Autism spectrum disorder (ASD) is a common neurodevelopmental disorder with high heritability. Here, we discuss data supporting the view that there are at least two distinct genetic etiologies for ASD: rare, private (de novo) single gene mutations that may have a large effect in causing ASD; and inherited, common functional variants of a combination of genes, each having a small to moderate effect in increasing ASD risk. It also is possible that a combination of the two mechanisms may occur in some individuals with ASD. We further discuss evidence from individuals with a number of different neurodevelopmental syndromes, in which there is a high prevalence of ASD, that some private mutations and common variants converge on dysfunctional ERK and PI3K signaling, which negatively impacts neurodevelopmental events regulated by some receptor tyrosine kinases.

Conflict of interest: The authors have declared that no conflict of interest exists.

Nonstandard abbreviations used: ASD, autism spectrum disorder; CNV, copy number variation; G X E, gene by environment; MET, met proto-oncogene; mTOR, mammalian target of rapamycin; RTK, receptor tyrosine kinase.

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other co-occurring mental health and physical conditions. Thus, to emphasize the functional importance of recognizing many different kinds of ASD, the term “autisms” has been used (14). The behaviors that are disrupted in ASD are complex and develop through a bottom-up assembly of simple to more complex brain circuits that control very basic processes such as physiological homeostasis and more complex tasks such as being motivated to pay attention to certain cues in the environment that regulate outward social behavior and verbal and nonverbal communication. The heterogeneity of ASD is entirely consistent with the concept that different genetic mechanisms may influence brain circuit development at different levels of the hierarchy (5). The field thus is moving away from defining the ASD genes to defining unique phenotypic features of stratified populations of children, adolescents, and adults that may relate to specific genetic etiologies, such as increased risk due to common allelic variations, rare mutations, or copy number variation (CNV) (Figure 1). Implicit in this view is that there will not be identification of genetic risks that map one-to-one with behavioral dysfunction; that is, while there are genetic variants that are enriched in populations with particular dysfunctions, such as language, there are no genes that directly regulate social behavior or language. Instead, genetic vulnerability resides in the disruption of cellular processes, due to the disruption of proteins encoded by genes, in specific brain circuits that may also be influenced by G X E mechanisms. Research findings emerging from human genetic and animal studies suggest that disruption of a key developmental process, synapse formation and stabilization (synaptogenesis), is a final common path in ASD etiology. Different molecular mechanisms may contribute to increasing ASD risk, including disturbances in the assembly of structural proteins needed to build synapses, such as the neuroligins and neurexins, and dysfunction of cellular signaling pathways that control synaptogenesis.

Distinct patterns of heritability of risk alleles in ASD
As noted above, ASD is highly heritable, and current studies suggest that there are multiple mechanisms through which different types of gene mutations increase risk of developing the disorder (15–17). There are a number of considerations that are key to successful genetic studies of ASD. First, because of the heterogeneity of the disorder, it is necessary to analyze large numbers of individuals with ASD. Second, each individual gene is likely to have very small effects on disease risk, but in combination with other genes and/or G X E factors, an individual gene may encode a protein that functions in a key cellular process, which, when disrupted, contributes to disease pathophysiology. Third, disorder emergence through de novo genetic mutations or heritability of gene-specific functional polymorphisms in the DNA sequence transmitted from parent to child may underlie distinct but equivalently valid ASD etiologies. Fourth, distinct genetic etiologies, together with different environmental factors, may be part of ASD heterogeneity. Last, the nature of the core behavioral dimensions that characterize ASD emerge through perturbation of developing brain circuits. Disruption at distinct levels of the organizational and functional hierarchy relate to the heterogeneity in social behavior and communication capabilities.

