Neuron
Article

Autism-Associated Promoter Variant in *MET* Impacts Functional and Structural Brain Networks

Jeffrey D. Rudie,[1,2] Leanna M. Hernandez,[1,3] Jesse A. Brown,[2] Devora Beck-Pancer,[1,3] Natalie L. Colich,[1] Philip Gorrindo,[4] Paul M. Thompson,[3,5] Daniel H. Geschwind,[3,6] Susan Y. Bookheimer,[3] Pat Levitt,[4] and Mirella Dapretto[1,3,*]
[1]Ahmanson-Lovelace Brain Mapping Center, University of California, Los Angeles, Los Angeles, CA 90095-7085, USA
[2]Interdepartmental Neuroscience Program, University of California, Los Angeles, Los Angeles, CA 90095-1761, USA
[3]Department of Psychiatry and Biobehavioral Sciences, University of California, Los Angeles, Los Angeles, CA 90095-1759, USA
[4]Zilkha Neurogenetic Institute, Department of Cell and Neurobiology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90089-9112, USA
[5]Department of Neurology
[6]Departments of Neurology and Human Genetics
University of California, Los Angeles, Los Angeles, CA 90095-7734, USA
*Correspondence: mirella@loni.ucla.edu
http://dx.doi.org/10.1016/j.neuron.2012.07.010

SUMMARY

As genes that confer increased risk for autism spectrum disorder (ASD) are identified, a crucial next step is to determine how these risk factors impact brain structure and function and contribute to disorder heterogeneity. With three converging lines of evidence, we show that a common, functional ASD risk variant in the Met Receptor Tyrosine Kinase (*MET*) gene is a potent modulator of key social brain circuitry in children and adolescents with and without ASD. *MET* risk genotype predicted atypical fMRI activation and deactivation patterns to social stimuli (i.e., emotional faces), as well as reduced functional and structural connectivity in temporo-parietal regions known to have high *MET* expression, particularly within the default mode network. Notably, these effects were more pronounced in individuals with ASD. These findings highlight how genetic stratification may reduce heterogeneity and help elucidate the biological basis of complex neuropsychiatric disorders such as ASD.

INTRODUCTION

Over the past decade significant strides have been made toward understanding the genetic basis of autism spectrum disorder (ASD) (see Geschwind, 2011 and State and Levitt, 2011 for review), a highly heritable psychiatric disorder (Bailey et al., 1995; Rosenberg et al., 2009; Hallmayer et al., 2011). Yet, due to the complexities of both ASD genetic architecture and brain-behavior relationships, great challenges remain in delineating how ASD risk genes shape the circuits underlying social behavior. Brain imaging studies have demonstrated that individual variation in task-based fMRI activation patterns, resting state functional connectivity (rs-fcMRI), and structural connectivity measures has a strong genetic component (Chiang et al., 2011; Kochunov et al., 2010; Fornito et al., 2011; Glahn et al., 2010; Koten et al., 2009) and is altered in ASD (see Di Martino et al., 2009 and Vissers et al., 2012 for review). Thus, neuroimaging endophenotypes are ideal for bridging the gap in our understanding of how genetic risk impacts brain circuitry. Yet, both behavioral and imaging phenotypes in ASD present significant heterogeneity and substantial overlap with typical populations, often leading to discrepant findings (e.g., Cheng et al., 2010). A critical question then is how genetic variability underlies phenotypic heterogeneity and, consequently, whether stratifying by genetic risk factors can improve our understanding of the neurobiology of ASD.

Although recent estimates suggest that hundreds of genes are likely to contribute to ASD risk (Buxbaum et al., 2012), the vast majority of evidence comes from rare mutations, such as the recently described copy number variants (CNVs) (Marshall et al., 2008; Pinto et al., 2010) and de novo single-nucleotide variants (SNVs) (Sanders et al., 2012; O'Roak et al., 2012; Neale et al., 2012; Iossifov et al., 2012). These mutations are rare (occurring in less than 1% of the population), may be unique to the individual, and are estimated to collectively impact 10%–20% of the ASD-diagnosed population. Therefore, while de novo events are conceptually important for understanding the many potential biological routes to ASD etiology, their utility for understanding phenotypic heterogeneity across the ASD population remains to be determined. Perhaps due to clinical heterogeneity, small estimated effect sizes, and limited statistical power, genome-wide association (GWA) studies focusing on common variants (>5% allele frequency) have failed to yield conclusive evidence for any specific common variants influencing ASD risk when pooling data across studies (Wang et al., 2009; Weiss et al., 2009; Anney et al., 2010). However, a few notable ASD candidate genes with common variants—namely, contactin-associated protein-like 2 (*CNTNAP2*) and Met Receptor Tyrosine Kinase (*MET*)—have been identified using large samples. Importantly, these variants have been replicated in independent cohorts, and follow-up studies have characterized the functional consequences of the genetic variant on gene or protein expression, providing additional support

(see State and Levitt, 2011, and independent autism risk gene databases: SFARI Gene Base, https://gene.sfari.org/autdb/GS_Home.do; and Autism Knowledge Base, Xu et al., 2012). Interestingly, common variation in *CNTNAP2* has been previously found to impact functional (Scott-Van Zeeland et al., 2010) and structural (Dennis et al., 2011) brain connectivity in healthy control participants. Despite a replicated common variant (*MET* rs1858830; Campbell et al., 2006, 2008; Jackson et al., 2009) and convergent lines of molecular and cellular evidence for autism risk (Judson et al., 2011b), the impact of *MET* on human brain circuitry has not yet been examined.

MET is one of multiple genes encoding proteins in the ERK/PI3K signaling pathway, including *PTEN*, *NF1*, and *TSC1*, that have been implicated in syndromic and idiopathic causes of ASD (Levitt and Campbell, 2009). In the forebrain, MET gene and protein expression is highly regulated in excitatory projection neurons during synapse formation (Judson et al., 2009, 2011a; Eagleson et al., 2011). MET is expressed widely in the mouse neocortex (Judson et al., 2009), but in monkeys (Judson et al., 2011a) and humans (Mukamel et al., 2011), it is far more limited, restricted to regions of temporal, occipital, and medial parietal cortex—regions that contain circuits underlying the processing of socially relevant information. The clinical relevance of MET cortical expression has been exemplified by postmortem brain studies, whereby individuals with ASD displayed 50% lower levels of MET protein in superior temporal gyrus (Campbell et al., 2007) and did not display the same temporo-frontal differential expression pattern as control subjects (Voineagu et al., 2011).

Three common variants in *MET* have been associated with ASD across independent cohorts (Campbell et al., 2006, 2008; Jackson et al., 2009; Sousa et al., 2009; Thanseem et al., 2010). The ''C'' variant of rs1858830 is particularly interesting because it is located in the promoter region of *MET* and is functional (Campbell et al., 2006, 2008; Jackson et al., 2009). The presence of the ''C'' variant reduces nuclear protein binding to the promoter region, and decreases gene transcription in vitro by 50% (Campbell et al., 2006). As expected for a common functional variant, the ''C'' allele correlates with lower levels of MET transcript and protein expression independent of diagnostic status (Campbell et al., 2007; Heuer et al., 2011). Common variants may increase risk but are not ''disorder-causing.'' Intriguingly, however, rs1858830 ''C'' allele moderates the severity of social symptoms in ASD, whereby individuals with ASD who carry this risk allele have more severe social and communication phenotypes than those who do not (Campbell et al., 2010).

The neurobiological correlates of the impact of reduced *MET* expression in humans have been examined in *Met* conditional knockout (*Met*-cKO) mice (Judson et al., 2009, 2010; Qiu et al., 2011). Neocortical pyramidal neurons in *Met*-cKO mice exhibited altered dendritic architecture and increased spine head volume (Judson et al., 2010), as well as a concomitant increase in local interlaminar excitatory drive onto corticostriatal neurons (Qiu et al., 2011). This finding of heightened local circuit connectivity is highly relevant to ASD risk and the current hypothesis regarding increased local circuit connectivity and decreased long-range connectivity of brain networks in individuals with ASD (Belmonte et al., 2004; Just et al., 2004; Courchesne and Pierce, 2005; Geschwind and Levitt, 2007). MRI evidence of long-distance underconnectivity in ASD using both structural and functional MRI is extensive, and although heterogeneity is common among ASD and even typically developing (TD) subjects, some consistent themes have emerged (Vissers et al., 2012). For example, reduced functional connectivity in distributed brain networks in ASD has been reported across a variety of cognitive tasks (e.g., Castelli et al., 2002; Just et al., 2004; Villalobos et al., 2005; Kleinhans et al., 2008) and when measuring task-independent (intrinsic) connectivity for interhemispheric (Dinstein et al., 2011; Anderson et al., 2011) and anterior-posterior connections (Cherkassky et al., 2006; Kennedy and Courchesne, 2008; Monk et al., 2009; Weng et al., 2010; Assaf et al., 2010; Rudie et al., 2012), particularly within the default mode network (DMN) (Raichle et al., 2001). The DMN is involved in socio-emotional processing including mentalizing and empathizing, which are classically impaired in individuals with ASD. Additionally, several diffusion tensor imaging (DTI) studies have reported reduced white matter (WM) integrity of anterior-posterior and interhemispheric tracts in ASD (Barnea-Goraly et al., 2004; Alexander et al., 2007; Sundaram et al., 2008; Shukla et al., 2011). However, DTI studies have been less consistent with regard to the precise tracts involved, with some studies even reporting tracts with higher fractional anisotropy (FA) in ASD (Cheung et al., 2009; Cheng et al., 2010; Bode et al., 2011). Interestingly, a recent study found that unaffected siblings of individuals with ASD have similar alterations in FA (Barnea-Goraly et al., 2010), suggesting that the alterations in WM integrity may represent a marker of genetic risk for ASD.

