Studying child development in genetic models of ASD

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Abstract
This chapter approaches the early development in autism spectrum disorder (ASD) through comparative study of some key monogenic syndromic models of ASD in humans. Using this method, as well as referring to relevant work in idiopathic ASD, we address three complimentary areas: (i) patterns of ASD behavioral phenotype expression across genetic syndromes, as a way of addressing gene-phenotype correlations; (ii) longitudinal developmental trajectories toward autism in early childhood, as a way of addressing developmental specificity; and (iii) experimental intervention trials, for treatment and mechanism discovery. The comparative approach does not highlight striking phenotypic specificity, but early studies were often limited and more methodologically sophisticated recent studies may suggest subtle distinctions. Longitudinal studies are at an early stage but can build on the substantive work on early prodromal development of idiopathic ASD. Translational intervention trials to date have not found candidate treatments and we argue that a new generation of more ambitious experimental mechanism trials is needed. This field now has the opportunity to combine comparative prospective longitudinal developmental studies with in-depth cross-syndrome phenotyping and linked ambitious targeted mechanistic interventions in a way that could be mutually informing and maximize the potential of syndromic models to illuminate the pathophysiology of ASD.

Keywords
Autism spectrum disorder, Syndromic ASD, Fragile X syndrome, Tuberous sclerosis complex, Neurofibromatosis 1, Phelan-McDermid syndrome, Behavioral phenotype, Developmental neuroscience, Experimental intervention trial
PHENOTYPE AND GENOTYPE IN ASD

Since first described by Leo Kanner in 1943, autism has been defined by its behavioral phenotype, a clustering of behavioral manifestations within children’s development emergent from second or third year of life. Core aspects of this phenotype have remained pretty consistent in the nosology since this time, but the most recent DSM 5 (American Psychiatric Association, 2013) includes some important adjustments consequent on subsequent investigation:

1) A collapsing of the original “triad of social impairments” (impairments in social communication, symbolic play, and repetitive and stereotyped behaviors) into two clusters (impairments in reciprocal social behavior and presence of repetitive stereotyped behaviors including sensory sensitivity). The empirical basis of this included new analyses from epidemiological cohorts in the United States and United Kingdom (Frazier et al., 2012; Mandy et al., 2012).

2) The removal of language disorder or delay per se from the autism phenotype. This is based on empirical data that language difficulties are generally orthogonal to autism symptoms; that absence of phrase speech by 3 years (previously necessary criteria for the diagnosis) was not necessary; and that autism could present itself in individuals with high levels of intellectual and structural language function—the fact that this language use was impaired in social contexts (a pragmatic difficulty) is a different matter and is included within current definitions.

In addition, beyond DSM 5, there is increasing recognition of more subtle forms of the phenotype and the notion of an “autistic spectrum,” which can under some circumstances incorporate the notion of “partial features” of autism (Lainhart et al., 2006) and gender-specific profiles.

Despite this evolution in clarification and definition, the core phenotypic characteristics have proved stable over time, and this is true also in ontogeny; there are typical developmental-phase specific manifestations but the core aspects of social impairment and restricted stereotyped behaviors are enduring and remain as usually lifelong characteristics. However, an accepted heterogeneity within the phenotype has led to a search for stratification within autism symptoms, with the hope that this may be illuminating for etiology. Despite the attraction of this notion, efforts empirically to substantiate subgroups have to date been notably unsuccessful. Formal cluster analyses within the phenotype have failed to be illuminating or replicative; and although clinical subtyping had been thought to be intuitive, in fact this has also been difficult to substantiate empirically. In an influential study prior to DSM 5, Lord et al. conducted a large survey across U.S. academic clinical specialist sites with regard to diagnostic practice, finding that reliability of use of subtype diagnostic labels such as “high functioning autism,” “Asperger syndrome,” “PDD,” across sites was low (Lord et al., 2012). Both sets of evidence led, within DSM 5, to official abandonment of these subsidiary clinical terms, reverting instead to a unified category of autism spectrum disorder (ASD).
The ultimate causes of this distinctive ASD phenotype and the neurological and cognitive process underlying it have been an object of fascination from its first identification. At an epidemiological level ASD has a similar prevalence wherever it has been studied globally (Elsabbagh et al., 2012) and research over many decades supports its high heritability. Heritability ($h^2$) estimates have ranged between 0.5 and 0.9, and more recent methodologically sophisticated studies have tended to support earlier higher-end estimates within this range. For instance a recent meta-analysis of twin studies (Tick et al., 2016) found near perfect concordance for MZ twins and 0.67 DZ correlation assuming low prevalence rate and 0.53 DZ correlations assuming high prevalence rate; giving a meta-analytic $h^2$ estimate of 64–91%. Another analysis of a large sample of pooled twin-pair data (Sandin et al., 2017) gave $h^2$ of 0.83, with evidence of the risk of ASD increasing with genetic relatedness. The sibling recurrence rate of ASD is estimated at 18.7% from a recent large prospective longitudinal study of combined data from infant sibling studies ($n = 664$) (Ozonoff et al., 2011) but sampling biases in the cohort may make this figure higher than a population-based recurrence, estimates of which have varied widely from 3% to 19%. Given this high heritability, there have been concerted efforts over more than 2 decades to identify specific causal genetic factors. Association studies suggest striking genetic heterogeneity with over 1000 candidate genes reported as related to ASD (Anney et al., 2012; Buxbaum et al., 2012). More recent studies have implicated an increasing number of rare, highly penetrant genetic variants ranging from chromosomal abnormalities, copy-number variations (CNVs), to single-nucleotide variations (SNV) (Yu et al., 2013). De novo CNVs are observed in 5–10% of screened ASD affected individuals; high-risk genes located in CNVs include NRXN1, 15q11.2-q13 duplications in Prader-Willi syndrome and Angelman syndrome, 16p11.2 deletion, 16p11.2 duplication, and certain X-linked deletions such as PTCHD1-PTCHD1AS (Pinto et al., 2010). One way of clarifying this heterogeneity is to look for patterns of functional gene networks. Pinto et al. (2014) looked at key ASD associated genes disrupted by CNVs and identified biological relationships and common pathways shared among those genes. They found that genes affected by de novo CNVs and SNVs converged on three broad functional networks relating to (i) intracellular signaling, including over and under expression of genes on the PI3-Ras-MAPK signaling pathway, which has an important function in cell cycle regulation; (ii) neuronal development and axon guidance; and (iii) chromatin modification and transcription regulation. In addition, known prenatal perturbations of epigenetic development and autism, for instance with maternal use of sodium valproate (Christensen et al., 2013) have been strongly empirically associated with later autism development in the child, as have mutation risks for instance related to paternal age (Goriely et al., 2013).

The fact that such etiological complexity at the genetic and epigenetic level as associated with such a consistent (albeit heterogeneous) behavioral phenotype, raises significant explanatory challenges. Some talk of multiple “autisms,” although, as above, meaningful stratification of the phenotype has not to date been successful. An alternative assumption is that there must be final common pathways or common adaptations of the neural system, consequent on heterogeneous primary factors that
mediate a common behavioral ASD phenotype. Efforts to delineate these common pathways, whether at a neural system or cognitive level, have met with only partial success. To further complicate the picture there are multiple overlaps between the risk-conferring genetic variants underlying ASD with those of other neurodevelopmental conditions such as schizophrenia, ADHD, and intellectual disability; although such apparent overlaps undoubtedly mask specific developmental differences in genetic expression over time in the different conditions (St Pourcain et al., 2018).

