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The promise and the pitfalls of autism research: an introductory note for new autism researchers

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The Promise

The last two decades have seen an explosive growth of research into the biological bases of autism spectrum disorders. A survey of PubMed citations using the search terms autism and autistic shows, that for the single year 1990, there were 213 papers published. In 2000 this grew to 441 and in 2009 this has more than tripled with 1522 papers published on this topic. The disorder that we now know as autism was first formally described in 1943 by the Austrian born child psychiatrist, Leo Kanner in his seminal paper *Autistic disturbances of affective contact* [25]. For many years after the publication of his paper, “infantile autism” was considered to be a very rare disorder affecting fewer than 5 in 10,000 individuals [26]. There was so little interest in this disorder, in fact, that Kanner’s paper was only referenced 34 times between 1943 and 1954. By contrast, it was referenced nearly 140 times in 2009 alone.

Clearly, recent estimates that 1:110 children in the United State are affected by some form of autism spectrum disorder [31] has galvanized both advocates and scientists alike to keep up the pressure for additional support and more intensive research. In addition to the emotional toll on family life, Ganz [18] has estimated that the lifetime societal cost of a child with autism is on the order of $3.2 million or $35 billion for all individuals diagnosed each year over their lifetimes. These modern statistics of the autism world have motivated political action culminating in the Combating Autism Act signed by President Bush in December of 2006. Under the new law, NIH funding for autism research is mandated to increase to $210 million by 2011 [39] and an additional $21 million will be provided to the Centers for Disease Control. In addition, the Defense Appropriations Bill set aside $7.5 million for autism research in fiscal year 2007 and similar appropriations have continued more recently.

But, this increased impetus for autism research comes in the midst of an ongoing process that has brought autism out of the darkness of psychiatric institutions onto the covers of major news magazines. Much of the credit for the increased research is due to the dedicated advocacy efforts of parents of children with autism throughout the world. One early example of this was the late Bernard Rimland, Ph.D., a psychologist and father of a son with autism. His influential book entitled, *Infantile Autism: The Syndrome and Its Implications for a*

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Neural Theory of Behavior (which had a forward by Leo Kanner) published in 1964, dismissed the myth that early psychodynamic influences, the “refrigerator mother”, caused autism. Rimland reasoned that if some of the co-morbid conditions of autism, such as epilepsy, were due to neural dysfunction, there was no reason not to think that the core features of autism might also be due to dysfunction of the nervous system.

Major impetus for the expansion of autism research in the United States came from the founding, in the mid 1990s, of two parent advocacy organizations, the National Alliance for Autism Research (NAAR) and Cure Autism Now (CAN), that promoted not only the awareness of autism spectrum disorders, but also the need for research into the biological bases of autism. With the merger of these fundraising and advocacy groups into Autism Speaks in 2005, a highly strategic program for worldwide autism research has been developed that has greatly expanded the scope and intensity of all levels of research concerning autism spectrum disorders. More recently, the Simons Foundation, which is focused heavily on genetic and neurobiological investigations, has also had a significant impact on the funding of basic science research in autism. A 2009 study by Singh et al., found that funding for autism research from the National Institutes of health increased fivefold between 1997 and 2006, from $22 to $108 million. Moreover, the number of autism research grants funded in the US increased 15% each year from 1997 to 2006, with the majority of grants focused on genetics and neuroscience. Based on an analysis conducted by the NIH Interagency Autism Coordinating Committee (IACC), by 2008 the US was spending over $222 million on autism research with 35% of the funding coming from private foundations (http://iacc.hhs.gov/portfolio-analysis/2008/index.shtml). In addition to basic science funding, there have also been efforts to increase support in the areas of translational and clinical research. This has been fostered, in part, through strategic planning by the IACC which has increasingly endeavored to encourage research directed at reducing disability now for persons with autism.

What this surge in interest and support for autism research has achieved is a wealth of new data into the biological features of autism. Gains in knowledge are both dramatic, given the need for many new autism centers and researchers to first establish the “infrastructure” to carry out autism research and, at the same time, frustrating for families who yearn for answers to the question, how do I solve the problem of autism for my child - now.

