Facilitating Autism Research

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Abstract

Early autism research focused on behavior and cognition. In recent decades, the pace of research has accelerated, and advances in imaging and genetics have allowed the accumulation of biological data. Nevertheless, a coherent picture of the syndrome at either phenotypic or biological level has not emerged. We see two fundamental obstacles to progress in basic understanding of autism. First, the two defining features (impairment in social interactions and communication, and restricted, repetitive behaviors and interests) are historically seen as integrally related. Others hold that these two major traits are fractionable and must be studied independently, casting doubt on autism as a coherent syndrome. Second, despite much recent research on brain structure and function, environmental factors, and genetics/genomics, findings on the biological level have not generally aligned well with those on the phenotypic level. In the first two sections, we explore these challenges, and in the third section, we review approaches that may facilitate progress, such as (1) including in studies all individuals defined by social impairment without regard to repetitive behaviors, (2) forming narrowly defined subtypes by thorough characterization on specific features, both diagnostic and non-diagnostic, (3) focusing on characteristics that may be relatively robust to environmental influence, (4) studying children as early as possible, minimizing environmental influence, and including longitudinal course as an important part of the phenotype, (5) subtyping by environmental risk factors, (6) distinguishing between what participants can do and what they typically do, and (7) aggregating large data sets across sites. (*JINS*, 2017, *23*, 903–915)

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INTRODUCTION

Approximately 70 years ago, two papers appeared in the psychiatric literature, describing "autistic disturbances of affective contact" (Kanner, 1943) and "autistic psychopathy" (Asperger, 1944). Asperger's description was published in German during WWII, and did not receive attention in the English speaking world until Lorna Wing described it in 1981 and Uta Frith translated it into English in 1991 (Asperger, 1991). Both syndromes are marked by poor social relationships and pragmatic language, obsession with specific interests and insistence on sameness. A third category in the DSM-IV, Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS; APA, 2002) shares social impairments, but lacks the same degree of restricted, repetitive behaviors (RRBs) or shows milder deficits.

In the most recent diagnostic manual of the American Psychiatric Association (DSM-5, APA, 2013), these entities are combined into "autism spectrum disorder" (ASD), defined by three social communication symptoms (poor nonverbal communication, poor social-emotional reciprocity, deficits in developing and maintaining relationships), plus two of four RRBs (stereotyped or repetitive speech or movements, resistance to change, highly restricted interests, and hypo- or hyper-reactivity to sensory input). Severity of the syndrome and intellectual or language impairment are coded separately. With decades of research, including an accelerating pace and a focus on biology in recent years, one might expect some definitive answers about the syndrome's clinical borders, pathophysiology, and etiology. Instead, enormous amounts of data have accumulated with little clarity emerging, which Rutter (2014) justifiably finds "odd."

There seem to be two fundamental obstacles to progress. First is a basic question about the coherence of the behavioral syndrome (explored by Hobson, 2014, and Rutter, 2014, in an issue of Autism [vol. 18] devoted to this topic, and Mandy and Skuse, 2008). If there is no coherence, then looking for a unified etiology or pathophysiology is pointless. The second obstacle is the failure of findings at one level (e.g., genetic) to map onto features of another (e.g.,

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phenotype) (Rapin, 2014; Waterhouse, 2013; Waterhouse, London, & Gillberg, 2016). Both questions raise doubts about even retaining the ASD diagnosis (Mueller & Amaral, 2017; Waterhouse, London, & Gillberg, 2017). We will explore these two obstacles in Sections A and B and then review potential avenues for facilitating research progress in Section C.

COHERENCE OF THE SYNDROME

Until recently, no one questioned ASD as a valid diagnosis (Rutter, 2013) although criteria, boundaries, and etiologies were much debated and changed over time. A valid syndrome is "a naturally occurring combination of deficits…there is an underlying [causal] factor" (Benton, 1961, p. 76).

Autism as such an entity, with a discoverable etiology and pathophysiology, is under question, as are other psychiatric and neurodevelopmental diagnoses (Cuthbert & Insel, 2013). The syndrome view assumes that social-communication deficits and RRBs co-occur at high rates, and bear some causal relationship. Recent theorists (Mandy and Skuse, 2008; Ronald, Happe, and Plomin, 2005) have argued that the two key features are not necessarily causally related and should be studied independently.

Four questions bearing on the coherence of autism will be briefly considered: (1) do the two defining traits co-occur in most cases; (2) is there substantial phenotypic overlap with other disorders; (3) how heterogeneous are autism phenotypes; and (4) how are theories about underlying causes shaped by the assumption of coherence?

Do Most Children Who Meet Social Criteria for Autism (as per DSM-IV, DSM-5, or ICD-10) also Have RRBs at Some Time in Their Development?

