2013 IACC Strategic Plan Update - Question 3 Draft

“What caused this to happen and can it be prevented?” – Volunteer drafters- Cindy Lawler, Joseph D. Buxbaum, Irva Hertz-Picciotto, Craig Newschaffer

Introduction

The aspirational goal for Question 3 is that “causes of autism will be discovered that inform prognosis and treatments and lead to prevention/preemption of the challenges and disabilities of ASD.” The original version of the IACC Strategic Plan, published in 2009, identified nine objectives focused on research to identify and deepen our understanding of genetic and environmental causes of ASD. In 2009 and 2010, several new objectives were added. The new objectives emphasized the need to study how environmental autism risks may differ in vulnerable subgroups, and encouraged research that capitalized on new opportunities and approaches in areas such as epigenetics, the microbiome, animal models of ASD and bioinformatics. Over the past 5 years, a total of $380 million dollars has been invested to support research under Question 3. Significant advances have occurred in identifying several new genetic and environmental risk factors, and in the initiation and/or expansion of large epidemiologic studies, many of which are responsive to multiple Strategic Plan objectives. Environmental exposure science within ASD studies, inclusion of diverse populations in etiological research, and the use of new animal models for gene/ environment research are areas that have not progressed as quickly and remain significant needs within the field. Continued investment is needed to follow-up on recent advances, to address a wide array of gap areas, and to determine the clinical and public health utility of both genetic and environmental findings. Leveraging the infrastructure of existing large population-based epidemiology studies that are well positioned to address several important questions about environmental risks for autism is a priority that will require significantly increased investment, although new cohort data remains important to capture environmental risks during the relevant windows of susceptibility.

Progress Toward the Strategic Plan Objectives

The 2011-2012 IACC ASD Research Portfolio Analysis reviewed projects funded by both government agencies and private foundations from 2008-2012. From 2008-2012, the total funding devoted to projects that address Question 3 was $380.82M, and if just the years since the publication of the first IACC Strategic Plan in 2009 are considered, the funding for projects related to Question 3 was $297.97M. Also in years 2009-2012, 4% of the total funding went toward research projects in the core/other category, while 96% of the total was invested in research gap areas identified by the 15 objectives in Question 3.

All of the 15 specific objectives under Question 3 have shown progress in funded projects since the publication of the first Strategic Plan. Four objectives addressing multi-site studies of prenatal environmental factors in high risk families, identification of genetic risk factors in people with ASD, large-scale gene-environment interaction studies, and a workshop on bioinformatics met or exceeded the recommended budget and fulfilled the recommended number of projects. Eleven objectives, concerning identification of genetic and epigenetic markers, environmental exposure risks, and study of special populations, partially met the recommended budget and had a number of projects underway.
Progress in the Field of Genetic Risk Factors

During the past few years there has been a major revolution in ASD genetics research. Using the newest molecular and epidemiological methods, recent data continues to strongly support the role of genes in ASD, and the understanding of this role has been greatly refined. The emerging picture is one of profound complexity. Rather than an exclusive focus on one kind of variation, genetic variation at all scales (from changes at a single DNA base-pair, through small genetic insertions and deletions and larger copy number variations (CNVs), all the way up to extra chromosomes) must be considered, as all can contribute to ASD risk\(^1\). In addition, both common and rare variation (both mutations that are commonly shared across many individuals with ASD and mutations that are very rare or unique) have been found to play a role in ASD risk\(^1\).\(^3\).

The emergence of new, high-throughput genetic sequencing (HTS) technologies has provided an opportunity to rapidly identify rare and extremely rare genetic variation that occurs spontaneously (not inherited) in single genes and can impact ASD risk. Data suggest that using these technologies, genetic changes that contribute to ASD risk could be identified in at least 30% of individuals with ASD \(^4\)\(-\)\(^11\). While the sample sizes analyzed using HTS to date are still too small to identify many new, individual ASD genes, six or seven have been unambiguously identified from the first 1,000 individuals sequenced\(^5\)\(-\)\(^8\)\(^,\)\(^12\)\(^,\)\(^11\)\(^,\)\(^13\), with several more likely candidate genes identified as well\(^14\). There are ongoing studies to examine data from a pool of 9,000 individuals with ASD, together with appropriate controls, for a total of at least 20,000 samples. It is anticipated that analysis of HTS data from this larger pool will double the number of known ASD genes, from the currently known genes\(^1\) that have been identified using other methods.

