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Receptor Tyrosine Kinase MET Interactome and Neurodevelopmental Disorder Partners at the Developing Synapse

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Abstract

Background—Atypical synapse development and plasticity are implicated in many neurodevelopmental disorders (NDDs). NDD-associated, high confidence risk genes have been identified, yet little is known about functional relationships at the level of protein-protein interactions, which are the dominant molecular bases responsible for mediating circuit development.

Methods—Proteomics in three independent developing neocortical synaptosomal preparations identified putative interacting proteins of the ligand-activated MET receptor tyrosine kinase, an autism risk gene that mediates synapse development. The candidates were translated into interactome networks and analyzed bioinformatically. Additionally, three independent quantitative proximity ligation assays (PLA) in cultured neurons and four independent immunoprecipitation analyses of synaptosomes validated protein interactions.

Results—Approximately 11% (8/72) of MET-interacting proteins, including SHANK3, SYNGAP1 and GRIN2B, are associated with NDDs. Proteins in the MET interactome were translated into a novel MET interactome network based on human protein-protein interaction

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databases. High confidence genes from different NDD datasets that encode synaptosomal proteins were analyzed for being enriched in MET interactome proteins. This was found for autism, but not schizophrenia, bipolar disorder, major depressive disorder or attentional deficit hyperactivity disorder. There is correlated gene expression between *MET* and its interactive partners in developing human temporal and visual neocortices, but not with highly expressed genes that are not in the interactome. PLA and biochemical analyses demonstrate that MET-protein partner interactions are dynamically regulated by receptor activation.

Conclusions—The results provide a novel molecular framework for deciphering the functional relations of key regulators of synaptogenesis that contribute to both typical cortical development and to NDDs.

Keywords

proteomics; synaptogenesis; neocortex; mental illnesses; autism; interactome

Introduction

Neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD), intellectual disability (ID), epilepsy (EP) and schizophrenia (SCZ), are characterized by impairments of cognition, social and emotional behavior, and communication (1, 2). NDDs are complex and clinically heterogeneous, with atypical brain development as the principle, overlapping etiology that is thought to result in different clinical symptoms (3–5). Genetic animal models, human genetics studies and network analyses of postmortem transcriptome datasets indicate the developing synapse is a key target disrupted in NDDs (4, 6–10). In particular, statistically-defined molecular networks of NDD-associated risk genes are enriched with those whose protein products are located at central synapses (11–14). Protein-protein, rather than gene-gene, interactions are the mediators of cellular functions, yet data regarding these interactions, particularly absent in neurodevelopmental contexts, are untapped paths of discovery for determining mechanism. Yet to date, only three studies, using either a yeast two-hybrid screen or mass spectrometry, have mapped SCZ and ASD risk protein interactome networks (15–17).

The present study reports novel discoveries on an ASD risk and developmentally regulated protein enriched at the synapse, the MET receptor tyrosine kinase. This proto-oncogene, activated by hepatocyte growth factor (HGF) (18, 19), is an ASD risk gene (20–28) of low effect size, yet it exhibits a significant reduction in expression in temporal neocortex of subjects with ASD (13, 29) and in Rett Syndrome (30). A family pedigree with siblings having a rare functional mutation that generates haploinsufficiency for *MET* have either ASD or social-communication deficits (31). The temporal patterns of MET expression in the mouse and primate telencephalon are conserved, with high expression during the beginning and peak of synaptogenesis, and limited expression during pruning (32–34). In contrast, cortical areal expression patterns vary significantly between rodent and primate (32, 34, 35). MET is expressed in excitatory neocortical neurons and enriched in growing forebrain axons and synapses (36, 37), with phosphorylation occurring mostly in the neuropil and not in axon tracts (38). Accumulating human and animal model data demonstrate an important role for MET in circuit development. For example, *Met* deletion alters dendritic and spine growth

and morphology, and interlaminar neocortical and CA1 hippocampal premature synapse maturation (20, 39–41). Neuroimaging studies demonstrate that the ASD ‘C’ risk allele for *MET*, which reduces gene transcription by 50%, modulates structural networks, resting state connectivity and functionally activated circuits that process social-emotional information (42). Conditional deletion of *Met* in different populations of neurons results in distinct behavioral disturbances (43, 44).