As pointed out by speakers at the recent Brain Research symposium, *The Emerging Neuroscience of Autism Spectrum Disorders: Etiologic Insights; Treatment Opportunities*, achieving consensus on the biological features of autism has been difficult though a number of areas of agreement are emerging. There is now substantial consensus, for example, that portions of the brain undergo precocious growth in children with autism spectrum disorder [10,13,19]. The causes for this regionally specific, rapid growth are not yet known but are appear to lead to a cascade of aberrant connection formation and dysfunction of networks that underlie the behavioral symptoms of autism. This aspect of autism reseach was highlighted in presentations by Coruchesne, Pierce and Murphy.

Microscopic analyses that might inform cellular processes leading to aberrant growth in the autistic brain are making progress but have not yet led to coherent theories of underlying mechanisms. In the amygdala, for example, there is consistent MRI data across several laboratories that, on average, the amygdala reaches an adult size earlier in children with autism than in typically developing controls [27,36]. Yet, as Schumann reviewed at the symposium, when postmortem studies of brains from older individuals with autism are carried out, there are actually significantly fewer neurons [35]. Hof provided additional insight into the location and types of cortical pathology in autism. Interestingly, the only other stereological study published to date has also shown fewer neurons in the fusiform

gyrus [43], a portion of the temporal lobe which is associated with face processing. This raises the question of whether there were fewer neurons in the amygdala and fusiform gyrus from birth or whether a neurodegenerative process takes place in autism. This won't be known until similar stereological studies can be carried out on the brains of much younger individuals with autism. Even the common findings, such as the enlarged amygdala in children with autism, do not appear to apply to all individuals with the diagnosis. In a large scale, longitudinal analysis of the developing brain in young children (2-4-years-old) with autism, we have found that there is indeed a subgroup in which the amygdala grows at an unusually rapid pace in this period [28] and this group accounts for about 40% of the children tested. The remaining 60% of children, however, either had amygdala growth rates that were similar to typically developing controls or were slower. We will return to the issue of heterogeneity in the "pitfalls" section but findings like these and those from genetic analyses provide substantial rationale for referring to the autismS rather than autism.

Another area of rapid advancement is in the determination of genetic risk factors for autism. Several decades of twin and family studies have indicated that there is a substantial genetic contribution to the etiology of autism [5,17,32]. However, as emphasized by Abrahams and Geschwind, [1] "in contrast to the complete absence of any biological understanding of the ASDs as recently as 30 years ago, we now know that defined mutations, genetic syndromes and *de novo* CNV account for about 10–20% of ASD cases. However, the striking finding that none of these known causes accounts for more than 1–2% of cases is reminiscent of mental retardation…for which there is no single major genetic cause, but rather many relatively rare mutations." The fact that there are no single or even a few genetic smoking guns for autism confirms the view that has emerged from many lines of evidence that autism spectrum disorders are, in fact, a large number of syndromes that manifest in behavioral alterations consistent with the diagnosis of autism. This genetic diversity has also spurred an interest in epigenetic factors that might influence the expression of DNA as a contributing factor to the etiology of autism [24,41]. The section on Genetics and Genomics of autism spectrum disorders in the Brain Research Meeting: The Emerging Neuroscience of Autism Spectrum Disorders: Etiologic Insights' Treatment Opportunities highlighted many of the most promising areas of genetic research. First, the genetics papers and presentations made the point that (a) there is currently little replicating evidence for common variants in autism and that (b) there is mounting evidence for rare, highly penetrant variants in autism. Presentations on medical genetic conditions in autism (Betancur) and on copy number variants in autism (Scherer) highlighted the positive findings, while a dissection of the lack of compelling evidence for common variants with affect sizes above 1.5 (Devlin) summarized other side of this story.

Second, the presence of convincing rare, penetrant variants leads to the ability to readily make model systems with very strong construct validity. There were presentations on such models with a deletion in a clear autism gene. This also leads to a way forward in genetics, i.e., the detailed cataloging of rare variants by high-throughput sequencing, another point that was made in the presentation by Buxbaum.