Recent data also suggest that approximately 40-60%\(^15\) of ASD risk can be attributed to inherited, common variation (single nucleotide polymorphisms/SNPs,) and it is likely that in the future it will be possible to estimate ASD risk based by analyzing multiple genetic markers\(^16\), as has been observed in other disorders. Common risk variants interact with other genes and also with the environment. Gene-environment (GxE) interactions are still suspected to be of major importance in ASD, but further work is needed to elucidate the nature of these interactions\(^17\)\(^,\)\(^18\). Based on the results of several studies, the number of genetic loci (or genetic “regions” that can include multiple genes) associated with ASD is now estimated to be near 1,000\(^5\)\(^,\)\(^14\)\(^,\)\(^19\), representing a surprisingly large proportion of genes in the genome (almost 5% of the 22,000 genes in the human genome). This suggests that many different genetic pathways may contribute to the development of ASD, and that further research on developmental trajectories could provide the potential to associate genetic changes with specific symptoms and behavioral phenotypes\(^20\)\(^,\)\(^21\).

New tools for identifying networks of functionally-related genes that influence brain development (i.e., those being used in the BrainSpan project\(^22\)\(^,\)\(^23\)) have been applied in ASD, and have shown that many of the identified risk genes map strongly to defined brain regions and cortical layers, act during specific developmental time windows within the prenatal period, and/or share the same functional pathways. The hope is that it will be possible to identify a smaller number of genes that are the “key drivers” of these pathways that could then become the priority targets for the development of new medicines.
Progress in the Field of Environmental Risk Factors

In 2008, little was known about environmental risk factors for ASD. Given what had been revealed about ASD's genetic complexity at that time, it was suspected that environmental exposures and gene-environment interaction would likely be important to fully understanding ASD risk. The California Autism Twin Study demonstrated that the environmental component of ASD etiology is probably quite substantial. More recent analysis of non-twin family data (from both simplex and multiplex families) also supports the idea that mechanisms beyond inherited gene mutations and de novo, or spontaneous, mutations or copy number variants will be necessary for understanding the complex causes of ASD. The time around conception and during pregnancy are likely the most important time windows of heightened vulnerability for the development of the brain, with supporting evidence from early reports linking autism symptoms to maternal ingestion of drugs such as thalidomide and valproic acid, and infection with congenital rubella, as well as more recent reports showing that maternal prenatal vitamin intake has a protective effect (i.e., reduces risk) for ASD and emerging work in brain gene expression patterns.

Over the past 5 years a modest investment has helped achieve good initial progress in identifying potential environmental ASD risk factors – especially considering the environment broadly as all influences beyond genetic predisposition. Along with the exposures listed above, factors associated with ASD protection or risk that have been replicated in two or more studies include: protective effect of prenatal vitamin intake, and risks from prenatal maternal infection, preterm birth, advanced maternal and paternal age at conception, and short inter-pregnancy interval. A recent study also showed elevated risk in mothers who used labor induction medications, but it is not clear whether the risk was associated with induction itself or with conditions that may have created the need for use of induction medications. Use of certain prescription medications by mothers during the prenatal period has also been suggested as a potential risk factor for ASD, although currently there are conflicts in data from different reports and underlying conditions in the parent might explain these associations. Ultrasound is another exposure that has been considered a possible risk, but recent studies have shown no association between ultrasound and ASD risk. Recent reviews about potential environmental risk factors have compiled lists of exposures of interest for future studies.