MET signaling mediates dendritic development through intracellular MAP Kinase and synapse formation via the Akt pathway. These pathways are implicated as targets in ASD and other NDDs (45, 46). There is thus the possibility that certain disorder-associated synaptic proteins are physically and functionally convergent and are targets of disruption during circuit development. This relation would reflect shared protein interactions, which, depending upon the genetic and/or environmental insults, contribute to NDDs and phenotypic heterogeneity. To address this knowledge gap, discovery-based co-immunoprecipitation and mass spectrometry (Co-IP/MS) was employed using synaptosomes isolated from the mouse neocortex at the peak of synaptogenesis. Using genetic consortia-defined high confidence associations for different NDDs, we report that approximately 11% of the MET-interacting proteins are associated with ASD and NDDs, including syndromic disorders, but not schizophrenia, bipolar and other common psychiatric disorders. The new findings highlight connectivity between MET and a molecular network that contributes to specific NDDs.

Materials and Methods

Co-immunoprecipitation (Co-IP) and Mass Spectrometry (MS) Screen

The MET Co-IP experiment and preparation of IP protein samples are described in detail in supplemental methods. The IP proteins were digested overnight by trypsin (Promega, V5111) and the peptides were extracted with 60% ACN/0.1% FA, dried out in a SpeedVac concentrator and reconstituted with 3% ACN/0.1% FA for nano LC-MS/MS analysis. Peptides were loaded on an Ultimate 3000 Nano/Capillary LC System (trap column: PepMapTM RSLC nano trap Column, DX164564; analytical column: C18 column, 75 μ m ID, 15cm length, in-house packed with Magic 5 μ 100Å C18AQ beads) and eluted during an 80 min linear gradient wash (flow rate 330 nL/min) with ACN concentration being increased from 8% to 35%. The eluate was directed into an LTQ-FT (FinniganTM) mass spectrometer set to data-dependent acquisition mode, with one MS survey scan, followed by five MS/MS scans. MS data were processed using Proteome Discoverer 1.4 (Thermo Scientific) and searched using both Sequest and Mascot V2.4 (Matrix science) against the corresponding mouse IPI sequence database. False discovery rates (FDR) were automatically calculated by the Percolator node of PD. A protein FDR of 0.01 and a peptide FDR of 0.01 were used for cutoff for the selection of high confidence true hits. Data reported were generated from three independent Co-IP/MS experiments using different animals.

MET-interactome Network and Sub Network

The strategies used to build the networks of putative interacting proteins are described in detail in supplemental methods. The members of the MET interactome were analyzed for

NDD enrichment against a specific list of high confidence genes defined by SFARIgene for ASD, Schizophrenia Working Group of the Psychiatric Genetics Consortium, BDgene consortium for bipolar disorder, ADHDgene consortium, PsyGenNet for major depressive disorder. The NDD category is comprised of 208 high confidence genes designated by these consortia, and the syndrome (S) category of SFARIgene.

In Situ Proximity Ligation Assays (PLA)

The PLA method and quantitative analysis is described in (37) and in detail in supplemental methods.

Western Blot Analysis

MET Co-IP samples were subjected to Western analysis as described previously (47) and in supplemental methods.

Statistical Analysis

Statistical analyses are provided in detail in supplemental methods.