Based on the convergent genetic, clinical, and neurobiological findings regarding MET as a candidate for mediating ASD risk, the dramatic restriction of primate neocortical expression to regions that are implicated in ASD dysfunction (Judson et al., 2011a; Mukamel et al., 2011), and the functional nature of the common risk allele in regulating levels of gene expression, we hypothesized that analysis of the *MET* promoter variant would be a powerful tool to examine functional heterogeneity in structural and functional neuroimaging endophenotypes. We tested this prediction by examining the relationship between *MET* risk genotype and functional activation patterns to social stimuli, DMN functional connectivity, and the integrity of major WM tracts. Additionally, we hypothesized that the *MET* promoter variant would help address ASD heterogeneity by clustering a unique subset of individuals with the diagnosis such that individuals with ASD and the rs1858830 *MET* risk allele would exhibit the greatest alterations in structural and functional endophenotypes. In addition to characterizing MET's role in these circuits, our findings support a basic strategy of population stratification with multimodal imaging and genetics that may reveal specific mechanisms underlying phenotypic heterogeneity.

RESULTS

A total of 162 children and adolescents including 75 with an ASD and 87 who were TD contributed data to one or more of the three neuroimaging experiments (see Table S1 available online). This included a task-based fMRI experiment involving the passive observation of emotional faces (n = 144), a resting state fMRI

scan (n = 71), and a diffusion-weighted scan (n = 84). DNA was extracted from saliva samples, and the *MET* variant, rs1858830, was genotyped by direct resequencing. Individuals carried zero, one, or two of the rs1858830 C "risk" alleles. There were three genotype groups: a CC homozygous risk group (30.2% of sample), a CG heterozygous intermediate-risk group (49.4% of the sample), and a GG homozygous nonrisk group (20.3% of the sample). Thus, the terminology (i.e., "risk" versus "nonrisk" group) used hereafter refers to both TD and ASD individuals with specific *MET* genotypes. Genotypes observed Hardy-Weinberg Equilibrium (χ^2 = 0.001; p = 0.973), and in this sample we did not observe an enrichment of the risk allele in individuals with ASD (Fisher's exact test, p = 0.654). However, it should be noted that our sample, like other neuroimaging studies, is small for standard genetic association testing, and the study sample consisted of high-functioning individuals with ASD. Prior studies have shown an enrichment of the *MET* risk allele in individuals with ASD, particularly in multiplex families (two or more children with ASD; Campbell et al., 2006) and in the most highly impaired individuals with ASD (Campbell et al., 2010).

In each of the three data sets, genotype groups did not differ by age, gender, head motion, IQ, or ASD diagnosis; similarly, there were no differences between diagnostic groups in age, gender, or head motion (Table S1). However, consistent with prior reports by Campbell et al. (2010), ASD homozygous risk and heterozygous risk groups had significantly higher levels of social impairment (Autism Diagnostic Observation Schedule [ADOS], Lord et al., 2000; social subscale, p = 0.001) than the ASD homozygous nonrisk group. IQ did not differ between the ASD homozygous nonrisk group and all TD groups (homozygous risk, heterozygous risk, and homozygous nonrisk) but was significantly lower in both ASD homozygous risk and heterozygous risk groups; thus, we included full-scale IQ as a covariate in all analyses examining the effect of an ASD diagnosis. Additionally, given that the inheritance pattern (additive, dominant, or recessive) of the genotype effect is not clearly established, for all data sets we first focused on a direct contrast between the homozygous risk (CC) and nonrisk (GG) groups collapsed across diagnostic status (with diagnostic status as a covariate). In addition, we performed whole-brain analyses comparing TD and ASD groups collapsed across genotype. Following these initial whole-brain analyses, we used the regions differing between the homozygous risk and nonrisk groups as a single region of interest (ROI) in analyses that included the intermediate genotype group and that were further stratified by diagnostic status. This approach allowed us to compare all possible subgroups in a sensitive and unbiased fashion.

Functional Activation Patterns to Emotional Faces

We performed fMRI in a cohort of 144 children and adolescents, including 78 TD (homozygous risk, n = 28; heterozygous risk, n = 34; homozygous nonrisk, n = 16) and 66 diagnosed with ASD (homozygous risk, n = 15; heterozygous risk, n = 39; homozygous nonrisk, n = 12; Table S1), during passive observation of faces displaying different emotions (angry, fearful, happy, sad, and neutral; with fixation crosses directing attention to the eye region as previously reported (Dapretto et al., 2006; Pfeifer et al., 2008, 2011). Across all subjects (independent of diagnosis), we observed strong correlations between the *MET* risk allele and unique patterns of functional brain activity. Remarkably, compared to the nonrisk group (n = 28), the risk group (n = 43) displayed a pattern of hyperactivation and reduced deactivation in the specific regions in which MET is expressed in primates and humans (Mukamel et al., 2011; Judson et al., 2011a; Figure 1A; Table S2). The risk and nonrisk groups both activated primary/secondary visual cortices, thalamus, and amygdala; however, the risk group activated amygdala and striatum more robustly than the nonrisk group. Additionally, the nonrisk group displayed widespread deactivation (i.e., reduced activity while viewing faces versus fixation crosses). The deactivation was most prominently displayed in midline structures of the DMN including the posterior cingulate cortex (PCC) and perisylvian regions centered on primary auditory cortex. In contrast, the intermediate-risk group did deactivate, but not to the same extent as the nonrisk group, and the risk group appeared to show slight activation in these regions on average (Figure 1B). In a whole-brain comparison between TD and ASD groups, there was also evidence for reduced deactivation in similar temporal, frontal, and subcortical regions in individuals with ASD (Figure S1A). To investigate the risk allele's inheritance pattern, we compared the average activity across regions differing between the risk and nonrisk groups for all three genotype groups stratified into either TD or ASD subgroups. We found that the *MET* promoter variant has a differential penetrance between neurotypical and autistic individuals. Specifically, TD individuals with one risk allele showed a similar deactivation pattern to those without a risk allele (Figure 1B). In contrast, in individuals with ASD, one *MET* risk allele was sufficient to give rise to the atypical pattern of functional activity, showing less deactivation than the nonrisk group. In fact, when comparing those with one risk allele, individuals with ASD exhibited significantly less deactivation in these regions compared to TD subjects, indicative of an even more atypical phenotype in the clinical population with the same *MET* risk genotype. Consistent with the ROI analysis, a whole-brain comparison of TD versus ASD subgroups within the heterozygous risk group found stronger and more widespread differences than those observed when comparing the TD and ASD groups across genotype (Figure S1B; Table S3).

DMN Functional Connectivity

Based on prior reports of altered DMN function in ASD (Kennedy et al., 2006; Kennedy and Courchesne, 2008) and MET's high expression in the PCC (Judson et al., 2011a), as well as our finding of atypical DMN deactivation in MET risk carriers, we next examined the extent to which the *MET* functional risk variant modulates intrinsic DMN functional connectivity. We used a seed centered in the PCC (Fox et al., 2005) for whole-brain functional connectivity analyses in rs-fcMRI data in a matched sample of 33 TD and 38 children and adolescents diagnosed with ASD. The results were remarkably consistent with the functional activation findings: the *MET* risk genotype significantly modulated functional connectivity, such that those in the highest risk group (CC; n = 16) had reduced intrinsic connectivity between the PCC and MPFC as well as other nearby regions in the PCC compared to the nonrisk group (n = 16; Figure 2A;

