Bridging the Gap between Genes and Behavior: Brain-Derived Neurotrophic Factor and the mTOR Pathway in Idiopathic Autism

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Background: Although autism is highly genetic, “idiopathic” cases, for which there is no known genetic basis, may be due to epigenetic or environmental factors. Indeed, recent efforts have been highly successful in identifying hundreds of genes, as well as interacting epigenetic and environmental factors that contribute to autism susceptibility, corroborating the importance of gene x environment interactions in the etiology of autism. Nevertheless, a more thorough understanding of the proteins and pathways that lead from genes to behavior is desperately needed.

Methods: Genetic studies have implicated molecules involved in synapse development and plasticity in autism pathogenesis. Among these are brain-derived neurotrophic factor (BDNF), its receptor, tropomyosin-related kinase B (TrkB), and their signaling pathways including mammalian target of rapamycin (mTOR), which is increased in most forms of syndromic autism. Notably, abnormalities in these molecules have also been found in idiopathic autism. Postmortem brain tissue of subjects with idiopathic autism exhibits imbalances in BDNF isoforms, reduced TrkB and downstream effectors PI3 kinase (PI3K), mTOR, Epidermal growth factor receptor pathway substrate 8 (Eps8) and the excitatory synaptic marker postsynaptic density protein 95 kDa (PSD-95). Furthermore, similar TrkB pathway deficits including reduced TrkB/mTOR signaling and PSD-95, along with autistic-like behavior, have been found in valproic acid-exposed rodents, a model of environmental/epigenetic causes of autism. Our studies in both human idiopathic autism and the valproic acid-induced rodent model suggest that decreased signaling through the mTOR pathway can be as damaging as its over-activation.

Results: Epidemiological and twin studies point to a major role for genetic factors in ASD [5,6], with a complex pattern of transmission thought to be the result of perhaps as many as 1000 interacting genes [7-9]. Hundreds of rare genetic events that carry increased risk for ASD, many of them arising de novo [10-14], have been identified [15-21]. However, genetic changes account for only half of all autistic cases; environmental influences are responsible for the remaining half [22]. Cases of autism without known genetic abnormality are termed “idiopathic”. Environmental factors causing idiopathic autism include in utero exposure to drugs such as valproic acid, environmental toxins such as pesticides, infection, paternal age, or other insults [23-29]. These environmental insults are thought to confer autism susceptibility by inducing epigenetic changes [30-36]. Involvement of epigenetic mechanisms in the etiology of idiopathic autism is supported by autistic behavior arising from epigenetic mutations (Fragile X syndrome) or disruption of key epigenetic regulatory factors (Rett syndrome) [37-39]. Further support for this hypothesis comes from recent exome sequencing studies showing that rare sequence variations in autism occur in genes coding for transcriptional and chromatin-remodeling proteins [7]. Together, these findings suggest that epigenetic dysregulation of gene expression might provide a link between gene and environment in autism pathogenesis.

The Synapse

Rare mutations and copy number variations in genes coding for synaptic proteins [40-45] suggest that alterations at synapses contribute to the behavioral and cognitive deficits of autistic subjects. Indeed, autism-linked mutations have been identified in genes involved in neurogenesis, neurite outgrowth and guidance, excitation-inhibition balance, and spine protein synthesis regulation [46]. Also, consistent with this evidence and supporting the hypothesis that synaptic dysfunction may play a major role in the etiology of ASD, animal models carrying autism-linked mutations in synaptic adhesion and scaffolding proteins, such as neuroligins and Shank, exhibit autistic-like behaviors [18,47]. These findings have focused attention on aberrant connectivity as an underlying cause of both genetically-linked ASD and idiopathic autism.
BDNF Biological Activity

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that plays a key role in neuronal development and differentiation, axonal and dendritic growth and connectivity, cell morphology, regulation of synaptogenesis and dendritic spine homeostasis, excitation/inhibition balance, and synaptic transmission and plasticity [48-56] via some of the pathways known to be affected in ASD [57,58]. It also modulates long-term potentiation and is involved in learning, memory and attention [49,59]. Because of its pivotal role in guiding synaptic development, plasticity and cortical organization [53], BDNF is an essential molecule in brain development and thus a prime candidate for involvement in ASD.