Results

Identification of NDD-associated Candidates in MET-interactome

To discover proteins that bind to unstimulated or HGF-activated MET receptor, we used anti-MET antibody to immunoprecipitate MET complexes in crude synaptosomes isolated during the peak of neocortical synaptogenesis in mice. The synaptosomes were treated with HGF for 5 minutes, as this time frame results in an abundant amount of phospho-MET receptor (38). Using Co-IP/MS, we identified 72 putative MET-interacting proteins. The candidates were classified into 4 categories based on 3 independent replicates: 1) class I: peptides present in MET Co-IP, but not control Co-IP, in all replicates (30 candidates); 2) class II: peptides present in MET Co-IP in all replicates and one peptide present in one or two control Co-IPs (18 candidates); 3) class III: peptides present in MET Co-IP in two replicates, without or with one peptide in control Co-IP (11 candidates); and 4) class IV: peptides present in MET Co-IP in at least two replicates, with more than one peptide in one control Co-IP (13 candidates) (Table S1). Including MET, 9 members of the interactome (12.5% -Table S1 and Table S2) are associated with NDDs, according to the high confidence criteria set by each of the genetic consortia. Specifically, 5 candidates (including MET) associated with ASD using Category 1 (high confidence) and Category 2 (strong candidate) on Simons Foundation Autism Research Initiative Gene (SFARIgene, https://gene.sfari.org/autdb/GS_Home.do), 2 associated with bipolar disorder (BD) based on the consortium's "hot gene list" from BDgene (<http://bdgene.psych.ac.cn/topGene.do>), 3 associated with schizophrenia (SCZ) using the 2014 Schizophrenia Working Group of the Psychiatric Genomics Consortium (48), and no candidates associated with attention deficit hyperactivity disorder (ADHD) and major depressive disorder (MDD) from ADHDgene consortium (<http://adhd.psych.ac.cn/topGene.do>) and PsyGenNet (<http://www.psygenet.org/>), respectively (Table S2).

Specific NDD Candidates Are Enriched in the MET-interactome

At the whole genome level, there is a significant enrichment in NDD-, ASD- and SCZ-associated candidates in the MET interactome (MET Co-IP; Table S2). The meaning of this enrichment is difficult to interpret, because the primary MET-interactome was generated from a synaptic fraction. Therefore, we focused our analyses on the more relevant enrichment of NDD-associated genes in the MET Co-IP at the synaptic level. Specifically, we used the genes expressed in a mouse neocortical synaptosome fraction, determined experimentally (49), as our background comparison (Table 1). There is a significant enrichment in total NDD-associated candidates in the MET Co-IP compared to the synaptosome (enrichment factor [EF]: 5.25), as well as a significant difference in the enrichment of NDD candidates associated with each NDD category (Chi-square = 102.3, $df = 7$, $p < 0.0001$). Specifically, ASD- (EF: 12.74), but not BD- (EF: 4.69) or SCZ- (EF: 4.95), associated candidates are significantly enriched in the MET Co-IP compared to the total synaptosome (Table 1). Note that no genes in the MDD or ADHD consortia datasets are represented in the MET interactome.

NDD-associated Candidates Are Present in the MET-interactome Networks

The protein sequences of MET and its primary interactome partners are highly conserved (Table S3). To translate our findings in mouse neocortex-generated data to a relevant human interactome, we performed network analyses based on human data. The 72 primary candidates in the MET Co-IP, together with their secondary interactive partners (proteins recorded in GeneMANIA that interact with proteins in MET Co-IP) and MET, were used to construct a full network, which includes 1253 nodes (interactive candidates) and 1756 edges (physical interactions) (Fig. 1A and Table S4). Five of the 1253 network proteins (0.4%) exceeded 100 interactions and thus were capped at this level. We calculated the distribution of the number of interactions for each primary candidate in the full network and found that one candidate has no interactive partner, 32 candidates have 1 to 10 interactive partners, 14 have 11 to 20 partners, 20 have 21 to 80 partners and 5 have over 100 partners (Table S5). Further, for 34 of the primary interactome candidates, at least half of their secondary partners also interact with at least one other primary candidate (Table S6), demonstrating a high degree of internal connectivity between primary candidates in the network. To further assess elements of the MET interactome, we built a sub network containing MET, the 8 NDD-associated candidates and their secondary protein interactors. This sub network has 210 nodes and 235 edges (Fig. 1B and Table S4).

Based on the NDD database defined here by the high confidence criteria set by each of the genetic consortia, as well as the syndromic genes in the SFARI database, 44 candidates (3.51%) in the full network are associated with at least one NDD (Table S7 and S8). In the sub network, 14 candidates (6.67%) are associated with NDDs (Table S7 and S8). Further, in the full MET-interactome network, 5 candidates (0.40%) are associated with ADHD, 12 (including MET, 0.96%) with ASD, 23 (1.84%) with BD, 5 (0.40%) with MDD and 17 (1.36%) (Table S8). In the sub MET-interactome network, 6 candidates (including MET, 2.86%) are associated with ASD, 6 (2.86%) with BD, 5 (2.38%) with SCZ and none with ADHD or MDD (Table S8).