There has been a recent re-emergence of the concept that at least some autism may have an immunological component to its etiology. Some of the earliest indications of an immune component to autism came from the work of the late Reed Warren and colleagues [45,46] who demonstrated a number of immunological irregularities in at least a subset of individuals with autism. These and more recent data indicate that immune dysfunction may play an important role in a subset of autism spectrum disorder cases [42]. Some individuals with autism spectrum disorders demonstrate abnormalities and/or deficits of immune system function leading to inappropriate or ineffective immune response to pathogen challenge [3,4]. For example, children with autism or pervasive developmental disorder often have

recurrent infections [38], peripheral immune abnormalities[2], or neuroinflammatory responses in the central nervous system[44]. There is also evidence that mothers and first-degree relatives of children with autism are more likely to have an autoimmune disorder than controls [12] although this relationship is far from certain [16]. Finally, antibodies directed against CNS proteins have been found in the sera of autistic children [9,48]. Given the increasingly prominent role identified for immune molecules in brain development [6], it is likely that increased research into the status of the maternal immune system and that of the child through gestation and early postnatal life will make important contributions to a segment of the autistic population, the proportion of which remains to be determined.

A final area that has begun to gain additional attention in autism research is environmental factors. Very early in the field of autism research it became clear that exogenous factors such as the antinausea drug thalidomide [40] or rubella virus [11] could increase the risk of autism. In the case of thalidomide exposure, a rate of 4% autism was observed in cases in which the exposure took place early in gestation. These early studies raised the prospect that at least some environmental factors could cause autism even on the background of low genetic risk. While environmental research is complex and expensive, ongoing studies [22] are beginning to clarify what of the myriad potential environmental factors may increase risk for autism [23,50]. As reviewed recently by Herbert [21], there are many routes including dietary factors, oxidative stress, neuroinflammation and mitochondrial dysfunction through which environmental factors may exert pathophysiological consequences leading to autism.

As emphasized in the section chaired by Sally Rogers entitled *Cutting edge research in behavioral interventions for ASD*, there has also been substantial progress in refining and systematizing behavioral approaches for the treatment of disabilities in autism throughout the lifespan. On the other hand, it became clear in the session chaired by McDougle on *Pharmacological interventions for autism spectrum disorders* attempts that much of current treatment remains directed at co-morbid symptoms such as irritability with drugs for treatment of core features still in the development phase.

The Pitfalls

Despite the achievements of the last two decades in deciphering the biology of autism, there remain a number of issues that continue to slow progress and prevent movement towards more effective treatments. A list of roadblocks could fill volumes but some of the most prominent include the following:

Heterogeneity

A hallmark of virtually every biological parameter assayed in individuals with autism is the enormous heterogeneity – far greater than in the general population. Many individuals with autism have big heads and big brains whereas others have small heads and brains. About 30% of individuals with autism have seizure disorders but the remainder do not. Many individuals have troubling gastrointestinal problems [8] although others do not. As one explores phenotype-genotype interactions, it may be possible to account for some of this variability. For example, Hazlett et al [20] have measured the volumes of various brain regions of children with fragile X and autism, idiopathic autism (without fragile X) and controls. They found that in the brains of the fragile X children with autism there was a robust enlargement of the caudate nucleus but a smaller amygdala. This contrasted with children with idiopathic autism who had a relatively modest enlargement of the caudate but a robust enlargement of the amgydala. Thus, there is a quite distinct pattern of brain pathology in two groups of children all of whom have the diagnosis of autism. Similar evidence of heterogeneity at a cellular level comes from a study of cell loss in the cerebellum in autism. For many years, Purkinje cell loss in the cerebellum was touted as one

of the most common features of autism. However, a recent quantitative stereological study, [47] from the Blatt laboratory examined Purkinje cell number in 6 brains from individuals with autism and 4 control brains. They found that the number of Purkinje cells in three of the autistic brains were clearly within the control range while three were lower than the control range. Thus, careful analysis even in this relatively small sample provides clear evidence of biological heterogeneity.