2 MONOGENIC SYNDROMIC ASD AND THE COMPARATIVE METHOD

In this broad context of etiological complexity and uncertain mechanism, there are clear benefits to the study of conditions with a known monogenic origin that expresses the ASD phenotype. The primary genetic etiology is much simpler to understand, knockout animal models can be bred allowing detailed study of neural system processes, alongside the possibility for experimental designs. Minimizing the genetic variance potentially enables systematic exploration of etiological pathways and neural system pathogenesis. The possibility of demonstrating distinct subtypes of ASD phenotype within separate monogenic syndromes might allow clarification of the phenotype and demonstration of specific genotype-behavior linkages. However, there are a number of concomitant challenges to this kind of work which include:

1) Pursuing the study of individual syndromes in isolation, resulting in attribution of potentially spurious “phenotypic specificity” to each.
2) Ignoring the fact that other features of these specific single gene neurodevelopmental models at a biological or neurodevelopmental level may confound interpretation of findings with regard to ASD.
3) Assuming findings from single gene models are representative of or generalizable to idiopathic or common familial autism.
4) The very rarity of each single gene problem in its own right creates difficulties for recruitment, sample size, and interpretation.

The comparative method—studies across different monogenic syndromic models of autism using common methodology—is an investigation strategy that can largely mitigate such difficulties; and this method will be reviewed in this chapter. We will take as exemplars a number of notable single gene conditions that have been associated with ASD; for instance, fragile X syndrome (FXS), neurofibromatosis I (NF1), tuberous sclerosis complex (TSC), and Phelan-McDermid syndrome (PMD). We then use a comparative method across these syndromes to see what illumination can be gained about general principles of child development within ASD and the neural pathophysiology of the condition. Within this comparative method, we tackle three key areas of enquiry in turn:
1. Comparative phenomenology of the behavioral phenotype across different single gene disorders.
2. Longitudinal developmental studies of behavior and neurodevelopment to address the comparative trajectories toward autism in each condition.
3. Experimental interventions, designed to make both treatment discovery and illuminate neurophysiological process.

3 THE ASD BEHAVIORAL PHENOTYPE ACROSS GENETIC MODELS OF ASD

Against the background of extreme genetic heterogeneity in idiopathic autism, the epidemiology of ASD within known monogenic disorders has become of great interest, both practically (since “syndromic autism” of this kind is thought to account for at least 10% of all diagnosed ASD), but also theoretically (because of how it might illuminate the complexity of the idiopathic disorder). Prevalence rates of autism in monogenic disorders are generally much increased over the ASD population base-rates of around 1.2% (Baird et al., 2006; Fombonne, 2018). FXS and TSC are the most well-known single gene disorders related to autism, with prevalence estimates of 24–47% (Bailey et al., 1998; Cornish et al., 2008) in the former, and 16–45% (Jeste et al., 2008; Vignoli et al., 2015; Wong, 2006) in the latter. More recently in NF1, Garg et al. using register-based epidemiological design and gold standard history and diagnostic assessment measures, showed a population prevalence of 25% ASD plus a further 20% with extended or partial phenotype (Garg et al., 2013a). A lower ASD prevalence of 13% and additional 26% sub-clinical symptoms were found within an international sample of 531 NF1 cases including adults and children, but this used largely clinic based sampling and only parent rated screening instruments (Morris et al., 2016). There is much higher prevalence of ASD in PMS, with 87% meeting threshold for autism and 55% for ASD (Richards et al., 2017). Other genetic syndromes where ASD have been reported include Down syndrome (DiGiuseppe et al., 2010), Cornelia de Lange syndrome (Berney et al., 1999; Moss et al., 2008), Smith-Magenis syndrome (Laje et al., 2010), Angelman’s syndrome (Steffenburg et al., 1996), Prader-Willi syndrome (Veltman et al., 2005), William’s syndrome (Klein-Tasman et al., 2007; Tordjman et al., 2012), Smith-Lemli-Opitz syndrome (Sikora et al., 2006), Cri-du-chat syndrome (Moss et al., 2008), and 22q11.2 deletion (Angkustsiri et al., 2014; Vorstman et al., 2006) (Table 1). However, there is considerable variability in the methodology behind these studies and thus confidence in their reported prevalence rates. There are often issues related to small sample size, lack of purposive design, inadequate measures used for autism phenotype identification (rarely standardized) and ascertainment bias, usually because the estimates are made on clinical referral rather than population-based grounds. Since clinical referral is often related to other aspects of the syndromes (for instance physical disorder), this may alter the
### Table 1 Prevalence of Autism Spectrum Disorder in Genetic Syndromes

<table>
<thead>
<tr>
<th>Genetic Syndrome</th>
<th>Prevalence Estimates</th>
<th>References</th>
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<tbody>
<tr>
<td>Fragile X</td>
<td>21–47%</td>
<td>Commonest cause of inherited intellectual impairment</td>
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<td>Hatton et al. (2006): cross-sectional sample of n = 179 children assessed using Childhood Autism Rating Scale (CARS); prevalence estimates of 21%</td>
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<td>Harris et al. (2008): clinical referred sample n = 63 assessed using ADI-R and ADOS; 30% met criteria for ASD</td>
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<td>Tuberous sclerosis</td>
<td>16–45%</td>
<td>Vignoli et al. (2015): clinic referred sample n = 42 assessed using SCQ; 40% met criteria for ASD</td>
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<td>Jeste et al. (2008): longitudinal study of infants with TSC; n = 20 assessed using ADOS; 33% met criteria for autism; and 13% for ASD at 36 months</td>
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<td>Wong (2006): retrospective case registry of TSC over 17-year period. 16% autism prevalence based on case notes</td>
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<td>Neurofibromatosis type 1</td>
<td>13–40%</td>
<td>Garg et al. (2013a, b): population-based ascertainment; two-stage sampling strategy; and n = 47 seen for in-depth phenotyping using ADI-R and ADOS; 25% prevalence of ASD and further 20% with partial phenotype</td>
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<td></td>
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<td>Morris et al. (2016): multisite retrospective study in children and adults with NF1 assessed using SRS questionnaire; ASD prevalence 13% and 26% partial phenotype</td>
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<td>Angelman’s syndrome</td>
<td>42–100%</td>
<td>Associated with severe intellectual impairment</td>
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<td>Peters et al. (2004): clinic referred study of n = 19 using ADOS</td>
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<td></td>
<td>Steffenburg et al. (1996): epidemiological study of children with intellectual impairment and epilepsy. Four children who met criteria for Angelman’s also met ASD criteria</td>
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<tr>
<td>Smith-Magenis syndrome</td>
<td>~90%</td>
<td>Laje et al. (2010): clinic referred sample n = 26 assessed using standardized questionnaire measure only</td>
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<tr>
<td>Prader-Willi syndrome</td>
<td>18–38%</td>
<td>Mild-moderate intellectual impairment</td>
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<td></td>
<td></td>
<td>Prevalence estimates drawn from a systematic review of nine studies of Prader-Willi syndrome by Veltman et al. (2005)</td>
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<tr>
<td>Down’s syndrome</td>
<td>5.1%</td>
<td>DiGuiseppi et al. (2010): population-based study using questionnaire measures only</td>
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<td></td>
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<td>Prevalence increased with greater cognitive impairment</td>
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representativeness of the estimates given. Inadequate phenotypic assessment similarly allows for the possibility of “diagnostic overshadowing,” where other known aspects of disorder mask the autism presentations (Garg et al., 2013b). As the field proceeds it will be important to re-assess syndrome-specific prevalence estimates with increasingly robust methodology.
In recent years, there has been interest in the possibility that particular genetic disorders give rise to characteristic patterns of ASD symptomatology. This is felt to be a potentially useful route of investigation into a possible genetic based stratification of the autism phenotype. Based on this hypothesis, an emerging body of work has investigated ASD symptom profiles across genetic syndromes in order to make inferences about the neurobiology, and as a means of stratifying patients, who may benefit from treatments targeted at specific pathways (Bruining et al., 2014). For example, the ASD symptom profile in toddlers with TSC is identical to non-syndromic autism on aspects of social communication and restrictive, repetitive behaviors (Jeste et al., 2016), but in FXS the symptom profile is marked by higher rates of restrictive repetitive behaviors (RRBs), lower rates of social impairment and less compulsive behavior as compared to non-syndromic ASD (Hall et al., 2010; Rogers et al., 2001; Wolff et al., 2012). In NF1, a study of 36 children with NF1 and ASD found that as compared to idiopathic ASD, the NF1 showed overall similarity broadly but with better eye contact and few restricted repetitive behaviors (Garg et al., 2015). A study comparing the behavioral phenotype of ASD in Cornelia de Lange (CdLS) syndrome with idiopathic ASD showed that the CdLS ASD group showed more eye contact and gestures, less repetitive behaviors but higher levels of anxiety (Moss et al., 2012). Fine-grained analysis of the ASD symptom profile in PMS suggests similarities with non-syndromic ASD in social communication impairments but fewer restricted repetitive behaviors (RRBs; Richards et al., 2017). Bruining et al. undertook a more ambitious cross-syndrome comparison approach through analyzing formal diagnostic ADI-R symptom profiles in six genetic disorders associated with ASD and using a machine-learning approach to analytic clustering. They found some behavioral specificity between different genotypes, with 63% chance of being classified correctly (Bruining et al., 2014). A larger study from the same research group of 601 participants with 8 different genetic syndromes (TSC, FXS, NF1, Down syndrome, 22q11.2, Prader-Willi, Klinefelter syndrome, and Supernumerary marker chromosome) used ADI-R symptom profiles and found machine-learning methods were able to classify each of the eight disorders 400% above chance level. Further, the study found that the phenotypic profile of most disorders showed greater similarities to TSC profile than to the FXS profile, a fact potentially linked by the authors to the complementary pathophysiology of these two disorders (Malki et al., 2018).