Expression and Correlation Enrichment of Interactive Partners in the MET-interactome in Human Brain Development

We explored the spatio-temporal expression patterns of interactive partners in the MET interactome using the BrainSpan RNAseq database (50). The relative expression levels of MET-interactive partners were analyzed in developing human brain regions that express MET, including ITC (inferolateral temporal cortex, area TEv, area 20), STC (superior temporal cortex, area 22c), and V1C (primary visual cortex, striate cortex, area V1/17). We focused on early postnatal time periods (4 to 36 months) during which rapid neocortical synapse formation occurs (51, 52) and there is prominent MET expression (32). Third trimester data are limited in BrainSpan, and thus, we did not include this time period. Examining the 72 members of the MET-interactome, relative expression of NDD candidates and most other partners gradually increased from 4 to 12 months, reaching a peak at 12 months in ITC, STC, V1C (Fig. 2A and S1A); expression decreases in samples from 2 years old and older (Fig. 2A and S1A), which corresponds to a time when the rate of net cortical synapse formation slows (51, 52). The analysis also revealed that relative expression of NDD candidates and most MET-interactive partners is positively and highly correlated with relative expression of *MET* in ITC, STC and V1C (Fig. 2B and S1B). To address the specificity of these correlations, we also analyzed the correlation of *MET* expression with highly expressed genes (with averaged RPKM > 200) that are not present in the MET interactome. These genes exhibited different expression patterns, with most having negative or no correlation to *MET* in ITC, STC and V1C (Fig. 2A and 2B).

The Wilcoxon rank-sum test was used to compare the following categories based on the ranked correlations of genes in each category to *MET*: 1) MET-interactive partners versus highly expressed genes not in the MET interactome; 2) NDD candidates in the MET interactome versus highly expressed genes not in the MET interactome; 3) NDD candidates versus non-NDD candidates in the MET interactome; and 4) for MET-interactive partners only, ITC versus STC, ITC versus V1C and STC versus V1C. The results show that the correlation of either the whole MET interactome or NDD candidates in the MET interactome to *MET* is ranked higher statistically compared to the correlation of highly expressed genes not in the MET interactome to *MET*. For completeness of comparison (Table S9), the analysis was done using four different cutoffs (RPKM > 100, 200, 300 or 400), which all show the same significance outcome. There is no significant difference for comparisons between NDD candidates and non-NDD candidates or between different brain structures (ITC, STC and V1C) (Table S9). Together, these data suggest that MET-interactive partners have a correlated expression pattern to *MET* in temporal and visual neocortex during human brain development, but not to non-partners.

HGF Regulates Interactions in the MET-interactome

The discovery-based Co-IP/MS approach defines MET as a component of a network of a subset of NDD-relevant proteins that interact at the developing synapse. MET Co-IP/MS identified 45 candidates present in both HGF-stimulated and unstimulated groups (Table S1 and S10). To assess HGF regulation of the MET-protein partner interactions, we used two complementary approaches on a subgroup of proteins. While there were many proteins to focus upon for this analysis, we selected five candidates for which high quality antibodies

were available, and that have been implicated functionally or genetically in NDDs (10, 53–57) and represent different classes in the initial Co-IP/MS screen (see Table S1). Experiments probed MET interactions with SYNGAP1 and SHANK3 (class III), GRIN2B (NMDA receptor 2B, NR2B) and GRM5 (mGluR5) (class IV), and NTRK3 (TrkC, class I).

In the first approach, we employed PLA on primary neocortical neuron cultures at the peak of synaptogenesis (14 DIV). Based on morphology and other criteria used in our laboratory, neurons at this time were healthy and fully capable of responding to HGF by increasing dendritic growth and synaptogenesis (Fig. S2A) (38). Experiments were performed using an antibody directed against MET in combination with antibodies specifically recognizing NTRK3, SYNGAP1, SHANK3, GRIN2B or GRM5. In this assay, a fluorescent PLA signal is generated only when the proteins of interest reside within 40 nm of each other. In control experiments, no PLA signal was detected using each antibody alone (Fig. S2B) or using antibody combinations targeting MET and Histone H3 (protein not present in MET Co-IP/MS screen) (Fig. S2C). In contrast, a positive PLA signal was generated for all antibody combinations (Fig. 3A–3E), validating the proteomics data indicating direct interactions or close association of each candidate and MET. Further, we counted PLA clusters (Fig. S2D) and found that there is a dynamic regulation of these interactions when MET is activated by HGF stimulation for 5, 10 or 30 minutes (Fig. 3F–3J). Three distinct interaction states were observed: 1) no change in the interaction following addition of the ligand (MET and NTRK3, MET and SHANK3, Fig. 3F and 3H); 2) a statistically significant decrease in the interaction following HGF addition (MET and SYNGAP1, Fig. 3G); and 3) a statistically significant increase in the interaction following HGF addition (MET and GRIN2B, MET and GRM5, Fig. 3I–3J). The increased interactions were maintained over the 30-minute assay period, whereas the HGF-induced decrease was transient, returning to control levels by 30 minutes.