BDNF Isoforms

BDNF is synthesized as a 32 kDa precursor, proBDNF, that can be processed to the 14 kDa mature form [60] either intracellularly [61] or extracellularly [62,63]. ProBDNF and BDNF are secreted molecules; proBDNF is thought to be the major form released at the dendrites, whereas in the soma it is secreted as mature BDNF after being processed in the Golgi [64]. ProBDNF exhibits distinct and opposite functions compared to mature BDNF, reducing dendritic spines and inducing apoptosis and long-term depression in cultured neurons. Conversely, mature BDNF promotes spine formation, neuronal survival and long-term potentiation [63,65-67]. This suggests that the correct balance between proBDNF and mature BDNF is crucial for synaptic development and plasticity, and that an imbalance of proBDNF and mature BDNF may contribute to synaptic abnormalities. Through a different cleavage, proBDNF also produces another BDNF isoform of 28 kDa called truncated BDNF, which is not further processed into mature BDNF [68]. The biological activities and roles of truncated BDNF are unknown.

Receptors

ProBDNF and BDNF bind to two different types of receptors. The first is TrkB, a receptor tyrosine kinase primarily responsible for transducing the survival, differentiation, spine maturation, and activity-dependent synapse strengthening functions of BDNF. TrkB is densely expressed on cortical and hippocampal neurons [54,69] and is involved in formation of both glutamatergic and GABAergic synapses during development [70-72]. With maturation, TrkB becomes enriched at excitatory synapses [73]. BDNF and proBDNF also bind to the low-affinity pan-neurotrophin receptor p75NTR, implicated in opposing functions to TrkB including apoptosis, neurite retraction, and LTD [74,75]. ProBDNF binds more strongly to p75NTR, whereas BDNF binds more strongly to TrkB [62], providing a balance in the brain between synaptic strengthening (BDNF/TrkB) and synaptic weakening (proBDNF/p75NTR). TrkB Signaling

TrkB is expressed in three splice variants [88]. Full-length receptors (TrkB-FL) contain an intracellular catalytic tyrosine kinase domain, are expressed almost exclusively by pyramidal neurons and interneurons and mediate classic neurotrophic signaling. Conversely, the two truncated TrkB isoforms, TrkB-T1 and TrkB-Shc, are located on both neurons and glia and are able to bind and sequester BDNF but, lacking kinase activity, cannot elicit the normal cellular response to BDNF [89]. They thus may act as negative regulators of BDNF signaling [90]. Neurotrophic effects of BDNF depend on the relative levels of TrkB isoforms [89,91,92].

BDNF binding to TrkB-FL leads to the activation of several intracellular pathways, including the phosphoinositide-3'-kinase (PI3K) pathway and the mitogen-activated protein kinase (ERK) pathway [74,93], which play key roles in the developing and adult brain. Pathways downstream of PI3K that play a pivotal role in synapse formation and function include the Epidermal growth factor receptor pathway substrate 8 (Eps8)-Rac pathway modulating Rac-dependent actin cytoskeletal remodeling at synapses [94,95] and the Akt-mammalian target of rapamycin (mTOR) pathway controlling spine protein synthesis [96] (Figure 1).

Figure 1. BDNF/TrkB/PI3K-activated intracellular signaling cascades involved in spine protein synthesis and actin remodeling at synapses. These include the Akt-mTOR pathway which regulates spine protein synthesis via two downstream signaling cascades, 4E-BP1/elf4E and S6 kinase/S6, and the Eps8-Rac pathway modulating Rac-dependent actin cytoskeletal remodeling at synapses [94,95] and the Akt-mammalian target of rapamycin (mTOR) pathway controlling spine protein synthesis [96] (Figure 1).