Syndromic specificity is also found in relation to the ASD “male bias”; well established in idiopathic ASD, with a 4.5:1 male:female (Idring et al., 2012). The mechanism of bias is still debated and probably includes a component of ascertainment bias; but a recent study also suggests naturally occurring sexually dimorphic processes, with genes expressed at higher levels in males significantly enriched for genes up-regulated in post-mortem autistic brains (Werling et al., 2016). In syndromic ASD, the male bias is evident but attenuated. For instance, in a study of 194 children with NF1, Garg et al. found a male bias in ASD prevalence of 2.68:1 male:female. Males with NF1 showed greater social communication impairments than females but no differences were found on the RRB domain (Garg et al., 2016).
Preliminary findings have been reported in other syndromic disorders including the other Rasopathies such as Noonan syndrome, Costello syndrome, and Cardio-facio-cutaneous (CFC) syndrome (Adviento et al., 2014). For instance, a study investigating microdeletions of SHANK1 in a four-generation family found that male carriers met the clinical criteria for ASD, whereas female carriers with the same mutation showed evidence of anxiety but not ASD (Sato et al., 2012). Similarly, ASD associated with Down’s syndrome shows significantly reduced penetrance in females with the disorder (Warner et al., 2014).

3.1 SUMMARY

Epidemiological estimates of autism prevalence are well established in the international literature (Elsabbagh et al., 2012), although varying estimates have been subject to ascertainment effects (Fombonne, 2018). We have reviewed the prevalence estimates across syndromic autism, and the search for behavioral phenotypic specificity between syndromes, as a strategy for genetic stratification of the disorder. There are significant methodological issues undermining strong inferences from many studies so far, although methodologies are improving. The essential messages from work to date are that in general, monogenic disorders have greatly increased prevalence of autism over the polygenic idiopathic form, and that studies with more rigorous methods tend not to find substantive overall disorder-specific phenotypic profiles. However there is emerging data using more fine grain approaches on larger pooled samples suggesting that some informative disorder-specific patterns may be discoverable in the future.

4 DEVELOPMENTAL TRAJECTORY OF ASD ACROSS GENETIC SYNDROMES

The last decade has been marked by an emerging body of work into prospective investigation of developmental trajectories of infants at-risk of ASD. Such studies have been inspired by a neuro-constructivist view of development, which argues against using adult phenotypic profiles to draw assumptions about early development. The neuro-constructivist framework considers a developmental perspective as central to understanding the origins of neurodevelopmental disorders, since development is fundamentally a dynamic process with the infant brain structuring itself over the course of ontogeny (Karmiloff-Smith, 1998). Mapping the developmental trajectory and understanding how the atypical development unfolds in terms of possible cascading developmental effects of early neurodevelopmental perturbation is therefore a central strategy for elucidating the pathological mechanisms leading to ASD symptom development in children (Farran and Karmiloff-Smith, 2012; Johnson et al., 2015a).

Most of this developmental trajectory work has focused on infant siblings of children with familial ASD (referred to as autism “at-risk” infants in the text), in whom
the pooled recurrence risk of ASD is about 18% (Ozonoff et al., 2011). Evidence from these studies suggests cognitive, motor, and behavioral markers in the first year of life that are associated with later autism (Johnson et al., 2015a). However, the genetic heterogeneity of familial ASD makes it difficult to draw conclusions about causal risk and therefore limits translational insights to developing new treatment strategies. Studying the developmental trajectory of ASD in monogenetic disorders provides an alternative strategy where the neurobiology is better understood through the use of animal models, allowing inferences about pathological mechanisms associated with ASD. Some disorders such as William’s syndrome, TSC, or NF1 can be diagnosed early in development or even prenatally, facilitating prospective investigation of emerging developmental trajectories. Such studies of syndromic autism are still in their infancy but have started to shed light on antecedent markers of ASD development. Here we review this emerging literature, organized into domains of brain structure, brain function, cognitive, and language function and behavior.

### 4.1 STRUCTURAL AND FUNCTIONAL BRAIN ABNORMALITIES

One of the early observations in the study of infant development prodromal to idiopathic ASD was the emergence, from late in the first year, of an overall pattern of enlarged head circumference compared to typically developing (TD) controls (Courchesne et al., 2003). Despite some contradictory data, this general finding has stood the test of time, although with the addition that both macrocephaly and microcephaly are now found as over-represented in ASD (Johnson et al., 2015a). Within syndromic ASD, macrocephaly is associated with FXS, PTEN (Phosphate and Tensin Homolog), PMS, NF1, and other Rasopathies such as Noonan syndrome. Microcephaly on the other hand has been reported in William’s syndrome, Rett’s syndrome, and Down’s syndrome. In Rett’s syndrome, there is deceleration of head growth with microcephaly evident by the second year of life (Jellinger, 2003).

Although the link between extreme head sizes and ASD is now well established, how macrocephaly and microcephaly contribute to autism risk remains unclear but perhaps relates to defects in neural progenitor proliferation and deficits in synaptic over or under-pruning (McCaffery and Deutsch, 2005; Nebel et al., 2015). A recent longitudinal neuroimaging study of 106 high-risk infants found that hyper-expansion of the cortical surface area between 6 and 12 months of age precedes brain volume overgrowth observed between 12 and 24 months in infants who are later diagnosed with autism at 24 months (Hazlett et al., 2017); these findings are suggestive of the early brain changes preceding the emergence of autistic symptomatology.