In the second approach, we performed semi-quantitative Western blot analyses of MET Co-IPs from crude synaptosomes isolated from P14 neocortex. Data from 4 independent experiments confirmed the proteomics data; MET interacts with NTRK3, SYNGAP1 and GRM5 (Fig. 4). HGF-stimulation for 5 minutes resulted in no statistically significant change in NTRK3-MET binding (mean fold change (HGF/PBS): 0.95, 95% CI: [0.80, 1.11]; Fig. 4A and 4D), a significant decrease in SYNGAP1-MET binding (mean fold change (HGF/PBS): 0.63, 95% CI: [0.36, 0.90]) and a significant increase in GRM5-MET binding (mean fold change (HGF/PBS): 1.35, 95% CI: [1.15, 1.55]; Fig. 4B–4D), consistent with the PLA data. Together, the biochemical and morphological data suggest that HGF differentially modulates MET-protein partner interactions, potentially influencing the development of synapses.

Cellular Co-expression of *Met* and Its Interactive Candidates in Developing Neocortex

Protein-protein interactions occur when there is cellular co-expression. To determine the extent of co-expression of *Met* with some of its interacting partners, we used RNAscope multiplex in situ hybridization. *Met* is expressed in its characteristic bilaminar pattern, with very dense labeling in layers 2–3 and sparser labeling of neurons in layers 5–6 (33). There was modest co-labeling of *Met* with *Ntrk3* and with *Grm5* in deep neocortical layers, with

far more co-expression in superficial layers (Fig. S3A–S3B and S3E–S3F). In contrast, there was dense bilaminar co-localization of *Met* and *Syngap1* in deep and superficial layers (Fig. S3C–S3D). The data, using 3 different analyses, place MET in a molecular and cellular context with protein partners that mediate specific neurodevelopmental events.

Discussion

Direct evidence from proteomics experiments is provided here to demonstrate that the MET receptor tyrosine kinase interacts with synaptic proteins implicated in both normal development and in disruption of synapses in specific NDDs. Human genetic studies have discovered high confidence NDD-associated risk genes, a major subset of which participates in synapse development and function (4, 58, 59). These findings have provided an important biological basis for causal models of NDDs, namely that related risk genes encode proteins within molecular signaling networks, which, when disrupted, cause atypical neurodevelopment that leads to specific disorders (45, 46, 60–62). These biological interactions, however, are largely inferred from genetic findings. Studies are now beginning to explore the connection among members of gene networks, evaluating putative protein interactions using predictive modeling or biochemical assays (15–17, 63).

Currently, many protein interactomes are mapped in silico. This approach has some important limitations. For example, there may be a high false positive rate for predictive modeling in humans because interactomes are defined in the absence of specific cell contexts (64), in which putative protein partners may not be co-expressed. Yeast-two hybrid discovery screens identify protein-protein interactions and map putative interactome networks (15, 16), but this approach does not address cell context limitations of interactions. To understand NDD-relevant protein-protein interactions in neuronal and developmental contexts, we performed Co-IP/MS using synaptosomes isolated at a time of peak synapse formation in the mouse neocortex. We chose this strategy because, while comparative proteomics, such as iTRAQ, provide direct comparisons of protein-protein interactions in different conditions, combining Co-IP with iTRAQ in an enriched cell fraction is technically challenging and cost-prohibitive. Specifically, when using developing neocortical synaptosomes, there is limited starting protein input after Co-IP, approximately 100-fold less than needed for high quality iTRAQ. The Co-IP/MS strategy is not directly quantitative, but is highly sensitive and has led to the discovery of novel MET-interacting partners. Further, we show that previously reported MET protein partners (GRB2, Gab1, EGFR) in cancer cells do not interact with MET in developing neocortical synapses. Thus, our data show the importance of determining functional protein networks in specific cell and developmental contexts (64). Moreover, limiting our analysis to isolated synaptosomes provides an opportunity to evaluate protein complexes that are relevant to specific physiological states (65–67). The co-expression analyses demonstrate a significant correlation of expression of MET interactome partners with *MET* in early postnatal human neocortex, as well as co-expression of specific candidates in developing rodent neocortical neurons. Such analyses (62) are essential to understand the heterogeneity of pathophysiological mechanisms.

