Regulation of MET by FOXP2, Genes Implicated in Higher Cognitive Dysfunction and Autism Risk

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Autism spectrum disorder (ASD) is a highly heritable, behaviorally defined, heterogeneous disorder of unknown pathogenesis. Several genetic risk genes have been identified, including the gene encoding the receptor tyrosine kinase MET, which regulates neuronal differentiation and growth. An ASD-associated polymorphism disrupts *MET* gene transcription, and there are reduced levels of MET protein expression in the mature temporal cortex of subjects with ASD. To address the possible neurodevelopmental contribution of *MET* to ASD pathogenesis, we examined the expression and transcriptional regulation of *MET* by a transcription factor, FOXP2, which is implicated in regulation of cognition and language, two functions altered in ASD. *MET* mRNA expression in the midgestation human fetal cerebral cortex is strikingly restricted, localized to portions of the temporal and occipital lobes. Within the cortical plate of the temporal lobe, the pattern of *MET* expression is highly complementary to the expression pattern of FOXP2, suggesting the latter may play a role in repression of gene expression. Consistent with this, MET and FOXP2 also are reciprocally expressed by differentiating normal human neuronal progenitor cells (NHNP) *in vitro*, leading us to assess whether FOXP2 transcriptionally regulates *MET*. Indeed, FOXP2 binds directly to the 5' regulatory region of *MET*, and overexpression of FOXP2 results in transcriptional repression of *MET*. The expression of *MET* in restricted human neocortical regions, and its regulation in part by FOXP2, is consistent with genetic evidence for *MET* contributing to ASD risk.

Introduction

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder. The genetic contribution to this syndrome is well documented (Abrahams and Geschwind, 2008), but the molecular pathways involved are just beginning to be elucidated (Bill and Geschwind, 2009). Although genetic variation in several genes has been implicated as potential risk factors in ASD, their regulation and neurodevelopmental functions are not well understood. *MET*, a gene belonging to the tyrosine kinase receptor family, is a candidate risk gene previously shown to be associated with ASD in four independent family cohorts (Campbell et al.,

2008; Jackson et al., 2009; Sousa et al., 2009). These studies identified two common risk alleles, one of which negatively regulates *MET* gene transcription (Campbell et al., 2006), consistent with a twofold reduction in MET expression in postmortem temporal cortex of subjects with ASD (Campbell et al., 2007). Overrepresented copy number variations (CNVs) that delete MET were reported in one study (Marshall et al., 2008), suggesting multiple genetic mechanisms for disrupting *MET* in ASD. MET was discovered as a proto-oncogene that is activated through its only known ligand (Birchmeier et al., 2003). MET expression in the forebrain is restricted to periods of dendritic growth and synapse formation in mice (Judson et al., 2009), and signaling via the receptor regulates these processes *in vitro* (Lim and Walikonis, 2008). Finally, signaling pathways that MET is known to interact with contain at least eight members that also show association with ASD (Bill and Geschwind, 2009).

There is limited information regarding the transcriptional regulation of *MET*. The *MET* promoter contains seven repeats of the consensus binding site for the Sp family of transcription factors, a number of potential binding sites for regulatory elements such as AP1, AP2, NF- κ B, IL-6RE (Liu, 1998), and a LINE regulator sequence in intron 2 (Mätlik et al., 2006). SP binding is important for promoting *MET* expression since there is reduced gene transcription and protein binding to the MET promoter in the presence of the ASD C risk allele rs1858830 compared to the G allele (Campbell et al., 2006). In searching for candidate regula-

Received Jan. 11, 2011; revised May 17, 2011; accepted June 6, 2011.

Author contributions: Z.M., G.K., E.W., P.L., and D.H.G. designed research; Z.M., G.K., G.E.O., H.D., and M.Y.B. performed research; M.Y.B. and P.L. contributed unpublished reagents/analytic tools; Z.M., G.K., E.W., G.E.O., P.L., and D.H.G. analyzed data; Z.M., G.K., and D.H.G. wrote the paper.