Wing and Gould (1979) reported that in a sample of 74 socially impaired children, *all* showed some degree of RRBs. However, others have identified PDD-NOS subgroups with low rates of RRBs (Brennan, Barton, Chen, Green, & Fein, 2015; Walker et al., 2004), although RRBs have variable ages of onset and might have appeared later (Barton, Robins, Jashar, Brennan, & Fein, 2013; Stone et al., 1999). Factor analyses (Frazier, Georgiades, Bishop, & Hardan, 2014; Mandy, Charman, Puura, & Skuse, 2014) suggest that a two-factor solution is the best fit, suggesting that RRBs and social-communication deficits are separable traits, although Constantino et al. (2004) found evidence for a single continuously distributed underlying factor.

Supporting the fractionation approach (studying the traits separately) is population based research suggesting that autism traits are associated with different genes (Happe & Frith, 2006). However, the logic of separating these traits because they lack genetic linkage and phenotypic correlation *in a general population* may not be sound. For example, there are several genetic disorders associated with wide set eyes and intellectual disability (Wolf-Hirschhorn, Cri-du-Chat, Angelman syndromes, etc.). Presumably, these two traits would show little correlation in the general population but still co-occur in specific syndromes.

Fountain, Winter and Bearman (2012), studying almost 7000 ASD children, found that those who were high functioning or rapidly improving in one domain tended to be so in other domains, consistent with linked development. However, other studies (see Leekham, Prior, & Uljarevic, 2011) suggest that social/communication and RRB symptoms can show different trajectories, consistent with fractionation. Alternatively, the traits may be linked, but diverge because one is more amenable to treatment.

This debate may be resolved by collecting large amounts of longitudinal data and reporting individual patterns of development. Instituting early treatment, however, may make this "natural history" difficult to observe. In the meantime, given the sparsity of data on this question, it seems unproductive to exclude from clinical or research activities children who show impaired social/communication, but have 0 or 1 RRB instead of the 2 required in DSM-5 (APA, 2013).

Phenotypic Overlap with Other Syndromes

Are RRBs *necessary features* of ASD, while other frequently comorbid symptoms are *ancillary* (as in DSM-5)? Comorbidities are the rule rather than the exception in ASD (e.g., Matthews, 2016), including intellectual disability (ID) (Blacher & Kasari, 2016), psychiatric and medical disorders (Bauman, 2010; Simonoff et al., 2008), and Specific Language Impairment (Bishop, 2010). Furthermore, these disorders share genetic risk factors with ASD (De Rubeis and Buxbaum, 2015; Elia et al., 2010). These and other behavioral comorbidities (e.g., self-injury, sleep problems, aggression) may be related to autism's defining symptoms in many possible ways (Gillberg & Billstedt, 2000).

Whether any of these "comorbidities" are as central to ASD as RRBs is obscured by evidence that young children are more likely to get an ASD diagnosis when they display comorbid symptoms (e.g., language disorder, intellectual disability, epilepsy) while "autism only" may be over-represented in studies of older children (Gillberg & Fernell, 2014). To ascertain whether comorbidity profiles indicate distinct pathophysiology and/or the need for distinct treatment, it will be helpful to routinely characterize participants with respect to comorbidities.

Phenotypic Heterogeneity

Another major obstacle to conceptualizing ASD as a syndrome is its unusually high level of phenotypic variability in symptoms and cognition (Boucher, 2012; Georgiades et al., 2013; Howlin, Goode, Hutton, & Rutter, 2004; Kjelgard & Tager-Flusberg, 2001; Rommelse et al., 2015; Waterhouse et al., 2016). Onset and course is also quite varied; symptoms may appear during the first year of life (Ozonoff et al., 2010; Zwaigenbaum et al., 2005) or as late as the third year (Ozonoff et al., 2015; Rogers, 2009); some children show regression in language and/or social attainments in the

second year (Hansen et al., 2008). A minority of children will lose their diagnosis (Anderson, Liang, & Lord, 2014; Fein et al., 2013; Helt et al., 2008), as will a higher proportion of children whose autism was due to environmental deprivation such as congenital blindness (Hobson & Lee, 2010; Hobson, 2014; Jure, Pogonza, & Rapin, 2016) or severe neglect (Rutter et al., 1999). In some children, RRBs will remit (Esbensen, Seltzer, Lam, & Bodfish, 2009), while other individuals will experience cognitive decline in adolescence or adulthood (Howlin, 2010).

