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AKAPs integrate genetic findings for autism spectrum disorders

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Autism spectrum disorders (ASDs) are highly heritable, and six genome-wide association studies (GWASs) of ASDs have been published to date. In this study, we have integrated the findings from these GWASs with other genetic data to identify enriched genetic networks that are associated with ASDs. We conducted bioinformatics and systematic literature analyses of 200 top-ranked ASD candidate genes from five published GWASs. The sixth GWAS was used for replication and validation of our findings. Further corroborating evidence was obtained through rare genetic variant studies, that is, exome sequencing and copy number variation (CNV) studies, and/or other genetic evidence, including candidate gene association, microRNA and gene expression, gene function and genetic animal studies. We found three signaling networks regulating steroidogenesis, neurite outgrowth and (glutamatergic) synaptic function to be enriched in the data. Most genes from the five GWASs were also implicated—independent of gene size—in ASDs by at least one other line of genomic evidence. Importantly, A-kinase anchor proteins (AKAPs) functionally integrate signaling cascades within and between these networks. The three identified protein networks provide an important contribution to increasing our understanding of the molecular basis of ASDs. In addition, our results point towards the AKAPs as promising targets for developing novel ASD treatments.

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Introduction

Autism spectrum disorders (ASDs) are a group of pervasive neurodevelopmental disorders that are characterized by qualitative impairments in reciprocal social interaction and communication as well as restricted, repetitive and stereotyped patterns of behavior, interests and activities. The ASDs include autism, Asperger's syndrome and pervasive developmental disorder, not otherwise specified (Diagnostic and Statistical Manual of Mental Disorders-fourth edition-text revision (DSM-IV-TR)). Individuals with autism show impairments in all three core symptom domains and an onset of symptoms before 3 years of age, whereas Asperger's syndrome is characterized by social and behavioral impairments in the presence of a normal language development before age 3 years. Pervasive developmental disorder, not otherwise specified, is diagnosed in people who do not meet autism criteria for all three symptom domains and/or show a later age of onset (DSM-IV-TR). The prevalence of ASDs is estimated to be 0.5–1%,^{1–3} with ~4 times more males than females being affected by these disorders.^{4,5} Family and twin studies show that ASDs are highly heritable, and ~90% of the phenotypic variance that is observed in these disorders can be explained by genetic factors.^{5–7} Despite this high heritability, the identification and replication of genetic susceptibility factors for ASDs has proven challenging, as the genetic architecture of these disorders is highly heterogeneous (see recent reviews^{8–10}). Rare genetic variants that

predispose individuals to ASDs are currently thought to account for 10–20% of all ASD cases.^{8,10} These variants include rare mutations in single genes that can lead to monogenic disorders (such as fragile X syndrome and tuberous sclerosis) associated with ASD symptoms, or genes identified through exome sequencing studies and copy number variations (CNVs), with the latter constituting deletions or duplications of a large variety of genes and potentially contributing to oligogenic forms of ASDs.^{8–10} Common genetic variants for ASDs—for example, single-nucleotide polymorphism (SNPs)—have also been identified through candidate gene and genome-wide association studies (GWASs), and each variant is assumed to contribute only a small increase in disease risk.^{7–9} To date, six GWASs for ASDs have been published.^{11–16} In addition to many protein-coding genes that are implicated in ASD pathophysiology, increasing evidence indicates that microRNAs may also be involved in ASD aetiology.^{17–20} In recent years, three main biological 'themes' have emerged from genetic ASD findings, namely steroid synthesis,^{21,22} growth and neurite outgrowth of developing neurons,^{9,15,21–23} and synaptic function.^{8,9,23–25} In this article, we integrate the most important findings from ASD GWASs into protein signaling networks that are linked to these biological themes, and provide corroborating evidence for these networks from exome sequencing/mutation, CNV, microRNA and other genetic data. Importantly, we find that signaling cascades within and between the three networks are functionally connected by A-kinase anchor proteins (AKAPs), which seem to

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be promising targets for the development of novel psychopharmacological treatment for ASDs.

Materials and methods

Identification of candidate genes and finding corroborating evidence. The main characteristics of the six published GWASs of ASDs published to date are presented in Table 1. We used SNP data from five of these studies to compile a list of top-ranked genes associated with ASDs (the cutoff for association was $P < 1 \times 10^{-4}$).^{11–15} The sixth GWAS¹⁶ was used for validation and replication processes (see below), as it was conducted on a discovery sample that was independent of the discovery samples that were used in the five other GWASs. The used SNPs included those that were located within exonic, intronic or untranslated regions of genes or found within 100 kilobases (kb) of downstream or upstream, potentially regulatory^{26–29} sequences flanking a gene. Of note, for the selection of SNPs from two of the GWASs, we used the complete and detailed association results for the combined discovery samples, rather than the lists of SNPs as they were published, which included replication samples.^{11,13} In addition, although our chosen statistical cutoff for association was essentially an arbitrary threshold, it was used in four of the six published ASD GWASs to designate ‘suggestive’ evidence of association,^{11–13,16} and application of this cutoff provided us with a manageable number of genes for our subsequent analyses. Applying the above described SNP selection criteria yielded a total of 200 genes from the five GWASs^{11–15} (Supplementary Table 1).