Particularly intriguing are the results on prenatal vitamin intake through supplements and diet, showing a 40% reduction in risk of ASD with prenatal vitamin supplements taken in the 3 months before or the first month of pregnancy, but not in pregnancy months 2-9. A trend of decreasing ASD risk as mothers consumed greater daily folic acid intake from foods, vitamins, and supplements in the first month of pregnancy was also reported. The 40% reduction in risk for women who used folic acid supplements in the time around conception based was replicated in a large Norwegian cohort study. These findings raise challenging issues for public health education, given that a sizable fraction of pregnancies are not planned. If they represent causal associations, then by the time a woman recognizes she is pregnant, it may be too late for folic acid supplementation for the purpose of reducing ASD risk in her offspring. They also invoke a number of hypotheses related to epigenetic mechanisms in ASDs.
Among modifiable exogenous exposures, the largest number of studies to date has addressed associations of increased ASD risk with air pollution exposure during gestation and/or early infancy. Multiple studies have reported significant associations; two studies examining ozone and three that examined NO₂ found significant associations with ASD. There is also now suggestive evidence that exposure to endocrine disrupting chemicals such as pesticides, including organophosphates and phthalates may be associated with ASD. The role of heavy metals in ASD remains an open question, as to date, too few studies have been done to assess exposure to heavy metals during pregnancy, which is the most etiologically relevant window. Further work is needed on all of these exposures to more clearly establish associations and ensure no residual confounding due to socioeconomic factors, and if the association is causal, to determine in which periods the fetus/infant might be most susceptible. Also, replication of findings with direct individual-level exposure measures, perhaps using biomarkers, is needed. A useful future direction in the area of environmental exposures could be to focus on how shared properties of exposures (such as endocrine disruption) map to specific phenotypes of ASD.

Exposure assessment represents an ongoing challenge for discerning a role for the environment in ASD causation. Evidence points to pregnancy and the early postnatal period as critical windows of vulnerability, yet, until recently, few studies were collecting relevant data in real time during this period. To address this challenge, two studies using enriched risk designs, the Early Autism Risk Longitudinal Investigation (EARLI) and Markers of Autism Risk in Babies-Learning Early Signs (MARBLES), were launched with NIH support. These studies have captured important and unique pregnancy and birth data and biosamples not possible in other cohorts. However, their ultimate success depends on continued funding of these complex longitudinal projects. The most recent plans proposed for the Main Study of the NIH National Children’s Study (NCS) may provide additional opportunities for exploring ASD risk with prevalent exposures, or ones that occur throughout pregnancy and can be measured at birth.

**Progress in the Field of Gene-Environment Interaction**

Although stressed as a critical area in ASD research, very few studies have focused explicitly on gene-environment interaction. This is in part due to lack of relevant and testable mechanistic hypotheses emerging from the basic sciences and also to the large logistical and resource challenges of assembling sufficiently-sized study populations with both adequate genetic and environmental data. Despite these obstacles, in the last 3 years several published examples provide empirical support for the long-suspected notion that the influence of environmental factors on ASD risk can be amplified in individuals with specific susceptibility genotypes. The first of these studies demonstrated the reduced ASD risk associated with folic acid intake during pregnancy is even stronger in populations with a specific carbon-metabolism genotype. Another study reported that ASD risk associated with prenatal traffic-related air pollution exposure was greater among children with specific genotypes. While these two findings suggest “proof of concept,” replication is needed. They also underscore the value of investments made to date in large and well-characterized study populations, and the parallel needs to continue expanding these efforts and to support infrastructure for specimen banking associated with such populations.

An area of both progress and opportunity for gene-environment research relates to epigenetics and ASD. The role of epigenetics in syndromic forms of autism is well established, and methylation analysis of blood and postmortem brain tissue now implicate epigenetics in the regulation of autism susceptibility genes. Most recently, a genome-wide examination of DNA methylation in a small sample
of postmortem brains revealed several regions with consistent differences in methylation in ASD cases compared to controls\textsuperscript{63}. Evidence continues to accrue regarding the ability of environmental factors such as nutrition\textsuperscript{64}, drugs\textsuperscript{65}, and psychosocial stress\textsuperscript{66} to regulate transcription through epigenetic modifications. This idea has not gained sufficient attention in ASD and merits additional research. Obstacles to further progress in elucidating the environmental epigenomics of ASD are the variability of epigenetic markers between and within tissues and over time. These factors make it difficult to use and interpret data from the kinds of biosamples that are typically available in human ASD studies (peripheral blood obtained after diagnosis), again highlighting the need for longitudinal pregnancy studies.