Therefore, ASD may represent dozens, or even hundreds, of disorders with overlapping phenotypes, but distinct biological etiologies (e.g., Abrahams, 2011; Rapin, 2014). Therefore, case-control studies classifying participants *only in regard to whether or not ASD criteria are met,* are likely reporting on groups of individuals with many different underlying biological conditions, perhaps as many as there are participants, limiting generalizability and replicability.

Implications for Theory

Mirroring the controversial independence of social and RRB symptoms, there are two classes of theory about underpinnings of autism traits: *The first class account for one trait*, usually the social impairment; these include poor *intersubjectivity* in early childhood (Hobson, 2014; Hobson and Meyer, 2005), *reduced social interest* and abnormal salience of social stimuli in early childhood (Fein, Pennington, Markowitz, Braverman, & Waterhouse, 1986; Uddin & Menon, 2009; Waterhouse, Fein, and Modahl, 1996), and an *inability to understand others' minds* (Baron-Cohen, 1997). Fewer theories attempt to explain the trait of RRBs in terms of *executive dysfunction or detail focus* (South, Ozonoff, & Mcmahon, 2007).

The second class are unifying theories that *attempt to explain both the social communication and the RRBs;* and implicitly accept the syndromic view of autism. These include the *"Intense World" hypothesis* of Markram and Markram (2010), in which stimuli experienced as painfully intense lead to selective attention and avoidance of social input. Similarly, Kinsbourne (2011) suggests that an *unstable arousal system* leads to avoidance of unpredictable social input, to restricted interests, and to inward focus of attention. This focus is associated with the default mode neural network (Menon & Uddin, 2010), which may not be normally deactivated by task demands in ASD (Kennedy, Redcay, & Courchesne, 2006). *Impaired attention shifting* can account for poor development of joint attention, resistance to change and perseverative interests (Harris, Courchesne, Townsend, Carper, & Lord, 1999). Another intriguing hypothesis (Gergely, 2003) suggests that at 0–3 months, the infant attends preferentially to perfectly contingent events, including visual input from his/her own movements, promoting the concept of self. At around 3 months, preference switches to imperfect contingency, attracting attention to social input. A *failure to make this genetically programmed switch* may lead to reduced social attention, stereotyped behavior, resistance to change, and preference for inanimate objects (e.g., Klin, Lin, Gorrindo, Ramsay, & Jones, 2009).

Syndrome coherence has implications for which class of autism theories are most tenable and for which individuals or subtypes.

Our own speculation is that ASD coheres at the behavioral (but not biological) level during a particular developmental window in the preschool years, representing a temporary final common pathway or "bottleneck" through which children with a wide range of genetic or environmental risk factors go (potentially due to missed experience-expectant social input resulting from each child's vulnerability), only to emerge differently, depending on brain maturation, available treatment, and response to treatment. This view is consistent with higher diagnostic reliability and stability at this age, relative to older and younger ages (e.g., Barbaro & Dissanayake, 2017; Brian et al., 2016). ASD secondary to environmental deprivation (severe, congenital blindness [Jure, Pogonza, & Rapin, 2016], or neglect [Rutter et al., 1999]) shows more improvement in later childhood than idiopathic autism (Hobson & Bishop, 2003; Rutter et al., 2007), whereas children with Rett syndrome and childhood disintegrative disorder fit ASD criteria early on but show a deteriorating course. This conceptualization of ASD as a developmental bottleneck is similar to the view of Williams and Bowler (2014) who argue for coherence at the behavioral level that may wax or wane with development.

Differences in behavioral coherence over development may also be a byproduct of flawed measurement (i.e., weak operational definitions, poor reliability) rather than a true phenomenon (Mandy et al., 2014). DSM-5 provides little guidance on how to adjust for developmental level. When operational definitions are murky, measurement of behaviors will be unreliable (Lord et al., 2012).

LACK OF CORRESPONDENCE AMONG LEVELS OF EXPLANATION

The second major problem facing autism research is the striking failure of findings at the etiological or physiological level to be closely associated to phenotypic features. In this section, we will briefly consider (1) the relationships between genotype and phenotype in autism, and (2) the contribution of brain imaging data.

Genotype–Phenotype Relationships

Many techniques have been used (see Persico and Napolioni, 2013) to identify rare or common autism vulnerability genes and variants, copy number variants (sections of the genome that are repeated, CNVs), linkage sites, or defined genetic syndromes with autistic features (e.g., Fragile X, tuberous sclerosis) (see reviews by Abrahams, 2011; Bourgeron, 2016). Concordance for ASD between monozygotic (MZ) and dizygotic (DZ) twins, and the existence of broad autism phenotype (BAP) in siblings and parents (Bishop et al., 2004; Piven, Palmer, Jacobi, Childless, & Arndt, 1997) confirm the high heritability of autism (estimated at 90% by Rutter, 2013,

although Bourgeron, 2016, finds it closer to 50%, with approximately 50% accounted for by non-shared environment).