A particular disease feature of NF1 is the presence of high intensity focal areas observed on T2-weighted brain MRI in up to 90% of children. These “T2 hyper intensities” (T2H) appear most commonly in the basal ganglia, cerebellum, thalamus, brain stem, and sub-cortical white matter. The relationship of the size, number, or location of the T2H with ASD is not yet known but the T2H in the thalamus or thalamo-striatal region is associated with more severe cognitive impairment
Similarly, cortical tubers are pathognomonic of TSC but there is no consistent correlation between the number and location of tubers and ASD expression (Numis et al., 2011).

Emerging evidence suggests that functional neuroimaging in infancy may be used to predict which infants will receive an autism diagnosis. A prospective neuroimaging study of 59 six-month-old infants, functional connectivity MRI correctly identified which individual children would receive a research diagnosis of ASD at 24 months. Furthermore, a machine-learning algorithm applied at 6 months reported a 100% positive predictive value of ASD diagnosis at 24 months (Greenwood et al., 2005; Pride et al., 2010). Aberrant development of white matter pathways precedes the manifestation of autistic symptoms in the first year of life in familial autism (Chabernaud et al., 2009, Hyman et al., 2007). Similar findings are emerging in syndromic ASD. For instance, a recent longitudinal diffusion tensor imaging (DTI) study of 22 infants with FXS, characterizing the development of white matter at 6, 12, and 24 months, demonstrated diminished white matter structural connectivity as compared to typical controls. Furthermore, these alterations in white matter structure were well established and relatively stable from 6 months of age (Numis et al., 2011). Similarly, a cross-sectional voxel-based morphometric study of toddlers with FXS (n = 52) and familial ASD (n = 63) found aberrant frontal/temporal gray and white matter changes both groups as compared to typical controls; but with direction of change in FXS opposite to familial ASD (Clements-Stephens et al., 2008). Changes in structural connectivity have also been reported in tuberous sclerosis; a case-control study of 20 individuals with TSC and 20 controls (age 3–24 years) showed significantly reduced inter-hemispheric connectivity and increased network clustering within hemispheres (Violante et al., 2013).

A growing body of work has used electroencephalography (EEG) as a low cost, relatively easy to use tool, to investigate atypical brain development in the infancy period. EEG studies in high-risk infants are suggestive of altered patterns of brain connectivity (O’Reilly et al., 2017) and changes in spectral power (Levin et al., 2017). Infant EEG activity is increasingly being considered as a biomarker for autism diagnosis. In a longitudinal study of 99 high-risk infants and 89 low-risk controls, Bosl et al. collected serial EEG measurement from 3 to 36 months. Using a data-driven approach, the study found that nonlinear analysis of EEG signals extracted at 3 months of age correlated highly with ASD diagnostic outcome at 36 months (Bosl et al., 2018). Event-related potential (ERP) studies in familial autism suggest both auditory and visual processing difficulties evident from the infancy period. Auditory processing paradigms are suggestive of aberrant neural circuits for processing of stimuli such as novelty detection, discrimination of stimulus features, and memory of previously presented stimulus in ASD (Jeste and Nelson, 2009). In FXS, auditory processing has shown to be abnormal both in humans and knockout mouse models; studies report reduced habituation to repeating trains of single frequency tones and enlarged amplitude of N1 component of ERP which is reflective of temporal lobe activity (Rotschafer and Razak, 2014). Schneider et al. used auditory ERP as an outcome measure in a small RCT (n = 12) of minocycline in children with FXS;
3 months of minocycline treatment was associated with reduced N1 amplitude and habituation to auditory stimuli (Schneider et al., 2013). Visual ERP studies consistently demonstrate atypical face processing in ASD (Elsabbagh et al., 2013b). Guy et al. examined the neural correlates of face processing in three groups of infants aged 12 months; \( N = 21 \) infants at high risk of familial autism, 15 infants with FXS, and 21 low-risk controls. The study found differences on the Nc component of the ERP between the three groups; however, the results of the high-risk infants and FXS were in the opposite direction suggesting that despite shared ASD behavioral outcomes, the two groups exhibit distinct neural patterns of attention and face processing in the infancy period (Guy et al., 2018). A study investigating face processing in young children (\( n = 19 \) TSC under 4 years) found longer N290 latency as compared to controls (Jeste et al., 2013). Interestingly the longest N290 latency was seen in children with autism and TSC.

### 4.2 NEUROCOGNITIVE MARKERS

Eye gaze deficits, known to be a hallmark of autism, have proved to be a useful paradigm to investigate atypicalities in the infancy period using eye tracking technology. Prospective longitudinal studies of high-risk infants suggest that infants later diagnosed with ASD show a decline in eye fixation within the first 2–6 months of life (Spurling Jeste et al., 2014) and reduced gaze fixation to people at 6 months (Chawarska et al., 2013). Other studies demonstrate disruption of attentional control in infancy/toddler period; toddlers who later develop ASD show longer habituation to static faces between 18 and 30 months (Webb et al., 2010) and difficulties with attentional disengagement (Elsabbagh et al., 2013a). Within syndromic autism, this approach is still in its infancy and therefore, it is unclear whether similar gaze processing impairments are present in the infancy period. Gaze avoidance is a hallmark feature of FXS; a study using eye tracking/pupilometry in an adolescent sample demonstrated significant differences in gaze patterns and increased pupillary reactivity in FXS as compared to controls (Farzin et al., 2009). A longitudinal study of FXS infants showed prolonged look durations at 12 months as compared to controls. Interestingly, look durations and latency to disengage attention in this study were correlated with autistic symptom severity but not mental age (Roberts et al., 2012).

Executive function and attentional impairments are well studied in familial ASD, specifically slow orientation and impairments in disengaging attention (Landry and Parker, 2013). Attention deficits are highly prevalent in most syndromic autism including FXS, NF1 (Garg et al., 2013b), and TSC (D’Agati et al., 2009). Attentional deficits in FXS are reported by 84% of parents as a major concern (Bailey et al., 2009). These deficits emerge early in development, beginning in toddlerhood and are a major predictor of longer-term cognitive outcomes in FXS (Cornish et al., 2012). Similarly in NF1, deficits in executive function and attention are commonly reported (Hyman et al., 2005; Pride et al., 2010). A detailed investigation into five domains of executive function (inhibition, cognitive flexibility, generativity, working memory, and planning) using an extended test battery in 42 NF1 children...
compared to normative and autistic controls found that executive deficits are a core feature of NF1 rather than a secondary effect of lower IQ or ASD symptoms (Plasschaert et al., 2016).

Longitudinal studies in FXS in the preschool period suggest that global developmental delay is common, particularly delays in fine motor and expressive language development. The severity of ASD symptomatology inversely correlates with development (Roberts et al., 2009). Similarly, global developmental delay is common in infants with TSC; infants later diagnosed with ASD show greater cognitive impairment by 12 months and a significant decline in non-verbal IQ between 12 and 36 months as compared to the non-ASD TS group (Spurling Jeste et al., 2014). A longitudinal study of infants with NF1 pilot study of 10 infants with NF1 suggested expressive and receptive language delay at 10 months both on parent report and Mullen’s Scale of Early Learning (Kolesnik et al., 2017); ASD outcomes from this group however are not yet reported.

