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Genetic Advances in Autism: Heterogeneity and Convergence on Shared Pathways

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Abstract

The autism spectrum disorders (ASD) are a heterogeneous set of developmental disorders characterized at their core by deficits in social interaction and communication. Current psychiatric nosology groups this broad set of disorders with strong genetic liability and multiple etiologies into the same diagnostic category. This heterogeneity has challenged genetic analyses. But shared patient resources, genomic technologies, more refined phenotypes, and novel computational approaches have begun to yield dividends in defining the genetic mechanisms at work. Over the last five years, a large number of autism susceptibility loci have emerged, redefining our notion of autism's etiologies, and reframing how we think about ASD.

Introduction

Autism is a neurodevelopmental disorder defined by three categories of deficits: 1) abnormal development or impairment of social interaction, 2) abnormal development or impairment of communication skills, and 3) stereotypic and repetitive behaviors [1]. Autism is part of a larger family of neurodevelopmental disorders categorized by the Diagnostic and Statistical Manual IV-R under the term Pervasive Developmental Disorders (PDD) [1]. PDD includes Asperger Syndrome (AS), where language appears normal, and pervasive developmental disorder not otherwise specified (PDD-NOS) in which children meet some but not all criteria for autism. Collectively, these disorders are known as the autism spectrum disorders (ASD) [1]. Rett syndrome and Childhood Disintegrative Disorder also are classified as a PDD, but are exclusionary for a diagnosis of autism.

Hypothesized by Kanner to be an innate or inborn disorder in his original description, autism was not formally determined to be a genetic disorder until Folstein and Rutter demonstrated a greater than 50% concordance for monozygotic, versus 0% for dizygotic twins. More recent twin studies have observed as high as 90% trait heritability for monozygotic twins [2], and family studies suggest a 22 fold increased risk over the general population for first-degree relatives [3], although this does not use the current CDC prevalence estimate of 1/152 for ASD [4]. Taken together, these studies indicate a high genetic liability, while leaving some room for environmental factors that may influence the penetrance or expressivity of these disorders with respect to genetic risk factors.

Although ASD is highly heritable, the identification of candidate genes has been hindered by the heterogeneity of the syndrome and insufficient numbers of participants, as compared to whole genome association studies in other complex genetic disorder. The establishment of collaborative groups, such as the International Molecular Genetic Study of Autism Consortium

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(IMGSAC) and Autism Genome Project Consortium [5,6], and shared resources, such as the Autism Genetic Resource Exchange Consortium (AGRE) [7], were therefore important steps in facilitating the identification of candidate genes. Linkage peaks on chromosomes 7q22–32 [5,8] and chromosome 17q21 [9–11] have been replicated. However most linkage signals have not been replicated, despite large increases in sample size, consistent with significant genetic heterogeneity [6,12]. Recently, a whole genome association study involving over 1500 cases and controls combined from different cohorts has identified and replicated at least one locus at genome-wide significance [13]. This demonstrates the promise of this approach, while at the same time, suggesting that very large sample sizes will be needed to identify additional genetic risk due to common alleles. Currently, there are over 25 different loci that may be considered autism susceptibility candidate genes (ASCG) and many more implicated loci are under investigation [12]. Most of these are rare, Mendelian mutations, including copy number variation (CNV) or syndromic forms of autism, and only a few are due to common genetic variation. In this brief overview, we will try to highlight some of the major advances in the study of autism, as well as discuss what the known ASCG can tell us about the neurodevelopmental mechanisms that may be causative.

A Phenotypic Renaissance

During the 1970s, psychiatric disorders were defined as deviations from the normal spectrum of behavior, but due to practical and economic factors, diagnostic schemes eventually became highly categorical. This categorization, while necessary from a clinical and educational standpoint, resulted in large groups of heterogeneous patients with diverse etiologies being defined by a single terminology. This clinical diagnostic schema based on the DSM was the primary means of phenotypic classification for early genetic work. But for modern genome-wide studies, clinical impression gave way to improved schema for classification using standardized and reliable research tools such as the ADOS and ADI [14,15]. Still the lack of formal replication of most autism linkage peaks [12], despite more than a 10-fold increase in sample size [6], indicates genetic heterogeneity. This, in conjunction with the wide range of phenotypes observed within the categorical classification of autism, suggests that methods reducing these two sources of complexity would increase power to identify ASCG [16].