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This study was supported by grants from the A. P. Giannini Foundation Medical Research Fellowship, National Alliance for Research on Schizophrenia and Depression Young Investigator Award, and NIH Grants K99MH090238-01 (G.K.), R37MH060233-06A1 (D.H.G.), R01MH081754-02R (D.H.G.), F30MH083474 (M.Y.B.), and R01MH067842 (P.L.). Human tissue was obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland (NICHD Contract numbers N01-HD-4-3368 and N01-HD-4-3383). The role of the NICHD Brain and Tissue Bank is to distribute tissue, and it therefore cannot endorse the studies performed or the interpretation of results. We thank Daning Lu for maintaining neuronal progenitor cells and Leslie Chen for technical assistance.

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DOI:10.1523/JNEUROSCI.0181-11.2011

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Figure 1. *MET* expression in the temporal cortex of human fetal brains. *In situ* hybridization is shown in the right panels and cresyl violet in the left panels. **A–C**, Sagittal sections from 18 GW (**A**, **B**) and from 20 GW (**C**) fetal brain. **D**, **E**, Coronal sections from 15 week fetal brain (**D**) and horizontal sections from 19 week fetal brain (**E**). **F**, **G**, Dark-field image (**F**) and bright-field image (**G**) of the cresyl violet staining of the cortical plate shown from the boxed area in **A**. The golden signal represents *MET* mRNA labeling. Temporal lobe enrichment of *MET* expression is evident in sections representing all cardinal planes. Layers II/III and V are marked by a horizontal line. Arrows mark the hippocampus, in which *MET* is expressed in CA1 subfield, and the temporal neocortex. Scale bars: **A–D**, 5 mm; **E**, 0.25 mm. Tctx, Temporal cortex; Hipp, hippocampus; GE, ganglionic eminence; BG, basal ganglia; SP, subplate; CP, cortical plate; MZ, marginal zone; a, anterior; p, posterior.

tory proteins of *MET* in neocortex, a preliminary screen of *MET* expression in the human fetal brain revealed a possible pattern reciprocal in nature to *FOXP2*, a gene encoding a repressor regulatory protein that has been implicated in regulating higher cognitive functions including language (Lai et al., 2001). While mutations in *FOXP2* are not implicated directly in increasing ASD risk, the language dysfunction that is central to ASD diagnosis may be influenced through downstream regulation by *FOXP2* of key gene networks that ultimately impact circuit wiring. Here we present evidence using a variety of methods to demonstrate a close relationship between *FOXP2* and *MET* in human neocortical development, providing additional evidence that a network of genes that includes these elements may be key targets in ASD risk etiology.

Materials and Methods

Tissue samples. Fresh-frozen human embryonic brains from 15 to 20 gestational weeks (GW) were obtained from the University of Maryland Brain and Tissue Bank. Demographics on the fetuses are as follows: Figures 1, **A** and **B**, and 2, **A** and **B**: #1057, 19 GW, female, African American, 1 h postmortem interval (PMI); Figure 1C: #1010, 20 GW, female, Caucasian, 3 h PMI; Figure 1D: #1075, 15 GW, female, African American, 3 h PMI; Figure 1E, #1926, 18 GW, female, African American, 2 h PMI;

Figure 2, **C** and **D**: #1110, 19 GW, female, African American, 2 h PMI. All fetuses were considered “normal” except for the one in Figure 1C, which had polyhydramnios, twin-to-twin transfusion syndrome in the fetus.

Primary cell culture. Normal human fetal neuronal progenitors (NHNP) were either purchased (Lonza; 17 GW, female, African American) or prepared from cortical tissue as previously described (Svendsen et al., 1998) (16 GW, male, race unknown, and 18 GW, sex and race unknown). Based on whole-genome genotyping analysis, these cells were deemed “normal” with a low number of CNVs >200 kb (0–2 per line) (Konopka et al., 2011). The cells were propagated in Neurobasal A, B27 (10%), FGF2 (20 ng/ml), EGF (20 ng/ml), LIF (10 ng/ml), and heparin (2 μg/ml). To induce differentiation, retinoic acid (500 ng/ml), NT-3 (10 ng/ml), and BDNF (10 ng/ml) were added.

Other methods. Information is available upon request.