However, the actual penetrance of autism-related genes, and the apparent complexity of gene–gene interactions and epigenetic influences, has not proved tractable. The Simons Foundation Autism Research Initiative (SFARI) Web site have large populations of affected families, and attempt to map phenotypic features onto established risk gene variants as well as CNVs. SFARI also lists over 800 genes, on virtually every chromosome, with 50 + ranked as "high confidence" or "strong candidate." Rare mutations have been identified in synapse-related genes, and genome-wide association (GWA) studies using single nucleotide polymorphisms have highlighted potential ASD risk loci on multiple chromosomes (Chaste et al., 2015), accounting for a small proportion of cases. CNVs, both de novo and familial, have been identified in 5–10% of individuals with ASD (Marshall et al., 2008; Sebat et al., 2007), which is a much higher rate than in the general population (1%), but not higher than in the ID population (Whibley et al., 2010). Some of the newly discovered CNVs contribute to neuronal migration and synaptic function but their presence in some unaffected family members (approximately 3%) leaves their contribution to autism uncertain (Gilman et al., 2011; Persico and Napolioni, 2013).

In a landmark study, Le Couteur et al. (1996) studied 28 MZ and 20 DZ twin pairs in whom at least one individual had autism; in the discordant pairs, especially the MZ pairs, the non-autistic twin often showed the broader phenotype. Amazingly, however, there was as much cognitive and language variation *within* concordant MZ pairs as *between* pairs, including up to 50 IQ point discrepancy; regression also fails to show concordance in multiplex families (Parr et al., 2011). The opposite problem has also been demonstrated: Yuen et al. (2015) did a GWA study of 85 families with two children affected by autism, focusing on genes previously associated with autism. They found that approximately 70% of the sibling pairs did *not* share an ASD-relevant mutation, but rather had different ones. Since there are presumably many autism-related genes not yet identified, this concordance may be underestimated. Nevertheless, the aggregation of ASD diagnoses in families and the lack of demonstrable genetic concordance within families, suggests a contribution from the shared uterine environment (Hallmayer et al., 2011). Another interpretation is that a non-genetic factor or a de novo mutation (e.g., in an older father, or with viral infection, etc.) may push an individual into the clinical manifestation of autism in the presence of familial genetic risk (Gaugler et al., 2014).

One approach to this seemingly intractable problem is to increase phenotypic homogeneity in subtypes in an effort to increase linkage signal or significance of association in GWA studies. The success of these efforts thus far is debated. Subtyping along a single or a few broad features often increases genetic homogeneity (even if only modestly) (Chaste et al., 2015; De Rubeis and Buxbaum, 2015; Liu, Paterson, & Szatmari, 2008; Loviglio et al., 2017; Shao et al.,

2003). Chaste et al. (2015) report that in a GWA study, stratifying a group of 2576 individuals by IQ, insistence on sameness, extent of RRBs, and overall severity of ASD, led to only a modest increase in association significance or estimates of heritability; for both, the most successful subtyping was for severity of RRBs. Heritability in the overall sample was approximately .4 and reached approximately .6 in groups with high RRBs. Note: if RRBs reflect anxiety, especially in individuals who cannot verbalize their emotional state, the heritability might be for anxiety.

Linkage studies have arguably fared somewhat better than GWA studies, with subtyping on specific features improving linkage signal (e.g., Liu et al., 2008, studied 976 multiplex families from the Autism Genome Project consortium, and LOD scores increased when sorting for delayed onset of phrases; Spence et al., 2006, also found increased linkage for language delay but not on the same chromosomes). Of course, to be confirmed, all of these associations need to be replicated on independent samples. See Chaste et al. (2015) for further discussion of linkage and GWA studies in relation to phenotypic subtyping.

Given this rather modestly successful set of efforts, subtyping along more numerous and fine-grained features may or may not yield more etiologically homogenous subgroups; the potential for success, of course, will require very large samples. GWA studies to date may have lacked sufficient sample size, due to the weak effect of individual common genetic variations, and hence have been underpowered (De Rubeis & Buxbaum, 2015). It is also possible that characteristics not considered central to autism, and therefore, not studied in large-scale genetic studies, such as improved behavior with high fever, might increase linkage; uncovering these relationships would necessitate rich phenotyping of all participants.

As described above, the overlap in phenotype and the shared genetic risk between autism and other neurodevelopmental disorders makes it even more difficult to identify genes specific to autism *versus* those that cause the common features. One assumes that the phenotype depends on interactions with other genes, epigenetic activation/suppression, imprinting, and the quality of pre and postnatal environmental conditions.

