2013 IACC Strategic Plan Update - Question 3

What caused this to happen and can it be prevented? – Volunteer drafters Cindy Lawler, Joe Buxbaum, Irva Hert-Picciotto, Craig Newschaffer

Introduction to the Question/Chapter

The aspirational goal for Question 3 is "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 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 objectives were added; the new objectives emphasized the need to study how autism risks from the environment may differ in vulnerable subgroups and encouraged research that capitalized on new opportunities and approaches in areas such as epigenetics, the microbiome, nonhuman models of ASD and bioinformatics. Over the past five years, a total of $380 million dollars has been invested to support research under Question 3. This investment has yielded uneven progress. Significant advances have occurred in the area of genetics and in the initiation and/or expansion of large epidemiologic studies, many of which are responsive to multiple Strategic Plan objectives. Less progress has been made in advancing exposure science within ASD studies, inclusion of diverse populations and making use of new animal models for gene environment research. Continued investment is needed to follow-up on recent advances through replication and mechanistic research, to address a wide array of gap areas, and to determine clinical and public health utility of both genetic and environmental findings. Leveraging the infrastructure of existing large population-based epidemiology studies, which 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 collection is also still important to capture environmental risks in the right windows of susceptibility.

Progress Toward Achieving the Strategic Plan Objectives for Question 3.

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 core research projects that were not aligned with the research gaps covered 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.

Genetic Risk Factors

During the past few years there has been a major revolution in ASD genetics. Using the newest molecular and epidemiological methods, the role of genes in ASD continues to be strongly supported while our understanding of the genomic architecture of ASD has been greatly refined, although the emerging picture is one of great complexity. Rather than an exclusive focus on one kind of variation, we now understand that genetic variation at all scales (from changes at a single base, through small genetic insertions and deletions and larger copy number variation/CNV, all the way up to extra chromosomes) can contribute to ASD risk[1,2]. Similarly, we now know that inherited, X-linked, and de novo variation all contribute to ASD. Whereas a great deal of attention has been focused on rare variation, recent evidence points to a contribution of both common and rare variation in ASD risk[1–3]. Finally, several studies have placed the number of genetic loci associated with ASD at somewhere near 1000[4–6], representing a surprisingly large proportion of genes in the genome (almost 5% of the 22,000 genes in the human genome). Parallel identification of discrete developmental trajectories in ASD could provide the potential to map genetic findings to specific behavioral outcomes[7,8].

The emergence of new, high throughput genetic sequencing technology (called variously massively parallel sequencing, next-generation sequencing, or high-throughput sequencing/HTS) has provided an opportunity to rapidly identify rare and extremely rare genetic variation that impacts single genes to alter risk for ASD. In the first papers making use of this technology, 4 types of risk variation have been found in upwards of 20% of individuals with ASD. These include de novo loss of function (LoF) mutations, recessive LoF mutations, X-linked LoF mutations, and small exonic CNV[6,9–15]. These findings are above and beyond the findings of larger CNV in 5-10% of individuals with ASD. These numbers indicate that using these technologies there will be rare genetic findings in at least 30% of individuals with ASD. This indicates that HTS will prove to be extremely valuable clinically in ASD, similar to what has recently been observed for neurological disorder, where a 25% diagnostic yield has recently been reported[16]. Beyond rare variation, a large proportion of ASD risk, on the order of 40-60%[17], lies in inherited, common variation (single nucleotide polymorphisms/SNPs) and it is likely that polygenic risk scores will be possible in ASD[18], as has been observed in other disorders.

While the sample sizes analyzed to date are still too small to identify many new ASD genes, 6 or 7 have been unambiguously identified from even the first 1000 individuals[6,9–12,15,19], with several more reaching criteria for genome wide significance when incorporating all rare variation[4]. There are ongoing initiatives to look at 9000 individuals with ASD using HTS, together with appropriate controls, for a total of at least 20,000 samples, and there is very good empirical evidence that this will double the number of known ASD loci, from the current 50-100 known genes[1]. It is important to remember that although the genes identified through these approaches are associated with substantial ASD risk increases, a mutation in any one of them may still not be sufficient to cause ASD and each of these mutations occurs very rarely. Nonetheless, these new gene findings could lead to a better understanding of aspects of ASD neurobiology, and might also inform the development of pharmacologic therapies. There is also emerging evidence that these genes may fall into a smaller number of common pathways. New tools for identifying networks of functionally-related genes influencing brain development (i.e., those being used in the BrainSpan project) have been applied in ASD and have shown that many of the identified risk genes map strongly to defined brain regions, act during specific developmental time windows within the prenatal period, and/or fall into a modest number of 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.

