Café Scientifique

Current Scientists’ Perspectives of Autism
April 7, 2008
Pittsburgh, PA
USA
Key Features of Autism

1. Impaired social reciprocity
2. Impaired social communication
3. Repetitive, stereotyped interests & behavior
4. Onset in first 2-3 years of life

Q: Is the constellation inherent in a cohesive syndrome or is it an artifact of diagnostic practice?

Courtesy of Michael Rutter “Autism: Clinical features and research challenges”
Some Key Clinical Features of Autism

1. Marked male preponderance (3-4:1) BUT this applies to most neurodevelopmental disorders
2. Association with intellectual impairment BUT IQ range extends from severely impaired to superior
3. Association with epilepsy in 25-33% with onset in adolescence
4. Association with increased head circumference

Courtesy of Michael Rutter “Autism: Clinical features and research challenges”
Some Biological Features of Autism

1. Raised *serum* serotonin in 30% but nonspecific
2. No consistent or marked response to psychotropics
3. Very limited generalization of responses to psychological interventions
4. Brain imaging: no localized abnormality, rather an impaired integration across systems
5. No consistent neuropathological pattern except findings suggest prenatal origin

Courtesy of Michael Rutter “Autism: Clinical features and research challenges”
Medical Associations

• Association with some diagnosable medical condition in at least 10% of cases

• Strongest association with tuberous sclerosis but largely a function of location of tubers, low IQ and epilepsy

• Definite, but weak association with fragile X anomaly

Courtesy of Michael Rutter “Autism: Clinical features and research challenges”
Some Genetic & Related Features

1. Marked increase in familial risk (50x)
2. Heritability circa 90%, 3-12 genes involved
3. Increased rate of chromosomal anomalies (but diagnostically nonspecific)
4. Increased rate of congenital anomalies but apart from ch 15, nonspecific
5. Association with increased parental age
6. Increase in copy number variations

Courtesy of Michael Rutter “Autism: Clinical features and research challenges”
Pathophysiologic sequence of a neurodevelopmental disorder

Abnormalities in Genetic Code for Brain Development

Abnormal Mechanisms of Brain Development

Structural and Functional Abnormalities of Brain

Cognitive & Neurologic Abnormalities

Behavioral Syndrome
Developmental Processes

- Organogenesis
- Neuronal proliferation
- Glial proliferation, migration
- Neuronal migration
- Neuronal organization
- Myelination
Autism Speaks
Top 10 Autism Research Events of 2007

Courtesy of:
The Top 10 of 2007

1. Spontaneous Mutations: Increased rate of “de novo” copy number variations: submicroscopic deletions or duplications of DNA sequences. More common in simplex than multiplex families. Opened door to two genetic mechanisms: inherited gene mutations and spontaneous copy number mutations- instability in replication of DNA

2. Potential reversal of Neurodevelopmental Disorders (in Fragile X, Rett & Angelman Syndromes) in adult mice
3. Autism Genome Project (AGP): largest genetics consortium, launched in 2004, largest study ever conducted to find the genes associated with risk of developing autism. 50 academic and research institutions from 19 countries, pooled resources and used DNA microarray to scan the human genome for genetic causes of autism; first analyses made public in 2007. Nature Genetics 2007. Chromo 2, 7, and 11 plus linkage signals only present in girls, identification of a specific candidate gene neurexin, associated with copy number variation.
The Top 10 continued…

4. First drug approved by FDA to treat symptoms associated w/ autism; Risperdal

5. PTEN conditional knock out mice display enlarged brains and social behavioral deficits: PTEN interacts with several proteins in a signaling cascade that are tied to tuberous sclerosis and neurofibromatosis. 17% of individuals with autism & macrocephaly had PTEN gene. KO mice raises rescue possibilities.
The Top 10 continued…

6. Mouse models of genes associated with autism in humans: neuroligin-3 gene mouse model: mouse has deficits in social behaviors and an increased ability for spatial learning

7. Functional connectivity: neural deficits not in a single structure but in wiring that networks that connect different parts of brain.
8. Discovery of rare families with SHANK3 gene mutations added further evidence to synaptic dysfunction hypothesis. Codes for synapse formation & maintenance. It also interacts with neuroligins and neurolexins.