The aim of genetic studies of ASD should be to identify functional variants that contribute to ASD risk. A thorough recent review provides a detailed listing of up-to-date genetic findings (16). One approach with great promise for the identification of candidate genes and pathways is analysis of CNV (see The basics of CNVs) (18, 19). However, as with single gene mutations and common variants, CNV analyses need to be interpreted with extreme caution for a number of reasons. First, the presence of a de novo CNV in an individual with ASD does not necessarily imply it is associated with increased risk of developing the disorder. Further, CNVs typically are not fully penetrant, meaning that they may be present in individuals who do not have an ASD. CNVs were first described in healthy control individuals, with more than 11 CNVs per individual (20), indicating that having multiple CNVs is not pathologic. Only formal genetic association analyses involving large sample sizes should be used to imply a particular CNV is associated with disorder risk. Second, the presence of a CNV does not necessarily imply functional disruption. Analyses of CNVs in the human adult cerebral cortex indicate that more than 50% of mature neurons are aneuploid (21, 22), and experiments in mice indicate that CNVs in cortical neurons may have little impact on function (23). Further, germline deletion of both copies of certain genes in experimental animals can result in mutants without a detectable phenotype. This suggests that due to adaptive processes, gene dosage in the form of CNV does not lead necessarily to dramatic functional changes in vivo. Third, CNV in peripheral blood cells, the cells typically analyzed in humans, may not relate in a one-to-one fashion to CNVs in neurons. Indeed, the number of CNVs in the human cerebral cortex is approximately 7-fold higher than in peripheral blood cells (21), and thus, analysis of peripheral blood may identify some, but not necessarily all, of the CNVs occurring in neurons that contribute to ASD-related disturbances of brain architecture and circuitry. Fourth, de novo CNVs are observed in 7%–10% of cases from simplex families (families with only one child with ASD), 2%–3% of cases from multiplex families

Figure 1
Current experimental approaches to determining genetic etiologies for ASD. These approaches include whole-genome analyses that identify disorder-related sequences or CNVs in genes that exhibit preferential inheritance patterns or de novo appearance in individuals with ASD. The current challenges include the translation of these genetic findings to define the biological consequences of the variations, to determine the influence on defined clinical phenotypes of ASD, and eventually to design new intervention strategies.
The basics of CNVs

CNVs are deletions or duplications of segments of chromosomes. Thus, rather than the normal two copies of a genetic sequence, CNVs are fewer or more copies of the particular sequence. CNVs are typically benign, either because no genes lie within the deleted or duplicated chromosomal region or because there is functional compensation for the amount of gene product produced due to the CNV. However, CNVs that are located in functionally important genes and disrupt regulatory or coding regions can contribute to disease processes. Large CNVs that eliminate or duplicate several genes are more likely to be pathophysiological than small CNVs that may miss important regulatory or coding regions of genes. CNVs may be inherited, arising in parental germ cells, or arise spontaneously (de novo) in offspring germ or somatic cells. In somatic cells, the developmental timing of when de novo CNVs arise (early versus late) may influence the degree of functional deficits.

(families with more than one child with ASD), and 1% of controls (24, 25). The de novo CNVs that occur in a subset of individuals with ASD in multiplex families may influence the severity of the disorder, rather than contributing directly to the expression of the disorder. Despite these cautions, CNV analysis can be used to identify candidate genes that can be tested further for functional effects that may contribute to ASD susceptibility (18, 19).

We are beginning to recognize that inheritance of rare or common functional alleles is only one genetic mechanism that increases disorder risk. Private (de novo) functional mutations also impart genetic risk. Analyses suggest that, at the genetic and behavioral levels, multiple of the same gene may be involved in the etiology of the same clinical syndrome and rare mutations point the way (24, 26–29). Furthermore, multiple genes or even multiple mutations in a single gene may be involved in the etiology of the same clinical syndrome and rare mutations point the way (24, 26–29). The de novo CNVs that occur in a subset of individuals with ASD in multiplex families may influence the severity of the disorder, rather than contributing directly to the expression of the disorder. Despite these cautions, CNV analysis can be used to identify candidate genes that can be tested further for functional effects that may contribute to ASD susceptibility (18, 19).