Specifically, mTOR influences protein translation at spines via two downstream pathways which are responsible for promoting translation of different pools of mRNAs [97]. One pathway involves S6 kinase and the eukaryotic initiation factor 4B (elf4E), while the other comprises the eukaryotic initiation factor 4E (elf4E) and its binding protein 1 (4E-BP1) [97-99]. A third pathway, the Erk signaling cascade, also contributes to regulation of local translation by BDNF through elf4E phosphorylation [100,101]. Lastly, proBDNF/p75NTR...
signals through several pathways, including RhôA [74,75], which opposes the Eps8-Rac pathway to promote spine or neurite retraction.

**BDNF and TrkB in ASD**

The molecular underpinnings of ASD remain unknown. Genetic, anatomical and functional imaging studies suggest that defects in synaptic development and plasticity, which impair establishment and maintenance of functional neuronal networks, underlie the clinical symptoms of autism. BDNF, TrkB and their signaling pathways, including Akt-mTOR and Eps8/Rac, play a key role in the development of the cortex and in synaptic function and plasticity [74,93]. It follows that altered BDNF/TrkB signaling through these pathways might be an important substrate of autism pathogenesis.

Multiple studies using ELISAs have reported elevated BDNF-immunoreactive protein in cord blood [102], serum [103,104] platelet-rich plasma [105] and brain [106,107] from ASD vs. control subjects. Specific BDNF SNP haplotypes are associated with autism [108], as are multiple SNPs and haplotypes of the TrkB gene, NTRK2 [105]. Furthermore, potential links between alterations in BDNF/TrkB-mediated signaling pathways and ASD are supported by reports of decreased phosphorylated and total Akt protein in the frontal cortex of autistic patients [109]. Also, mutations in Akt-mTOR cascade components, including hamartin (TSC1), tuberin (TSC2), phosphatase and tensin homolog on chromosome ten (PTEN) and methyl-CpG-binding protein 2 (MeCP2), cause monogenic disorders with high rates of autism [40,110-112]. In addition, single nucleotide insertions in the promoter of mTOR downstream effector eIF4E have been found in autistic patients [113], and autism-like phenotypes have been observed in eIF4EBP2 knockout and eIF4E-overexpressing mice [114]. Collectively, these findings strengthen the hypothesis that defective BDNF/TrkB-mediated pathways contribute to autism pathology, likely by disrupting mTOR-dependent protein synthesis.

**De-Regulated mTOR, Dendritic Spines and Synaptic Transmission**

mTOR is a key “hub” in the control of spine protein synthesis and neuronal survival, and either too much or too little signaling through this pathway can result in autistic behavior. For example, mutations in TSC1/2 and PTEN, which activate the PI3K-Akt-mTOR pathway, lead to tuberous sclerosis complex and macrocephaly, respectively, disorders exhibiting a high prevalence of autism [40,111]. Conversely, mutations in MeCP2, a transcriptional regulator that acts through epigenetic changes in chromatin structure, reduce BDNF expression and mTOR pathway signaling and cause Rett syndrome, which like tuberous sclerosis complex and macrocephaly, is another monogenic disorder associated with high rates of autism [115,116]. This suggests that either hypo- or hyper-activation of mTOR, leading to deficient or excessive protein synthesis at synapses, may be equally disruptive; optimal synaptic function appears to occur within a narrow range [110,117].

mTOR regulates dendrite growth and dendritic spine morphogenesis [96,118,119]. Both up- and down-regulation of the mTOR pathway result in abnormal spine morphology and density as seen in tuberous sclerosis complex (increased mTOR), fragile X (increased mTOR) and Rett (decreased mTOR) syndrome animal models and patients [112]. Specifically, both tuberous sclerosis complex and Rett syndrome are characterized by a decreased number of spines, with the spines that are present having elongated necks [119-122]. However, spine heads are enlarged in tuberous sclerosis complex [119], while they are reduced in Rett syndrome [121]. Lastly, patients and rodent models of fragile X syndrome have an increased density of long and thin (immature) spines [123-127]. Alterations in dendritic spines, which are the principle site of excitatory synapses [128-131], are likely to impair establishment and remodelling of cortical networks that subserve higher cognitive functions and behavior. Hence, by perturbing dendritic spines (excitatory synapses), a hypo- or hyper-activated mTOR pathway impairs connectivity in neuronal circuits and thus contributes to autism etiology. Further support for this hypothesis comes from the evidence that reduced TrkB-mTOR signaling is associated with a significant decrease in protein expression of the excitatory synaptic marker PSD-95 in patients with idiopathic autism [132], suggesting fewer excitatory synapses. Lastly, knockout of the TrkB-activated actin-capping molecule Eps8 results in widespread abnormalities in spine morphology and function, decreased LTP and autism-like symptoms [115]. Taken together, these data support the model that defective TrkB-mTOR signaling disrupts spine density, morphology and function, hence playing a key role in aberrant connectivity during development and thereby in the etiology of autistic traits.