Results

MET expression is enriched in the temporal cortex of the developing human brain

To identify the expression pattern of *MET* at midgestation in the human fetal brain, we performed *in situ* hybridization mapping. We used two non-overlapping probes that specifically recognize different regions of the *MET* transcript. High expression of *MET* was detected specifically in the temporal cerebral cortex of the

fetal brain during the 15th to 20th gestational weeks, a period when neuronal migration is almost complete and there is a major influx of axons into the reorganizing subplate and cortical plate (Kostovic and Rakic, 1990). Changes in gene expression at these embryonic ages are critical for later developmental processes, and thus characterizing modifications in gene expression at these time points provides insight into developmental disorders such as ASD. *MET* expression was also detected at lower levels in the developing hippocampus and in the occipital cortex, which is a similar developmental state as the temporal neocortex. In contrast, *MET* expression was not detected in the more rostral regions of the developing cerebral hemispheres, including somatosensory, motor, and frontal regions (Fig. 1*A–E*). By analysis of postemulsion slides, we detected restricted expression of *MET* in the cortical plate (Fig. 1*F, G*). Here, labeling appeared to be most dense in supragranular layers II and III. At the ages examined, the expression of *MET* was not detected in other developing brain areas such as the striatum or cerebellum.

MET and *FOXP2* expression are complementary

It has been shown previously in human fetal brain that *FOXP2* is expressed in the frontal and temporal cortex, specifically in layer VI and also in the thalamus (Teramitsu et al., 2004), two regions in which *MET* was not detected in the current study. To examine the possible complementary expression of the two transcripts more directly, we examined *MET* and *FOXP2* in sections prepared from the same brains (Fig. 2). *In situ* hybridization analysis of adjacent brain sections revealed that *MET* and *FOXP2* are expressed in different layers of the cortical plate in the posterior parietal region and in the temporal region (Fig. 2). Whereas *MET* transcript is expressed most densely in supragranular layers II–III, *FOXP2* is located mostly in infragranular layer VI. This non-overlapping pattern of cortical plate expression was intriguing given the primary role of *FOXP2* as a negative regulator of transcription and its regulation of another ASD risk gene, *CNTNAP2* (Vernes et al., 2008).

To examine the potential regulatory influence by *FOXP2* on *MET* transcription, we used NHNPs as a model to examine more precisely the complementary expression of each protein. We induced differentiation of NHNP cells, confirming differentiation by reduction of nestin expression, a marker of undifferentiated neuroepithelial cells (Dahlstrand et al., 1995). Indeed, after 4 weeks of differentiation, anti-nestin immunostaining revealed greatly reduced protein expression, while the expression of Tuj1, a marker of early neuronal differentiation (Lee et al., 1990), increased significantly (Fig. 3*A, B* and Konopka et al., 2011). We have also conducted genome-wide gene expression analysis of these cell lines and find significant upregulation of neuronal markers such as *MAP1B*, *PAX6*, and *SNAP25*, and downregulation of proliferation and progenitor markers such as nestin, *CXCR4*, and *CDC20* (Konopka et al., 2011). Though *MET* in primate and rodent neocortex is not expressed in neural progenitor cells, expression of *MET* in undifferentiated NHNPs was detected using gene microarrays (data not shown). However, as evident in both species (Judson et al., 2009, 2011), initially high *MET* expression becomes greatly reduced over time. To confirm these results in the NHNPs, we performed qRT-PCR using three biological replicates. We compared the cells differentiated for 4 weeks to the undifferentiated cells and found a 2.4-fold reduction in the *MET* transcript (Fig. 3*C*). In contrast, *FOXP2* mRNA exhibited a dramatic increase in expression between 0 and 4 weeks of differentiation, from almost undetectable baseline levels to a >100-fold increase (Fig. 3*D*).

Figure 2. *MET* and *FOXP2* are expressed in largely non-overlapping areas of the brain. ***A, B***, *In situ* hybridization analysis of sagittal sections from 19 week fetal brain (left panels) and cortical plate magnification of corresponding box insets (right panels) (***A***). Same analysis of coronal sections with corresponding cortical plate magnification box insets (bottom panels) (***B***). Thal, Thalamus; SVZ, subventricular zone; Cau, caudate. Other abbreviations are as in Figure 1.

FOXP2 negatively regulates *MET* expression

The anatomical and molecular analyses suggest that *FOXP2* is a candidate to negatively regulate *MET* gene transcription. To test this directly, we overexpressed *FOXP2* in undifferentiated NHNP cells and examined *MET* expression 4 d later. There was a dramatic reduction of both *MET* transcript and protein expression by cells overexpressing *FOXP2* (Fig. 3*E, F*). We also confirmed this inverse expression of *FOXP2* and *MET* using two additional human neuronal cell lines (SH-SY5Y cells) overexpressing *FOXP2* (Fig. 3*G*) (Konopka et al., 2009). These data extend the results from the NHNP differentiation experiments and the complementary pattern of expression in the fetal human brain, indicating that *FOXP2* negatively regulates *MET* expression.