Using MET and the primary candidates as seeds in a human protein-protein interaction database, we mapped the full MET-interactome network and a sub network defined by MET

and 8 NDD risk proteins. There are two key limitations. First, the Co-IP/MS screen identified candidates from developing mouse neocortex, but the networks are based on a human database. Second, the human database used for network generation was not produced in a neurodevelopmental context, which to our knowledge currently does not exist. Thus, the interactome networks may not define all the protein interactions of MET at developing human neocortical synapses or may define secondary protein interactions that do not occur at the synapse, a general challenge because of limitations in fresh human brain tissue. Protein-protein interactions also likely change over developmental time, though for MET, neocortical expression across species is limited mostly to the period of synaptogenesis.

As with other common alleles, the functional 'C' allele of the *MET* gene underlies very modest to low risk for an ASD diagnosis (27), and is not significant at the genome-wide level. However, the present proteomics data, together with functional neuroimaging (42) and postmortem human brain studies (13, 29, 30), and behavioral (43, 44), electrophysiology and morphological analyses of synapse development (20, 38–40) in mutant mice, suggest that the MET interactome contributes to NDD expression. This does not mean that disrupting MET alone is causal for NDDs. Rather, given its interactions with proteins such as SYNGAP1, SHANK3 and GRM5, modulation of MET may contribute to intermediate phenotypes of different NDDs (68–70). Defining functional protein networks provides a relevant molecular framework for addressing pathophysiological aspects of NDDs in a neurobiological context. Gaining an understanding of interactome dynamics may address heterogeneity of clinical phenotypes that is characteristic of single gene, syndromic and genetically complex NDDs.

We used primary and multiple comparison-corrected post-hoc tests to examine possible enrichment of NDD-associated candidates in the developing MET interactome. While the analysis did reveal enrichment with ASD but not the other NDDs analyzed (ADHD, BD, MDD and SCZ), permutation testing of our dataset against appropriate random or background datasets would improve the analysis of enrichment. However, methodologies for determining enrichment have focused on genetic data in which variants, CNVs and other rare events are examined in the context of the whole genome. Thus, we present the enrichment analysis with a note of caution, and rely on the outcomes together with cellular data and expression mapping as a way of determining convergence of evidence that the MET synaptic interactome is enriched with specific NDD-related proteins. The ligand-modulated interaction of MET with GRM5 and SYNGAP1 are of particular interest. GRM5 is implicated as a dysfunctional receptor and therapeutic target in Fragile X Syndrome (71), and the transcripts encoding MET and GRM5 are FMRP targets (72). *Syngap1* deletion causes premature maturation of functional synapses in the developing mouse hippocampus (73), an unusual phenotype that also occurs in the hippocampus due to *Met* deletion (20). Here, we discovered that MET and SYNGAP1 interact physically, which decreases with MET activation. It remains to be discovered how MET-SYNGAP1 interactions may regulate synapse maturation, but it is of significant interest because CAMKII-dependent SYNGAP1 phosphorylation impacts its dispersion in spines and AMPA receptor insertion (74).

Finally, it is important to emphasize that the current study includes an initial analysis of the dynamics of synaptic protein-interacting partners with MET activation. The data, even with

just a limited number of protein partners of MET, reveal that synaptic protein networks should be viewed as dynamic in nature. Future studies of other receptor-intracellular signaling components, together with functional analyses of the MET-NDD interactome, provide a framework for determining the molecular basis of NDD causes and, as important, a potential basis for heterogeneity of clinical phenotypes related to overlapping impact on the development of relevant brain circuits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. MET interactome full network and sub network

Protein-protein interaction networks were created using MET, the primary candidates in the MET Co-IP and their secondary interactions in human as defined in the GeneMania database. The full network (A) includes all primary candidates, while the sub network (B) includes only those associated with NDDs. Each category for nodes is labeled by different colors and shapes as indicated.