Language deficits are common across both syndromic and non-syndromic ASD. In FXS, language delays are part of the global developmental delay but visual attention at 12 months predicts language development during the second year of life (Kover et al., 2015). In NF1, at least a third of children present with functional language impairments which impact on social communication; these may include deficits in language structure, semantic language, receptive and expressive language, verbal working memory, and comprehension (Brei et al., 2014; Dilts et al., 1996; Thompson et al., 2010). A cross-sectional case-control study of 39 NF1 toddlers aged 21–30 months suggested delayed language development, reduced productive vocabulary, and sentence complexity in almost three quarters of the sample on parental report (Lorenzo et al., 2011).

4.3 MOTOR FUNCTION

Delays in achievement of motor milestones have been observed across different disorders; for instance in infants of mothers with schizophrenia (Henriksson and McNeil, 2004) and in infants who are later diagnosed with ADHD and ASD. In the first year of life, ASD symptoms are difficult to detect, however delays in motor milestones are more likely to be present. Six-month-old infants later diagnosed with ASD are likely to show poor head control (Flanagan et al., 2012). At-risk infants as a group show poor postural control (Nickel et al., 2013) and more limited and grasping skills (Libertus et al., 2014). Baranek et al. used retrospective video analyses to compare sensory-motor patterns in infants with FXS and autism and found unusual motor patterns such as posturing and repetitive leg movements as distinguishing FXS features (Baranek et al., 2005). Children with FXS who also have ASD have lower fine motor skills than those without ASD (Zingerevich et al., 2009). A cross-sectional study of 69 children with NF1 (aged 4–16 years) found that over 60% were clinically impaired on detailed assessment of motor function. In addition, motor impairments were associated with measures of ASD, ADHD, and externalizing disorders but not with age, IQ, scoliosis, hypotonia, or hypermobility (Rietman et al., 2017).
4.4 BEHAVIOR DEVELOPMENT

Behavior phenotype symptomatology of ASD is not evident in the first 6 months of the infancy period, but emerges during the latter part of the first and second years. Prospective longitudinal studies report decline in eye contact, social smiling, and examiner rated social responsiveness from 6 months onward and continue to develop for several years (Bryson et al., 2007; Ozonoff et al., 2010). Infants at-risk of familial autism also differs from controls in emerging language abilities and repetitive behaviors. The emerging behavioral phenotype of FXS has been described in some studies has identical to non-syndromic autism (Rogers et al., 2001). Individuals with FXS and ASD display greater social communication impairments and higher levels of repetitive and challenging behaviors (Smith et al., 2012) as compared to individuals with FXS only. Self-injurious behaviors are common in both FXS (Symons et al., 2003) and TSC (Eden et al., 2014), but in the context of significant learning impairments.

Sleep disturbance is a commonly reported behavioral comorbidity associated with neurodevelopmental disorder but has not yet been investigated in detail by research studies. A large cross-sectional study of 129 NF1 children and their unaffected siblings found significantly more disturbances in initiating and maintaining sleep, arousal, sleep-wake transitions, and hyperhidrosis in the NF1 group. In this study, the sleep problems did not correlate with IQ or ADHD (Licis et al., 2013). Similarly a large national survey of FXS children found that a third of parents reported sleep problems particularly with falling asleep and frequent night awakenings (Kronk et al., 2010). A recent study used sleep EEG in 28 children (aged 4–11 years) with Angelman syndrome and found that the sleep EEGs contained fewer sleep spindles of shorted duration as compared to controls (Den Bakker et al., 2018).

4.5 SUMMARY

The studies across syndromes are suggestive of atypical brain early in development preceding the emergence of ASD behavioral symptoms. Understanding infant and child development in this way are critical if we are to identify neural “markers” of ASD in the prodromal phase. Screening ASD solely based on behavioral symptomatology is likely to miss a critical developmental window for early intervention approaches, which may likely alter the trajectory of development (Bosl et al., 2018).

It is also clear that these markers are not disorder specific but are common across risk groups. This gives then some emerging clue as to signals of final common pathways of effect consequent on differing primary genetic variation. What we have not yet achieved is an indication of the mechanism through which the known individual primary pathologies at a cell or protein expression level combine into a common pathway. Much focus is currently at the level of synaptic protein expression, but this is hard to study in human subjects. Additionally, how may these subtle early domains of impairment interact dynamically during development to result in an ASD behavioral profile? Another aspect that needs detailed examination is the high level of
comorbidity of ASD and ADHD across the genetics syndromes. Here, Johnson proposes that ASD may represent the end result of early brain adaptation rather than the direct consequence of ongoing neural pathology (Johnson et al., 2015b). This view suggests the possibility that the primary neural impairments that lead to ASD may be transitory and may be difficult to detect after a critical developmental period.

5 EXPERIMENTAL INTERVENTION

We have seen that, in common idiopathic autism, a high level of genetic heterogeneity is the rule, with association studies showing the influence of up to 1000 genetic variants of small effect in different combinations. We have also noted heterogeneity in the behavioral phenotype. These two kinds of heterogeneity lead to the appealing (if over-literal) notion that the behavioral phenotype should be amenable to genetic stratification. Such an idea proves simplistic. Genetic variants associated with autism have been mapped into functional networks relating to intracellular signaling and neural development (Pinto et al., 2014) but there is no work yet in humans that links such proximal functional domains to specific cognitive or behavioral outcomes. We have seen that emerging data from prospective neurodevelopmental studies of the prodrome of autism indicate a similarly heterogeneous pattern of natural history neurodevelopmental markers anticipating later autism development (Johnson et al., 2015a; Szatmari et al., 2016).

The advantages of monogenic models mentioned above are particularly pertinent to the area experimental intervention. The purposes of well-designed experimental interventions are: (1) as a method of treatment discovery and (2) as a method of illuminating basic developmental processes. These two aims are complimentary and can be pursued together within a single trial, if appropriately designed. To allow convincing inferences, such a trial in the developmental neuroscience context needs some or all of a number of characteristics:

i) That the intervention is theoretically targeted at key aspects of a hypothesized mechanism or biomarker.

ii) That the trial contains an embedded mechanism study to test this proximal intervention effect.

iii) That it be adequately powered against a primary outcome that appropriately captures the autism phenotype.

iv) That it contains repeated measures of developmental outcomes to allow testing of downstream effects.

The work to date reviewed here rarely meets these criteria. Most trials in this area in humans to date are exploratory, small-scale, and underpowered, often with insufficient characterization and testing of putative mechanisms, and rarely containing repeated measures developmental outcomes. They have characteristically translated hypotheses based on findings from the animal literature directly into human studies, and focused efforts on treatment discovery while implicitly assuming that the same
mechanisms are at play and in the same way in the human as well as animal contexts. This clearly may or may not be the case. Nevertheless, this kind of work is developing fast in ambition, and a great advantage of the models studied and reviewed below (FXS, TSC, NF1, and to lesser extent Rett syndrome and PMS) is that they have interestingly specific and yet importantly convergent pathways in relation to neural system pathophysiology, with various aspect of synaptic function and synaptic protein expression common between them. This makes the comparative method potentially very informative about heterogeneity as well as final common pathways to the phenotype (Table 2).