FOXP2 regulation of *MET* is via direct interaction

To address the possibility that *FOXP2* physically interacts with the *MET* gene to reduce transcription, we searched for canonical *FOXP2* binding sites (AATTTG or CAAATT) within the *MET* gene. Potential *FOXP2* binding sites were identified within the 5' promoter and intron 3 (Fig. 4*A*). Two plausible sites of regulation were examined: site I in the promoter area at position –538 [where zero is defined as the transcription start site, NM_000245 (Liu, 1998)], and site II with two potential binding sites in close proximity in the third intron at chr7:116165987 and chr7:116166013, respectively. To test whether these human-specific sites bind *FOXP2*, we conducted EMSA assays. Probes I and II correspond to nucleotides –556 to –518 in the *MET* promoter and chr7:116165984–116166013 in intron 3 of the *MET* gene. Both probes robustly bound to a protein complex in the nuclear extract of cells overexpressing FLAG-tagged *FOXP2* (Fig. 4*B*, lanes 1 and 7). Mutant forms of these probes (Fig. 4*B*, lanes 2 and

8) failed to bind this complex. Adding unlabeled probe led to competition with the labeled probe and reduced binding to the protein complex (Fig. 4*B*, lanes 3 and 9). Finally, preincubation of the nuclear extract with an anti-FLAG antibody led to a detectable shifted DNA–protein band on the gel (Fig. 4*B*, lanes 4 and 10, upper band). This shift confirms the presence of FOXP2 in the nuclear protein complex that binds to these putative regulatory regions of *MET*. To confirm *in vivo* binding of these putative regulatory elements by FOXP2, we performed chromatin immunoprecipitation (ChIP) with NHNP cells transduced with FOXP2. Anti-FOXP2 specifically precipitates *MET* DNA, whereas control IgG yields no enrichment of the *MET* sequence (Fig. 4*C*), supporting direct *in vivo* binding of FOXP2 to the *MET* promoter region as demonstrated *in vitro* by EMSA.

Discussion

The current study provides the first evidence that *MET*—an ASD risk gene—is predominantly expressed in the temporal lobes of the fetal human brain, and is specifically enriched in the supragranular layers of the developing cortical plate around midgestation. In addition, unpublished data from the Allen Brain Atlas (<http://human.brain-map.org/>) demonstrate that *MET* is the most differentially expressed transcript in temporal cortex compared to all other cortical areas in adult human brain. This regional specificity is especially relevant to ASDs because the temporal lobes play a critical role in language processing, emotional control, and affective perception, the clinical spheres most affected by these disorders (Courchesne et al., 2007). The restricted pattern of *MET* transcript expression is different from the more widespread distribution of *MET* in the developing mouse neocortex (Judson et al., 2009). Whereas the distribution of *MET* within the layers of cortical areas and the timing of *MET* expression is conserved, the expression of *MET* within specific cortical regions is not conserved (Judson et al., 2011). Thus, these data provide evidence for primate-restricted expression of *MET* that parallels our findings in human brain at midgestation.

The present study further strengthens the possible connection between aberrant *MET* signaling and complex cognitive development, including language, by demonstrating that the protein encoded by *FOXP2*, a gene involved in the development of human speech and language, negatively regulates *MET* expression and does so in untransformed neural progenitors derived from midgestation human fetal brain. This negative regulation is also implied *in vivo*; however, there it seems to be uncoupled to differentiation. The early-born neurons in the deeper cortical layers express FOXP2 and thus, we postulate that *MET* expression is repressed. In later-born neurons of the more superficial layers, FOXP2 is not expressed, and therefore *MET* expression is

Figure 3. Overexpression of FOXP2 leads to downregulation of *MET* expression. *A, B*, Immunocytochemistry of undifferentiated NHNPs (*A*) and NHNPs differentiated for 4 weeks (*B*). Green is nestin-positive staining, red is Tuj1-positive staining, and blue is DAPI. *C, D*, qRT-PCR analysis of *MET* and *FOXP2* in NHNP cells differentiated for 4 weeks (4W) compared to undifferentiated cells (0W). *E*, Western blot analysis of *MET* and *FOXP2* protein expression in NHNP extract. GAPDH indicates equal loading. *F*, qRT-PCR analysis of NHNPs overexpressing FOXP2. * $p \leq 0.05$, ** $p \leq 0.01$; error bars are $\pm$ SEM (Student's *t* test, $n = 3$). *G*, Western blot analysis of *MET* protein expression in two different lines of SH-SY5Y cells overexpressing FOXP2. GAPDH indicates equal loading.