One final breakthrough is a mechanistic link between the environmental risk factor of paternal age and ASD risk, where a mechanism has been identified around the rate of de novo mutations. Several studies have shown that advanced paternal age at conception is associated with greater risk of ASD\textsuperscript{39,67,42–45,68}. Separate studies have shown that older fathers produce sperm with greater numbers of de novo mutations\textsuperscript{69}, while studies in animals have suggested that there are more profound epigenetic changes in sperm from older fathers\textsuperscript{68}. The causes of these age-related changes in sperm are not yet known, but further exploration could provide key insights about the interface between genes and the environment.

**Progress Toward the Aspirational Goal**

Investments in the past 5 years have led to identification of new genetic and environmental factors contributing to ASD risk and identified the importance of the conception and gestational periods for the development of ASD. The new gene findings hold promise for a better understanding of the neurobiology of ASD and the development of novel pharmacotherapies. Rare variants, as they are discovered, create both clinical and research opportunities. Genetic tests are now being routinely carried out in individuals with unexplained developmental delays. Chromosome microarray (CMA) is already recommended by the American Academy of Pediatrics (AAP) and the American College of Medical Genetics (ACMG)\textsuperscript{70}, and can inform some families about some ASD causes, rare but potentially serious comorbid medical conditions, and, in certain cases, the risk of ASD recurrence in future offspring.

In addition, because these rare variants are often associated with major neurophysiologic effects, they provide the opportunity to develop model systems in cells and in animals, where the basic pathobiology of ASD can be worked out, and where potential new medicines can be examined. This approach, carried out in cells in culture --- including human neurons induced from individuals with genetic lesions --- and in mice and rats, has led to novel treatment approaches. One of the most exciting developments in the past several years in ASD is the emergence of neurobiologically-defined new medicines for subtypes of ASD. Examples include ongoing trials in Fragile X syndrome, Rett syndrome, and Phelan-McDermid syndrome. All three of these result from clearly identified genetic lesions, and all are associated with very high risk of ASD. In some cases, the same drug is being tried in idiopathic ASD (ASD with unknown cause). Families that have a genetic diagnosis can now identify advocacy and family groups with similar mutations and can also choose to participate in relevant clinical trials. While these first trials are still at early stages, we are seeing the beginning of individualized medicine in ASD, based on genetic findings.

The most recent findings available about the sibling recurrence risk for ASD have important clinical implications for families. Whereas earlier results from pooled baby siblings research samples suggest a recurrence risk of approximately 18\%\textsuperscript{71}, a more recent population-based registry study in Denmark
found a substantively lower risk of about 7\% \textsuperscript{72}. The research sample rate may be an over-estimate due to selection bias, while the registry study may under-identify affected siblings (especially milder phenotypes). Consequently, the best estimate may lie somewhere between 7 and 18\%. Notably, the Denmark study also found elevated recurrence among maternal half-siblings, which supports the idea of an etiologic role for maternal genetic factors, maternal intrauterine environment, and other prenatal environmental factors common across pregnancies.

Collectively, the candidate exposures studied to date in ASD represent the “first wave” of findings that were made possible by the initiation or continuation of large autism-focused studies such as Childhood Autism Risk from Genes and Environment (CHARGE) and the CDC’s Study to Explore Early Development (SEED). The careful extension of existing population-based cohorts has also been important in understanding candidate exposures because these cohorts have enabled linkage to prospectively collected clinical records and biospecimens. In many cases, early research investments have focused on establishing study infrastructure and have provided only limited support for analyses. The need to continue cultivating existing investments in this area cannot be overstated. While findings to date are not yet robust enough to inform public health action, the field is now well positioned to address questions regarding ASD-exposure relationships, as well as the identification of genetically, metabolically, or immunologically susceptible subsets of the population. SEED has completed the first phase of data collection for over 2,700 enrolled children, including genetic and environmental exposure data. These data are expected to be available for analyses in early 2014.