Newer work has returned to the concept of disease-related spectrums in a more refined manner based on the concept of endophenotypes [17]. In autism, endophenotypes such as presence or lack of language, age at first word, age at first phrase, social cognition, gender, restrictive repetitive behaviors, and best estimate IQ, have been used in an attempt to increase power [9, 16,18,19]. Alarcón and coworkers initially utilized this approach to identify a locus related to language delay on chromosome 7q [16,20]. Subsequent linkage directed association identified CNTNAP2 as an ASCG using an age at first word endophenotype, one of the first ASCG identified from a comprehensive evaluation of common variants within a linkage region defined by a whole genome linkage scan [21]. Other support for the more general involvement of this gene in language development were the findings that it is associated with Specific Language Impairment and is related to monogenic disorders affecting language [22,23]. Thus, the further identification and use of endophenotypes related to other autism-related domains will likely increase power to detect ASCG.

The Genomic Revolution

Structural chromosomal variations, including CNVs, have been shown to play an important role in the etiology of ASD [24]. *De novo* CNVs, hypothesized to be ASD-specific, have been identified in up to 7–10% of sporadic ASD [24,25] D*e novo* CNVs are less frequent in multiplex families, occurring only in about 2% of families screened [24,26], possibly suggesting different genetic liabilities in simplex and multiplex ASD. Recurrent CNVs at 15q11–13 (1–3% of ASD

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patients), 16p11 (~1% of ASD patients), and 22q11–13 [6,24,25,27,28] have been confirmed in multiple studies. Many of the CNVs identified overlap with previously identified mental retardation (MR) loci or chromosomal syndromes. For example, Phelan-McDermid Syndrome defined by deletions of 22q13 overlaps with deletions and duplications of the gene SHANK3, an ASCG [29]. This is not a surprise as 94% of the original Phelan-McDermid Syndrome patients met ASD criteria via the CARS phenotyping tool. Therefore, analyses of genomic syndromes like Phelan-McDermid Syndrome or other syndromes that are comorbid with ASD diagnoses indicate important genes in the etiology of ASD [12,30].

The syndromes of structural chromosomal abnormalities also may provide insight into the mechanism behind sporadic ASD. For example, advanced paternal age is an established risk factor for ASD [31], and genetic instability through altered recombination efficiency increases as a parent ages. The increased recombination acts as a mechanism for multiple MR-associated syndromes [32]. Therefore, while it is unknown if paternal-age related recombination is a causative agent in CNV formation in autism, it is an attractive hypothesis. Our preliminary analysis of AGRE and other cohorts suggests that this may be the case (Abrahams and Geschwind, unpublished data).

Candidates and Compatriots

Epistasis is a basic and ubiquitous genetic paradigm well known within the developmental biology community. With the advent of large protein-protein interaction maps, full genome expression profiles and large-scale computing resources, network and pathway analyses offer promise of dealing with autism's complexities. Iossifov and coworkers [33] utilized the idea that interacting proteins involved in linear signaling pathways would have a similar chance of being involved in the etiology of ASD. By overlaying protein interaction datasets with linkage analysis, they were able to identify 24 putative novel ASCG [33], which need further confirmation.

Pathway analysis has also been used to identify novel associations with ASD utilizing a more standard biological approach. In 2006, Campbell and coworkers identified MET as an ASCG [34]. Utilizing knowledge of the MET signaling pathway, they investigated genes involved with this pathway and identified that SERPINE and PLAUR, two components critical for HGF (MET's ligand) regulation, potentially increase the risk of ASD [35]. Furthermore, MET signals through the PI3K-AKT pathway that contains two known ASCG (PTEN and CYFIP1) and contains three genes that are known to be causative for syndromes that co-occur with ASD [Tuberous Sclerosis (20% diagnosed with ASD) and Fragile X (25% of the males and 6% of females diagnosed with ASD)] [12] (Figure 1). Therefore, it is possible that alleles of SERPINE, PLAUR, MET, PTEN, TSC complex, FMR1, and CYFIP1 might contribute to autism via epistatic interactions. Other than autism, there is not a large overlap of the phenotypes related to each of these genes, but this could be explained by the pleiotropic nature of each of these genes. Therefore, one could hypothesize that the ASD phenotype is common to a large number of epistatic interactions, but due to pleiotropy of the individual loci, a heterogeneous phenotype emerges.