This complexity pervades the study of psychiatric and neurodevelopmental disorders. Indeed, central to their study are equifinality (syndrome coherence at the behavioral level despite multiple biological causes) and multifinality (a single cause can produce highly variable symptoms) (Williams & Bowler, 2014). This complexity may indicate that there are phenotype–genotype links that are simply out of our grasp with the current state of knowledge, data, and technique. Although difficult and expensive, large samples with rich phenotyping may be the only way forward.

Brain Imaging Data

The difficulty in relating genetic variants to symptoms also holds for the intermediate levels of brain imaging and post-mortem data. Autopsy and in vivo studies have indicated

differences in the organization of mini columns in the neocortex (Casanova, El-Baz, Vanbogaert, Narahari, & Switala, 2010), inflammatory processes (Pardo et al., 2005 but see non-replication in Pardo et al., 2017), abnormalities in neurotransmitter balance (Lam, Aman, & Arnold, 2006), and abnormal connectivity (Belmonte et al., 2004) but details vary substantially. It is unclear whether inconsistent findings are due to methodological differences, developmental stage, or individual variation (Waterhouse et al., 2016).

One somewhat consistent anatomical finding is accelerated brain growth in the first 2 years of life (e.g., Courchesne, Carper, & Akshoomoff, 2003; Hazlett et al., 2005). We compared medical records for head growth in a group of high functioning autistic individuals and a group who moved off the spectrum; we found accelerated head growth from 12 to 24 months in both groups, failing to correlate an important outcome variation with a biological marker (Mraz, Dixon, Dumont-Mathieu, & Fein, 2009). However, head growth may be too coarse a measure; a study of high risk infant siblings (Hazlett et al., 2017) found that an above-average increase in the surface area of the brain in the first year and in whole brain volume in the second year predicted (with 80%) accuracy which infants went on to develop autism. This positive result, together with the moderately promising results in phenotyping, suggests that more fine-grained measurement in both biology and phenotype may lead to progress.

SUGGESTIONS FOR RESEARCH PROGRESS

As the complexity of autism has become increasingly apparent, some new directions have been considered. Here, we speculate about which approaches might be most fruitful, based in large part on overcoming the obstacles discussed above.

The Initial Pool from Which Participants Can Be Drawn Should Represent the Entire Autism Spectrum

Even after decades of research, we do not know whether RRBs *must* accompany autistic social deficits. The DSM-5 (APA, 2013) requires two RRBs for an ASD diagnosis. In addition to lack of continuity with the body of DSM-IV based research, the social symptoms in DSM-V are difficult to apply to young or cognitively impaired children. Most importantly, these criteria have prematurely closed the category to individuals with autistic social deficits and no (or one) RRBs. Adding PDD-NOS (APA, 2000) or atypical autism (WHO, 1993) to autistic disorder or childhood autism would capture individuals with ASD's core social domain, who can then be further characterized. In contrast, starting with ASD as defined in the DSM-5 will guarantee that all individuals enrolled in research have both social impairments and RRB but will not reveal whether they cohere as a syndrome.

All Research Participants Should Be Characterized for Detailed Clinical Features, Both Diagnostic and Non-diagnostic

Given the heterogeneity of autism, and the modest success in mapping diagnosis onto biological findings, it is perhaps time to largely *abandon simple case control studies,* without thoroughly characterizing participants. Waterhouse and Gillberg (2014) argue that very *narrowly defined subgroups*, both at the phenotypic and biological levels, will increase the probability of being able to link the two. Studies starting with a single diagnostic group of doubtful validity will be uninformative (Uher & Rutter, 2012).

Furthermore, *detailed phenotypes must not be based solely on the defining features of ASD*. As discussed, ASD may be a multifinal endpoint of potentially numerous starting states. Disruption in social communication may come about from lack of experience expectant input, which, in turn, may have been caused by a genetic factor (e.g., decreased proclivity to seek eye contact, reduced ability to perceive biological motion, attention shifting impairment, sensory processing impairment, etc.) or an actual lack of input (as in blindness and severe environmental deprivation). Therefore, classifying by severity in social communication may not increase etiological homogeneity.

Several systems for detailed phenotyping have been proposed: First is Gillberg's ESSENCE system (Gillberg, 2010; Pettersson, Anckarsäter, Gillberg, & Lichtenstein, 2013), which includes motor abnormalities, general delays, speech/language delay, social functioning, hyperactivity/ inattention, sleep disturbances, mood, and feeding difficulties. Second is a comprehensive list by Lai, Lombardo, Chakrabarti, and Baron-Cohen (2013), with over 100 variables including developmental course, gender, comorbidities (medical and psychiatric), cognitive level and profile (including social cognition), as well as genetics (including syndromes, multiplex/simplex family, and gene level variants associated with autism), and environmental risk factors. Third is the Research Domain Criteria (RDOC; Cuthbert & Insel, 2013), in which the targets of study are psychological processes rather than diagnostic syndromes. Individuals with ASD show deficits in many RDOC domains (positive and negative emotions, memory, language, perception, social communication, understanding of self and others, arousal, self-regulation, sleep rhythms, etc.)