Figure 2. Transcript expression of MET interactome partners and highly expressed genes not in the MET interactome in the human brain based on BrainSpan

(A) Heatmap illustrates the relative transcript expression of MET interactome partners and highly expressed genes not in the MET interactome in the human brain during development. Numbers represent postnatal age in months. For specific genes in the MET interactome, see Supplemental Figure 1. Note that the expression within the MET interactome appears to correlate to a greater extent at 4, 10 and 12 months compared to later ages. There also appear to be subgroups of genes not in the MET interactome that show correlation patterns. (B) Heatmap illustrates the correlation of transcript expression summarized across the postnatal age range (4–36 months) of MET interactome partners and highly expressed genes not in the MET interactome with *MET*. Note the consistent correlation in the 3 cortical areas for the MET interactome members, with limited correlation with non-members. In both A and B, the MET interactome partners and highly expressed genes not in the MET interactome are

tagged with yellow and cyan, respectively. ITC: inferolateral temporal cortex; STC: superior temporal cortex; V1C: primary visual cortex.

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Figure 3. HGF dynamically regulates MET interactions in neocortical neurons

Photomicrographs show confocal images of neocortical neurons at 14 days *in vitro* following treatment with PBS or HGF for 5, 10 and 30 minutes. Red fluorescent profiles represent regions of PLA signal amplification denoting close proximity (40nm) of MET and NTRK3 (A), SYNGAP1 (B), SHANK3 (C), GRIN2B (D), or GRM5 (E). Green fluorescent profiles represent MET immunoreactivity in the same field. Quantitative analysis reveals that there are stable (NTRK3 (F), SHANK3 (H)), and changing (SYNGAP1 (G), GRIN2B (I), GRM5 (J)) states of proximity with MET following HGF receptor activation. Error bars represent standard error of the mean, N = 3 independent culturing sessions in each group. *p < 0.05; **p < 0.01. Scale bar, 5 μm in E (applies to all other images).

Figure 4. HGF modulates MET interactions in crude neocortical synaptosomes

An anti-MET antibody was used to IP the MET complex from solubilized P14 crude neocortical synaptosomes. Western blot analyses detect NTRK3 (A), SYNGAP1 (B) and GRM5 (C) in the MET complex (α-MET, PBS lane), but not in control IgG IPs (α-IgG, PBS lane). HGF activation of the receptor does not alter MET/NTRK3 interactions, down-regulates MET-SYNGAP1 interactions and up-regulates MET-GRM5 interactions (α-MET, HGF versus PBS lanes). Phospho-MET (pMET) and MET antibodies confirmed the efficiency of MET activation and MET IP, respectively. The fold change of HGF treated-group as compared to PBS-treated group for each IP is presented as box-and-whisker plots (Whiskers: 5–95 percentile) (D). The line bisecting the box represents the median. The horizontal dash line in D indicates unchanged level (1.0) for comparison between HGF and PBS. N = 4 independent Co-IP experiments for each interaction.

Table 1

NDD-associated candidates are enriched in MET-interactome

Name	ASD			BD		SCZ		NDDs	
	MET Co-IP	Synaptosome	MET Co-IP	MET Co-IP	Synaptosome	MET Co-IP	Synaptosome	MET Co-IP	Synaptosome
NDDs associated	4	14	2	19	2389	2	18	6	51
NDDs unassociated	50	2394	52	2408	2408	54	2408	48	2357
Total	54	2408	54	2408	2408	54	2408	54	2408
Percentage (%NDDs)	7.41	0.58	3.70	0.79	0.75	3.70	0.75	11.11	2.12
Enrichment factor	12.74		4.69			4.95		5.25	
p value [#]	0.014 [*]			> 1.0		> 1.0		0.036 [*]	

[#] Multiple comparison-adjusted p values;

^{*} significant at the 0.05 level.

Note that there are no proteins in the MET Co-IP that are listed as high confidence gene from the MDD and ADHD genetic consortia.

Synaptosome dataset from reference (49).