It is has been demonstrated recently that mTOR not only plays a role in regulating development and plasticity of excitatory synapses, but also in controlling GABAergic transmission [133]. It is thus possible that de-regulated mTOR might adversely affect GABAergic synaptic activity. Notably, abnormal GABAergic transmission has been observed in Fmr1 knockout (KO) mice [134-136] and MeCP2 null mice [137,138]. In particular, Fmr1-KO mice have enhanced GABA-mediated synaptic transmission [134], while GABAergic synaptic transmission is greatly reduced in MeCP2 deficient mice [137,138]. This suggests that either too much or too little mTOR perturbs neuronal networks and contributes to autistic behavior by altering both glutamatergic and GABAergic synapses.

Interestingly, autistic-like behaviors in mouse models can be reversed in the adult by administration of mTOR inhibitors [139,140] or activators [141]. Tsc2−/− mice have increased Akt-mTOR signaling and impaired social behavior, both of which were rescued by treatment with the mTOR inhibitor rapamycin [139]. MeCP2 (Rett) mice have reduced BDNF, which has been linked to abnormal excitability, as well as reduced Akt-mTOR signaling and protein synthesis, which are associated with disease progression [142,143]. Increasing BDNF expression in these mice rescues TrkB/Akt signaling and breathing dysfunction [143]. Also, treatment with insulin-like growth factor 1 (IGF-1) which, like BDNF, activates the mTOR pathway [144], rescues synaptic deficits and social and anxiety behavior in MeCP2 mice and in human Rett syndrome [141,145]. Reversal of autistic behavior in these genetic models of ASD, particularly in Rett syndrome, which arises from a mutation in an epigenetic regulatory gene, supports the exciting possibility that autistic behavior can also be reversed in idiopathic autism.

**Molecular Studies in Human Postmortem Brain**

Distinct areas of the brain are associated with the behavioral deficits of ASD patients [146]. For example, disruptions of the fusiform gyrus area of cortex are associated with poor social skills and difficulty with face perception [147-152]. Therefore, we examined fusiform gyrus using a molecular approach [106]. Postmortem brain samples from subjects with autism and matched controls were provided to us by the Autism Speaks’ Autism Tissue Program (ATP, Princeton, NJ), the
Harvard Brain Tissue Resource Center (Belmont, MA) and the NICHD Brain and Tissue Bank (University of Maryland, Baltimore). To reduce heterogeneity, we selected subjects with idiopathic autism and excluded autism spectrum and related genetic disorders such as Asperger’s, Rett syndrome, and PDD-NOS.

**BDNF Isoform Imbalance in Idiopathic Autism**

In agreement with previous studies, using ELISAs, we found elevated BDNF-immunoreactivity in postmortem fusiform gyrus of subjects with autism compared to controls [106]. These data confirm a neurochemical abnormality in a brain region implicated in social interaction deficits in ASD. However, ELISAs cannot distinguish between the three BDNF protein isoforms. Thus, we quantified BDNF isoforms in autistic and control fusiform gyrus using Western blotting. We found elevated proBDNF and decreased truncated BDNF protein levels but no difference in BDNF mRNA levels [106] in fusiform gyrus of autism subjects compared to controls.