What are the implications of the enormous heterogeneity of autism? One is that it is unlikely that a single diagnostic biomarker will be discovered that identifies individuals at risk for autism. There are clearly very different genetic and biological processes that underlie autism in different individuals and there is no apriori reason to expect that etiologies related to immune dysfunction, for example, would manifest in biological perturbations that would overlap with known genetic etiologies. There is currently some hope that final common pathways, such as the biology of the synapse [7,29], will be found across various autism etiologies but this remains to be experimentally validated. A likely productive strategy is to define more homogenous subgroups of individuals with autism (eg. those with deletions or duplications on chromosome 16p or those with rapidly growing amygdalas) and then carry out exhaustive biomedical analyses to look for common and distinguishing features. While this is a time consuming effort, it may more quickly provide targets for selective diagnostic markers and more personalized therapeutic interventions. Without first selecting phenotypes to study, it becomes very important to have a sufficiently large sample size to insure adequate representation of all variants of whatever autism feature is being evaluated.

Lack of postmortem brain material

Postmortem human brain research on autism is still very much in its infancy. Newcomers to autism research are often surprised by how little is known about the neuropathology of autism. Attempts at applying powerful new scientific techniques are often frustrated by the modest amount and quality of tissue available. Efforts have historically been hindered by poor tissue quality and small sample sizes, with fewer than *100* autism cases studied to date and a typical sample size of *5* – 10 autism cases per published study. Moreover, nearly all of the brains studied have been from adults with autism. Therefore, there is virtually no information on the characteristics of the brain in young subjects with autism, during the critical time period in which MRI studies suggest strikingly aberrant enlargement of brain structures.

The current system for autism tissue donation has developed semi-autonomously through public and private efforts. The private effort has been spearheaded by the Autism Tissue Program that was initially coordinated by the National Alliance for Autism Research and now is a component of the scientific program of Autism Speaks. The Autism Tissue Program (ATP) http://www.autismtissueprogram.org/site/c.nlKUL7MQIsG/b. 5183271/k.BD86/Home.htm has for a number of years carried out a nationwide advertising campaign to increase the awareness of families of individuals with autism about the value of brain donations. The ATP has managed a collection of brains that has been donated to its program that are stored at a central repository. The ATP has a Tissue Advisory Committee that evaluates applications for tissue and maintains a portal for dissemination of clinical information and an archive of scientific information gathered through analysis of its brain specimens.

One product of the ATP/Autism Speaks effort is the Autism Celloidin Library, that consists of a series of autistic and control brains embedded in celloid and sectioned from the rostral to caudal extents of the brain. This provides researchers a standardized resource of brains for stereological studies. This series does contain young cases (Santos et al., in this issue) and

Van Kooten et al. [43] indicating that it is feasible to acquire critical brains for defining the developmental neuropathology associated with autism.

On the public side, autism brain donations find their way to one of several national brain banks, now mainly the NICHD funded bank at the University of Maryland. These brains are then made available through tissue dissemination procedures developed by the brain banks.

In order for progress on the cellular and molecular level to advance, there is clearly a need for a far more vigorous program of brain acquisition and distribution. This is an effort, however, that must be done with the highest level of sensitivity and through a centralized national (or even international) organization. Hopefully, this will become a very high priority both for public and private funders of autism research.

Need for longitudinal studies

Autism is a developmental disorder. But, the lifespan trajectory of autism has barely begun to be studied. The interesting finding which has already been described of precocious brain growth, for example, is only observable through a narrow span of postnatal development [30]. At birth, the brains of individuals with autism are either at the same size or even slightly smaller than age-matched typically developing controls. During the next 1-2 years, on average the brains of individuals with autism demonstrate a precocious growth which exceeds that of the controls. But, within the next few years, brain size in typically developing controls catches up so that by adolescence there is no statistical difference in total brain size between individuals with autism and age-matched controls [36].

There is a critical need to follow children from the earliest possible age through diagnosis to identify the temporal correlations between alterations in brain structure and function (or other biological correlates) and the emergence of autistic symptomatology; infant sibling studies of this type are now under way [33,49].

Need for adequate animal models

The session chaired by Dr. Jacki Crawley in the recent Brain Research symposium, *The Emerging Neuroscience of Autism Spectrum Disorders: Etiologic Insights; Treatment Opportunities*, provided an excellent overview of current attempts to develop rodent models relevant to autism. And, Crawley has written extensively on this topic [14,15,37]. It is fair to say that at this time, there is still substantial work that must be done to achieve a highly useful animal model of autism. The strength of an animal model depends, in large part, on its resemblance to the human disorder in question. Three criteria are commonly used to evaluate animal models: 1) Construct validity – the extent to which the model reproduces the etiology and/or pathophysiology of the disorder, 2) Face validity – the degree to which the model resembles symptoms of the disorder and 3) Predictive validity – the extent to which treatment of the animal model provides insight into therapeutic options for the human condition.