However, none of these lists focus explicitly on *quality of behavior,* especially social behavior. We need to agree upon measures of *quality* and for *changes across development.* Foss-Feig, McPartland, Anticevic, & Wolf (2016) had the interesting idea of sorting symptoms as negative, positive, or cognitive (as with schizophrenia). Wing and Gould (1979) suggested typing children by their predominant social attitude (aloof, passive, active but odd). A somewhat more detailed system of qualifying social behavior that we have found very clinically useful is to identify simple social processes and partners that may be present, emerging, or not yet present. In the grid (Figure 1), we expect development to

	Mother/first caregiver	Second caregiver	Siblings	Teachers/other familiar adults	Familiar children	others
aware						
respond						
initiate						
sustain						

Fig. 1. Characterization of social interaction.

proceed more or less from top to bottom and left to right; this system allows clinicians to track development, to target next developmental steps, and perhaps to identify social subtypes.

We also suggest adding variables that have distinguished groups in previous studies, or are candidate endophenotypes: *pretend play* with peers (Kasari, Chang, & Patterson, 2013), *spontaneous imitation* (e.g., Toth, Munson, Meltzoff, & Dawson, 2006), *temperament* (e.g., Garon et al., 2009), *psychiatric comorbidities* (Hutton, Goode, Murphy, Le Couteur, & Rutter, 2008), *medical comorbidities* (Bauman, 2010), *savant skills* (Hermelin, 2001; Howlin, 2010), and *family history* of psychiatric/neurodevelopmental disorders, including the BAP (Bailey & Parr, 2003).

Furthermore, It May Be Productive to Study Characteristics That Are Likely to Be Less Influenced by the Environment

The more a behavioral feature can be influenced by the environment, the less we can expect that stratifying ASD groups along that feature will yield increased genetic homogeneity. Rett's syndrome was discovered by studying the subgroup of ASD children who are female, show excessive hand wringing, marked regression of hand skills, and head growth deceleration in the second year (Percy, 2001), all of which are likely to be minimally influenced by environmental factors. The unusual cry in infancy may have been crucial in identifying Cri-du-Chat as a specific syndrome (in combination with variable dysmorphic features) with the genetic underpinnings elucidated later (Lejeune et al., 1963; German, Lejeune, Macintyre, & De Grouchy, 1964).

One factor that seems relatively independent of environment is improvement of emotional and social responsiveness with a high fever (Curran et al., 2007), perhaps implicating changes in brain chemistry or the operation of the locus coeruleus in a subset of autism cases (Mehler & Purpura 2009). Possible high pain tolerance (although the possibility has been raised that it is the expression rather than the experience of pain that differs) (Allely, 2013) and distinctive physical parameters (e.g., head and body growth, dysmorphology) are other features likely to be relatively uninfluenced by the environment. Freedman and Foxe (2017) suggest impaired visual saccade and saccade adaptation as an early autism endophenotype, which may be correlated with cerebellar vermis dysfunction, may be measurable as early as

10 months, and may result in abnormal cortical mapping of space, with over-representation of the peripheral visual field, an intriguing idea since some affected children seem to attend preferentially to the visual periphery.

When Considering the Need to Minimize Environmental Influence, the Issue of Timing Is of Utmost Importance

Enrolling children before any intervention is started is especially important for variables that show increasing sensitivity to environment over time. For example, social attention in infancy is strongly correlated with genetic factors (Constantino et al., 2017); however, environmentally determined factors such as amount of face to face interaction (affected by parenting and intervention) will probably play a larger and larger role in their social attention as development unfolds and so will only be likely to yield more genetically homogenous groups if measured early in development (e.g., Jones and Klin, 2013).

Gathering detailed behavioral data in the first year or 2 of life, given ASD's prevalence, is a logistical challenge that may be met in part by studying high-risk samples (premature babies, baby sibs of affected children). Some groups are attempting to do structural and even functional imaging with very young children with ASD or risk for ASD during sleep; a recent study reported 80% correct prediction of the ultimate diagnostic status of baby siblings by monitoring early brain growth (Hazlett et al., 2017). Two limitations of this high-risk strategy are that large samples will still be needed, although much smaller than general population studies, and that autism unfolding in these groups may not be wholly representative of autism in other individuals.