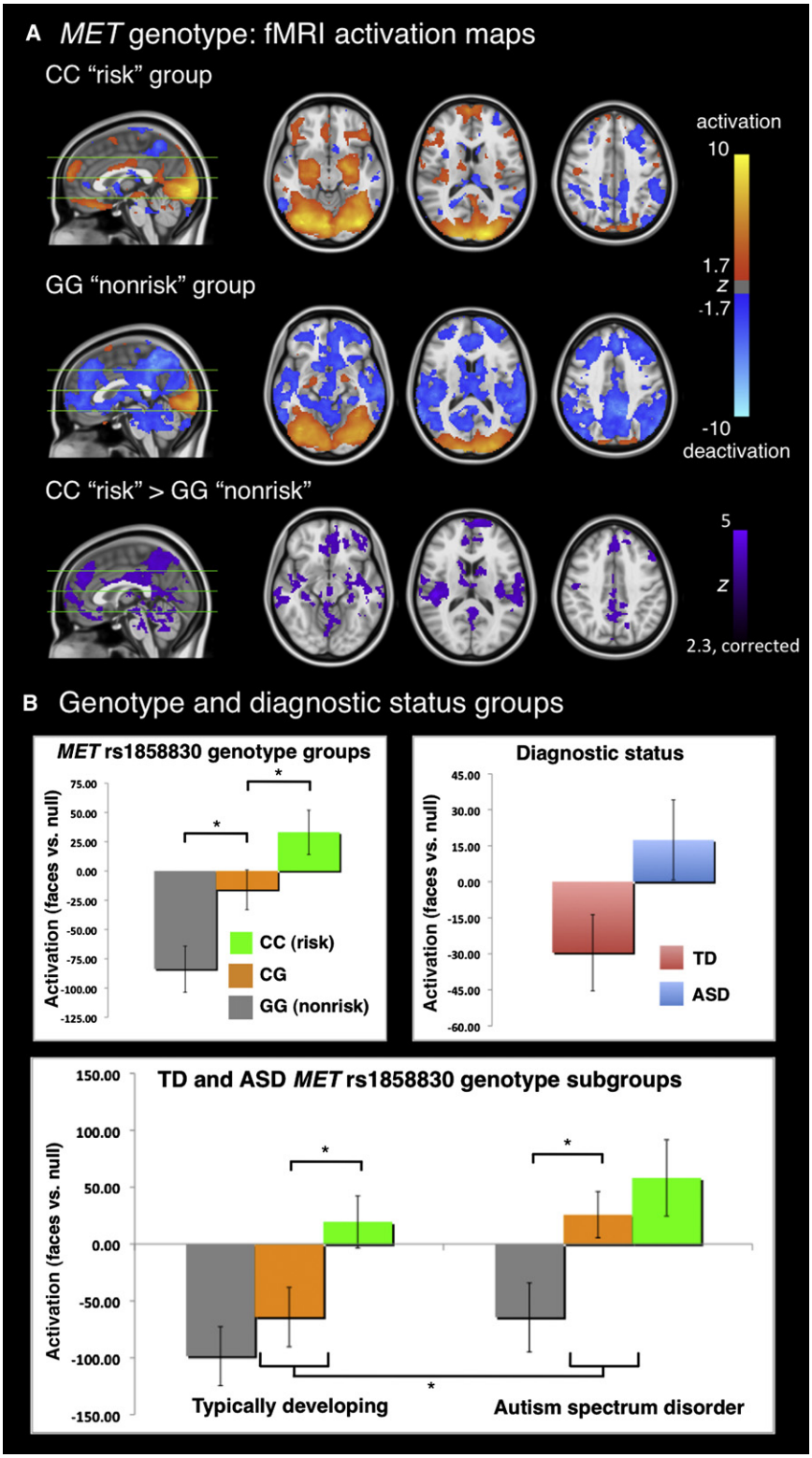

Figure 1. fMRI Activation Patterns to Emotional Faces in *MET* Risk Carriers

(A) Within group whole-brain activation (orange) and deactivation (blue) maps for CC "risk" group, GG "nonrisk" group, and between groups (risk > nonrisk; purple).

(B) Averages and SEs for functional activation parameter estimates from regions in risk > nonrisk contrast for each genotype phenotype subgroup (full-scale IQ and MRI scanner included as covariates in 2X3 ANOVA model). *p < 0.05.

See also Figure S1.

Table S4). In agreement with the functional activation analyses, the heterozygous risk group diagnosed with ASD (n = 24) showed a pattern of functional connectivity similar to that observed in the homozygous risk group, whereas functional connectivity in the TD heterozygous risk group (n = 15) was no different than the homozygous nonrisk group. Collapsed across genotype, the ASD group exhibited reduced PCC-MPFC connectivity relative to the TD group (Figure 2B). A whole-brain analysis comparing TD and ASD groups independent of genotype revealed similar, and even more extensive, reductions in DMN connectivity as a function of ASD diagnosis (Figure S2B). This diagnostic effect appeared to be partially driven by a stronger penetrance of the *MET* risk allele in the ASD group, as significant differences between TD and ASD subgroups were only observed in risk carriers (Figure 2B); indeed, *MET* genotype explained 1.7 times as much variance in DMN connectivity in autistic relative to neurotypical individuals. Using an additional seed within the MPFC, we confirmed that both short- and long-range intrinsic DMN functional connectivity was reduced as a function of both MET risk genotype and ASD diagnosis (Figure S2D; Table S5).

WM Structural Connectivity

To obtain a third line of evidence for the impact of the *MET* risk allele on brain circuitry, we examined the structural integrity of WM tracts across the whole brain in a cohort of 84 children and adolescents (TD, n = 38; ASD, n = 46). Notably, the *MET* risk genotype predicted marked reductions in FA across a restricted number of major WM tracts known to connect the very same regions previously implicated in our functional connectivity analyses. Compared to nonrisk allele homozygotes (n = 19), risk allele homozygotes (n = 23) displayed lower FA in multiple major tracts in temporo-parieto-occipital regions that

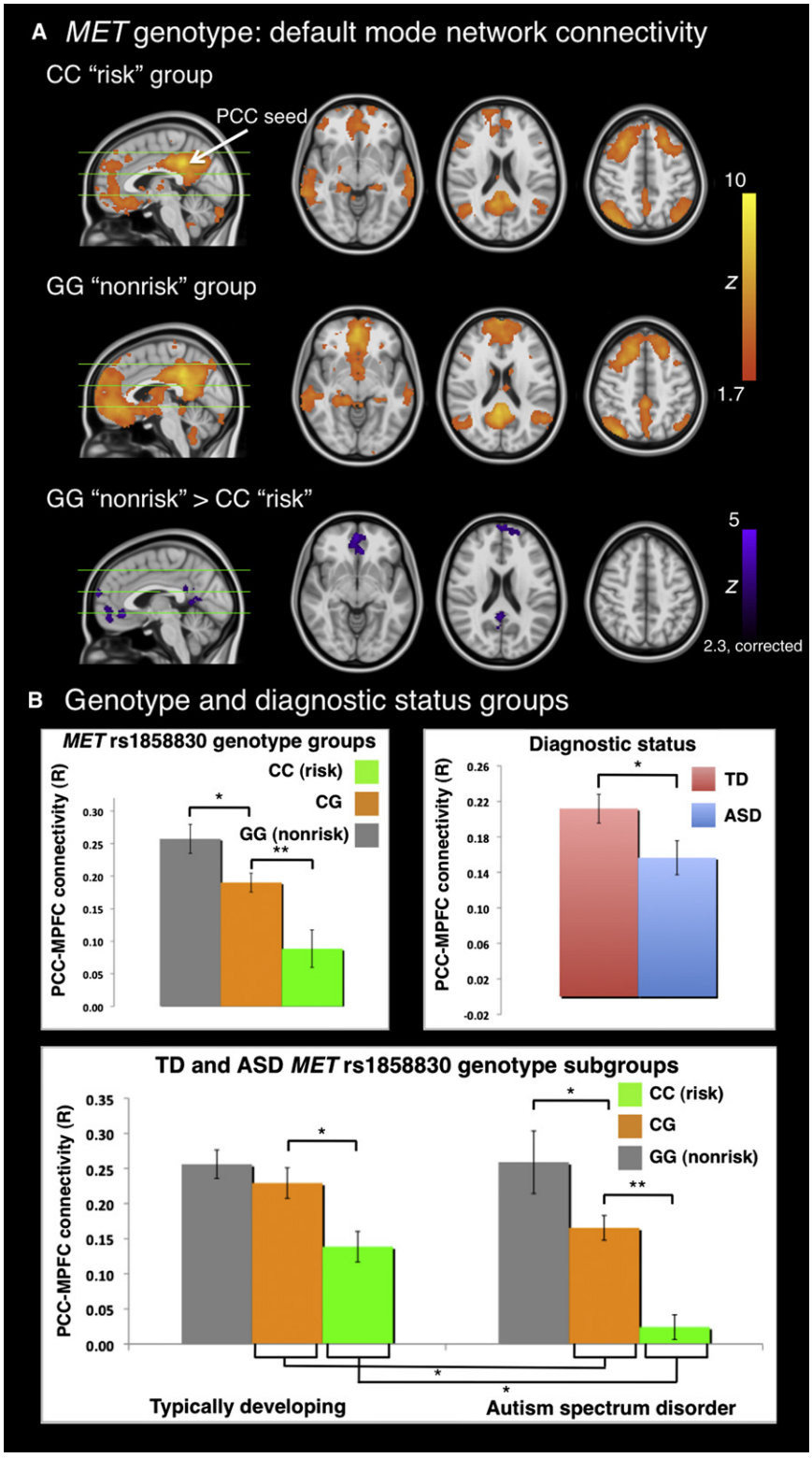

Figure 2. Reduced DMN Functional Connectivity in *MET* Risk Carriers

(A) DMN connectivity within CC "risk" group, GG "nonrisk" group, and between groups (risk > nonrisk; purple).

(B) Averages and SEs for functional connectivity between posterior cingulate seed and medial prefrontal and frontal orbital clusters from GG > CC contrast for each genotype phenotype subgroup (age and IQ included as covariates in 2X3 ANOVA). *p < 0.05, **p < 0.01.

See also Figure S2.

exhibit high *MET* expression developmentally (i.e., splenium of the corpus callosum, superior/inferior longitudinal fasciculus, and cingulum; Figure 3A; Table S6). Consistent with the observed functional connectivity patterns, in these tracts the *MET* risk allele had a stronger impact in individuals with ASD (Figure 3B), explaining nearly twice (1.9 times) as much variance in the ASD group. More specifically, ASD heterozygous risk allele carriers (n = 25) and homozygous risk allele carriers (n = 12) both exhibited strong reductions in FA, whereas structural connectivity was only significantly impacted in TD homozygous risk carriers (n = 11). This was also true for follow-up whole-brain analyses looking at the additive effect of the MET risk allele in the TD and ASD groups independently (Figure S3). Somewhat surprisingly, whole-brain analyses directly comparing TD and ASD groups, independent of genotype, found relatively minimal reductions in FA for the ASD compared to TD group (Figure S3; Table S6).

Correlation between Imaging and Behavioral Measures

Within the ASD group, we correlated scores on the ADOS social subscale (Lord et al., 2000), with measures derived from the imaging analyses. Lower levels of deactivation while viewing emotional expressions, as well as functional and structural connectivity, were significantly associated with higher levels of social impairment in the ASD group overall (Figure S4). However, as previously noted, we also found a direct relationship between *MET* risk genotype and increased symptom severity within individuals with ASD. Indeed, the relationship between brain circuitry and symptom severity was no longer significant when covarying for *MET* risk genotype, suggesting that *MET* risk genotype may contribute to both alterations in brain circuitry and disrupted social behavior.

