chartered to advise and make recommendations regarding the National Vaccine Program.

Epigenomics provides a ready mechanism for understanding how genes and environment may act jointly to affect autism risk. Studies are needed to investigate whether candidate environmental exposures alter epigenetic mechanisms that modify the expression of suspected autism susceptibility genes or genomic regions. Such studies should incorporate examination of time or stage of development as an important factor determining the impact of environmental agents on epigenetic programming. Finally, studies are needed to understand how changes in epigenetic tags in response to environmental stimuli could lead to specific phenotypic characteristics associated with autism.

Another approach for studying risk factors for ASD requires large sample sizes to disentangle the many possible genetic and environmental factors that contribute to and help explain ASD and the frequently co-occurring conditions. For other complex disorders, large DNA collections, i.e. >20,000 samples, have been necessary to detect the full genetic risk architecture. There are no genetic repositories of this size for ASD. Similarly, large birth cohort studies, in which biological samples have been collected throughout pregnancy and early postnatal life may be essential for detecting the interplay of environmental exposures and genetic factors that lead to ASD. As a complement to these large-scale studies, research on critical sub-populations that may be at higher risk could provide leverage in identifying genetic and environmental risk factors.

ASPIRATIONAL GOAL: CAUSES OF ASD WILL BE DISCOVERED THAT INFORM PROGNOSIS AND TREATMENTS AND LEAD TO PREVENTION/PREEMPTION OF THE CHALLENGES AND DISABILITIES OF ASD.

Research Opportunities

- Genetic and epigenetic variations in ASD and the symptom profiles associated with these variations.
- Environmental influences in ASD and the symptom profiles associated with these influences.
- Family studies of the broader autism phenotype that can inform and define the heritability of ASD.
- Studies in simplex families that inform and define de novo genetic differences and focus on what role the environment might play in inducing these differences.

- Standardized methods for collecting and storing biospecimen resources from well-characterized people with ASD as well as a comparison group for use in biologic, environmental and genetic studies of ASD.

- Case-control studies of unique subpopulations of people with ASD that identify novel risk factors.

- Monitor the scientific literature regarding possible associations of vaccines and other environmental factors (e.g., ultrasound, pesticides, pollutants) with ASD to identify emerging opportunities for research and indicated studies.

- Better understanding environmental and biological risk factors during pre- and early post-natal development in "at risk" samples.

- Cross-disciplinary collaborative efforts to identify and analyze biological mechanisms that underlie the interplay of genetic and environmental factors relevant to the risk and development of ASD, including co-occurring conditions.

- Convene ASD researchers on a regular basis to develop strategies and approaches for improving data standards and sharing, understanding gene – environment interactions, improving the speed of replication of findings, and enhancing the translation of research on potential causative factors to prevention and treatment studies.

- Measures of key exposures for use in population and clinic based studies and standards for sample collection, storage, and analysis of biological materials.

- Studies of behavioral, developmental, and medical variations across those with ASD who share common genetic factors.

- Studies of clinically meaningful subgroups to examine common genetic and environmental factors, as well as unique epigenomic signatures.

Short-Term Objectives

A. Coordinate and implement the inclusion of approximately 20,000 subjects for genome-wide association studies, as well as a sample of 1,200 for sequencing studies to examine more than 50 candidate genes by 2011. Studies should investigate factors contributing to phenotypic variation across individuals that share an identified genetic variant and stratify subjects according to behavioral, cognitive, and clinical features. *IACC Recommended Budget: $43,700,000 over 4 years.*

B. Within the highest priority categories of exposures for ASD, identify and standardize at least three measures for identifying markers of environmental exposure in biospecimens by 2011. *IACC Recommended Budget: $3,500,000 over 3 years.*

C. Initiate efforts to expand existing large case-control and other studies to enhance capabilities for targeted gene – environment research by 2011. *IACC Recommended Budget: $27,800,000 over 5 years.*

D. Enhance existing case-control studies to enroll racially and ethnically diverse populations affected by ASD by 2011. *IACC Recommended Budget: $3,300,000 over 5 years.*

New Objective

E. Support at least two studies to determine if there are subpopulations that are more susceptible to environmental exposures (e.g., immune challenges related to infections, vaccinations, or underlying autoimmune problems) by 2012. *IACC Recommended Budget: $8,000,000 over 2 years.*

New Objective

F. Initiate studies on at least 10 environmental factors identified in the recommendations from the 2007 IOM report "Autism and the Environment: Challenges and Opportunities for Research" as potential causes of ASD by 2012. *Estimated cost $56,000,000 over 2 years.*

Long-Term Objectives

A. Conduct a multi-site study of the subsequent pregnancies of 1,000 women with a child with ASD to assess the impact of environmental factors in a period most relevant to the progression of ASD by 2014. *IACC Recommended Budget: $11,100,000 over 5 years.*

B. Identify genetic risk factors in at least 50% of people with ASD by 2014. *IACC Recommended Budget: $33,900,000 over 6 years.*

C. Determine the effect of at least five environmental factors on the risk for subtypes of ASD in the pre- and early postnatal period of development by 2015. *IACC Recommended Budget: $25,100,000 over 7 years.*

D. Support ancillary studies within one or more large-scale, population-based surveillance and epidemiological studies, including U.S. populations, to collect data on environmental factors during preconception, and during prenatal and early postnatal development, as well as genetic data, that could be pooled (as needed), to analyze targets for potential gene/environment interactions by 2015. *IACC Recommended Budget: $44,400,000 over 5 years.*

What Progress is Being Made in Fulfilling the Objectives?

(Please provide 1-2 paragraphs to summarize progress.)

***Note:** Objectives labeled "New Objective" are either entirely new additions to the 2010 Strategic Plan or significantly modified objectives from the 2009 Strategic Plan. Objectives from the 2009 Strategic Plan that did not change or that have been slightly modified for clarification purposes are unmarked.*