Syndromic disorders and rare mutations point the way

Although not understood from an etiological or pathophysiological perspective, it is now clear that rare neurodevelopmental disorders (<1 in 10,000) are becoming increasingly important to study in greater detail because of their relationship to ASD. Higher penetrance of ASD diagnosis (far greater than the 0.75% observed in the general population) is reported in children who have genetically diverse neurodevelopmental syndromic disorders, including Angelman syndrome, Fragile X syndrome (FraX), Rett syndrome, Smith-Lemli-Opitz syndrome, Timothy syndrome, neurofibromatosis, and tuberous sclerosis. It is important to emphasize that each syndrome is characterized by fundamentally different gene mutations, which presumably impart distinct molecular pathophysiologies (Table 1). However, there are few studies that examine closely the similarities and differences in phenotypic characteristics between single gene (syndromic) and multigenic (idiopathic) ASD (36). The neurodevelopmental syndromic disorders listed in Table 1 are characterized in part by intellectual disability (ID; formally termed mental retardation). A large minority (25%–40%) of individuals with ASD has ID, but ASD is not synonymous with ID. A recent structural MRI study suggests that individuals with FraX, with or without ASD diagnosis, are more closely related in the context of the size of brain structures than those with idiopathic ASD (37). Microarray analysis of lymphocytes from patients with FraX, chromosome 15q deletion, or idiopathic ASD reveal unique patterns of gene expression that may serve as a signature for each disorder, but with a potentially important small subset of overlapping changes in mRNA expression (38). Moreover, detailed neuropathological studies are lacking to compare these syndromes and idiopathic ASD. Although there is likely to be diversity in the pathological targets in each syndrome, there are suggestions of some commonalities. The triad of overlapping dysfunctions (social behavior, communication, and repetitive behavior) across ASD and the syndromes, together with the known brain neuropathology of some of the syndromes, suggests that later neurodevelopmental events, such as synapse formation and maturation, dendritic growth, and myelination, are probably most vulnerable. In addition to the evidence from syndromic disorders, the focus on later events in this Review is supported by the discovery of rare mutations in certain genes that regulate synaptogenesis. A substantial focus has been on the adhesive and structural elements needed for synapse formation, stability, and physiologic maturation. In ASD cases, rare mutations and CNVs have been identified in genes encoding neurelins, neurexins, contactin-associated protein-2 (CNTNAP2), and SH3 and multiple ankyrin repeat domains 3 (SHANK3). The disruptions are likely to occur in shared forebrain and cerebral cortical circuits. Thus, while not identical to idiopathic ASD, biological and behavioral analyses of syndromic disorders and rare mutations provide a sound approach to discern potential overlapping molecular and brain targets (Table 1).

Intracellular kinase signaling in ASD-associated disorders

The potential contribution of defects in adhesion and structural proteins that build synapses to the etiology of ASD has been the subject of many reviews of ASD (16, 17, 39–41). In this Review, we suggest that some neurodevelopmental syndromic disorders and rare mutations point to an additional set of molecular targets. Thus, recognizing that we are attempting to resolve a spectrum of
disorders that will not have a single, underlying etiology, findings from studies of certain syndromic disorders with high penetrance of ASD converge on the ERK and PI3K intracellular signaling pathways that we believe deserve increased scrutiny in all forms of ASD. ERK and PI3K activate mammalian target of rapamycin (mTOR), which through other kinases will increase mRNA translation to influence developmental functions as diverse as the cell cycle, cell survival, differentiation, and motility. Receptor tyrosine kinases (RTKs) can signal through either of these intracellular kinase pathways, with cell type and cellular milieu defining the intracellular response (Figure 2). Table 1 reports several syndromic disorders with high penetrance of ASD that involve a primary disruption in signaling through these pathways specifically and others that would disrupt RTK signaling, the primary membrane receptor class that transduces signals through ERK and PI3K. The most convincing connections between ERK/PI3K signaling disruption and ASD are evident in tuberous sclerosis and neurofibromatosis type 1, in which different elements of the ERK/PI3K pathway are disrupted genetically, leading to enhanced mTOR downstream activation (Figure 2). In addition, ERK and PI3K signaling is dependent in part on normal cholesterol biosynthesis, which is absent in Smith-Lemli-Opitz syndrome. For example, Ras signaling, a key upstream mediator of ERK activation, requires cholesterolization. Rare gene mutations of another element of the PI3K signaling pathway, phosphatase and tensin homolog (PTEN), which encodes a protein that binds to specific regulatory regions of certain genes (based on DNA methylation patterns) that control gene transcription. Methylation status and/or MECP2 binding directly regulates transcription of key genes involved in met proto-oncogene–RTK signaling (MET RTK signaling; MET is also known as HGFR), which our laboratory has implicated in ASD risk (see below). Those genes include those encoding MET, the MET coreceptor CD44, the MET transcriptional regulator SP1, and several proteins in the ERK/PI3K downstream signaling pathway (42).

The various neurodevelopmental syndromic disorders and rare mutations described thus far along the ERK/PI3K pathways result in an increased state of activation of mTOR (Figure 2). Additional evidence for involvement of these intracellular kinase pathways in ASD comes from recent treatment studies in genetically engineered mice that exhibit behavioral and neuropathologic phenotypes that are common in the human neurodevelopmental syndromic disorders. For example, systemic administration of drugs that reduce mTOR activation, such as rapamycin, wortmannin, and RAD001, can reverse behavioral and structural pathology in mice with Pten (43), tuberous sclerosis 1 (Tsc1) (44, 45), and neurofibromin 1 (Nf1) (46, 47) mutations, with no reported side effects.