Following this logic, we utilized the Ingenuity pathway analysis software to identify published direct binding partners, transcriptional regulators, and translational regulators for a set of ASCG [12,26,35–37] to determine if the connections might suggest epistatic relationships. Of 33 candidate genes, direct or indirect interactions (2 degrees of separation at most) can be established, with the obvious caveat that some genes have been recently identified and display a reduced connectivity due to reduced publication volume (Figure 2). The limitations to this analysis lie in validation of the interactions and a realization that interactions are often temporally and spatially specific events. Therefore, while the interaction may be possible, it

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may not occur in the right tissue or at the right time to affect the pathology of ASD. Despite its limitations, this preliminary network of genes suggests true interconnectedness of several currently known ASCG. The potential for non-linearity and feedback loops suggested by even these simple relationships may obscure phenotypic effects from single polymorphisms or mutations.

Continuing Quandaries

Despite great advances in the genetics of ASD, there are still at least two major unanswered quandaries: the basis of the skewed sex ratio and the effect of the environment on ASD. Early studies demonstrated that males are diagnosed with ASD four times as often as females, and gender skewing becomes more pronounced in high-functioning autistic and Aspergers patients as the ratio approaches ten males to every one female [38]. Some of the gender bias might be explained by a larger than average number of ASCG on the X chromosome [12], as is the case with candidate loci for other forms of mental retardation. Marshall and coworkers observe that inherited X-linked CNVs are maternally transmitted and suggest this as a possible mechanism for the gender bias [25]. Sebat and coworkers found equal numbers of *de novo* CNVs between the genders [24], suggesting the bias is not due to increased mutability of the sex chromosomes, at least in terms of *de novo* structural variation. A second factor that may lead to the increased gender ratio is influence of autosomal loci that preferentially affect males or females, as originally demonstrated by Stone and coworkers [9]. In the largest linkage study to date, the AGP also found evidence for autosomal, but not X chromosome, sex related autism loci [6].

A non-genetic hypothesis has also been proposed to explain the gender difference. “The extreme male brain hypothesis” [39] is based on the fact that fetal testosterone acts as an environmental agent and alters sex-specific neuronal development *in utero* causing a decrease in function within the social and communication spectrums, thereby predisposing an individual with a susceptible genotype toward the diagnosis of ASD [39]. Our Ingenuity network, presented here, may be consistent with this hypothesis or at least suggests a role for the androgen receptor as it is regulated by PTEN, directly binds UBE3a, and inhibits the transcription of SERPINE, three ASCG (Figure 2, orange lines). However, there is as of yet no direct evidence for “the extreme male brain hypothesis”, as most of the evidence is correlative in nature.

The second quandary is the role of the environment. Given that the concordance of monozygotic twins is not 100%, and certain perinatal factors increase the risk for ASD, a role for environmental factors needs to be considered, eg. parental age [31], premature birth [40], or immune interaction [41]. Herbert and coworkers overlaid the linkage peaks identified from several ASD full genome scans with environmental, toxicologic, and immune related gene databases, finding an overlap of 135 genes [42]. While this analysis was perhaps overly inclusive in the scope of the ASCG genes selected and lacks rigorous statistical substantiation, it does indicate a potential set of gene-environment interactions to test.

Synthesis of an Integrated Model

We have recently discussed genetic models for the etiology of ASD [43] and provided a neurodevelopmental synthesis of autism that is based on altered connectivity between higher order cortical association areas, especially anterior frontal and temporal lobes [44]. Anatomical evidence suggests that during the first three years of life, the trajectory of brain growth is elevated in ASD, head circumference increasing from approximately normal to 10% larger than age matched peers [45]. During this time period, ages two to four, this growth appears to localize in the frontal cortex, temporal lobes, and amygdala [45]. Patients with Fragile X and co-occurring ASD also have an increase in head circumference size compared to those with Fragile X alone [46]. Diffusion tensor imaging shows that the axon tracts to these areas are

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disrupted [45], consistent with the notion of a developmental disconnection. It is notable that rare mutations in PTEN cause syndromic forms of macrocephaly and autism in humans [47]. Mouse knockouts of PTEN also display macrocephaly and display differences in axon and dendrite arborization. It is not yet know whether PTEN causes ASD directly via changes in brain size, or via effects on axon and dendritic arborization, and the changes in brain size are secondary.