Several aspects of clinical course have some specificity for autism (Rutter, 2011), including early regression of language or social milestones in approximately a quarter of cases (Baird et al., 2008; Pickles et al., 2009), savant skills in approximately one third of cases (Howlin, 2010), early accelerated brain growth in a minority of cases (Libero, Nordahl, Li, & Amaral 2017; Woodhouse et al., 1996), onset of epilepsy at distinctive times in approximately one third of cases (Bolton et al., 2011), extreme variability in adult outcome (Howlin et al., 2004), with onset of psychiatric comorbidity at puberty in 20% of cases (Hutton et al., 2008),

and loss of diagnosis in a significant minority (Fein et al., 2013). Therefore, as suggested by Rutter (2011), and Uher & Rutter (2012), *adding longitudinal course* to characterization of participants is also likely to reduce etiological heterogeneity. Clinical course should include age of onset of symptoms in early childhood, as a third to a half of parents of children with autism recall abnormalities beginning in the first year (Yirmiya & Charman, 2010).

Furthermore, If There Is a True Increase in the Incidence of Autism (not Simply an Increase in Those Diagnosed, and This Issue Is yet to Be Resolved), then Changing Environmental Exposures May Be Serving as Epigenetic Triggers in the Presence of Background Familial Risk

Prospective longitudinal studies, such as the Norwegian mother and baby study of 100,000 individuals hold the greatest promise for shedding light on this question (Rønningen et al., 2006). Other biological studies can be done even earlier, such as studies of placental abnormalities at birth (Anderson et al., 2007), midpregnancy variables (Kolevzon, Gross, & Reichenberg, 2001) such as maternal autoantibodies to fetal brain protein (Croen et al., 2008), and bleeding (Gardener, Spiegelman, & Buka, 2009). Cases with environmental factors such as toxins (Lyall, Schmidtt, & Hertz-Picciotto, 2014) or medication during pregnancy (Christensen et al., 2013) and early sensory deprivation (Jure et al., 2016; Rutter et al., 1999) are clearly needed for subtyping, and perhaps should be excluded from genetic studies. Although just beginning, recent studies have suggested that the gut microbiome, influenced by mode of delivery, feeding, and mother's health status, may be abnormal in autism (Li et al., 2017) and may represent a future target of treatment.

We Also Need to Distinguish What Individuals with ASD Cannot Do and What They Do not Do.

In many tightly controlled lab or clinic settings, individuals with ASD demonstrate proficiency on a task, but they do not deploy these skills in natural circumstances (Dalton et al., 2005; Kinsbourne and Helt, 2011; Mueller and Amaral, 2017; Schultz et al., 2000). Although participants with ASD often display typical social behaviors such as mimicry (McIntosh et al., 2006), imitation (Hamilton, Brindley, & Frith, 2007), and eye contact (Hadjikhani et al., 2004) in experiments where these behaviors are specifically cued, ASD severity consistently correlates with what these individuals *typically* do when observed in *natural* settings (e.g., McIntosh, Reichmann-Decker, Winkielman, & Wilbarger, 2006).

For example, fMRI studies of face perception have shown that individuals with ASD fail to look at the eyes of target stimuli if they are adult strangers (thus failing to significantly activate the fusiform face area (FFA), e.g., Schultz et al., 2000) but *will* look at the eyes (and activate FFA) if the stimuli involve familiar people (Pierce, Haist, Sedaghat, & Courchesne, 2004), or if they are explicitly cued to do so (Zürcher et al., 2014). In addition,

individuals may perform well on a task but use a different process; for instance, individuals with a history of ASD but who have achieved "optimal outcomes," perform as well on tests of language and cognition as their TD peers, but their brain scans suggest that they are using compensatory strategies, activating more areas of the brain to complete these tasks normally (Eigsti et al., 2016). These factors make study design especially delicate in regards to ASD. We must be careful not to conclude that underlying neural systems are "not intact" in individuals with ASD simply because they do not display a specific behavior in specific circumstances.

Since Biological Causes for Autism Are Numerous and Complex, and Narrowly Defined Subgroups Will Also Be Numerous, There Will Be a Need for Very Large Samples, with Data Aggregated across Many Sites to Make Significant Progress

Sophisticated and exploratory big data analyses should be used to produce phenotypic subtypes and correlate them with etiological possibilities, or to identify gene variants and correlate them with phenotypic characteristics (see Campbell, Kohane, & Kong, 2013, for an example of exploratory analysis of biological data). Such attempts at aggregation have been started by the National Database for Autism Research, and by networks of brain imaging researchers (Di Martino et al., 2011). The MSSNG ("Missing") project is a collaboration of Autism Speaks and Google, which hosts the database in the cloud, and is directed by Toronto's Hospital for Sick Children. As of March 2017, they had enrolled and analyzed data from over 5000 families with an autistic member, with data contributed by pre-existing databases such as the Autism Genetic Research Exchange, and had identified many more autism-associated gene variants, some of which were also associated with comorbidities such as epilepsy. The Simons Foundation SPARK study aims to collect genetic and medical information from 50,000 affected individuals and their family members, and is collecting data from multiple autism research centers around the country.