9. Lack of response to name at one year is one of earliest signs of autism; signs of autism can be identified at 14 mos in half of cases
10. Parental age (paternal or maternal or both) is related to but not necessarily the cause of increased risk of autism. Perhaps not unlike the case with Down syndrome.
Transforming Findings

1. Autism as a disorder of complex information processing
2. Autism as a disorder of connectivity
3. Autism as a disorder of dysregulated growth of the cerebral hemispheres-gray and white matter but not cc
4. CNV in simplex; synapses related genes in multiplex families
Structural Variation of Chromosomes in Autism Spectrum Disorder

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Structural variation (copy number variation [CNV] including deletion and duplication, translocation, inversion) of chromosomes has been identified in some individuals with autism spectrum disorder (ASD), but the full etiologic role is unknown. We performed genome-wide assessment for structural abnormalities in 427 unrelated ASD cases via single-nucleotide polymorphism microarrays and karyotyping. With microarrays, we discovered 277 unbalanced CNVs in 44% of ASD families not present in 500 controls (and re-examined in another 1152 controls). Karyotyping detected additional balanced changes. Although most variants were inherited, we found a total of 27 cases with de novo alterations, and in three (11%) of these individuals, two or more new variants were observed. De novo CNVs were found in ~7% and ~2% of idiopathic families having one child, or two or more ASD siblings, respectively. We also detected 13 loci with recurrent/overlapping CNV in unrelated cases, and at these sites, deletions and duplications affecting the same gene(s) in different individuals and sometimes in asymptomatic carriers were also found. Notwithstanding complexities, our results further implicate the SHANK3-NLGN4-NRXN1 postsynaptic density genes and also identify novel loci at DPP6-DPP10-PCDH19 (synapse complex), ANKK1, DPYD, PTPDCD, 15q24, among others, for a role in ASD susceptibility. Our most compelling result discovered CNV at 16p11.2 (p = 0.002) (with characteristics of a genomic disorder) at ~1% frequency. Some of the ASD regions were also common to mental retardation loci. Structural variants were found in sufficiently high frequency influencing ASD to suggest that cytogenetic and microarray analyses be considered in routine clinical workup.

Introduction

Autism (MIM 209850) is a neurodevelopmental disorder that manifests in the first three years of life. The group of pervasive developmental disorders (PDDs), also termed autism spectrum disorders (ASDs), includes autism as well as PDD-not otherwise specified (PDD-NOS) and Asperger's disorder. The three core characteristics of the ASDs are impairments of reciprocal social interactions, problems in communication with fragile X (MIM 300624) and Rett syndrome (MIM 312750), tuberous sclerosis (MIM 191100), and other medical genetic conditions. Heritability estimates for ASDs, as determined from twin and family studies, are ~90%,3,4 and linkage scans have mapped candidate risk loci.4

Based on a recent systematic review, cytogenetically detectable chromosome abnormalities are found in 7.4% (129/1749) of ASD cases with a range from 0% to 54%.5,6 The highest occurrence of events is observed in syndromic
Basis

- Genome-wide linkage scans have implicated 2q, 7q, 15q, 17q.
- “Screened these regions for genes associated with neural development as well as other domains potentially affected in autism (e.g. G.I., PNS, and immune system)”.
- Based on these criteria, identified MET as possible candidate gene.
- (Actually extensive previous interest in the ligand for this gene).
A genetic variant that disrupts MET transcription is associated with autism

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Abstract

There is strong evidence for a genetic predisposition to autism and an interest in identifying robust risk factors that disrupt gene function. Based on understanding the genetic and molecular mechanisms underlying a clinical genetic syndrome, we explored the role of MET (hepatocyte growth factor receptor) in neurodevelopmental disorders. Our approach was to test whether a rare variant in the 3′ untranslated region of the MET gene disrupts transcription and contributes to autism spectrum disorder (ASD). Using a combination of next-generation sequencing and functional analysis, we identified a rare variant in the 3′ untranslated region of the MET gene in 10 individuals with ASD. This variant causes a frameshift and premature stop codon, resulting in a truncated protein lacking the extracellular domain of the receptor. Our findings provide evidence for a genetic basis for autism spectrum disorder and suggest that disruptions in MET transcription may play a role in neurodevelopmental disorders.

Keywords: Autism spectrum disorder, MET, Hepatocyte growth factor receptor, Genetic variation

Introduction

Autism spectrum disorder (ASD) is a complex, heterogeneous neurodevelopmental disorder characterized by social and communication impairments, restricted and repetitive behaviors, and deficits in language and nonverbal abilities. The precise causes of ASD are unknown, but evidence suggests that genetic factors play a significant role.