A second major model for autism pathogenesis is that ASD is caused by a disruption of the formation or maintenance of synaptic connections. This model is driven by the identification of the synaptic proteins NLGN3, NLGN4X, NRXN1, and SHANK3 as ASCG [48]. As predicted by this model, the gene ontology terms appear to suggest a significant enrichment of genes that have a synaptic function annotation (Figure 3a,b). On the other hand, a model proposing that ASD is a synaptic disorder may be too limiting. The distribution of gene ontology functions spanning the entirety of nervous system development and function for the 33 ASCG analyzed suggests that broader biological functions may be involved (Figure 3a,b), and many of these genes cause other neurodevelopmental syndromes. So, the broad notion of synaptic dysfunction while clearly contributory may not be sufficient to account for the specificity autism.

Conclusion

In this brief overview, we have outlined how genetic advances have led to a new level in understanding ASD etiologies. Genomic tools allowing for the identification of *de novo* and heritable CNVs have so far contributed the most to our understanding of ASD, explaining about 10% of sporadic ASD. The analysis of co-morbid phenotypes and endophenotypes also provides a promising avenue of investigation, as evidenced by the association of common variants in CNTNAP2 with language endophenotypes in ASD and SLI. Surprisingly, many of the syndromic or rare ASCG appear to potentially interact at the level of molecular pathways, making it likely that mutations of one ASCG may affect the expression and function of others during development. This also provides hope that common treatments could be developed for those with etiologically distinct genetic forms of ASD. Any useful model of ASD pathogenesis will need to combine data from genetic, neurodevelopmental, and cell biological studies in model systems with functional and anatomical studies in human populations. This is especially true, because the majority of ASCG are not ASD specific and have been implicated in other neurodevelopmental disorders such as intellectual disability, epilepsy or psychiatric conditions [12,23,30,33,49]. The mechanism of the male gender bias and the degree and manner in which environmental influences will contribute to the etiology of ASD remain open questions.

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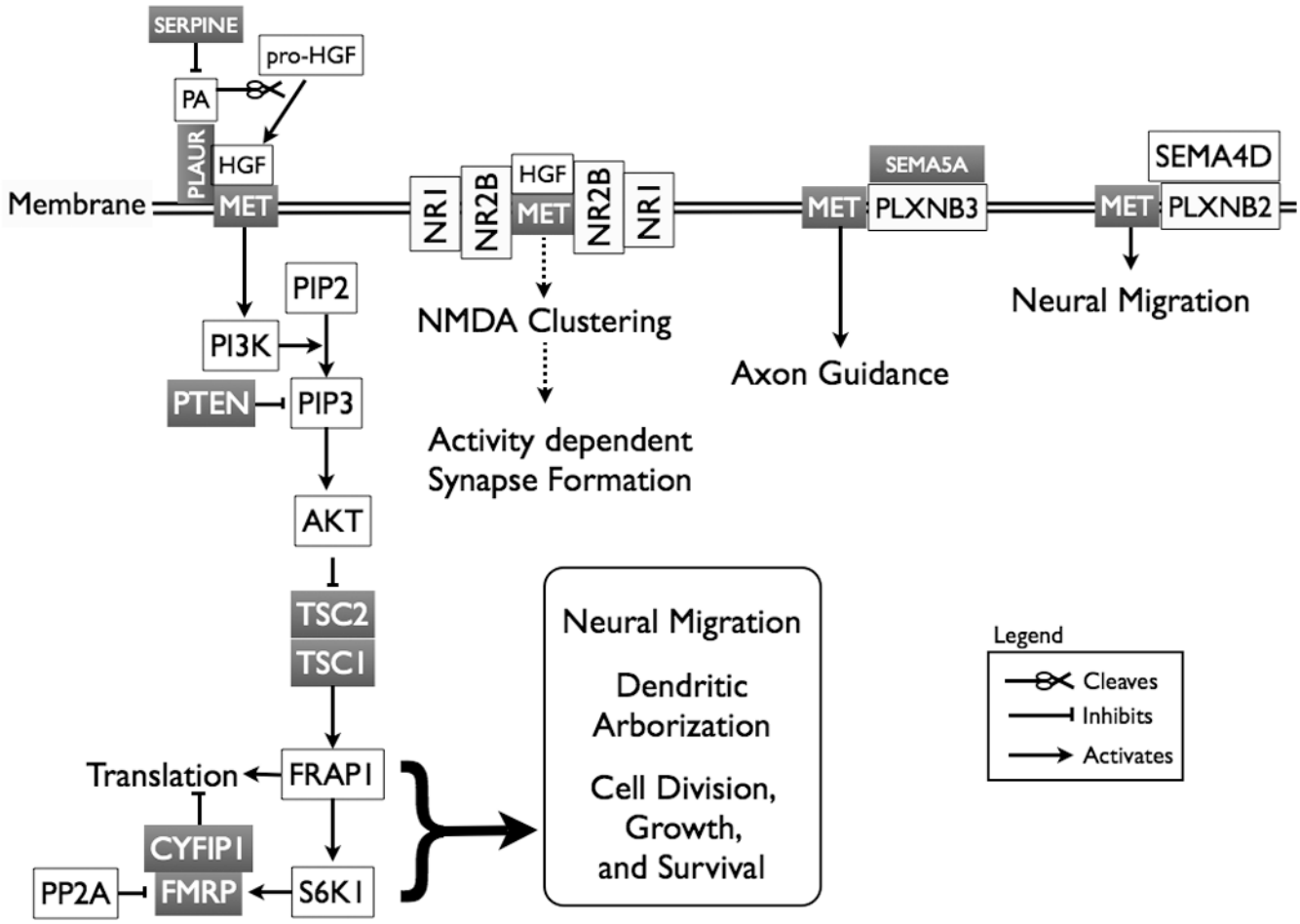