unrepressed and elevated. While we observed *MET* expression in proliferating NHNPs but not in the GE *in vivo*, this is likely due to the semicommitted fate of the cells in culture based upon the location and timing of their origin. We further show that FOXP2 directly binds to regulatory sequences in *MET* genomic DNA *in vitro* and *in vivo* and forced expression of FOXP2 leads to downregulation of *MET* expression in multiple neuronal cell lines, indicating that FOXP2 can directly regulate *MET* expression.

There has been increasing success in identifying ASD susceptibility candidate genes and the biological mechanisms by which they increase risk for ASDs. Here we focused on the *MET* gene, which has common variants associated with ASD. *MET* is a receptor tyrosine kinase that has been shown to modulate dendritic development (Lim and Walikonis, 2008), synapse maturation (Tyndall and Walikonis, 2007), and LTP (Akimoto et al., 2004). Moreover, the expression in rodent is enriched in the forebrain just before and during the peak of synapse formation, particularly in the cerebral cortex (Judson et al., 2009). The results presented here in the human indicate that while the temporal patterns may

Figure 4. FOXP2 binds regulatory regions of the *MET* gene *in vitro* and *in vivo*. **A**, Schematic of the FOXP2 binding sites within the genomic structure of human *MET*. Exons are black boxes, an asterisk marks the transcription start site, and an arrow marks the translation start site. **B**, Probes I and II bind to a single transcription factor complex (lanes 1 and 7, middle arrow). An antibody to the FLAG epitope yielded a slower migrating (supershifted) band (lanes 4 and 10; upper arrow). Competition with 100× molar excess of unlabeled probe reduces probe–complex formation (lanes 3 and 9). Mutant forms of the FOXP2 sites disrupt interactions (lanes 2 and 8). Unlabeled probe is detected in lanes 5 and 6. The asterisk indicates a nonspecific band. The lower arrow marks unbound probe. **C**, ChIP assay in NHNP cells. DNA fragments immunoprecipitated with IgG or anti-FOXP2 antibodies were analyzed by PCR with primers specific for the potential FOXP2 binding site. N.E., Nuclear extract; m, mutated oligo.

be conserved between rodents and human, the neocortical expression is far more restricted in primates. In fact, this is also evident in a detailed analysis of the developing macaque brain prenatally and postnatally (Judson et al., 2011). There is a dramatic expansion of both the frontal and temporal lobes in the primate, the latter being most relevant for language processing (Spiroski et al., 2009) and social cognition (including face recognition) (Amaral et al., 2008). These differences in *MET* expression between mouse and primates suggest that it will be important to understand genetic differences that have emerged on the primate lineage. *MET* has been highly conserved during mammalian evolution, as the mouse and the human gene share 86.6% homology at the nucleotide level, and the predicted promoter is also conserved (Seol et al., 1999). Interestingly, alignment of human and mouse genomic DNA shows that the core binding site (AAT) in two of the three potential FOXP2 binding sites studied here differs by one nucleotide in the mouse. Based on our data regarding the relationship between *MET* and FOXP2,

we speculate that while the developmental neurobiological functions of *MET* are likely to be highly conserved, the regulation of human *MET* in the development of specific neural circuitry has diverged from the rodent. It is important to be clear that these data do not exclude a role for Foxp2 regulation of *Met* in mouse. Preliminary observations suggest that Foxp2 can bind the mouse *Met* promoter (M. Y. Bergman and P. Levitt, unpublished observations). However, the data presented here show that there are likely key functional differences in the *MET* promoter sequence between human and mouse, specifically in regions where we show FOXP2 to bind and regulate *MET* expression in humans. Since FOXP2 is repressing expression of *MET* throughout deeper cortical layers, clearly other undetermined transcription factors are responsible for primate-specific enrichment of *MET* in temporal regions.