These collaborative databases will no doubt accelerate the pace of autism discoveries, particularly in genetics. However, in our view, *large scale data aggregation must occur in concert with agreed-upon phenotypic measures with a minimum of key features as described above, and not just a positive diagnosis*. As mentioned above, studies of linkage and GWA studies require very large samples and adding longitudinal factors may make these prohibitively expensive. Alternatively, if autism risk genes, or specific CNV's are used to genetically characterize a group which is then intensively phenotyped, such studies may be productive and more feasible.

An example of such a "genotype first" study with more fine-grained subtyping as well as animal modelling is that of Bernier et al. (2014); they focused on mutations in a gene (CHD8) which previous studies had identified in some autistic individuals. They resequenced the gene in approximately 8000 individuals, identifying additional mutations affecting function

(all de novo except one maternally inherited). None of these mutations were found in unaffected siblings or in the general population. Clinical information was available from 15 affected individuals to correlate with the mutations. Features that were present in more than half of the participants, in addition to diagnosed ASD (13/15), were ID or borderline cognition (11/15), macrocephaly (12/15), tall stature (12/14), a characteristic facial feature (8/9), gastrointestinal problems (12/15), and sleep problems (10/15). Finally, the team studied the expression over development of CHD8 (highly conserved across species) and its disruption in zebrafish, which resulted in increased head size and impaired gut motility, both reported in a subset of individuals with autism.

CONCLUSION

There is widespread agreement that ASD represents a complex behavioral endpoint with hundreds of possible biological and environmental vulnerabilities in potentially endless combinations. Truly recognizing the massive heterogeneity within ASD means that *case-control studies* of heterogeneous groups (i.e., those that only require that ASD is present) have limited utility, and, in many cases, are simply muddying the waters. It is likely that even GWS involving thousands of participants are underpowered due to the sheer number of genetic risk factor combinations for autism.

Given this level of complexity, we propose that autism research going forward must adopt several principles that will allow us to move forward. We should *include in ASD samples all individuals who struggle with social communication* (of the type described in DSM-IV and 5 and ICD-10) *regardless of whether RRBs are present*. Genotyping, brain imaging, and environmental contributions should then be studied for *groups who are homogeneous on particular phenotypic variables,* by characterizing each individual on an expansive number of behavioral and medical variables, *with a focus on those most likely to be robust to environmental influence* (e.g., high pain threshold, improvement with fever, poor GI motility, impaired visual saccades, decreasing eye contact in the first year). *The field as a whole must agree upon a minimal standardized set of history and clinical phenotype measures and begin to relate these measures to genetic and environmental factors.*

In "genotype-first" studies, where the participants share a probable genetic contributor, these same variables should be used. Studying these variables *before any intervention* will increase the independence from the environment. Phenotyping measures should also include *longitudinal course, including onset of symptoms and delays, regression and response to treatment.* The fact that sensory deprivation such as blindness or neglect can lead to autism suggests that a lack of social input from any of multiple causes disrupts the sense of self and other and validates the idea of *environmental causes*, which need to be vigorously pursued.

Advances in neuroimaging, genetic/genomic analyses, and exploratory statistical methods (e.g., Campbell et al., 2013) will possibly accelerate the discovery of etiologies and pathophysiology, especially with very large data sets accrued by cross-site aggregation. Even if, in many cases, narrowly defined subgroups fail to link genotype to phenotype, the possibility of linking the two for even one subgroup of ASD is worth pursuing; if we could learn how ASD behaviors result from underlying biology for one subgroup, it may shed light on how behavior and biology are linked in other subgroups. Furthermore, defining phenotypic and biological subgroups will likely lead to better predictions about treatment and prognosis.

We believe that defining subgroups of ASD on behavioral variables that show minimal sensitivity to environmental input is our best chance of connecting ASD symptoms to underlying biology. However, given the disconnect between genotype and phenotype thus far, we acknowledge the possibility that even this type of subtyping will not prove ultimately successful. In this case, the most fruitful way forward is still to begin defining subgroups on phenotypic variables, including symptom emergence, response to medical and behavioral treatments, comorbidities, and longitudinal course. This should allow clinical advancements, with earlier and earlier detection and better and better treatment improving outcomes.

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