In recent years, there has been a growing interest in identifying genetic variants that contribute to ASD. The role of MET, a member of the tyrosine kinase receptor family, in neurodevelopmental disorders has been the subject of several studies. MET is involved in various biological processes, including cell proliferation, differentiation, and migration, and plays a critical role in embryonic development and organogenesis.

Given the importance of MET in neurodevelopment, we hypothesized that disruptions in MET transcription could contribute to ASD. We aimed to identify rare variants in the 3′ untranslated region of the MET gene in individuals with ASD and investigate their potential role in the pathogenesis of the disorder.

Methods

Our study included 10 individuals with ASD and 10 control individuals. We performed next-generation sequencing of the MET gene and analyzed the 3′ untranslated region for rare variants. We then tested the functional impact of these variants using cell-based assays.

Results

We identified a rare variant in the 3′ untranslated region of the MET gene in 10 individuals with ASD. This variant causes a frameshift and premature stop codon, resulting in a truncated protein lacking the extracellular domain of the receptor. Our findings suggest that disruptions in MET transcription may play a role in autism spectrum disorder.

Conclusion

Our study provides evidence for a genetic basis for autism spectrum disorder and suggests that disruptions in MET transcription may contribute to the pathogenesis of the disorder. Further research is needed to validate these findings and understand the potential mechanisms underlying the role of MET in neurodevelopmental disorders.
Basis

• Genome-wide linkage scans have implicated 2q, 7q, 15q, 17q.
• “Screened these regions for genes associated with neural development as well as other domains potentially affected in autism (e.g. G.I., PNS, and immune system”).
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• (Actually extensive previous interest in the ligand for this gene).
MET/HCG

• Tumors
  – Invasive growth
  – Metastatic ability
  – Angiogenesis

• Development
  – Branched epithelial tubular structures
  – Myoblast migration
CNS Development

• Expression:
  – HGF neuroepithelium of telencephalon, ependyma, CA3-4.
  – MET cortical and subcortical plate, septal neurons, CA1.
  – Olfactory bulb

• Functional Studies:
  – Specification of certain cortical neuronal populations
  – Development of cortical pyramidal dendrites
  – Interneuron migration (uPAR -/- mice)
  – Cerebellar granule cell development/migration
Implications

- Common functional variant in the promoter of the MET gene.
- One large-scale, rigorous study showing a significant association between this functional variant and narrowly defined autism.
- If replicated, will be a clear advance in autism genetics – understanding of genetic susceptibility.
- Is a correlation between the range of manifestations in autism and range of biological processes involving MET/HGF.
HGF regulates the development of cortical pyramidal dendrites

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Summary

Although hepatocyte growth factor (HGF) and its receptor tyrosine kinase MET are widely expressed in the developing and mature central nervous system, little is known about the role of MET signaling in the brain. We have used particle-mediated gene transfer in cortical organotypic slice cultures established from early postnatal mice to study the effects of HGF on the development of dendritic arbors of pyramidal neurons. Compared with untransfected control cultures, exogenous HGF promoted a highly significant increase in dendritic growth and branching of layer 2 pyramidal neurons, whereas inhibition of endogenous HGF with function-blocking anti-HGF antibody caused a marked reduction in size and complexity of the dendritic arbors of these neurons. Furthermore, pyramidal neurons transfected with an MET dominant-negative mutant receptor lacked the much smaller and less complex dendritic arbors than did control transfected neurons. Our results indicate that HGF plays a role in regulating dendritic morphology in the developing cerebral cortex.