Figure 1. Epistatic and Pleiotropic Relationships of Autism Susceptibility Candidate Genes

Analyzing the MET-PI3K-AKT pathway can identify epistatic relationships of ASCG. Genes that are in gray have been associated with ASD, Tuberous Sclerosis (TSC1 and 2) or Fragile X (FMRP), syndromes that are comorbid with ASD. In the left most pathway, MET is seen interacting with PLAUR. PLAUR is the receptor for the tissue specific plasminogen activator (PA), a protease that cleaves HGF into its active form. SERPINE inhibits PA from activating HGF. After HGF binds MET, MET signals downstream through the canonical PI3K-AKT pathway that has a potential terminal endpoint of translational regulation by FMR1 and CYFIP. We propose that the core ASD phenotype may be represented by biological pathway convergence, while the pleiotropic nature of each gene provides the overlying phenotypic variability. For example, MET can signal through multiple receptor complexes that define the functional outcome. For example, MET clustering of NMDA receptors can affect long-term potentiation; while MET binding with members of the plexin receptor family dictate neural migration or axon guidance functions.

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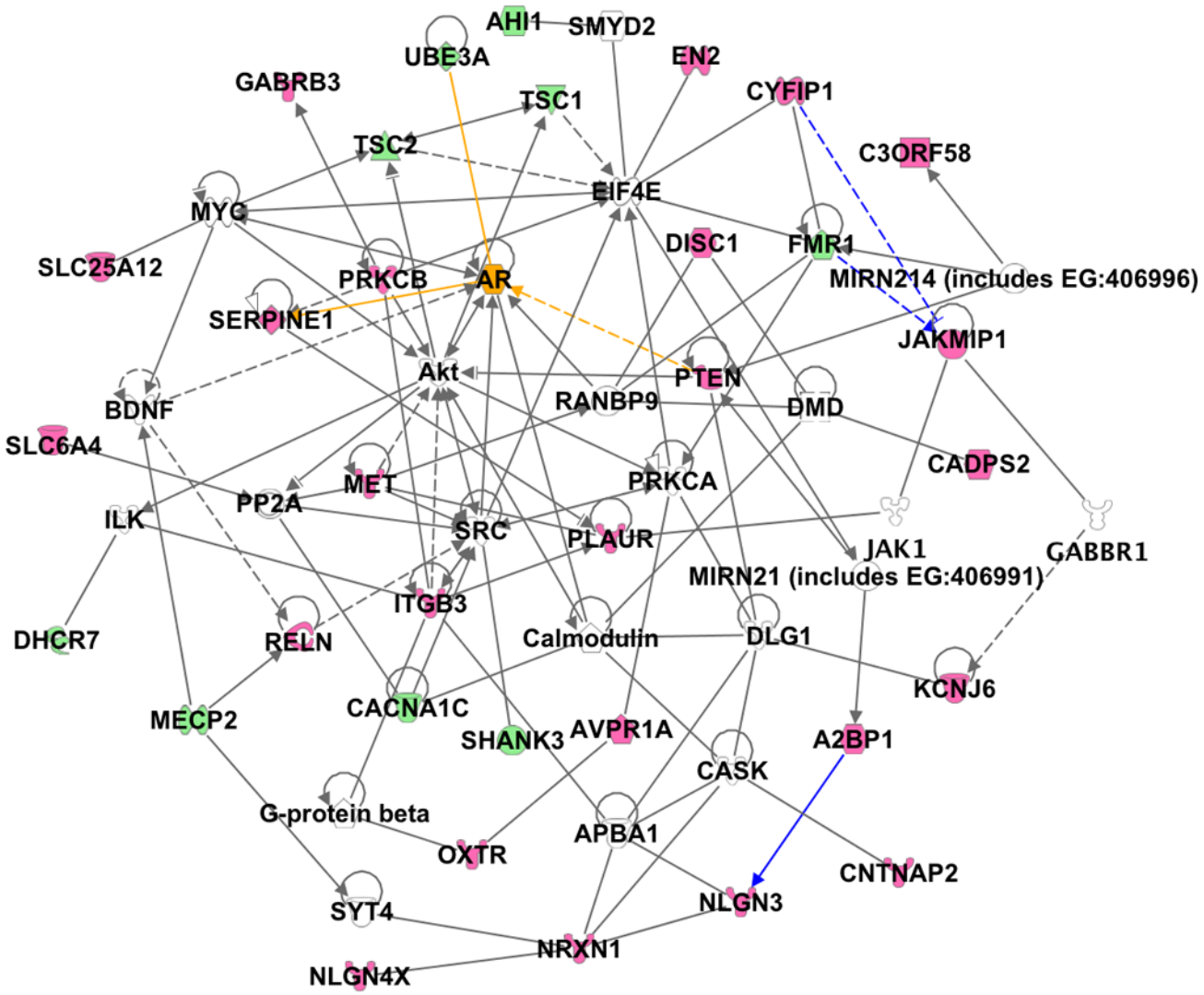

Figure 2. A social network for autism susceptibility candidate genes

Analysis of the relationships between 33 ASCG (pink emblems) and associated syndrome genes (green emblems) were analyzed using the Ingenuity Pathway Analysis (http://www.ingenuity.com). Direct (solid lines) and indirect (dashed lines) associations between the ASCG demonstrate the close relationship, less than 2 interactions between each ASCG. For clarity, we did not show all of the associations identified, but focus primarily on direct associations that link ASCG. Recently published interactions linking FMR1, CYFIP1, and JAKMIP1 by gene regulation, and A2BP1 (FOX1) with NLGN3 by splicing regulation were added as custom interactions (blue lines). The androgen receptor (orange emblem) and three of its interactions with ASCG (orange lines) are highlighted to demonstrate the correlation with the extreme male brain hypothesis. This is a first attempt to explore the connectivity between these genes, and therefore is not comprehensive. We do not mean to imply a single pathway as causative, as there may be many pathways that could be implicated.