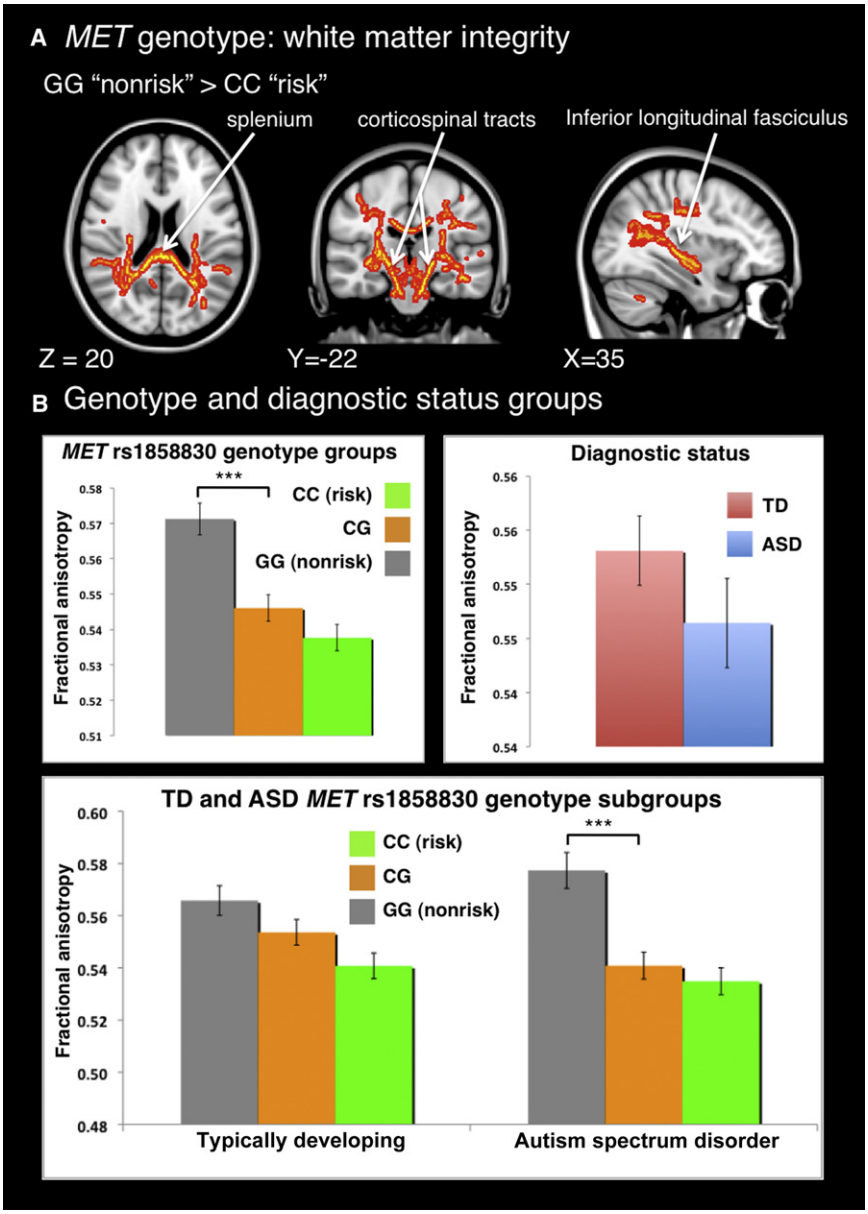

Figure 3. Reduced WM Integrity in *MET* Risk Carriers

(A) Results of TBSS analysis comparing FA in GG "nonrisk" group versus CC "risk" group ($p < 0.05$, corrected).

(B) Averages and SEs for FA values in tracts from nonrisk > risk contrast for each genotype phenotype subgroup (age and IQ included as covariates in 2X3 ANOVA). ***$p < 0.001$.

See also Figure S3.

DISCUSSION

In the present study, we used a multimodal imaging genetics approach to examine the impact of a common functional variant in *MET* on neuroimaging endophenotypes known to be disrupted in ASD. First, we found that, irrespective of clinical diagnosis, the functional promoter "C" allele of *MET* alters functional activity patterns to social stimuli, DMN functional connectivity, and WM integrity. Second, individuals with ASD exhibited similar circuit alterations for all three measures. Third, the *MET* risk allele appeared to have a stronger impact across individuals with ASD, especially within the heterozygous risk group. Fourth, the most impacted circuits in our study included the very regions that exhibit the greatest *MET* expression in the developing neocortex, including circuits that subserve processing of socially relevant information. And lastly, measures of structural and functional circuitry correlated with symptom severity in the expected direction, although this correlation was driven by the fact that *MET* risk genotype was associated with both increased symptom severity and alterations in brain circuitry. These findings highlight a key principle that is consistent with the concept of endophenotypes (Gottesman and Gould, 2003), whereby a functional risk allele predisposing to a disorder will have a larger impact on disorder-relevant phenotypes (i.e., relevant to the function of the gene) than the disorder itself. Thus, the present data suggest that taking into account *MET* risk genotype will serve as a sound strategy to stratify individuals with ASD and gain insight into the neurobiological bases of the functional heterogeneity that characterizes ASD (Figure 4).

Functional Activation Patterns

In our analyses, we first focused on functional activation patterns in response to the passive observation of emotional facial expressions in a large sample of 66 ASD and 78 TD subjects. The high expression of MET in ventral temporal cortex, including the amygdala and fusiform gyrus, prompted us to test whether the "C" risk allele might impact activity in these regions in response to socially relevant and affect-laden stimuli. While early studies of emotional face processing documented amygdala and fusiform hypoactivation in ASD (Baron-Cohen et al., 2000; Critchley et al., 2000; Schultz et al., 2000), later studies that better controlled for eye gaze (such as a fixation cross that directs gaze at the eyes, similar to the one used in the present study) found either no differences or hyperactivation in these regions (Hadjikhani et al., 2004; Pierce et al., 2004; Dalton et al., 2005; Monk et al., 2010). Here, we found that *MET* risk genotype was associated with hyperactivation of amygdala and striatum, as well as the relatively unexpected finding of reduced deactivation in temporal and midline neocortex. These latter areas comprise circuits that have the highest MET

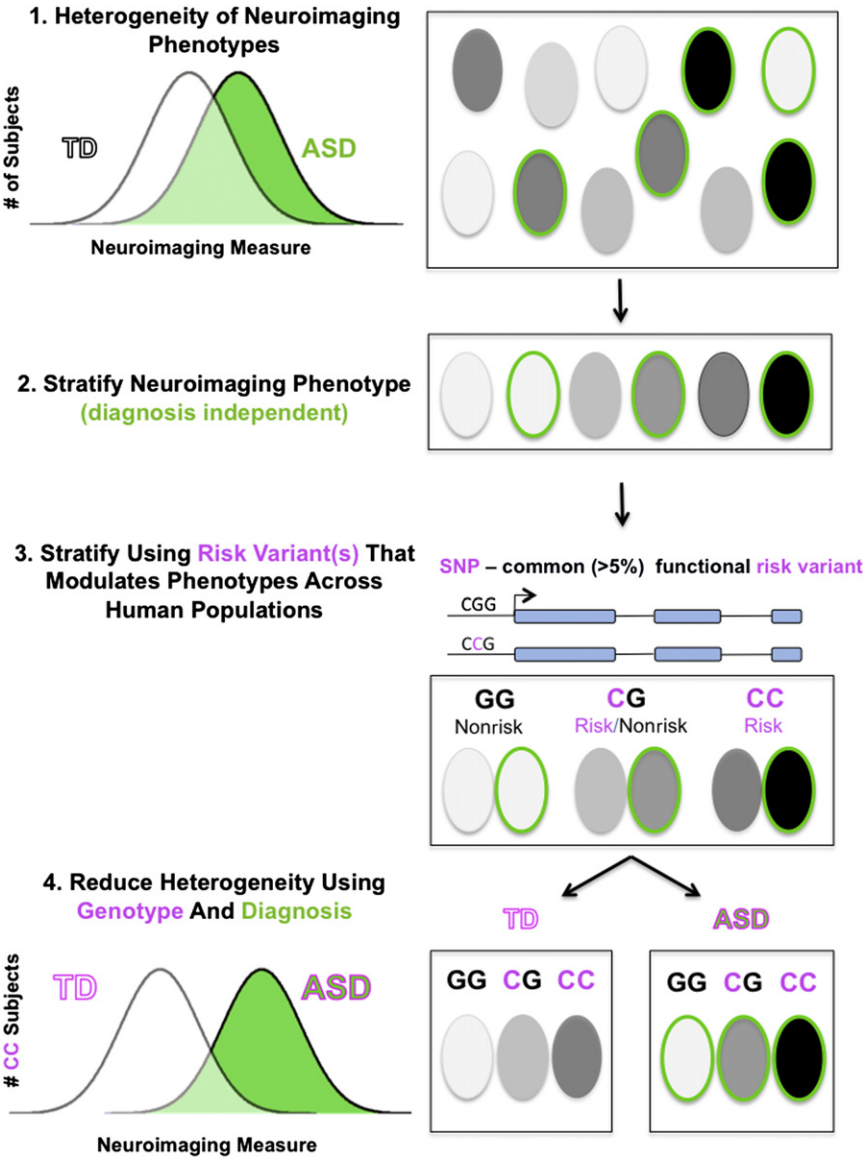

Figure 4. Schematic Depicting a Strategy for Addressing Phenotypic Heterogeneity

The shading of the ovals indicates variability in a given phenotypic measure (e.g., brain connectivity). The green outline of the ovals indicates individuals with a clinical diagnosis (e.g., ASD) relative to TD controls. Although group differences on a phenotypic measure may be detected between a clinical sample and matched controls, considerable overlap often exists (1). Stratifying individuals by neuroimaging endophenotypes independent of diagnosis reveals a continuum of phenotypes (2). Common risk variants (>5% of the population) for a disorder (e.g., MET rs1858830 C/G SNP) may impact brain circuitry and thus offer a means to stratify populations, particularly when these variants are functional in nature (3). Sample stratification by diagnosis and genotype allows for enhanced parsing of phenotypic heterogeneity (4), ultimately providing new insights into the neural mechanisms underlying psychiatric disorders.

expression in developing humans and monkeys (Judson et al., 2011a; Mukamel et al., 2011). In whole-brain analyses comparing TD and ASD groups, we also found evidence for reduced deactivation in temporal and DMN regions in ASD subjects, although there were no significant differences in the amygdala and regions of occipital fusiform gyrus corresponding to the fusiform face area.