5.1 FRAGILE X DISORDER (FXS): GABA-GLUTAMATE BALANCE

FXS is the syndrome by far the most studied to date within experimental treatment trials in humans. A trinucleotide (CGG) repeat expansion on the X chromosome of variable length effects the FMR1 gene function in a dose-response fashion, leading to reduction in production of the fragile X mental retardation protein (FMRP) (Fu et al., 1991; Pieretti et al., 1991). Studies in animal models suggest that the pathophysiology of the protein change leads to an overabundance of metabotropic glutamate receptor (MGLuR1/5) controlled synaptic proteins, which have important roles within synaptic plasticity, learning and memory, particularly at excitatory glutamnergic synapses. In the influential MGLuR theory of FXS, exaggerated MGLuR signaling induces enhanced hippocampal long-term suppression and results in the behavioral phenotype (Bear et al., 2004). Therapeutic excitement followed the finding that this system was much more malleable than might have been anticipated, for instance multiple FX phenotypes are corrected in the Fmr1-KO mouse by genetic reduction of mGluR5 protein production (Dolen et al., 2007). Experimental intervention studies targeting the MGLuR receptor function therefore fulfill the theoretical criteria of targeting core aspect of pathophysiology; they created much anticipation regarding the potential efficacy of in human translational treatment studies (Bhakar et al., 2012).

Two approaches have been used to target mGluR compensatory reduction in humans. An initial small \((n = 12)\) single dose single arm open-label study of Fenobam, an mGluR receptor antagonist, indicated some evidence of reduction in anxiety and hyperactivity measured on lab tasks in humans (Berry-Kravis et al., 2009). A larger study was of Mavoglurant, a non-competitive mGluR5 inhibitor, which had shown promise in FXS mouse models in improving dendritic spine morphology and behavioral phenotype. Mavoglurant was trialed in a 12-week multicenter phase 2b RCT of 175 adults and 139 adolescents, and disappointingly showed no significant treatment effects between Mavoglurant and placebo on primary behavioral endpoints (Berry-Kravis et al., 2016). In relation to the criteria noted above, however, it is notable that in trials like this, there is no direct measure of the putative effect of treatment on the target pathophysiology (viz mGluR signaling). This and similar kinds of design therefore raises the possibility of type II error, since it may be that the treatment is affecting aspects of core pathophysiology but that these did not within the study context read through into endpoint behavioral phenotype change. A concern is that this may lead to
<table>
<thead>
<tr>
<th>Target</th>
<th>Autism Group</th>
<th>Primary Outcome Measures</th>
<th>Intervention Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FXS</strong></td>
<td><strong>Glutamate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fenobam (mGluR antagonist) Single dose, single arm, open-label study, (n = 12) (Berry-Kravis et al., 2009)</td>
<td>FXS</td>
<td>Prepulse inhibition and continuous performance test to measure sensory gating and attention</td>
<td>Reduction in anxiety and hyperactivity</td>
</tr>
<tr>
<td>Mavoglurant (non-competitive mGluR inhibitor). Phase 2b multicenter RCT, (n = 175) adults and 139 adolescents stratified by methylation status (Berry-Kravis et al., 2016)</td>
<td>FXS</td>
<td>ABC</td>
<td>No effect on primary or secondary outcome measures</td>
</tr>
<tr>
<td>Memantine (NMDA receptor antagonist) Open-label study, (n = 6) (Erickson et al., 2009)</td>
<td>FXS</td>
<td>CGI</td>
<td>Improvements on CGI but no improvements on symptom specific rating scales</td>
</tr>
<tr>
<td><strong>GABA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arbaclofen (GABA agonist) (Berry-Kravis et al., 2017), (n = 125) adolescents/adults and 172 children. Phase 3 RCT</td>
<td>FXS</td>
<td>Social avoidance subscale of FXS specific ABC</td>
<td>No effect on primary outcome measure. In child study, significant effect in treatment group noted in irritability subscale of ABC and parenting stress index</td>
</tr>
<tr>
<td>Acamprosate (GABA agonist and mGluR antagonist) (Erickson et al., 2010a), (n = 12) Open-label study</td>
<td>FXS</td>
<td>CGI</td>
<td>Some improvement in social behavior, inattention, and hyperactivity</td>
</tr>
<tr>
<td>Minocycline (Leigh et al., 2013), (n = 55) Double-blind crossover trial</td>
<td>FXS</td>
<td>CGI, visual analog scale for rating behavioral symptoms</td>
<td>Treatment effect noted on clinician rated measures but not on behavioral measures</td>
</tr>
<tr>
<td>Methylphenidate (dopamine re-uptake inhibitor) (Hagerman et al., 1988), (n = 15), double-blind crossover design</td>
<td>FXS</td>
<td>Parent and teacher behavior checklists</td>
<td>Improvement noted in attention, social skills, and hyperactivity on Methylphenidate</td>
</tr>
</tbody>
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*Continued*
Table 2  Experimental Intervention Studies—cont’d

<table>
<thead>
<tr>
<th>Target</th>
<th>Autism Group</th>
<th>Primary Outcome Measures</th>
<th>Intervention Effects</th>
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<tr>
<td>FXS</td>
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<td>Improvement in irritability</td>
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<td>Glutamate</td>
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<tr>
<td>Aripiprazole (partial D2 agonist) (Erickson et al., 2010b), n = 12, open-label study</td>
<td>FXS</td>
<td>CGI</td>
<td>Improvement in irritability</td>
</tr>
<tr>
<td>NF1</td>
<td></td>
<td>CBCL, WISC</td>
<td>No effect on the primary outcome measures</td>
</tr>
<tr>
<td>Simvastatin (down-regulates Ras/MAPK pathway activity) (Van Der Vaart et al., 2013), n = 84, double-blind RCT</td>
<td>NF1</td>
<td>CBCL, WISC</td>
<td>No effect on the primary outcome measures</td>
</tr>
<tr>
<td>12 weeks simvastatin vs placebo in young children (Stivaros et al., 2018), n = 30, 4.5–9.5 years, triple-blind RCT</td>
<td>NF1-autism</td>
<td>Peripheral pMAPK, MRS, ASL, ADC, rsfMRI, ABC, CGI</td>
<td>Simvastatin effects in brain areas previously associated with NF1 pathophysiology and the social brain network</td>
</tr>
<tr>
<td>Lovastatin (down-regulates Ras/MAPK pathway activity) (Payne et al., 2016), n = 146, double-blind RCT</td>
<td>NF1</td>
<td>Paired associate learning task</td>
<td>No effect</td>
</tr>
<tr>
<td>TSC</td>
<td></td>
<td>Trial evaluated reduction in seizure frequency, quality of life measures</td>
<td>Reduction in seizure frequency and improvement in ASD-related symptoms</td>
</tr>
<tr>
<td>Everolimus (mTOR inhibitor) (Krueger et al., 2013), n = 20 open-label trial</td>
<td>TSC</td>
<td>Trial evaluated reduction in seizure frequency, quality of life measures</td>
<td>Reduction in seizure frequency and improvement in ASD-related symptoms</td>
</tr>
<tr>
<td>Growth and Neurotrophic Factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin-like growth factor 1 (IGF-1) (Kolevzon et al., 2014), n = 9, double-blind crossover study design</td>
<td>Phelan-McDermid syndrome</td>
<td>ABC</td>
<td>Significant improvement in social impairments and restricted behaviors associated with treatment</td>
</tr>
<tr>
<td>IGF-1 (Khwaja et al., 2014), n = 12, single arm open label</td>
<td>Rett syndrome</td>
<td>Cardiorespiratory measures, EEG, and behavioral outcomes CGI and parental ratings of symptoms</td>
<td>Improvement in apnea, anxiety, and mood</td>
</tr>
</tbody>
</table>

Summary of key Biological Mechanism targets and Intervention Outcomes (see text).
FXS: fragile X; NF1: neurofibromatosis type 1; TSC: tuberous sclerosis complex, ABC: aberrant behavior questionnaire; CARS: Childhood Autism Rating Scale; CGI: clinical global improvement; CBCL: child behavior checklist; SRS: Social Responsiveness Scale; WISC: Wechsler intelligence scale for children; pMAPK: phosphorylated MAPKinase assay; MRS: magnetic resonance imaging spectroscopy; ASL: arterial spin labeling; ADC: apparent diffusion; rsfMRI: resting-state functional MRI.
abandonment of further testing of agents such as Mavoglurant, when in fact this may have more potential if mechanism studies in humans were done.