ASD etiologies also are likely to include environmental factors that work together with genetic risk to drive neurodevelopmental systems over the threshold for disorder expression (Figure 3). We therefore hypothesize that different genetic routes to altered RTK function, by way of modulation of ERK/PI3K signaling pathways, combine with environmental factors, such as biochemical stressors, that also modulate these signaling pathways. The G X E interactions either modulate the degree of dysfunction of the core clinical features of ASD or have an impact on neurobiological circuits that are at greater risk for dysfunction, because genetic vulnerability pushes the system closer to disorder threshold.

Given that ERK/PI3K signaling is widely distributed throughout multiple organ systems, where does disorder specificity arise? One way to think about the issue of specificity is to recognize that signaling through ERK/PI3K is highly influenced by cell type and timing of activation of the RTK signaling systems. For example, there is a potential dichotomy in the molecular mechanisms of ASD and cancer that would involve different genetic risk factors affecting ERK/PI3K signaling. Unequivocal evidence implicates hyperactivated PI3K signaling in a number of malignant cancer types (48–50). In contrast, decreased PI3K activation may contribute in some instances to ASD (26, 51, 52). We are unaware of any studies of cancer frequencies in individuals with ASD. However, disruption of PI3K signaling also has been implicated in other psychiatric disorders of neurodevelopmental origin, such as schizophrenia (53, 54). An altered incidence of various cancers in individuals with schizophrenia is debated (55, 56), but reduced cancer incidence is observed consistently in parents and siblings of individuals with schizophrenia compared with the general population (57–59). These data support

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### Table 1

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>ASD co-diagnosis</th>
<th>Gene mutation</th>
<th>Cell function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angelman</td>
<td>&gt;50%</td>
<td>UBE3A (maternal)</td>
<td>Protein degradation</td>
<td>96–98</td>
</tr>
<tr>
<td>Down syndrome</td>
<td>5%–15%</td>
<td>Chromosome 21 triplication</td>
<td>Multiple</td>
<td>99, 100</td>
</tr>
<tr>
<td>Fragile X</td>
<td>&gt;45%–70%</td>
<td>FMR1</td>
<td>RNA trafficking</td>
<td>101, 102</td>
</tr>
<tr>
<td>Neurofibromatosis</td>
<td>&gt;4%</td>
<td>NF1/NF2</td>
<td>PI3K signaling activity</td>
<td>103</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>&gt;7%</td>
<td>PTEN</td>
<td>PI3K signaling activity</td>
<td>104</td>
</tr>
<tr>
<td>Potocki-Lupski</td>
<td>&gt;9%</td>
<td>17p duplications</td>
<td>Unknown</td>
<td>105</td>
</tr>
<tr>
<td>Rett</td>
<td>&gt;5%</td>
<td>MECP2</td>
<td>Transcriptional regulation, including</td>
<td>106, 107</td>
</tr>
<tr>
<td>Smith-Lemli-Opitz</td>
<td>&gt;50%–75%</td>
<td>DHCRL7</td>
<td>Cholesterol biosynthesis; Ras-mediated</td>
<td>108, 109</td>
</tr>
<tr>
<td>Timothy</td>
<td>&gt;75%</td>
<td>CACNA1C</td>
<td>ERK signaling; PI3K signaling</td>
<td>110</td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>&gt;40%–50%</td>
<td>TSC1/TSC2</td>
<td>Calcium signaling</td>
<td>111, 112</td>
</tr>
<tr>
<td>22q13 deletion</td>
<td>&gt;90% PDD-NOS</td>
<td>Microdeletions</td>
<td>Multiple</td>
<td>113</td>
</tr>
</tbody>
</table>