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Gene	GO Function	GO Process	GO Component
A2BP1 (FOX1)	RNA binding, protein C-terminus binding	RNA splicing, RNA transport, mRNA processing	cytoplasm, nucleus, trans-Golgi network
AHI	None listed	None listed	None listed
AVPR1A	V1A vasopressin G-protein coupled receptor activity, Protein Kinase C signaling	G-protein coupled receptor protein signaling pathway, activation of phospholipase C activity, elevation of cytosolic calcium ion concentration, generation of precursor metabolites and energy, social behavior	Plasma membrane, endosome
CACNA1C	Calcium ion and Calmodulin binding, dihydropyridine-sensitive calcium channel activity, voltage-gated ion channel activity	Calcium ion transport into cytosol	Plasma membrane, postsynaptic density, cytoplasm, voltage-gated calcium channel complex
CADPS2	Calcium ion and Lipid binding	Protein transport and exocytosis	Cell junction, cytoplasmic vesicle membrane, membrane, synapse
CNTNAP2	Receptor binding	Cell adhesion, neuron recognition, signal transduction, transmission of nerve impulse	Membrane
CYFIP1	Protein binding	Axon extension, cell differentiation, multicellular organismal development, nervous system development, regulation of cell shape	Cell junction, cytoplasm, synapse
C3orf58	None listed	None listed	None listed
DHCR7	7-dehydrocholesterol reductase activity, oxidoreductase activity, protein binding	Cell differentiation, cholesterol biosynthetic process, multicellular organism growth, post-embryonic development, regulation of cell proliferation	Endoplasmic reticulum membrane, nuclear outer membrane, integral to membrane
DISC1	Protein binding	None listed	Centrosome, cytoplasm, cytoskeleton, microtubule
EN2	Sequence-specific DNA binding, transcription factor activity	Midbrain and hindbrain development, neural development	Nucleus
FMR1	mRNA and protein binding	mRNA transport	Nucleus, cytoplasm, nucleolus, ribosome
GABRB3	GABA-A Receptor Activity, Chloride binding and channel activity, extracellular ligand gated channel	Ion transport, signal transduction	Cell junction, postsynaptic membrane
ITGB3	Integrin binding, identical protein binding	Cell adhesion, cell-matrix adhesion, intergin mediated signaling, negative regulator of lipid transport, lipoprotein metabolism, and low density lipoprotein receptor biosynthetic process.	External side of plasma membrane, integrin complex, plasma membrane
JAKMIP1	GABA receptor and RNA binding	Protein transport	Cytoplasm, cytoskeleton, microtubule, membrane, ribonucleoprotein complex
KCNJ6 (GRIK2)	G-protein activated inward rectifier potassium channel activity	Potassium ion transport	Golgi apparatus, plasma membrane, voltage-gated potassium channel complex
MECP2	Double-stranded methylated DNA binding, protein binding, corepressor activity	DNA-dependent negative regulation of transcription from RNA polymerase II promoter	Mitochondria and nucleus
MET	Hepatocyte Growth factor receptor, ATP binding, nucleotide binding, Protein binding, transferase activity	Activation of MAPK, brain development, adult behavior, multicellular development, neuron migration, auto phosphorylation	Plasma membrane, flagella
NLGN3	Protein binding	Cell adhesion, social behavior, synapse organization	Plasma membrane, cytoplasm
NLGN4X	Chloride binding, protein homodimerization activity, neurexin binding	Cell adhesion, cell-cell junction, social behavior, synapse organization	Plasma membrane, nucleus
NRXN1	Calcium ion and protein binding, receptor activity	Axon guidance, cell adhesion	Plasma membrane
OXTR	G-protein coupled receptor activity, oxytocin receptor activity, vasopressin receptor activity	G-protein coupled receptor protein signaling pathway	Plasma membrane