Overall, the *MET* risk group and ASD subjects (particularly the intermediate-risk group) showed less deactivation in multiple cortical and subcortical regions. Deactivation is a less well-characterized phenomenon in fMRI, but the DMN is known to show signal decreases in response to a variety of tasks requiring externally directed attention (Raichle et al., 2001). Interestingly, task-induced DMN deactivation was shown to have a neuronal origin (Lin et al., 2011), so it may relate to intrinsic inhibitory properties of local cortical circuits. Few studies have focused on differences in deactivation in ASD, but our findings are highly consistent with those of Kennedy et al. (2006), who reported that individuals with ASD exhibit less deactivation within regions of the DMN. The auditory cortex is also known to deactivate during visual tasks (Laurienti et al., 2002; Mozolic et al., 2008), and in our study the auditory cortex exhibited the strongest deactivation differences between genotype groups during this visual task. These findings of reduced deactivation of perisylvian and DMN regions in *MET* risk carriers may relate to a failure to appropriately suppress neuronal activity, perhaps through an enhancement of local connectivity that was influenced by MET during development, as reported in the *Met* mutant mouse by Qiu et al. (2011). Future imaging and neurophysiological studies are needed to test this hypothesis.

Functional and Structural Connectivity

The fact that *MET* risk carriers displayed altered DMN deactivation patterns further prompted us to test whether the risk allele impacts intrinsic functional connectivity in this network, particularly since DMN connectivity has consistently been shown to be disrupted in ASD (Cherkassky et al., 2006; Kennedy and Courchesne, 2008; Monk et al., 2009; Weng et al., 2010; Assaf et al., 2010; Rudie et al., 2012). Indeed, we found that *MET* risk carriers and individuals with ASD exhibited reductions in long- as well as short-range DMN connectivity. The combination of reduced deactivation and connectivity supports the notion that the DMN is both less integrated with itself and less segregated from other neural systems in both *MET* risk carriers

and individuals with ASD (Rudie et al., 2012). Additionally, these findings suggest that functional alterations in the DMN represent a trait marker shared in those with, or at risk for, ASD. Future work should characterize functional connectivity alterations in other networks as a function of the MET risk allele.

Next, we examined whether structural connectivity was altered in *MET* risk carriers, as the MET protein is highly expressed during axon outgrowth in specific WM tracts in primates (Judson et al., 2011a). Remarkably, the presence of the *MET* risk allele was associated with much stronger disruptions in WM integrity than having an ASD diagnosis. The effects were most pronounced in temporo-parietal regions of high *MET* expression and especially within the splenium, which includes fiber pathways originating from the posterior cingulate/precuneus of the DMN. This hub region, implicated in all three imaging analyses, has been characterized as the structural core of the human connectome (Hagmann et al., 2008). The combined array of imaging findings is consistent with experiments showing the involvement of *MET* in neurodevelopmental processes including dendritic and axon growth and synaptogenesis that underlie circuit development (Judson et al., 2011b for review). The reduction in *MET* expression due to the functional promoter polymorphism may affect structure formation and ongoing synaptic function independently. Additional work is needed to clarify structure-function relationships with regard to both MET-mediated and ASD-general alterations in connectivity.

Enhanced Effect of *MET* Risk Allele in ASD

Perhaps most surprisingly, the cumulative data suggest that the *MET* "C" risk allele has a greater effect in individuals with ASD. Beyond the rare, highly penetrant SNVs and CNVs, ASD appears to have a combinatorial etiology (Geschwind, 2011), likely due to the influence of other factors that shape circuits underlying social behavior and communication. Across all three imaging measures, the neuroimaging endophenotypes of the ASD intermediate-risk (heterozygote) group were similar to those observed in the high-risk (homozygote) group, whereas the neuroimaging phenotypes of the TD intermediate-risk group resembled those of the nonrisk group. This is consistent with the notion that multiple genetic and/or environmental factors contribute to both disrupted *MET* expression and atypical circuitry in individuals with ASD. In fact, we previously found that carriers of a common risk allele in *CNTNAP2* also display alterations in functional and structural connectivity (Scott-Van Zeeland et al., 2010; Dennis et al., 2011). In addition to *CNTNAP2* and *MET* modulating brain connectivity, transcription of both genes is regulated by FOXP2 (Vernes et al., 2008; Mukamel et al., 2011), which is known to pattern speech and language circuits in humans (Konopka et al., 2009). Consistent with a multiple-hit model, these findings collectively indicate that in individuals with ASD, who likely have additional alterations in the MET signaling pathway, the presence of the *MET* promoter risk allele results in more severely impacted brain circuitry and social behavior.

Relevance to ASD Connectivity Theories

The converging imaging findings reported here provide a mechanistic link, through MET disruption, to the previously hypothesized relationship between altered local circuit and long-range network connectivity in ASD (Belmonte et al., 2004; Courchesne and Pierce, 2005; Geschwind and Levitt, 2007; Qiu et al., 2011). Moreover, the present results draw a striking parallel with alterations in neuronal architecture and synaptic functioning abnormalities found in Met-disrupted mice (Judson et al., 2010; Qiu et al., 2011). Local circuit hyperconnectivity at the neocortical microcircuit level seen in conditional Met null/heterozygous mice may lead to the hyperactivation/reduced deactivation we observed in humans with *MET* risk alleles. While speculative at this point, this may in part account for the presence of enhanced visual and auditory discrimination (Baron-Cohen et al., 2009; Jones et al., 2009; Ashwin et al., 2009) or sensory overresponsivity, observed in some individuals with ASD (Ben-Sasson et al., 2007; Baranek et al., 2006). Alterations in local circuit connectivity and/or structural connections may ultimately hinder the typical formation of long-range connectivity (Dosenbach et al., 2010) as observed in both *MET* risk allele carriers and individuals with ASD.

Addressing Phenotype Overlap and Heterogeneity in Neurodevelopmental Disorders

We found that structural and functional connectivity was related to autism symptom severity, particularly in the social domain. However, this relationship was mediated by the fact that the *MET* risk allele was associated with increased symptom severity and reduced functional and structural connectivity. This result, in combination with the finding that, across all imaging measures, TD individuals with two risk alleles exhibited more "atypical" brain circuitry than individuals with ASD carrying no risk alleles, reveals one possible generalized mechanism for phenotype overlap that is observed across nonclinical and clinical groups (Figure 4). This raises critical issues regarding the causal nature of altered connectivity findings in ASD, and the role of a combination of genetic and environmental factors that may contribute to phenotypes that collectively lead to a clinical diagnosis. The idea that functional and structural alterations may at least in part reflect genetic vulnerability is also supported by recent studies showing greater similarity in brain measures between individuals with ASD and their unaffected siblings than between controls and unaffected siblings (Kaiser et al., 2010; Spencer et al., 2011), which is particularly the case for DTI measures (Barnea-Goraly et al., 2010). The present study highlights the critical need for future research to take into consideration relevant genetic factors to parse the heterogeneity present in neurodevelopmental disorders and behavioral phenotypes (Figure 4) to ultimately improve diagnostic or prognostic tools (Fox and Greicius, 2010).

Limitations and Future Directions

Although these findings are useful for developing a more mechanistic understanding of the neurobiology of ASD, the present study focuses on common variation in a single candidate gene. Future work should characterize the additive effects of, and interactions between, multiple risk alleles in the context of both typical and atypical development. Future research should also attempt to combine different genetic, structural, and functional measures to test the direction of influence that these may have

on one another at the individual level. These types of analyses will require much larger data sets likely available only through large-scale collaborative efforts such as the human connectome project (HCP) (Marcus et al., 2011) and the autism brain imaging data exchange (ABIDE), a grass roots initiative under the international neuroimaging data-sharing initiative (INDI) (Biswal et al., 2010). Additionally, given that some network alterations are present in typical individuals who simply carry risk alleles, future study designs should include unaffected siblings to tease apart alterations that are related to genetic risk for ASD (i.e., present in both affected and unaffected siblings) from those that are specific to actually having the disorder (i.e., present only in sibling with an ASD diagnosis).

Conclusions

Here, we show how a functional ASD risk allele predisposes to ASD by affecting functional activity, connectivity, and WM tract integrity in regions involved in social cognition. This study reports converging evidence of altered brain function and connectivity across three different brain measures, both in individuals with a disorder and those carrying a genetic risk factor for that disorder. These findings have a number of broad implications. First, these results reveal an enhanced penetrance of a risk allele within individuals with ASD, reflecting a mechanism whereby a common functional variant that is not disorder causing, but in the context of other factors related to ASD etiology, has a larger effect on network structure and function than in neurotypical individuals. Second, given that differences between ASD and controls were moderated by *MET* risk genotype and in the case of functional activity were only revealed when the cohort was stratified by *MET* genotype, these data demonstrate the power of utilizing genetic data for understanding and parsing phenotypic heterogeneity in ASD as well as other neuropsychiatric disorders characterized by considerable heterogeneity (e.g., Rasetti and Weinberger, 2011; Figure 4). This approach may provide a more sensitive means to identify subgroups of individuals with particular risk alleles and brain circuitry for whom targeted treatments may be developed. Finally, expanding upon our prior findings linking a *CNTNAP2* common variant to brain connectivity (Scott-Van Zeeland et al., 2010; Dennis et al., 2011), the discovery that the *MET* risk allele has large effect sizes on structural and functional brain circuitry in both typical and atypical development indicates that some alterations in brain networks in ASD may, in part, reflect genetic vulnerability, or liability, rather than causal mechanisms. Taken together, the current results indicate that considering relevant genetic factors when interpreting neuroimaging data will greatly aid in understanding, and ultimately treating, ASD and other clinically and genetically heterogeneous neuropsychiatric disorders.