Environmental Risk Factors

In 2008, little was known about environmental risk factors for ASD. Given what had been revealed about ASD's genetic complexity even 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 is probably quite substantial in ASD etiology. More recent analysis of non-twin family data (from both simplex and multiplex families) also supports the idea that mechanisms beyond heritable genetics and de novo mutations or copy number variants will be necessary for understanding the complex causes of ASD. The time around conception and the prenatal period are likely time windows of heightened vulnerability for the developing brain, with supporting evidence from early reports linking autism symptoms to maternal ingestion of thalidomide[20] or congenital rubella[21,22] , more recent reports on maternal prenatal vitamin intake [23–25], and emerging work in brain gene expression patterns.

Over the past five years a modest investment has helped achieve good initial progress in identifying potential environmental ASD risk factors – especially when we consider the environment broadly as all influences beyond genetic predisposition. Factors associated with ASD risk that have been replicated in two or more studies include: prenatal vitamin intake (protective)[23–25], prenatal maternal infection[26,27], preterm birth, advanced maternal and paternal age at conception, short interpregnancy interval [28,29], and use of certain prescription medications by mothers during the prenatal period[30,31], although underlying conditions in the parent such as depression, might explain these associations.

Among modifiable exogenous exposures, the largest number of studies to date has addressed associations of increased ASD risk with air pollution exposure in prenatal and/or early postnatal life. Over half a dozen studies have reported significant associations[32–37]; both studies examining ozone found significant associations with ASD[35,37],and all three that examined NO_2 found significant associations[34,36,37] . Further work is needed to ensure no residual confounding from socioeconomic factors, and if the association is causal, then to determine in which periods the fetus/infant might be most susceptible. Replication of findings with direct individual-level exposure measures, perhaps via biomarkers, is needed.

Particularly intriguing are the results on prenatal vitamin intake through supplements and diet, showing a 40% reduction in risk for ASD for prenatal vitamin supplements taken in the three months before or the first month of pregnancy, but not in pregnancy months 2-9[23]. In further work, these authors reported a trend of decreasing ASD risk as mothers consumed greater daily folic acid from foods, vitamins, and supplements in the first month of pregnancy[24]. Suren and colleagues replicated the 40% reduction in risk for women who used folic acid supplements in the time around conception based on a large Norwegian cohort study[25]. 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 epigenetics.

Other aspects of the environment where there is now suggestive evidence for an association with ASD risk include pesticides and phthalates, two types of chemicals with endocrine disrupting properties. Evidence of elevated autism-related symptoms or increased risk of an ASD diagnosis has been reported

in relation to organophosphate pesticide exposures during pregnancy[38–40]. Moreover, a neuroimaging study observed alterations in cortical volume of brain regions associated with attention, receptive language processing, social cognition and inhibitory control in children with high prenatal exposures to chlorpyrifos, an organophosphate insecticide[41].

Phthalates are environmental chemicals that act as anti-androgens and have widespread use in personal care products as well as in food, plastics and building materials in the home[42,43] . Poorer executive function, emotional control, and scores in social cognition, social communication and social awareness on the Social Responsiveness Scale, were associated with prenatal exposures to the form of phthalates most frequently found in personal care products[44] . Other support for a link to ASDs comes from a study of health and homes in Sweden, in which vinyl flooring (noted for off-gassing high levels of phthalates) was more commonly reported in homes of affected children relative to the population[45] . 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 as the critical window 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. 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 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.