**Decreased Catalytic TrkB and TrkB Signaling in Idiopathic Autism**

We also found decreased full-length TrkB (TrkB-FL) isoform levels in autism postmortem fusiform gyrus [132]. This decrease in TrkB-FL protein levels is not due to changes in relative amounts of neurons vs. glia. Indeed, we observed no difference in the protein levels of neuronal (βIII-Tubulin) or glial (GFAP) markers between autism and control samples [132]. Furthermore, we demonstrated reduced BDNF/TrkB downstream effectors P13K, Akt, mTOR, S6 kinase (p70 S6K) and eIF4B protein expression [132], suggesting decreased translation of mRNAs mainly encoding components of the translational machinery (Figure 2).

![Figure 2. Schematic diagram of BDNF/TrkB and downstream pathways through Ras-Mek-Erk and PI3K-Akt-mTOR. Yellow indicates proteins implicated in single-gene disorders associated with high rates of autism. Green indicates proteins implicated in our study of idiopathic autism. White indicates proteins that do not exhibit differences between idiopathic autism and control (Erk, 4E-BP, eIF4E) or that have not yet been tested.](image)

Signaling is reduced through this pathway: reduced phosphorylation of mTOR is evident in fusiform gyrus of autism vs. control subjects [132]. No significant changes in 4E-BP1 or eIF4E protein levels were found in the same cohort [132], nor was there a difference between groups in Erk protein expression [115], demonstrating the specificity of the pathway deficit. These findings are consistent with the model that the key role BDNF plays in development of the cortex is in part mediated by activation of the PI3K-Akt-mTOR signaling pathway, which affects dendritic arborization and local protein synthesis in dendrites [96]. Additionally, these results support the idea that hypo-activation of the mTOR pathway may, like hyper-activation, contribute to autistic behavior [110,117].

A separate pathway downstream of PI3K activates Eps8, an actin-capping molecule that controls spine stability and filopodial motility in response to BDNF [153]. The Eps8 pathway signals through Rac, which stabilizes dendritic spines, and is opposed by Rho, which destabilizes spines [154]. We found significantly decreased Eps8 in autism vs. control fusiform gyrus [115], suggesting an imbalance in the Rac/Rho pathway influencing spine density and maturation. Together with decreased Eps8/Rac signaling, increased proBDNF in autism, acting through p75NTR to activate Rho, may unbalance the Rac/Rho pathway. This may destabilize spines and reduce neuritogenesis (Figure 1). The importance of this pathway for autism is corroborated by the fact that Eps8 knockout mice exhibit autistic-like behavior and aberrant LTP and spine morphology [115].

Notably, disruptions of BDNF/TrkB signaling cascades are associated with significant reductions in the excitatory postsynaptic marker PSD-95 in autism vs. control fusiform gyrus [132]. PSD-95 is a
scaffolding protein essential for synaptic organization and function. It forms a complex with TrkB, thereby promoting signaling through PI3K-Akt, which in turn induces synaptic localization of PSD-95, crucial for both synapse formation and synaptic plasticity [93,155]. Taken together, these findings suggest that imbalances in BDNF/TrkB signaling may ultimately affect excitatory synapses and consequently the development and maintenance of cortical circuits, thus contributing to autism’s cognitive and behavioral deficits.

Consistent with this hypothesis, our studies showed that abnormalities in BDNF isoforms [106], TrkB and their downstream signaling pathways [132] are widespread in the brains of subjects with idiopathic autism (excluding subjects with other disorders on the spectrum or with known genetic causes of autism). In idiopathic autism, there is decreased TrkB signaling through the PI3K/Akt/mTOR pathway [132] and through the Eps8/Rac pathway [115], whereas proBDNF is increased [106]. This is in contrast to neurodevelopmental disorders with high rates of autism such as tuberous sclerosis, neurofibromatosis type I and Fragile X syndrome. The mutated genes in these disorders normally apply “brakes” to the mTOR pathway. Their mutation causes increased mTOR pathway signaling, the opposite of the decrease we see when there are deficits in the “accelerator”, TrkB (Figure 2). Thus, an imbalance in either direction in this pathway appears to result in autistic behavior.