PLAUR	U-plasminogen activator receptor activity, enzyme binding	GPI anchored protein, cell motion, chemotaxis, regulation of proteolysis, signal transduction	anchored to membrane, extrinsic to membrane, plasma membrane
PRKCB	ATP binding, calcium channel regulator activity, calcium ion binding, diacylglycerol binding, protein binding, protein kinase C activity, transferase activity, zinc ion binding	calcium ion transport, cellular calcium ion homeostasis, intracellular signaling cascade, lipoprotein transport, protein amino acid phosphorylation	Plasma membrane, cytoplasm, nucleus
PTEN	inositol-1,3,4,5-tetrakisphosphate 3-phosphatase activity, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase activity, phosphatidylinositol-3,4-bisphosphate 3-phosphatase activity, phosphatidylinositol-3-phosphatase activity, platelet-derived growth factor receptor binding, protein tyrosine/serine/threonine phosphatase activity, PDZ domain binding, hydrolase activity, lipid binding, magnesium ion binding	inositol phosphate dephosphorylation, lipid metabolic process, phosphoinositide dephosphorylation, negative regulation of (NR) apoptosis, NR cell cycle, NR cell migration, NR cell proliferation, NR focal adhesion formation, NR protein kinase B cascade, neurite development, platelet-derived growth factor receptor signaling pathway, protein amino acid dephosphorylation, regulation of cyclin-dependent protein kinase activity, regulation of protein stability	Cytoplasm, nucleus
RELN	Serine-type peptidase activity, protein serine/threonine/tyrosine kinase activity, calcium ion binding, zinc ion binding	Multicellular organismal development, brain development, spinal cord patterning, cell adhesion, neuron migration, cell morphogenesis involved in differentiation, cell morphogenesis involved in differentiation, axon guidance, glial cell differentiation, locomotory behavior, response to pain, peptidyl-tyrosine phosphorylation, positive regulation of protein kinase activity, positive regulation of small GTPase mediated signal transduction, thyroid hormone metabolic process	Cytoplasm, dendrite, extracellular region
SERPINE	mRNA 3'-UTR binding, protein binding	Regulation of mRNA stability	Cytoplasm, nucleus
SHANK3	SH3 domain binding, protein binding	MapKKK cascade signaling	Cell junction, post synaptic membrane
SLC6A4	Norepinephrine:sodium symporter transmembrane activity, protein binding	Monoamine transport, synaptic transmission	Cytoplasm, membrane, mitochondrion
SLC25A12	L-aspartate transmembrane transporter activity, L-glutamate transmembrane transporter activity, Ca binding, protein binding	L-glutamate transport, aspartate transport, malate-aspartate shuttle, response to calcium ion,	Membrane, mitochondrial inner membrane, mitochondrion
TSC1	Chaperone binding, protein n-terminus binding	Activation of Rho GTPase activity, NR Tor signaling pathway, NR cell cycle, NR cell proliferation, NR protein ubiquitination, NR of translation, positive regulation of focal adhesion formation, rRNA export from nucleus, regulation of cell-matrix adhesion, regulation of phosphoprotein phosphatase activity, regulation of stress fiber formation	TSC1-TSC2 complex, colocalizes with actin filaments, cytoplasm, lamellipodium, membrane
TSC2	GTPase activator activity, protein binding, protein homodimerization activity	Negative regulation of cell cycle, positive regulation of GTPase activity	TSC1-TSC2 complex, perinuclear region of cytoplasm, cytoplasm, membrane
UBE3A	Acid-amino acid ligase activity, ubiquitin-protein ligase activity, protein binding	Brain development, protein modification and proteolysis	Cytoplasm, nucleus