GABA/glutamate balance seems to be of significant importance within autism neurodevelopment. The mGluR theory of FXS postulates a deficiency in GABA receptor expression and GABA-mediated inhibition, alongside the glutamate enhancement. A complimentary approach therefore has been the testing of GABA agonists. The GABAb agonist Arbaclofen has been tested in a number of studies and has given perhaps the most promising treatment signal in this area. A double-blind RCT of Arbaclofen in 63 FXS patients aged 6–40 years found significant treatment effects in social behavior and function (Berry-Kravis et al., 2012). A more recent double-blind placebo-controlled RCT of Arbaclofen in 125 adolescents/adults aged 12–50 years and 172 children aged 5–11 years by contrast found no significant overall effect in either group on the primary outcome of social avoidance, however an incidental finding of reduced behavioral irritability in the child subsample suggested a possibility of moderated treatment effect by age (Berry-Kravis et al., 2017). A small study testing Acamprosate, a compound which is both a GABAa receptor agonist and a MGlurR5 receptor antagonist, in children aged 6–17 years, did suggest some improvement in social behaviors, inattention, and hyperactivity (Erickson et al., 2010a), although the sample size ($n=12$) was too small for any robust inference. Other studies targeting the GABA/glutamate system in FXS are underway, but it is clear that as yet no solid signal of treatment effect has been found. Again it is worth noting that none of these studies have taken a mechanistic approach nor have they tested downstream developmental effects over time. The GABA system is a target for disorders beyond FXS and Arbaclofen is currently being further tested in familial autism.

FXS also shows deficits in dopaminergic functioning and a double-blind crossover trial of Methylphenidate in 15 children with FXS has demonstrated response to the dopamine re-uptake inhibitor results date with evidence of improvements in social skills hyperactivity and attention (Hagerman et al., 1988). This kind of effect with Methylphenidate is of course seen across children with many other disorders such as non-syndromic ADHD and so is not specific to FXS.

The basic biology of FXS implicates mGluR5-mediated increase in synaptic protein expression; but it also seems likely that effects of this change are further mediated through alterations in post-synaptic cell signaling pathways, specifically the Ras-ERK and mTOR pathways. This biology emphasizes the complexity of cell signaling pathways, but also links FXS biology to two other monogenic disorders that also highly express ASD; NF1 and TSC, and which provide other specific treatment targets linked to cell signaling.

### 5.2 NF1: THE MAPK/ERK CELL SIGNALING PATHWAY

Mutations within the NF1 gene region cause reduction in the protein neurofibromin, one of whose functions is to regulate the RasMapKinase intracellular signaling pathway. NF1 is thus associated with Ras pathway overactivity, which in animal
knockout models leads to altered GABA function, impairments in synaptic protein expression, plasticity and long-term potentiation as well as cognitive, social and other behavioral impairments. In humans, mutations in NF1 and a number of other genes directly affecting the Ras pathway (so-called “rasopathies”) have been shown to be associated with ASD in epidemiological studies (see above). It also happens that dysregulation of the Ras pathway is prominent in the functional networks of familial idiopathic autism-related genes (Pinto et al., 2014). From animal studies, statins downregulate the Ras pathway through inhibiting farnyslization and produce a cascade of effects that rescues the cognitive and behavioral phenotype (Li et al., 2005), a result that is also achieved through the alternative strategy of co-deletion (Molosh et al., 2014). This latter study also showed detailed and localized mechanism evidence, with effects on synaptic protein expression in the amygdala, which gives important insight into what are likely to be frequent regional effects in the impact of interventions on the brain. This animal work is thus detailed, elegant, and has been replicated, similar in its own way to the parallel work in FXS, and suggests a robust pathophysiological mechanism in NF1 that can be targeted with intervention, as well as providing proof of principle that neural system alteration can result in behavior phenotype change.

As in the case of FXS, however, translation of this work into human subjects has not shown such clear effects. Initial smaller studies were promising, for instance an observational study of lovastatin in 23 children aged 10–17 years (Acosta et al., 2011) and in a 14-week RCT of lovastatin in 44 of 10–15-year-olds (Bearden et al., 2016). Four days of high dose lovastatin improved synaptic plasticity and phasic alertness measured with transcranial magnetic stimulation in a case-control study of 11 adults with NF1 (Mainberger et al., 2013). However larger statin trials in human subjects have shown less effect on cognitive or behavioral outcomes. A 12-week double-blind placebo-controlled RCT of simvastatin in 62 children with NF1 aged 8–16 years found no group differences on visual cognitive tasks (Krab et al., 2008) and another RCT of simvastatin on 84 children aged 8–16 years found no improvements in cognitive deficits or parent reported behaviors (Van Der Vaart et al., 2013). A 16-week RCT of lovastatin in 146 of 8–15-year-olds with NF1 found no improvements in the cognitive outcome on paired associate learning (Payne et al., 2016). These essentially negative large-scale trials are disappointing but again it is notable that none of them have studied any intermediate pathophysiological mechanism; and they may be underpowered to detect endpoint behavioral outcomes. Type II errors are a real potential issue in intervention into complex neural systems with the downstream outcome measured on behavior. An example of an experimental intervention trial that did by design look at neural system mechanism as well as behavioral outcome was a data-rich study RCT (n = 30) of simvastatin in young children with NF1 autism aged 4.5–10.5 years. The selection for this trial thus further constrained the phenotypic variance by specifically selecting children with co-occurring NF1 and autism. It also studied for the first time a complete hypothesized pathway from the proximal post-synaptic RasMAPKinase cell signaling target (measured by proxy through peripheral lymphocyte pMAPK activation); through multi-parametric imaging of neural system function (using structural and functional
imaging, spectroscopy, and perfusion studies); as well as autism phenotype-related outcome behaviors (Stivaros et al., 2018). With caveats for the small sample size, the study found theoretically relevant statin effects toward normalizing function at each hypothesized level of pathophysiology. There was evidence of simvastatin treatment associated with: (i) increased frontal white matter MRS GABA, GABA/Glx ratio, and reduced deep gray nuclei Glx; (ii) increased ASL perfusion in ventral diencephalon; and (iii) decreased ADC in cingulate gyrus. Machine-learning classification of the combined imaging outcomes achieved 79% ($P < 0.05$) accuracy in differentiating groups at endpoint against chance level (64%, $P = 0.25$) at baseline, suggesting a value of a combined multimodal imaging approach as a potential biomarker. Three of 12 (25%) simvastatin cases compared to none in placebo met “clinical responder” criteria for behavioral outcome. Despite being underpowered for definitive outcome estimation, particularly at the behavioral phenotype level, this study is perhaps more positive in its implications than some of the negative studies above; testing the full hypothesized pathway within an investigative mechanism-focused design may be a more sensitive indicator of treatment effect, and more illuminating for basic science, than studies focused primarily on treatment discovery for early exploitation in therapeutics.