One could argue that the value of an animal model of autism is related to how closely the experimental manipulation used to create the model are related to know etiologies of the condition – to its construct validity. In other words, does the model stem from a putative etiology of human autism? This has proven particularly challenging for the field of autism where the underlying cause(s) of the disease for most cases remain unknown. However, as potential causes of autism are identified, animal models will play an increasingly critical role in translating results from human autism research into testable hypotheses. This must be done carefully, however, since the hallmark features of autism, such as impairment of social interaction, can be affected by so many manipulations. Harlow and colleagues, for example, showed that nonhuman primate behavior could be seriously impaired if the postnatal infant

was deprived of maternal interaction [34]. But, one would not seriously consider maternal separation as a primary cause of human autism.

Animal models are produced by changing gene expression or brain anatomy and chemistry and ultimately lead to alterations of behavior. Face validity is the extent to which these changes resemble the phenotype of the human disorder. For behaviorally defined disorders such as autism, it is important to relate the behavioral outcome of the animal model to the hallmark features of the human disorder. Diagnosis of autism is based on qualitative impairments in social interaction and communication, with the presence of restricted repetitive and stereotyped patterns of behavior, interests, and activities. While some features of autism can not be successfully modeled in animal species, an ideal animal model with high face validity would produce behavioral changes in the three diagnostic domains of autism: 1) social interaction, 2) communication and 3) repetitive behaviors. Of course, how one tests each of these domains will depend on the species under analysis. Social interest in the mouse, for example, is demonstrated by immediate exploration of a novel conspecific with habituation over time. By contrast, rhesus monkeys are initially hesitant to engage a conspecific and species-typical social behaviors only emerge over time as confidence in the affiliative nature of the other animal is ascertained. One also must be mindful of the differences in neural systems that might underlie these candidate behaviors in different species. It is highly likely that the frontal lobe in general and portions of the orbitofrontal cortex in particular plays a major role in mediating social behavior. While the organization of the human frontal lobe can be seen in the rhesus monkey brain, there are major portions of this brain region that do not seem to have a homologue in the mouse brain. This may pose a difficulty to the mouse model if it is determined, as seems to be the case, that abnormal development of the frontal lobe is a key feature of the pathology of autism.

The ultimate goal of any animal model is to develop a test bed for evaluating strategies for preventing or treating a human disorder. Predictive validity refers to how successful an animal model is to lead to treatment discoveries for the human condition. Although we are just beginning to develop valid animal models of autism, the information gained through these models will ultimately help us move towards the goal of developing preventative strategies and/or novel therapies to reduce the disability of autism.

CONCLUSION

As attested to by the excellent presentations and articles emerging from the recent Brain Research symposium, *The Emerging Neuroscience of Autism Spectrum Disorders: Etiologic Insights; Treatment Opportunities*, much progress has been made on the analysis of the biological features of autism. But, many fundamental questions remain: What are the causes of autism? What brain regions are most impacted by autism and how? Are the co-morbid components of autism, such as gastrointestinal disorders, a clue to etiology or a side effect of the core disorder? Why are there four times as many males with autism as females? Are the signs, symptoms and biology of autism similar in males and females? What happens to individuals with autism as they age?

The answers to these questions will come from collaborative, interdisciplinary research fueled by the desire to help lessen disability for individuals and families with autism. It is probably wise at this point to remain open minded to potential etiologies, some of which may be out of the mainstream of current medical practice. It is inevitable that autism researchers will interact with the autism lay community. It is a wonderful, dynamic community that is at the same time pushy and respectful, optimistic and frustrated, hopeful but pragmatic. The autism community is desperate for useful information and it is incumbent on the autism researcher to make every effort to translate their research to this

receptive lay audience. Despite the temptation, it is important not to promise too much. And, given the incredible heterogeneity of this disorder, understanding that one size will never fit all is a reasonable perspective to frame all future findings.

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