The progress achieved to date in the field of environmental epidemiology of ASD has occurred despite significant challenges in exposure measurement. Autism Speaks funded the development and validation of a brief exposure questionnaire that was created by consensus by leading autism environmental science researchers that can be incorporated into a wide range of studies. When available, banked biospecimens are often limited in amount and timing of collection. Analysis of non-persistent chemicals in samples collected after diagnosis does not reflect exposures occurring during early development. Consequently, many studies have relied on maternal recall of exposures, information available in medical records or various indirect methods of assigning exposures, such as using information about the known exposures associated with the residential location(s) where the mother resided during pregnancy.

These exposure assessment challenges are not unique to the field of ASD, and researchers in many other fields are working toward advancing exposure science. As these advances are made, it will be critically important to ensure that they are rapidly incorporated into ASD studies. Research on technologies to precisely monitor exposures in individuals, to accurately measure markers of exposure in banked blood and brain samples, and to consider the totality of an individual’s exposures should be harnessed whenever possible to improve detection of environment-ASD relationships. Advances in the development and application of persistent biomarkers of exposure are especially needed so that analysis of current biospecimens can be used as a record for exposures occurring much earlier in development. Specimen banks such as newborn blood spots by some state governments, and cord blood or stem cells by commercial entities have tremendous potential for use in ASD research where they are available. Similarly, the development of biomarkers for exposure using strands from the often-saved first haircut, or using deciduous teeth may be worthwhile strategies for research investments.

There are several additional areas where more work is needed to meet Question 3 objectives. The first is use of animal models to explore gene-environment interaction. As noted under Question 2, there are now a substantial number of genetic mouse models that exhibit neuropathological and behavioral
phenotypes that can be used to study ASDs. Work on environmentally-induced models of ASD-like symptoms has been limited primarily to valproate and maternal infection, however, and there have been few efforts to use animal models to explore gene-environment interaction. Another strategy that warrants attention is the systematic evaluation/screening of candidate exposures for their effect(s) on molecular pathways that have been implicated by ASD genetic studies. For example, a recent study reported that defects in topoisomerases (enzymes involved in DNA replication, repair and epigenetic changes) may contribute to ASD by virtue of their importance to the expression of extremely long genes, which are overrepresented among known ASD risk genes. This finding provides new leads for identifying exposures that may affect ASD risk through their impact on topoisomerase function. Further development and use of integrative bioinformatics tools that combine information about toxicants with the genes and pathways implicated in ASD provide another means for identifying and prioritizing candidate exposures for further study in ASD studies.

Finally, additional efforts are needed to address barriers to enrollment and retention of racially and ethnically diverse populations and ensure their representation in both epidemiologic and clinical studies. This information is critical for identifying vulnerable subgroups and informing public health prevention efforts. Enhancing the overall diversity of study populations should also prove helpful in detecting environment-ASD associations, as groups underrepresented in clinical studies are often those with disproportionate exposures.

Overall, while many advances have been made in the past five years in autism genetics and the study of environmental risk factors, including studies that have confirmed mid-pregnancy as a key timepoint of vulnerability for development of ASD, much work remains to be done to identify additional contributors and fully understand their complex interaction. Communication between researchers in the autism field and in other fields will be essential in order for advances in exposure science and genetic studies of other disorders to benefit the autism field. In addition, it will be critically important to maintain investment in and build upon existing infrastructure including birth cohorts, networks and databases to accelerate progress, and to ensure that risk is studied in underrepresented groups, in both girls and boys, and in relation to such factors as socioeconomic background and geography. A strengthened research base to address these issues will be essential to achieve the aspirational goal of discovering “causes of autism...that inform prognosis and treatments and lead to prevention/preemption of the challenges and disabilities of ASD.”

References

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