EXPERIMENTAL PROCEDURES

Subjects

High-functioning children and adolescents with ASD and TD children were recruited from the greater Los Angeles area to participate in this study. Informed consent and assent to participate were obtained prior to assessment under our institutional review board-approved protocols. Details regarding recruitment, consent, and sample demographics are included in Supplemental Experimental Procedures and Table S1.

Genotyping

Subjects provided saliva samples for genetic analysis. DNA was isolated from saliva using standard protocols from the OraGene Collection Kit (DNA Genotek, Ontario, Canada). Genotypes at rs1858830 were determined by direct sequencing, as described elsewhere by Campbell et al. (2007) and detailed in the Supplemental Experimental Procedures.

MRI Data Acquisition

A total of 75 individuals with ASD and 87 TD individuals were included in at least one of the three data sets (fMRI, rs-fcMRI, and DTI) detailed in Table S1. The fMRI data were collected across two different scanners (Siemens 3T Trio and Siemens 3T Allegra), while all of the DTI and rs-fcMRI data were collected on a Siemens 3T Trio scanner. See Supplemental Experimental Procedures for MRI acquisition details.

fMRI Task Data Analysis

Participants underwent a rapid event-related fMRI paradigm in which they simply observed faces displaying different emotions (see Dapretto et al., 2006; Pfeifer et al., 2008, 2011). These data underwent standard fMRI preprocessing including motion correction, brain extraction, spatial smoothing, and normalization to standard space. The contrast of all emotional faces versus null events was examined at the group level using a mixed effects model. See Supplemental Experimental Procedures for further details.

rs-fcMRI Data Analysis

In a single resting state session, subjects were told to relax and keep their eyes open while a fixation cross was displayed on a white background for 6 min. In addition to all of the preprocessing steps described above for the task-related fMRI scan, we band-pass filtered ($0.1\ \text{Hz} > t > 0.01\ \text{Hz}$) the data and regressed out nuisance covariates, including six rigid body motion parameters, volumes corresponding to motion spikes, and average WM, cerebrospinal fluid (CSF), and global time series. Average time series from 5 mm radius spheres in the PCC and MPFC within the DMN (Fox et al., 2005) were correlated with every voxel in the brain to generate connectivity maps for each subject, which were compared between participants using ordinary least-squares regression. See Supplemental Experimental Procedures for further details.

DTI Data Analysis

We examined FA across the whole brain using Tract-Based Spatial Statistics (TBSS version 1.2; Smith et al., 2006). Data analysis consisted of removal of images with gross artifacts, motion and eddy current correction, brain extraction, fitting a tensor model and calculating FA at each voxel, nonlinear registration to a template brain in standard space, skeletonization of tracts, and voxel-wise inference testing through permutation testing as implemented with Randomise. See Supplemental Experimental Procedures for further details.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, six tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2012.07.010.

ACKNOWLEDGMENTS

This work was supported by NICHD Grant P50 HD055784 (to S.Y.B.), NIMH Grants R01 HD06528001 (to S.Y.B.), NIMH 1R01 MH080759 (to P.L.), T32 GM008044 (to J.D.R.), T32 MH073526-05 (to J.D.R.), NIH Grants (RR12169, RR13642, and RR00865), and Autism Speaks. We thank Z. Shehzad, B. Abrahams, and K. Eagleson for commenting on the manuscript as well as J. Pfiefer, K. McNeally, L. Borofsky, A. Martin, and B. Way for help with data collection.

Accepted: July 5, 2012
Published: September 5, 2012

REFERENCES

Alexander, A.L., Lee, J.E., Lazar, M., Boudos, R., DuBray, M.B., Oakes, T.R., Miller, J.N., Lu, J., Jeong, E.K., McMahon, W.M., et al. (2007). Diffusion tensor imaging of the corpus callosum in Autism. Neuroimage *34*, 61–73.

Anderson, J.S., Druzgal, T.J., Froehlich, A., DuBray, M.B., Lange, N., Alexander, A.L., Abildskov, T., Nielsen, J.A., Cariello, A.N., Cooperrider, J.R., et al. (2011). Decreased interhemispheric functional connectivity in autism. Cereb. Cortex *21*, 1134–1146.

Anney, R., Klei, L., Pinto, D., Regan, R., Conroy, J., Magalhaes, T.R., Correia, C., Abrahams, B.S., Sykes, N., Pagnamenta, A.T., et al. (2010). A genome-wide scan for common alleles affecting risk for autism. Hum. Mol. Genet. *19*, 4072–4082.

Ashwin, E., Ashwin, C., Rhydderch, D., Howells, J., and Baron-Cohen, S. (2009). Eagle-eyed visual acuity: an experimental investigation of enhanced perception in autism. Biol. Psychiatry *65*, 17–21.

Assaf, M., Jagannathan, K., Calhoun, V.D., Miller, L., Stevens, M.C., Sahl, R., O'Boyle, J.G., Schultz, R.T., and Pearlson, G.D. (2010). Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients. Neuroimage *53*, 247–256.

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E., and Rutter, M. (1995). Autism as a strongly genetic disorder: evidence from a British twin study. Psychol. Med. *25*, 63–77.

Baranek, G.T., David, F.J., Poe, M.D., Stone, W.L., and Watson, L.R. (2006). Sensory Experiences Questionnaire: discriminating sensory features in young children with autism, developmental delays, and typical development. J. Child Psychol. Psychiatry *47*, 591–601.

Barnea-Goraly, N., Kwon, H., Menon, V., Eliez, S., Lotspeich, L., and Reiss, A.L. (2004). White matter structure in autism: preliminary evidence from diffusion tensor imaging. Biol. Psychiatry *55*, 323–326.

Barnea-Goraly, N., Lotspeich, L.J., and Reiss, A.L. (2010). Similar white matter aberrations in children with autism and their unaffected siblings: a diffusion tensor imaging study using tract-based spatial statistics. Arch. Gen. Psychiatry *67*, 1052–1060.

Baron-Cohen, S., Ring, H.A., Bullmore, E.T., Wheelwright, S., Ashwin, C., and Williams, S.C. (2000). The amygdala theory of autism. Neurosci. Biobehav. Rev. *24*, 355–364.

Baron-Cohen, S., Ashwin, E., Ashwin, C., Tavassoli, T., and Chakrabarti, B. (2009). Talent in autism: hyper-systemizing, hyper-attention to detail and sensory hypersensitivity. Philos. Trans. R. Soc. Lond. B Biol. Sci. *364*, 1377–1383.

Belmonte, M.K., Allen, G., Beckel-Mitchener, A., Boulanger, L.M., Carper, R.A., and Webb, S.J. (2004). Autism and abnormal development of brain connectivity. J. Neurosci. *24*, 9228–9231.

Ben-Sasson, A., Cermak, S.A., Orsmond, G.I., Tager-Flusberg, H., Carter, A.S., Kadlec, M.B., and Dunn, W. (2007). Extreme sensory modulation behaviors in toddlers with autism spectrum disorders. Am. J. Occup. Ther. *61*, 584–592.

Biswal, B.B., Mennes, M., Zuo, X.N., Gohel, S., Kelly, C., Smith, S.M., Beckmann, C.F., Adelstein, J.S., Buckner, R.L., Colcombe, S., et al. (2010). Toward discovery science of human brain function. Proc. Natl. Acad. Sci. USA *107*, 4734–4739.

Bode, M.K., Mattila, M.L., Kiviniemi, V., Rahko, J., Moilanen, I., Ebeling, H., Tervonen, O., and Nikkinen, J. (2011). White matter in autism spectrum disorders—evidence of impaired fiber formation. Acta Radiol. *52*, 1169–1174.

Buxbaum, J.D., Betancur, C., Bozdagi, O., Dorr, N.P., Elder, G.A., and Hof, P.R. (2012). Optimizing the phenotyping of rodent ASD models: enrichment analysis of mouse and human neurobiological phenotypes associated with high-risk autism genes identifies morphological, electrophysiological, neurological, and behavioral features. Mol. Autism *3*, 1.

Campbell, D.B., Sutcliffe, J.S., Ebert, P.J., Militerni, R., Bravaccio, C., Trillo, S., Elia, M., Schneider, C., Melmed, R., Sacco, R., et al. (2006). A genetic variant that disrupts MET transcription is associated with autism. Proc. Natl. Acad. Sci. USA *103*, 16834–16839.

Campbell, D.B., D'Oronzio, R., Garbett, K., Ebert, P.J., Mirnics, K., Levitt, P., and Persico, A.M. (2007). Disruption of cerebral cortex MET signaling in autism spectrum disorder. Ann. Neurol. *62*, 243–250.

Campbell, D.B., Li, C., Sutcliffe, J.S., Persico, A.M., and Levitt, P. (2008). Genetic evidence implicating multiple genes in the MET receptor tyrosine kinase pathway in autism spectrum disorder. Autism Res. *1*, 159–168.

Campbell, D.B., Warren, D., Sutcliffe, J.S., Lee, E.B., and Levitt, P. (2010). Association of MET with social and communication phenotypes in individuals with autism spectrum disorder. Am. J. Med. Genet. B. Neuropsychiatr. Genet. *153B*, 438–446.

Castelli, F., Frith, C., Happé, F., and Frith, U. (2002). Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. Brain *125*, 1839–1849.

Cheng, Y., Chou, K.H., Chen, I.Y., Fan, Y.T., Decety, J., and Lin, C.P. (2010). Atypical development of white matter microstructure in adolescents with autism spectrum disorders. Neuroimage *50*, 873–882.

Cherkassky, V.L., Kana, R.K., Keller, T.A., and Just, M.A. (2006). Functional connectivity in a baseline resting-state network in autism. Neuroreport *17*, 1687–1690.