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 attributable to lack of relevant and testable mechanistic hypotheses emerging from the basic sciences and also to the substantive logistical and resource challenges of assembling sufficiently-sized study populations with both adequate genetic and environmental data. Despite these obstacles, in just the last three 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 an interaction of ASD risk associated with intake of prenatal vitamin supplements and genetic variation in one carbon metabolism[24] . Another study reported that ASD risk associated with prenatal traffic-related air pollution exposure was greater among children with specific *MET* gene promoter variants[46]. While these specific findings suggest we are closer to '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 now implicate epigenetics in the regulation of autism susceptibility genes such as oxytocin. 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[47]. Evidence continues to accrue regarding the ability of environmental factors such as nutrition, drugs and psychosocial stress to regulate transcription through epigenetic modifications, yet this idea has not gained sufficient traction in ASD and merits additional attention. Obstacles to further progress in elucidating the environmental epigenomics of ASD include transcriptional heterogeneity both between and within tissues as well as the lability of epigenomic marks. 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 the potential link between paternal age, ASD risk and rate of de novo mutations. Several studies have shown that advanced paternal age at conception is associated with greater risk of ASD. Separate studies have shown that older fathers produce sperm with greater numbers of de novo mutations, while studies in animals have suggested that there are more profound epigenetic changes in sperm from older fathers. The potential role of exogenous exposures or endogenously dysregulated pathways contributing to loss of fidelity in DNA replication in germ cells and ultimately, de novo copy number variants or base-pair mutations, has yet to be explored, but targeted research may provide clues to a key component of the interface between environment and genes.

Progress Toward Achieving the Question 3 Aspirational Goal

Investments in the past five years have led to identification of new factors contributing to ASD risk and a corresponding revolution in our thinking about the genetic architecture underlying risk. The new gene findings hold promise for a better understanding of the neurobiology of ASD and the development of novel pharmacotherapeutics. 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) in instances of unexplained ASD and developmental delay, and can inform some families about causes, rare but potentially serious comorbid medical conditions, and, in certain cases, 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 skin samples from individuals with genetic lesions --- and in mice and even rats, has led to novel treatment approaches (see Table 1). This is one of the most exciting developments in the past several years in ASD, with 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, three types of mutations associated with very high risk of ASD. In some cases, the same drug is being tried in idiopathic ASD. 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.

ASD-associated syndrome	Primary genetic lesions	Treatment approach	Preclinical studies	Patient population	ClinicalTrials.Gov Identified
Fragile X syndrome (FXS)	FMR1	Arbaclofen	Reversal of deficits in Fmr1-deficient mice [19]	FXS, ASD	NCT01282268, NCT01288716
Rett syndrome (RS)	MECP2	IGF-1	Reversal of deficits in Mecp2-deficient mice and iPSC [20,21]	RS	NCT01777542
Phelan-McDermid syndrome (PMS)	SHANK3	IGF-1	Reversal of deficits in Shank3-deficient mice [22]	PMS, ASD	NCT01525901, NCT01970345

Table 1. Select clinical trials in ASD and associated disorders, based on gene discovery and model systems.

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%, a more recent population-based registry study in Denmark found a substantively lower risk of about 7%. 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 Studies to Explore Early Development (SEED) as well as by the careful extension of existing population-based cohorts that enabled linkage to prospectively collected clinical records and biospecimens. In many cases, early 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 or metabolically susceptible subsets of the population.

The progress achieved to date in the field of environmental epidemiology of ASD has occurred despite significant challenges in exposure measurement. 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 geocoding to link residential location(s) during pregnancy with regional data for specific exposures.

These exposure assessment challenges are not unique to the field of ASD, and are unlikely to be solved through the collective efforts of ASD researchers. There is, however, a pressing need to ensure that improvements in the larger field of exposure science are rapidly incorporated into ASD studies so that research on ASD is well positioned to take advantage of new developments. Research in areas such as personal sensors for more precise measures of individual-level exposures, new analytic methods for measurement of relevant analytes in small volumes of banked biospecimens and conceptual advances in 'exposomics'-characterizing 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 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. Efforts to introduce higher quality and more extensive exposure characterization in large ASD genetic sample collections could also rapidly reap benefits, and early efforts to develop suitable data collection instruments should be redoubled, while recognizing that challenges related to validly assessing exposure during the etiologically relevant windows are formidable.

There are several additional areas where more work is needed to meet Goal 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 endophenotypes that are linked to ASDs. Work on environmentally-induced models 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 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 prove helpful also in detecting environment-ASD associations, as groups underrepresented in clinical studies are often those with disproportionate exposures.

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