Given the intimate relationship between FOXP2 and *MET*, one can speculate that the repression of *MET* expression in specific frontal, temporal, and striatal circuits is key for the successful development of language. FOXP2 is critical for speech and language, and also directly regulates other language-related and/or ASD genes, such as *CNTNAP2* (Vernes et al., 2008), the sushi-repeat protein *SRPX2*, and the plasminogen activator urokinase receptor *PLAUR* (or *uPAR*) (Roll et al., 2010), which is also in the *MET* signal transduction cascade (Campbell et al., 2008; Bill and Geschwind, 2009). *CNTNAP2* has also been shown to be involved in specific language impairment and has focal cortical expression (Vernes et al., 2008). The regulation of *CNTNAP2*, *PLAUR*, and *MET* by FOXP2 provides an interesting connection to ASD candidate genes that are part of an interactive network that mediate neurobiological events involved in circuit formation and maturation (Bill and Geschwind, 2009). Moreover, mutations in *SRPX2* are associated with a form of epilepsy in which the seizures originate in the speech areas of the brain, and *SRPX2* interacts with *PLAUR* to form a complex (Roll et al., 2010). Thus, our data add an additional player, *MET*, into the molecular pathways that are related to speech disorders. On a molecular level, repression of *MET* by FOXP2 may be critical for regulation of dendritic or axonal outgrowth in deeper cortical layers during early brain development, as *MET* is typically highly expressed in these subcellular compartments and involved in their function (Judson et al., 2010). Thus, FOXP2-mediated regulation of *MET* may be important for regulation of subcortical efferent pathway signaling that may underlie some aspects of language and cognition.

These data together suggest that *MET* may be an important molecular component of human temporal lobe development. Both the positive and negative regulation of *MET* will be critical to define, because both will contribute to the specificity of expression in temporal lobe and related circuitry that are critical for the development of human higher cognition functions, some of which are dysfunctional in ASD, including social cognition and language. However, there is no genetic evidence directly linking FOXP2 with ASD. These current results further strengthen an indirect connection of FOXP2 with ASD and suggest that assessment of the relationship between ASD-related genetic variation in *MET* and human temporal lobe structure and function using MRI will be of significant value.

References

- Abrahams BS, Geschwind DH (2008) Advances in autism genetics: on the threshold of a new neurobiology. *Nat Rev Genet* 9:341–355.
- Akimoto M, Baba A, Ikeda-Matsuo Y, Yamada MK, Itamura R, Nishiyama N, Ikegaya Y, Matsuki N (2004) Hepatocyte growth factor as an enhancer of NMDA currents and synaptic plasticity in the hippocampus. *Neuroscience* 128:155–162.