Cheung, C., Chua, S.E., Cheung, V., Khong, P.L., Tai, K.S., Wong, T.K., Ho, T.P., and McAlonan, G.M. (2009). White matter fractional anisotrophy differences and correlates of diagnostic symptoms in autism. J. Child Psychol. Psychiatry *50*, 1102–1112.

Chiang, M.C., McMahon, K.L., de Zubicaray, G.I., Martin, N.G., Hickie, I., Toga, A.W., Wright, M.J., and Thompson, P.M. (2011). Genetics of white matter development: a DTI study of 705 twins and their siblings aged 12 to 29. Neuroimage *54*, 2308–2317.

Courchesne, E., and Pierce, K. (2005). Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. Curr. Opin. Neurobiol. *15*, 225–230.

Critchley, H.D., Daly, E.M., Bullmore, E.T., Williams, S.C., Van Amelsvoort, T., Robertson, D.M., Rowe, A., Phillips, M., McAlonan, G., Howlin, P., and Murphy, D.G. (2000). The functional neuroanatomy of social behaviour: changes in cerebral blood flow when people with autistic disorder process facial expressions. Brain *123*, 2203–2212.

Dalton, K.M., Nacewicz, B.M., Johnstone, T., Schaefer, H.S., Gernsbacher, M.A., Goldsmith, H.H., Alexander, A.L., and Davidson, R.J. (2005). Gaze fixation and the neural circuitry of face processing in autism. Nat. Neurosci. *8*, 519–526.

Dapretto, M., Davies, M.S., Pfeifer, J.H., Scott, A.A., Sigman, M., Bookheimer, S.Y., and Iacoboni, M. (2006). Understanding emotions in others: mirror neuron dysfunction in children with autism spectrum disorders. Nat. Neurosci. *9*, 28–30.

Dennis, E.L., Jahanshad, N., Rudie, J.D., Brown, J.A., Johnson, K., McMahon, K.L., de Zubicaray, G.I., Montgomery, G., Martin, N.G., Wright, M.J., et al. (2011). Altered structural brain connectivity in healthy carriers of the autism risk gene, CNTNAP2. Brain Connect. *1*, 447–459.

Di Martino, A., Ross, K., Uddin, L.Q., Sklar, A.B., Castellanos, F.X., and Milham, M.P. (2009). Functional brain correlates of social and nonsocial processes in autism spectrum disorders: an activation likelihood estimation meta-analysis. Biol. Psychiatry *65*, 63–74.

Dinstein, I., Pierce, K., Eyler, L., Solso, S., Malach, R., Behrmann, M., and Courchesne, E. (2011). Disrupted neural synchronization in toddlers with autism. Neuron *70*, 1218–1225.

Dosenbach, N.U., Nardos, B., Cohen, A.L., Fair, D.A., Power, J.D., Church, J.A., Nelson, S.M., Wig, G.S., Vogel, A.C., Lessov-Schlaggar, C.N., et al. (2010). Prediction of individual brain maturity using fMRI. Science *329*, 1358–1361.

Eagleson, K.L., Campbell, D.B., Thompson, B.L., Bergman, M.Y., and Levitt, P. (2011). The autism risk genes MET and PLAUR differentially impact cortical development. Autism Res. *4*, 68–83.

Fornito, A., Zalesky, A., Bassett, D.S., Meunier, D., Ellison-Wright, I., Yücel, M., Wood, S.J., Shaw, K., O'Connor, J., Nertney, D., et al. (2011). Genetic influences on cost-efficient organization of human cortical functional networks. J. Neurosci. *31*, 3261–3270.

Fox, M.D., and Greicius, M. (2010). Clinical applications of resting state functional connectivity. Front. Syst. Neurosci. *4*, 19.

Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., and Raichle, M.E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc. Natl. Acad. Sci. USA *102*, 9673–9678.

Geschwind, D.H. (2011). Genetics of autism spectrum disorders. Trends Cogn. Sci. *15*, 409–416.

Geschwind, D.H., and Levitt, P. (2007). Autism spectrum disorders: developmental disconnection syndromes. Curr. Opin. Neurobiol. *17*, 103–111.

Glahn, D.C., Winkler, A.M., Kochunov, P., Almasy, L., Duggirala, R., Carless, M.A., Curran, J.C., Olvera, R.L., Laird, A.R., Smith, S.M., et al. (2010). Genetic control over the resting brain. Proc. Natl. Acad. Sci. USA *107*, 1223–1228.

Gottesman, I.I., and Gould, T.D. (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. Am. J. Psychiatry *160*, 636–645.

Hadjikhani, N., Joseph, R.M., Snyder, J., Chabris, C.F., Clark, J., Steele, S., McGrath, L., Vangel, M., Aharon, I., Feczko, E., et al. (2004). Activation of the fusiform gyrus when individuals with autism spectrum disorder view faces. Neuroimage *22*, 1141–1150.

Hagmann, P., Cammoun, L., Gigandet, X., Meuli, R., Honey, C.J., Wedeen, V.J., and Sporns, O. (2008). Mapping the structural core of human cerebral cortex. PLoS Biol. *6*, e159.

Hallmayer, J., Cleveland, S., Torres, A., Phillips, J., Cohen, B., Torigoe, T., Miller, J., Fedele, A., Collins, J., Smith, K., et al. (2011). Genetic heritability and shared environmental factors among twin pairs with autism. Arch. Gen. Psychiatry *68*, 1095–1102.

Heuer, L., Braunschweig, D., Ashwood, P., Van de Water, J., and Campbell, D.B. (2011). Association of a MET genetic variant with autism-associated maternal autoantibodies to fetal brain proteins and cytokine expression. Transl. Psychiatry *1*, e48.

Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., Yamrom, B., Lee, Y.H., Narzisi, G., Leotta, A., et al. (2012). De novo gene disruptions in children on the autistic spectrum. Neuron *74*, 285–299.

Jackson, P.B., Boccuto, L., Skinner, C., Collins, J.S., Neri, G., Gurrieri, F., and Schwartz, C.E. (2009). Further evidence that the rs1858830 C variant in the promoter region of the MET gene is associated with autistic disorder. Autism Res. *2*, 232–236.

Jones, C.R., Happé, F., Baird, G., Simonoff, E., Marsden, A.J., Tregay, J., Phillips, R.J., Goswami, U., Thomson, J.M., and Charman, T. (2009). Auditory discrimination and auditory sensory behaviours in autism spectrum disorders. Neuropsychologia *47*, 2850–2858.

Judson, M.C., Bergman, M.Y., Campbell, D.B., Eagleson, K.L., and Levitt, P. (2009). Dynamic gene and protein expression patterns of the autism-associated met receptor tyrosine kinase in the developing mouse forebrain. J. Comp. Neurol. *513*, 511–531.

Judson, M.C., Eagleson, K.L., Wang, L., and Levitt, P. (2010). Evidence of cell-nonautonomous changes in dendrite and dendritic spine morphology in the met-signaling-deficient mouse forebrain. J. Comp. Neurol. *518*, 4463–4478.

Judson, M.C., Amaral, D.G., and Levitt, P. (2011a). Conserved subcortical and divergent cortical expression of proteins encoded by orthologs of the autism risk gene MET. Cereb. Cortex *21*, 1613–1626.

Judson, M.C., Eagleson, K.L., and Levitt, P. (2011b). A new synaptic player leading to autism risk: Met receptor tyrosine kinase. J. Neurodev. Disord. *3*, 282–292.

Just, M.A., Cherkassky, V.L., Keller, T.A., and Minshew, N.J. (2004). Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain *127*, 1811–1821.

Kaiser, M.D., Hudac, C.M., Shultz, S., Lee, S.M., Cheung, C., Berken, A.M., Deen, B., Pitskel, N.B., Sugrue, D.R., Voos, A.C., et al. (2010). Neural signatures of autism. Proc. Natl. Acad. Sci. USA *107*, 21223–21228.

Kennedy, D.P., and Courchesne, E. (2008). The intrinsic functional organization of the brain is altered in autism. Neuroimage *39*, 1877–1885.

Kennedy, D.P., Redcay, E., and Courchesne, E. (2006). Failing to deactivate: resting functional abnormalities in autism. Proc. Natl. Acad. Sci. USA *103*, 8275–8280.

Kleinhans, N.M., Richards, T., Sterling, L., Stegbauer, K.C., Mahurin, R., Johnson, L.C., Greenson, J., Dawson, G., and Aylward, E. (2008). Abnormal functional connectivity in autism spectrum disorders during face processing. Brain *131*, 1000–1012.

Kochunov, P., Glahn, D.C., Lancaster, J.L., Winkler, A.M., Smith, S., Thompson, P.M., Almasy, L., Duggirala, R., Fox, P.T., and Blangero, J. (2010). Genetics of microstructure of cerebral white matter using diffusion tensor imaging. Neuroimage *53*, 1109–1116.

Konopka, G., Bomar, J.M., Winden, K., Coppola, G., Jonsson, Z.O., Gao, F., Peng, S., Preuss, T.M., Wohlschlegel, J.A., and Geschwind, D.H. (2009). Human-specific transcriptional regulation of CNS development genes by FOXP2. Nature *462*, 213–217.

Koten, J.W., Jr., Wood, G., Hagoort, P., Goebel, R., Propping, P., Willmes, K., and Boomsma, D.I. (2009). Genetic contribution to variation in cognitive function: an FMRI study in twins. Science *323*, 1737–1740.

Laurienti, P.J., Burdette, J.H., Wallace, M.T., Yen, Y.F., Field, A.S., and Stein, B.E. (2002). Deactivation of sensory-specific cortex by cross-modal stimuli. J. Cogn. Neurosci. *14*, 420–429.

Levitt, P., and Campbell, D.B. (2009). The genetic and neurobiologic compass points toward common signaling dysfunctions in autism spectrum disorders. J. Clin. Invest. *119*, 747–754.