Although many of the genes in these and related pathways have been implicated in recent genetic studies of ASD, their protein products had not been examined in autistic brain. The importance of fully documenting changes in these signaling cascades in postmortem tissue cannot be understated. It is difficult, if not impossible, to move to translational work (targeting specific molecules for therapy) without full knowledge of the underlying molecular dysfunction in the human brain, which, as demonstrated here, may differ between genetic and epigenetic syndromes. To further investigate the relationship of TrkB signaling deficits to autistic behavior, we have now moved to an animal model that mirrors the molecular changes described above. We have chosen the valproic acid (VPA)-exposed rodent because of its validity as a model for the pathways and behavior under study, and as a model for possible epigenetic origins of idiopathic autism.

**Maternal Challenge with VPA as a Model of Idiopathic Autism**

Valproic acid (VPA) is a known risk factor for autism. This fatty acid is used widely as an antiepileptic drug [156] and for the treatment of mood disorders [157,158]. VPA regulates gene expression through (epigenetic) chromatin remodeling by inhibition of histone deacetylase activity [159]. Maternal exposure to VPA at the time of neural tube closure increases the risk of autism in humans [160-163] and causes autistic-like symptoms in rodents [164,165]. A single exposure to VPA in *utero* causes impaired social interactions, stereotypical hyperactivity and sensory/communication deficits in rodents’ offspring [164-167]. Rodents prenatally exposed to VPA also show anatomical and molecular alterations similar to human autism including decreased cerebellar volume and Purkinje cell number [163,168,169], disrupted spine density and morphology [170-172], decreased expression of the autism-associated postsynaptic adhesion molecule Neulolin 3 [173], and increased NR2A and NR2B NMDA receptor subunits [171]. Furthermore, rats prenatally exposed to VPA prior to formation of the neocortex have perturbed cortical connectivity along with abnormal synaptic formation and pruning [174,175]. Additionally, in line with the evidence that postulates a disruption of the excitatory/inhibitory circuit balance in human autism [176], VPA-exposed mice exhibit a decrease in parvalbumin (PV)-positive interneurons in parietal and occipital cortices [177]. Lastly, like humans, VPA-treated rats respond successfully to early environmental enrichment [178]. Taken together, these findings support face, construct and predictive validity of the VPA-induced rodent model. Although much of today’s ASD research uses transgenic mouse models carrying mutations in single genes known to cause autism or related developmental disorders, a model of epigenetic changes such as the VPA-exposed rodent might better represent the many idiopathic autism cases having no known genetic basis.

We have determined that molecular abnormalities similar to those we demonstrated in the brain of subjects with idiopathic autism are detectable in postnatal day (PND) 35-38 rats whose dams were injected with VPA on embryonic day E12.5 [132]. VPA-exposed offspring have Akt-mTOR signaling deficits consistent with human autistic brain tissue [132]. They also exhibit ASD-like behaviors including decreased social play behavior [179-180]. These data suggest that VPA-treated rodents are a suitable animal model for further studies of autism and can be used to determine the contribution of BDNF/TrkB/mTOR signaling abnormalities to the behavioral symptoms and synaptic deficits of autism. Furthermore, the VPA rodent model appears to be a valuable tool to investigate whether pharmacological intervention of the TrkB-mTOR pathway can ameliorate autistic behavior.

**Conclusions**

Recent years have seen major advances in both the genetics and the behavioral aspects of autism. Yet there is still relatively little research on the molecular mechanisms that link genes to behavior. Our analysis of protein expression and post-translational regulation in postmortem human brain tissue has led to some unique insights. We demonstrated that, unlike most single-gene disorders with high rates of autism, postmortem brain tissue from subjects with idiopathic autism exhibits decreased mTOR pathway signaling. This supports a growing realization that either too much or too little signaling can lead to similar synaptic deficits. In addition, we corroborated the validity of the VPA-exposed rodent as an experimental model of idiopathic autism, since it reflects molecular changes evident in idiopathic cases of autism and is epigenetic in origin as opposed to genetic models. Understanding the molecular and synaptic dysfunction associated with autism by studying both human and rodent brain may lead to a better understanding of the heterogeneity of ASD and to the identification of new therapeutic targets.

**References**