B

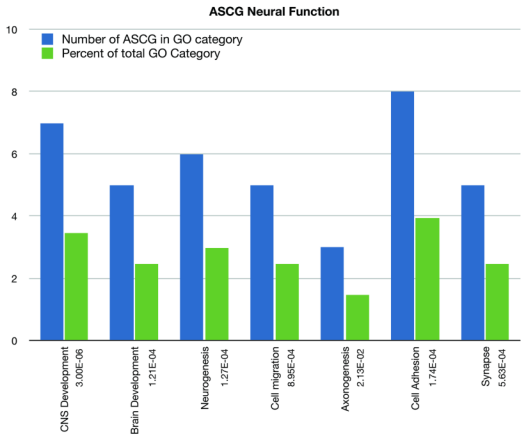

Figure 3. Functional annotation of the autism susceptibility candidate genes

Annotation was performed using the gene ontology (GO) nomenclature assignments from the National Center for Biotechnology Information (NCBI) for the 33 ASCG used for the Ingenuity analysis (A). GO terminologies are divided into function, process and component. For easier visualization, neuro-specific annotations were compiled using the DAVID Bioinformatics database (http://david.abcc.ncifcrf.gov/) as a histogram (B). The 33 genes from the previous analysis were divided by their GO category (blue bars), and as a percentage of the total genes annotated within the same GO category (green bars). The significance of the enrichment is denoted by p-values, shown adjacent to the GO category. The limitations of the GO terminology are that the categories utilize general descriptors to categorize protein function;

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some functions are not present in the database, and some protein lack annotation, excluding them from analysis. For example, FMR1 is known to function in dendritic morphogenesis; but this function is not described in its GO annotation. Despite limitations, the GO annotations clearly show that ASD affects multiple processes in the brain suggesting models that address a single aspect of biological function are likely to be too limiting.