*Co-diagnosis (4%) of narrowly-defined autistic disorder with neurofibromatosis type I; the co-occurrence of ASD is likely to be higher. Co-diagnosis of neurodevelopmental disorders with neurofibromatosis type I is 70%. CACNA1C, calcium channel, voltage-dependent, L type, alpha 1C subunit; DHCR7, 7-dehydrocholesterol reductase; FMR1, fragile X mental retardation syndrome 1; MECP2, methyl CpG binding protein 2; NF1, neurofibromin 1; PDD-NOS, pervasive developmental disorder—not otherwise specified; PTEN, phosphatase and tensin homolog; TSC1, tuberous sclerosis 1; UBE3A, ubiquitin protein ligase E3A.
Figure 2
The MET RTK signaling pathway and genes implicated in ASD risk. Intracellular signaling of MET and other RTKs occurs via the PI3K or ERK1/2 pathways. Rare mutations and CNVs (which are both designated by ‡) or associated common alleles (which are designated by *) have been identified in individuals with ASD in seven genes encoding proteins involved in these signaling pathways. Of note, an association between common MET variants and ASD has been reported for five independent family cohorts. PLAUR and SERPINE1 associations with ASD have been determined in single, large family cohorts (>600 families). Ras disruption in Smith-Lemli-Opitz syndrome is due to alterations in cholesterol biosynthesis (which is designated by †). Also depicted are other proteins that interact with the MET signaling pathway, such as semaphorins, plexins, and other RTKs. MET can signal via the PI3K and the ERK pathway. RTKs, including MET, are involved in key neurodevelopmental processes, including axon guidance, synapse formation, and plasticity. Convergence of many different genetic etiologies suggests that risk via ERK/PI3K signaling may be common in ASD. Risk, severity of the pathophysiology (i.e., intellectual disability), and disorder heterogeneity may relate to differences in genetic and epigenetic points of entry to the pathways. Thus, the impact due to genetic risk, via regulators of ligand availability or RTKs such as MET, may be less severe than the more severe clinical impact (i.e., intellectual disability) from disruption downstream along the intracellular signaling pathways. c-cbl, E3 ubiquitin-protein ligase c-Cbl; rheb, Ras homolog enriched in brain; RSK, ribosomal S6 kinase; uPA, urokinase plasminogen activator.

MET in PI3K signaling and ASD
Our own genetic and neuropathological studies of ASD have focused on one of the upstream activators of both ERK and PI3K in various cell types, MET. Much is known about its role in PI3K signaling. Specifically, HGF activation of MET causes phosphorylation of AKT that can be blocked by the PI3K inhibitors LY294002 andwortmannin (63–65). Most relevant to the current discussion, MET activation of PI3K signaling has been demonstrated in neuronal cells, resulting in neuroprotection of cerebellar granule cells (63), cell motility in striatal progenitor cells (66), and protection of cortical neurons from hypoxia-induced insult (67).

It is again important to emphasize that the phenotypic heterogeneity of ASD makes it unlikely that any individual gene will contribute to more than a subset of cases. This creates a natural tension in the field that is attempting to translate genetic findings into plausible biological models of ASD. Thus, there currently is legitimate skepticism regarding any particular candidate gene (16, 17). However, both convergent neurobiological and genetic evidence is emerging to suggest ASD vulnerability may lie, in part, in the well-defined MET signaling pathway. Our initial decision to examine the RTK MET gene as an ASD risk candidate was based on several factors, including the location of the gene under a broad linkage peak on chromosome 7 that has been replicated multiple times (68–72) as a region carrying ASD risk genes, as well as a number of developmental neurobiology findings that implicate MET signaling in forebrain circuit development. MET activation by HGF modulates forebrain interneuron motility in vitro (73). Excitatory/inhibitory imbalance has been postulated to occur in ASD (74, 75). Moreover, in mice gene targeting of the MET signaling pathway, through deletion of the gene encoding plasminogen activator, urokinase receptor (Plaur), which controls levels of HGF, results in reduced numbers of neocortical interneurons, spontaneous seizures (which occur in 20%–30% of children with ASD), increased anxiety, and reduced social interactions (76–78). Additionally, MET signaling participates in autonomic nervous system and cerebellar development, immune function, and gastrointestinal function and repair (79–84). Disruptions of these neural and peripheral elements have been reported in ASD (85–90).