- Amaral DG, Schumann CM, Nordahl CW (2008) Neuroanatomy of autism. *Trends Neurosci* 31:137–145.
- Bill BR, Geschwind DH (2009) Genetic advances in autism: heterogeneity and convergence on shared pathways. *Curr Opin Genet Dev* 19:271–278.
- Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF (2003) Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 4:915–925.
- Campbell DB, Sutcliffe JS, Ebert PJ, Militeri R, Bravaccio C, Trillo S, Elia M, Schneider C, Melmed R, Sacco R, Persico AM, Levitt P (2006) A genetic variant that disrupts MET transcription is associated with autism. *Proc Natl Acad Sci U S A* 103:16834–16839.
- Campbell DB, D'Oronzio R, Garbett K, Ebert PJ, Mirnics K, Levitt P, Persico AM (2007) Disruption of cerebral cortex MET signaling in autism spectrum disorder. *Ann Neurol* 62:243–250.
- Campbell DB, Li C, Sutcliffe JS, Persico AM, Levitt P (2008) Genetic evidence implicating multiple genes in the MET receptor tyrosine kinase pathway in autism spectrum disorder. *Autism Res* 1:159–168.
- Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP, Morgan J (2007) Mapping early brain development in autism. *Neuron* 56:399–413.
- Dahlstrand J, Lardelli M, Lendahl U (1995) Nestin mRNA expression correlates with the central nervous system progenitor cell state in many, but not all, regions of developing central nervous system. *Brain Res Dev Brain Res* 84:109–129.
- Jackson PB, Boccuto L, Skinner C, Collins JS, Neri G, Gurrieri F, Schwartz CE (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.
- Judson MC, Bergman MY, Campbell DB, Eagleson KL, 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 MC, Eagleson KL, Wang L, Levitt P (2010) Evidence of cell-autonomous changes in dendrite and dendritic spine morphology in the met-signaling-deficient mouse forebrain. *J Comp Neurol* 518:4463–4478.
- Judson MC, Amaral DG, Levitt P (2011) Conserved subcortical and divergent cortical expression of proteins encoded by orthologs of the autism risk gene MET. *Cereb Cortex* 21:1613–1626.
- Konopka G, Bomar JM, Winden K, Coppola G, Jonsson ZO, Gao F, Peng S, Preuss TM, Wohlschlegel JA, Geschwind DH (2009) Human-specific transcriptional regulation of CNS development genes by FOXP2. *Nature* 462:213–217.
- Konopka G, Wexler E, Rosen E, Mukamel Z, Osborn GE, Chen L, Lu D, Gao F, Gao K, Lowe JK, Geschwind DH (2011) Modeling the functional genomics of autism using human neurons. *Mol Psychiatry*. Advance online publication. doi:10.1038/mp.2011.60.
- Kostovic I, Rakic P (1990) Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *J Comp Neurol* 297:441–470.
- Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP (2001) A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* 413:519–523.
- Lee MK, Tuttle JB, Rebhun LI, Cleveland DW, Frankfurter A (1990) The expression and posttranslational modification of a neuron-specific beta-tubulin isotype during chick embryogenesis. *Cell Motil Cytoskeleton* 17:118–132.
- Lim CS, Walikonis RS (2008) Hepatocyte growth factor and c-Met promote dendritic maturation during hippocampal neuron differentiation via the Akt pathway. *Cell Signal* 20:825–835.
- Liu Y (1998) The human hepatocyte growth factor receptor gene: complete structural organization and promoter characterization. *Gene* 215:159–169.
- Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, Thiruvahindrapadam B, Fiebig A, Schreiber S, Friedman J, Ketelaars CE, Vos YJ, Ficicioglu C, Kirkpatrick S, Nicolson R, Sloman L, et al. (2008) Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 82:477–488.
- Mätlik K, Redik K, Speck M (2006) L1 antisense promoter drives tissue-specific transcription of human genes. *J Biomed Biotechnol* 2006:71753.
- Roll P, Vernes SC, Bruneau N, Cillario J, Ponsolle-Lenfant M, Massacrier A, Rudolf G, Khalife M, Hirsch E, Fisher SE, Szepietowski P (2010) Molecular networks implicated in speech-related disorders: FOXP2 regulates the SRPX2/uPAR complex. *Hum Mol Genet* 19:4848–4860.
- Seol DW, Chen Q, Smith ML, Zarnegar R (1999) Regulation of the c-met proto-oncogene promoter by p53. *J Biol Chem* 274:3565–3572.
- Sousa I, Clark TG, Toma C, Kobayashi K, Choma M, Holt R, Sykes NH, Lamb JA, Bailey AJ, Battaglia A, Maestrini E, Monaco AP (2009) MET and autism susceptibility: family and case-control studies. *Eur J Hum Genet* 17:749–758.
- Spiroski I, Kedev S, Antov S, Trajkov D, Petlichkovski A, Dzhekova-Stojkova S, Kostovska S, Spiroski M (2009) Investigation of SERPINE1 genetic polymorphism in Macedonian patients with occlusive artery disease and deep vein thrombosis. *Kardiol Pol* 67:1088–1094.
- Svendsen CN, ter Borg MG, Armstrong RJ, Rosser AE, Chandran S, Ostfeld T, Caldwell MA (1998) A new method for the rapid and long term growth of human neural precursor cells. *J Neurosci Methods* 85:141–152.
- Teramitsu I, Kudo LC, London SE, Geschwind DH, White SA (2004) Parallel FoxP1 and FoxP2 expression in songbird and human brain predicts functional interaction. *J Neurosci* 24:3152–3163.
- Tyndall SJ, Walikonis RS (2007) Signaling by hepatocyte growth factor in neurons is induced by pharmacological stimulation of synaptic activity. *Synapse* 61:199–204.
- Vernes SC, Newbury DF, Abrahams BS, Winchester L, Nicod J, Groszer M, Alarcón M, Oliver PL, Davies KE, Geschwind DH, Monaco AP, Fisher SE (2008) A functional genetic link between distinct developmental language disorders. *N Engl J Med* 359:2337–2345.