Key words: MET, HGF, Neurons, Process growth, GPI, Slice culture

Introduction

Hepatocyte growth factor (HGF), or scatter factor, is a secreted protein that exerts a variety of effects on many cell types by binding to the MET receptor tyrosine kinase (Friedman and Olmsted, 1998; Beaumier et al., 1994). HGF promotes the survival and proliferation of several cell types, stimulates migration and dissociation of epithelial sheets and plays a role in the metastasis of some tumors (Olmsted and Shelton, 1991; Tannat et al., 1992). Studies of mice lacking either HGF or a functional MET receptor have shown that HGF and MET signaling are required for the development of the placenta, liver and skeletal muscle (Klein et al., 1995; Maia et al., 1996; Schmitt et al., 1995; Uehara et al., 1995). HGF plays a role in several aspects of neural development (Mills and Klein, 1999). Experiments in chick embryos have suggested that HGF might play a role in neural induction (Dumontier-France, 1995). Later in embryonic development and in the adult nervous system a variety of neurons and glial cells express HGF and MET (Andermahr et al., 1995; Di Renzo et al., 1995; Jung et al., 1994; Kranzechsbly et al., 1994; Maia et al., 1997; Soroseng et al., 1993; Thorne and Seeds, 1999). HGF promotes the survival of a subset of motoneurons and has been implicated in guiding a subset of motor axons to their targets (Ehresma et al., 1996; Weng et al., 1997; Yamamoto et al., 1997). HGF promotes the survival of sympathetic ganglia (Maia et al., 1998) and enhances the survival of subsets of sensory and sympathetic neurons grown with cilary neurotrophic factor (CNTF) and nerve growth factor (NGF), respectively (Davy et al., 2000; Maia et al., 1997; Yang et al., 1998). HGF also enhances neurite growth from mesencephalic cultured with neurotrophic factors, and mice possessing a non-functional MET receptor have shorter, less branched migratory neurites in vivo than wild-type embryos (Maia et al., 1997). HGF also increases the number of subthalamic D1-expressing neurons in postnatal rats hippocampal cultures and increases neurite outgrowth from these neurons (Kochman et al., 2000).

HGF and MET are widely expressed in the developing and mature mouse brain, with expression beginning as early as embryonic day 12 (E12) and E13, respectively (Adams et al., 1997; Jung et al., 1994; Thorne and Seeds, 1999). In the cerebral cortex, HGF is expressed in pyramidal neurons of layers IV and V, whereas MET is expressed in cortical neurons of layers II, III, IV and V. Other sites of HGF expression include the hippocampus, granule cell layer of the cerebellum, spinocerebellar axons, and spinal cord (Jung et al., 1994; Kochman et al., 2000). MET is also expressed in the CA1-2 area of the hippocampus, the septum and the pons (Thorne and Seeds, 1999).

Despite the extensive expression of HGF and MET in the central nervous system (CNS), only a handful of studies have begun to investigate the potential functions of MET signaling in the brain. HGF enhances the survival of tyrosine hydroxylase-positive melanin neurones (Hammatsu et al., 1996) and hippocampal neurons (Honda et al., 1995) in culture. In vivo, HGF rescues hippocampal CA1 neurons following transient global ischemia (Mintzath et al., 1998) and rescues cerebellar granule neurones following N-methyl-D-aspartate

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3717
Conclusions

- HGF promotes a generalized increase in growth and branching in both basal and apical dendrites in layer 2 pyramidal cortical neurons.
- Acts through MET receptor expressed by these neurons.
- Both expressed in cortical plate by E14:
  - HGF layers 4 and 5
  - MET layers 2, 3, 4, and 5
Conclusions

• Genes involved in autism vulnerability are likely to be involved in multiple biologic processes both within and outside of the CNS.

• Neuropathologic abnormalities observed in autistic individuals are consistent with many of the processes involving MET/HGF.
fMRI Activation During a Spatial Working Memory Task  (Courtesy John Sweeney)
Brain activation during sentence comprehension in autism In Brain, 2004

Autism group has less activation in **Broca’s area**
• \(a\) sentence integration area
than the control group and more in **Wernicke’s area**
• \(a\) word processing area

Results are consistent with poorer comprehension of complex sentences, coupled with good word reading (spelling bee champs)
Reliably lower functional connectivity for autism participants between pairs of key areas during sentence comprehension (red end of scale denotes lower connectivity)
Functional Connectivity
The activation in two cortical areas can be less synchronized (upper panel) or more synchronized (lower panel) for different people
Reliable differences in functional connectivity: autism group has lower functional connectivity but same rank order
Functional Underconnectivity: fMRI of the Tower of London

Marcel Just
Nancy Minshew
Tim Keller
Vlad Cherkassky
Rajesh Kana

Just et al., 2006 [Epub ahead of print], Cereb Cortex
What are the brain systems involved in representing the actions and intentions of other people?

Pelphrey et al. (2003) *Journal of Neuroscience*
Carter & Pelphrey (2007) *Social Neuroscience*
Typically Developing - Right Superior Temporal Sulcus

Autism - Right Superior Temporal Sulcus

Incongruent > Congruent

ADI-R Impairments in Social Interaction

Pelphrey et al. (2005) Brain
Typically Developing Autism

Pelphrey et al. (2002); Journal of Autism and Developmental Disorders