Candidate gene analyses often fail to generate replicable findings due to small effects in a limited number of samples. In the case of the MET signaling cascade (Figure 2), however, the pathophysiologic and genetic evidence of its contribution to ASD risk is now considerable. First, the expression of MET protein is reduced or proteins that regulate growth factor availability would place signaling through this pathway at risk but require additional genetic and environmental insults to cause neurodevelopmental disruption. Disruption of the development of specific brain circuits would occur, because, unlike their intracellular mediators, upstream signaling elements are not distributed uniformly. Rather, RTKs and growth factors may be concentrated in developing circuits at key periods of development that mediate the maturation of connections underlying specific functions. This hypothesis is consistent with the identification of neuregulin 1 (the ligand for the RTK ERBB4) as a factor for schizophrenia susceptibility (60) and the RTK MET as a factor for ASD risk (26, 27, 52). Although both ERBB4 and MET activate PI3K signaling, the differential timing and patterns of expression of each of these RTKs in developing cerebral cortex (61) may account for the distinct neurodevelopmental disruptions characteristic of each disorder. We have shown that MET is enriched in neocortex, amygdala, septum, and cerebellum, regions implicated in ASD (62).
identified functional mutations that are more prevalent in the cases compared with controls (26). The mutations alter the juxtamembrane region of MET, which regulates receptor activation (92). Last, five other genes in the MET signaling pathway were examined in a large family cohort. Two of the genes, PLAUR and serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (SERPINE1), are associated with increased ASD risk (27), and each mRNA exhibits altered expression in the postmortem cerebral cortex of individuals with ASD compared with age- and gender-matched controls (52).

The genetic findings from our own studies and those gathered from analysis of defined neurodevelopmental syndromic disorders implicate PI3K signal disruption in both multigenic and syndromic ASD. The observation that de novo CNV is substantially more common in simplex families than multiplex families (24, 25) suggests that private mutations, along with other rare functional mutations, may contribute to ASD. In contrast, association of the risk alleles in MET (26, 91) and the MET-regulating genes PLAUR and SERPINE1 (27) is found only in multiplex families and is therefore linked to multigenic, idiopathic ASD. Heritability of common risk alleles in multiplex families also is reflected in behavioral profiles in parents of children with ASD. Thus, the broader autism phenotype is found to a far greater extent in parents from multiplex compared with simplex families (28, 29), suggesting that heritable, rather than de novo mechanisms for ASD expression occur in multiplex families. Collectively, there seem to be a number of different genetic etiologies that can contribute to altering MET signaling in many individuals with ASD.

**Figure 3**
Contributions of the PI3K pathway to ASD risk threshold. The degree of genetic risk is indicated by shading, with darker color indicating increased risk. The model presents common functional variants in the MET, PLAUR, and SERPINE1 genes that, along with other genetic risk alleles, contribute to risk of developing ASD. Adaptive processes may prevent presentation of ASD, but additional environmental factors or the presence of multiple risk alleles result in idiopathic (multiple genes, each having a small effect) ASD. Mutations further down the PI3K pathway result in syndromic disorders, with penetrance and phenotype severity determined by a decreasing availability of adaptive processes.

**G X E interactions in ASD etiology**
Beyond multiple genetic elements implicated in ASD risk, the MET/PI3K pathway also is highly vulnerable to environmental perturbations. For example, increasing the redox state of oligodendrocyte progenitor cells by brief exposure to lead or mercury activates c-Kit–regulated internalization and degradation of certain RTKs, including MET, EGFR, and PDGFR (93). Not all RTKs are affected by Stressing cells through altering redox state. The reason for selective vulnerability of MET and other RTKs is not known. Irrespective of the mechanism, the cell stressor results in reduced signaling through ERK/PI3K. Benzo(a)pyrene (BaP), a common chemical in vehicle exhaust, paper and wood processing, and trash incineration, disrupts the binding of transcription factors such as SP1 to DNA targets (94, 95). This is relevant to MET expression and perhaps ASD, because the normal level of binding of SP1 is reduced by the ASD-associated rs1858830 C allele (25). A testable hypothesis would be to combine the MET risk allele with exposure to BaP to examine how the double hit affects expression levels. The findings from the studies involving cell stressors and toxic chemicals suggest additional ways in which genetic risk due to regulatory alleles may combine with environmental factors to shift a system closer to disease threshold (Figure 3).

**Final thoughts**
ASD heterogeneity needs to be considered more seriously in developing strategies to investigate underlying biological etiologies. Within syndromic and multigenic ASDs, functional profiles are
diverse. Heterogeneity at the genetic level may be probed more strategically by using much larger sample populations, as has been done for diabetes and cardiovascular disease. Technology development will continue to facilitate the larger scale association analyses that have been done for diabetes and cardiovascular disease. Technology that can lead to earlier diagnosis and individualized treatments.

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