Lin, P., Hasson, U., Jovicich, J., and Robinson, S. (2011). A neuronal basis for task-negative responses in the human brain. Cereb. Cortex *21*, 821–830.

Lord, C., Risi, S., Lambrecht, L., Cook, E.H., Jr., Leventhal, B.L., DiLavore, P.C., Pickles, A., and Rutter, M. (2000). The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J. Autism Dev. Disord. *30*, 205–223.

Marcus, D.S., Harwell, J., Olsen, T., Hodge, M., Glasser, M.F., Prior, F., Jenkinson, M., Laumann, T., Curtiss, S.W., and Van Essen, D.C. (2011). Informatics and data mining tools and strategies for the human connectome project. Front. Neuroinform. *5*, 4.

Marshall, C.R., Noor, A., Vincent, J.B., Lionel, A.C., Feuk, L., Skaug, J., Shago, M., Moessner, R., Pinto, D., Ren, Y., et al. (2008). Structural variation of chromosomes in autism spectrum disorder. Am. J. Hum. Genet. *82*, 477–488.

Monk, C.S., Peltier, S.J., Wiggins, J.L., Weng, S.J., Carrasco, M., Risi, S., and Lord, C. (2009). Abnormalities of intrinsic functional connectivity in autism spectrum disorders. Neuroimage *47*, 764–772.

Monk, C.S., Weng, S.J., Wiggins, J.L., Kurapati, N., Louro, H.M., Carrasco, M., Maslowsky, J., Risi, S., and Lord, C. (2010). Neural circuitry of emotional face processing in autism spectrum disorders. J. Psychiatry Neurosci. *35*, 105–114.

Mozolic, J.L., Joyner, D., Hugenschmidt, C.E., Peiffer, A.M., Kraft, R.A., Maldjian, J.A., and Laurienti, P.J. (2008). Cross-modal deactivations during modality-specific selective attention. BMC Neurol. *8*, 35.

Mukamel, Z., Konopka, G., Wexler, E., Osborn, G.E., Dong, H., Bergman, M.Y., Levitt, P., and Geschwind, D.H. (2011). Regulation of MET by FOXP2, genes implicated in higher cognitive dysfunction and autism risk. J. Neurosci. *31*, 11437–11442.

Neale, B.M., Kou, Y., Liu, L., Ma'ayan, A., Samocha, K.E., Sabo, A., Lin, C.F., Stevens, C., Wang, L.S., Makarov, V., et al. (2012). Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature *485*, 242–245.

O'Roak, B.J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B.P., Levy, R., Ko, A., Lee, C., Smith, J.D., et al. (2012). Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature *485*, 246–250.

Pfeifer, J.H., Iacoboni, M., Mazziotta, J.C., and Dapretto, M. (2008). Mirroring others' emotions relates to empathy and interpersonal competence in children. Neuroimage *39*, 2076–2085.

Pfeifer, J.H., Masten, C.L., Moore, W.E., 3rd, Oswald, T.M., Mazziotta, J.C., Iacoboni, M., and Dapretto, M. (2011). Entering adolescence: resistance to peer influence, risky behavior, and neural changes in emotion reactivity. Neuron *69*, 1029–1036.

Pierce, K., Haist, F., Sedaghat, F., and Courchesne, E. (2004). The brain response to personally familiar faces in autism: findings of fusiform activity and beyond. Brain *127*, 2703–2716.

Pinto, D., Pagnamenta, A.T., Klei, L., Anney, R., Merico, D., Regan, R., Conroy, J., Magalhaes, T.R., Correia, C., Abrahams, B.S., et al. (2010). Functional impact of global rare copy number variation in autism spectrum disorders. Nature *466*, 368–372.

Qiu, S., Anderson, C.T., Levitt, P., and Shepherd, G.M. (2011). Circuit-specific intracortical hyperconnectivity in mice with deletion of the autism-associated Met receptor tyrosine kinase. J. Neurosci. *31*, 5855–5864.

Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., and Shulman, G.L. (2001). A default mode of brain function. Proc. Natl. Acad. Sci. USA *98*, 676–682.

Rasetti, R., and Weinberger, D.R. (2011). Intermediate phenotypes in psychiatric disorders. Curr. Opin. Genet. Dev. *21*, 340–348.

Rosenberg, R.E., Law, J.K., Yenokyan, G., McGready, J., Kaufmann, W.E., and Law, P.A. (2009). Characteristics and concordance of autism spectrum disorders among 277 twin pairs. Arch. Pediatr. Adolesc. Med. *163*, 907–914.

Rudie, J.D., Shehzad, Z., Hernandez, L.M., Colich, N.L., Bookheimer, S.Y., Iacoboni, M., and Dapretto, M. (2012). Reduced functional integration and segregation of distributed neural systems underlying social and emotional information processing in autism spectrum disorders. Cereb. Cortex *22*, 1025–1037.

Sanders, S.J., Murtha, M.T., Gupta, A.R., Murdoch, J.D., Raubeson, M.J., Willsey, A.J., Ercan-Sencicek, A.G., DiLullo, N.M., Parikshak, N.N., Stein, J.L., et al. (2012). De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature *485*, 237–241.

Schultz, R.T., Gauthier, I., Klin, A., Fulbright, R.K., Anderson, A.W., Volkmar, F., Skudlarski, P., Lacadie, C., Cohen, D.J., and Gore, J.C. (2000). Abnormal ventral temporal cortical activity during face discrimination among individuals with autism and Asperger syndrome. Arch. Gen. Psychiatry *57*, 331–340.

Scott-Van Zeeland, A.A., Abrahams, B.S., Alvarez-Retuerto, A.I., Sonnenblick, L.I., Rudie, J.D., Ghahremani, D., Mumford, J.A., Poldrack, R.A., Dapretto, M., Geschwind, D.H., and Bookheimer, S.Y. (2010). Altered functional connectivity in frontal lobe circuits is associated with variation in the autism risk gene CNTNAP2. Sci. Transl. Med. *2*, 56ra80.

Shukla, D.K., Keehn, B., Smylie, D.M., and Müller, R.A. (2011). Microstructural abnormalities of short-distance white matter tracts in autism spectrum disorder. Neuropsychologia *49*, 1378–1382.

Smith, S.M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T.E., Mackay, C.E., Watkins, K.E., Ciccarelli, O., Cader, M.Z., Matthews, P.M., and Behrens, T.E. (2006). Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage *31*, 1487–1505.

Sousa, I., Clark, T.G., Toma, C., Kobayashi, K., Choma, M., Holt, R., Sykes, N.H., Lamb, J.A., Bailey, A.J., Battaglia, A., et al; International Molecular Genetic Study of Autism Consortium (IMGSAC). (2009). MET and autism susceptibility: family and case-control studies. Eur. J. Hum. Genet. *17*, 749–758.

Spencer, M.D., Holt, R.J., Chura, L.R., Suckling, J., Calder, A.J., Bullmore, E.T., and Baron-Cohen, S. (2011). A novel functional brain imaging endophenotype of autism: the neural response to facial expression of emotion. Transl. Psychiatry *1*, e19.

State, M.W., and Levitt, P. (2011). The conundrums of understanding genetic risks for autism spectrum disorders. Nat. Neurosci. *14*, 1499–1506.

Sundaram, S.K., Kumar, A., Makki, M.I., Behen, M.E., Chugani, H.T., and Chugani, D.C. (2008). Diffusion tensor imaging of frontal lobe in autism spectrum disorder. Cereb. Cortex *18*, 2659–2665.

Thanseem, I., Nakamura, K., Miyachi, T., Toyota, T., Yamada, S., Tsujii, M., Tsuchiya, K.J., Anitha, A., Iwayama, Y., Yamada, K., et al. (2010). Further evidence for the role of MET in autism susceptibility. Neurosci. Res. *68*, 137–141.

Vernes, S.C., Newbury, D.F., Abrahams, B.S., Winchester, L., Nicod, J., Groszer, M., Alarcón, M., Oliver, P.L., Davies, K.E., Geschwind, D.H., et al. (2008). A functional genetic link between distinct developmental language disorders. N. Engl. J. Med. *359*, 2337–2345.

Villalobos, M.E., Mizuno, A., Dahl, B.C., Kemmotsu, N., and Müller, R.A. (2005). Reduced functional connectivity between V1 and inferior frontal cortex associated with visuomotor performance in autism. Neuroimage *25*, 916–925.

Vissers, M.E., Cohen, M.X., and Geurts, H.M. (2012). Brain connectivity and high functioning autism: a promising path of research that needs refined models, methodological convergence, and stronger behavioral links. Neurosci. Biobehav. Rev. *36*, 604–625.

Voineagu, I., Wang, X., Johnston, P., Lowe, J.K., Tian, Y., Horvath, S., Mill, J., Cantor, R.M., Blencowe, B.J., and Geschwind, D.H. (2011). Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature *474*, 380–384.

Wang, K., Zhang, H., Ma, D., Bucan, M., Glessner, J.T., Abrahams, B.S., Salyakina, D., Imielinski, M., Bradfield, J.P., Sleiman, P.M., et al. (2009). Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature *459*, 528–533.

Weiss, L.A., Arking, D.E., Daly, M.J., and Chakravarti, A.; Gene Discovery Project of Johns Hopkins & the Autism Consortium. (2009). A genome-wide linkage and association scan reveals novel loci for autism. Nature *461*, 802–808.

Weng, S.J., Wiggins, J.L., Peltier, S.J., Carrasco, M., Risi, S., Lord, C., and Monk, C.S. (2010). Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. Brain Res. *1313*, 202–214.

Xu, L.M., Li, J.R., Huang, Y., Zhao, M., Tang, X., and Wei, L. (2012). AutismKB: an evidence-based knowledgebase of autism genetics. Nucleic Acids Res. *40* (Database issue), D1016–D1022.