5.3 TSC: THE mTOR PATHWAY
As NF1 is associated with Ras pathway, so TSC shows a pathophysiology particularly related to alteration of the mTOR pathway. These pathways show many inter-relationships, and the alteration of FMRP in FraX, for instance, also relates to impacts on both Ras and mTOR post-synaptic pathways. Although there is a high incidence of ASD in TSC (see above) there have not as yet been an intervention trial published that has targeted ASD symptoms as an outcome, nor selected comorbid TSC autism for sampling. The nearest are preliminary trials of mTOR inhibitors Sir-olimus which found improved executive function in TSC (Davies et al., 2011) and Everolimus which showed reduced seizure frequency and ASD-related symptoms on quality of life measures (Krueger et al., 2013). Based on these initial results, several more TSC RCTs are currently underway testing mTOR pathway inhibitors for neurocognitive and ASD-related deficits.

5.4 RETT AND PHELAN-McDERMID SYNDROMES: GROWTH AND NEUROTROPHIC FACTORS
Deletion/mutation of the SHANK3 gene in PMS (also known as 22q13 deletion syndrome) results in reduced expression of scaffolding proteins in the post-synaptic density of excitatory synapses, impairing glutaminergic transmission, and synaptic plasticity (Moessner et al., 2007). PMS is associated with 0.5–2.0% of all ASD cases. Similarly loss of function mutations of the X-linked gene MECP2 in Rett syndrome affect the structure and function of synapses at the microcircuit level critical for synaptic transmission and plasticity (Guy et al., 2001). Recent studies suggest that
insulin-like growth factor-1 (IGF-1) can have a beneficial effect on synaptic development by promoting neuronal cell survival, synaptic maturation, and synaptic plasticity (Bozdagi et al., 2013). A pilot placebo-controlled double-blind RCT using insulin-like growth factor in nine children with PMS was associated with significant improvements in social impairments and repetitive behaviors (Kolevzon et al., 2014). In Rett syndrome, an open-label phase 1 study of IGF-1 showed good safety, tolerability, and improvements in behavioral abnormalities (Khwaja et al., 2014). Studies of IGF-1 in FXS are also underway.

6 SUMMARY

Although there is a long history of comparative studies aiming to use genetic models of ASD to illuminate its phenotype and pathogenesis, our review will have highlighted that a truly rigorous and multifaceted approach to the issue is only really just beginning. It is too early to draw definitive conclusions.

Too often in the past generalized claims have been made from the study of single monogenic disorders, claims further undermined by the small sample sizes often employed. Associations reported between specific genetic variants and phenotypic profiles have on comparative study found to be less convincing. Broadly speaking at the level of gross behavioral phenotype, comparative descriptions, and epidemiology have not yet suggested strong evidence for specific phenotype-genotype correlations within ASD; rather the phenotypic profile across syndromes looks broadly comparable. Newer and more fine grain comparisons using larger samples across syndrome and a more item level analysis using computational modeling has suggested the possibility of some discrete and meaningful patterns within phenotypic data across genetic variance (Malki et al., 2018). However this kind of analysis is at an early stage and if there are syndrome-specific variations they are likely to be quite subtle and may be difficult to replicate. There has in other words been a failure to show what was initially thought promising from phenotypic studies across syndromic autism; that genetic syndrome-specific phenotypes would be found in a way that would allow specific gene-behavior associations and mechanistic illumination. Perhaps two conclusions are pertinent at this stage of the work in this area. First, the ASD behavioral phenotype is a behavioral outcome that is on the one hand quite stable across groups and within individual ontogeny but is also quite non-specific in relation to etiology. In this it is similar to most symptom complexes in child psychopathology; overdetermined in relation to cause. Rather than looking for over-simple gene-behavior correlations, we need to be more developmentally process orientated, asking the question as to how this phenotype (and at what level of mechanism) comes to be a final common pathway for many different perturbations in early development. Second, as the upstream neural system processes disrupted in neurodevelopmental disorder become clearer, the complex inter-relations of different transcription, cell signaling, synaptic function disruptions within different disorders becomes clearer. This will in turn require revision of overly simple or linear pathophysiological models.
Recent prospective longitudinal neurodevelopmental and behavior developmental studies offer an important new and complimentary approach to these descriptive accounts, particularly since they will build on a decade and more of work of this kind in idiopathic autism, which has hugely increased our understanding of the autism prodrome. However comparative cohort studies of this kind with syndromic autism models are only just beginning. Early evidence from one suggests some initial striking specificity in NF1 trajectory compared to idiopathic autism but this is based on a small initial sample, thus at best can only be indicative. Further studies of this kind will emerge and have considerable potential.

Experimental intervention work in syndromic ASD is also at a relatively early stage. Elegant experiments with knockout animal models have been highly suggestive in showing that manipulation of key cellular pathways implicated in single gene models can produce predicted (and dramatic) downstream neural system and behavioral effects. These provide a test of theory and practical demonstration of potential treatment discovery. A number of molecules have been derived from such animal experiments and tested on humans, none of which has yet achieved phase 3 translation potential. The obvious conclusion at this phase of work is that human translation in this area will not be simple or straightforward. There have been no “quick wins” in terms of treatment discovery, and this has unfortunately led some commercially focused efforts to re-evaluate their investments in this area. On the other hand we have argued that experimental invention work in humans in this area has really hardly yet begun. Many of the studies to date have been inadequately designed and underpowered, which could have led to type II errors. We have argued that the complexity of the systems involved mean that we now need a phase of ambitious within-human mechanism trials, that work up to be properly powered for developmental outcomes. Just one published study to date (Stivaros et al., 2018) has attempted a data-rich prior hypothesized mechanism design of the kind undertaken in animal work. The sample size here was small but at least as proof of principle the study demonstrated that upstream manipulation of a physiological pathway can produce downstream effects in the brain that were theoretically interpretable and potentially illuminating. More studies of this kind are currently underway internationally and will be valuable in testing putative etiological pathways as well as having potential for treatment discovery in humans.

A further iteration of experimental intervention work will then be to mount comparative treatment trials. The pathophysiology of the monogenic syndromic ASD models described here is beginning to be understood as interestingly interlinked; common neural system pathways, particularly at the level of synaptic protein expression and function, are emerging; which are potentially interesting in the context of final common pathways to ASD phenotype. As this emerges, there will be a logic for treatment trials targeting common pathways across different disorders; a comparative approach to intervention that, if hypothesis driven, may be highly illuminating for process. For instance, animal work looking at complementary FXS and TSC pathophysiology within mTOR and glutamate signaling found
that positive modulation of MGluR improved TSC cognition and behavior, in contrast to the opposite effect in FXS; a result that illuminates the causal pathways involved (Auerbach et al., 2011).

Further, synergistic combinations of the three areas of inquiry described here—the comparison of outcome behavioral phenotypes, the longitudinal description of emerging developmental pathways, and the manipulation of these via targeted intervention strategies—should provide further added value. More syndrome-specific behavioral description will better identify the target ASD syndrome in each disorder. Longitudinal studies will demonstrate natural history biomarkers of autism emergence in these particular developmental syndromes, and comparison of these biomarkers across different syndromes compared to outcome should be highly valuable. The addition of idiopathic autism cohorts into the comparative design clearly further adds to this potential. Finally, these natural history biomarkers within development can be tested as targets of intervention trials. An intervention considered as a developmental perturbation can be studied for its downstream effects using repeated measures designs that can demonstrate, as no other design really can do, causal links between cellular signaling disruption, neural system perturbation, and cognitive and behavioral phenotypic outcomes. In this way, over time there is then a strategy to identify whether there are indeed final common pathways leading to ASD behavioral phenotypic symptomatology—a key goal in autism research.

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