Subsequently, we searched the literature for rare genetic variants—mutations and/or CNVs—that have been found in people with ASDs to find genes that were also identified through the five GWASs (Supplementary Table 1). We searched for mutations that were identified in one or more recently published exome sequencing studies^{30–34} and/or candidate gene mutation studies in people with ASDs. In addition, we searched for overlapping CNVs that were identified in people with ASDs through a genome-wide array-based hybridization approach and/or that were ‘recurrent’, in that they were observed in case studies of at least two unrelated people with ASDs. Furthermore, we searched for GWAS candidate genes that are targeted by ASD-implicated microRNAs. MicroRNAs are very small RNAs that negatively regulate the expression of multiple genes.^{17–20} We compiled a list of microRNAs that have been repeatedly implicated in the aetiology of ASDs (Supplementary Table 2), after which we used Targetscan to identify which of the 200 ASD candidate genes are targeted and hence downregulated by ASD-implicated microRNAs. Targetscan is a web-based tool (<http://www.targetscan.org/>) that predicts possible gene targets of microRNAs and ranks the predicted efficacy of microRNA targeting using a calculated context score.

Finally, we searched the literature for ‘other’ genetic evidence implicating the GWAS candidate genes in ASDs, including data from candidate gene association studies, gene expression studies, gene/protein function studies and genetic animal studies. In addition, we compiled a list of 33 additional ASD candidate genes that were not directly observed in the top-ranked GWAS findings but are implicated in ASDs

through at least two independent lines of (strong) genetic evidence (see Supplementary Table 3).

Bioinformatics analysis. To detect enriched gene categories in the 200 top-ranked candidate genes from the five GWASs, we performed a bioinformatics analysis using the Ingenuity Pathway Analysis software package (<http://www.ingenuity.com>) (see Supplementary Table 4). Based on the Ingenuity Knowledge Base—which draws on information from the published literature as well as many other sources, including gene expression and gene annotation databases—genes are assigned to different categories and subcategories of functionally related genes. The Ingenuity Pathway Analysis program calculates single *P*-values for the enrichment of each gene category and subcategory using the right-tailed Fisher’s exact test. For each category and subcategory, a multiple testing corrected *P*-value, calculated using the Benjamini–Hochberg correction, is also provided.

Literature analysis and identification of protein networks. Subsequently, we systematically searched the literature for the (proposed) function of all proteins derived from the ASD candidate genes using two databases: the Uniprot Protein Knowledgebase (UniProtKB) (<http://www.uniprot.org/uniprot>) and PubMed (<http://www.ncbi.nlm.nih.gov/sites/entrez>).

For each ASD candidate from the five GWASs, we first looked at the available information in UniProtKB, which in most cases already provided a general indication of the (putative) function of the gene or encoded protein in question. Based on the Ingenuity analysis, UniProtKB and the three main biological ‘themes’ linking the genetic ASD findings (see Introduction), we subsequently searched PubMed using the search terms ‘Leydig’, ‘steroid’, ‘steroid synthesis’, ‘steroidogenesis’, ‘testosterone’, ‘estradiol’, ‘estrogen’, ‘brain’, ‘neuron’, ‘neuronal growth’, ‘neurite’, ‘neurite outgrowth’, ‘synapse’, ‘synaptic’, ‘glutamate’ and ‘glutamate receptor’ in combination with the name of each candidate gene (or encoded protein). Guided by the literature we found, we also searched PubMed for functional interactions between the candidate genes/encoded proteins.

Replication and validation. Using the above described SNP selection criteria, we compiled a list of top-ranked genes from GWAS 6,¹⁶ and we subsequently searched for overlapping genes between this study and the five GWASs that were considered in the aforementioned analyses.^{11–15} We also systematically searched the literature using UniProtKB and PubMed (see above) for the (proposed) function of the proteins encoded by all top-ranked genes from the sixth GWAS, and based on this information, we then tried to (putatively) place each protein in one or more of the three identified networks.

Results

Identification of candidate genes and finding corroborating evidence. As stated, six GWASs of ASDs have been published to date (Table 1). Applying the SNP selection criteria described in the Materials and methods section to

Table 1 Overview of the clinical samples from six published GWASs of ASDs^{11–16}

GWAS	Clinical sample	Genotyping platform	Phenotype	Diagnosis
Wang <i>et al.</i> ¹¹	Combined discovery sample (AE): AGRE (943 families) ^a + ACC cohort (1453 cases and 7070 controls) from CHOP and several other US clinical sites	Illumina	ASD	ADOS + ADI-R
Ma <i>et al.</i> ¹²	CAP discovery sample (AE): four US clinical sites (438 families)	Illumina	Autism	ADI-R + DSM-IV
Weiss <i>et al.</i> ¹³	Combined discovery sample (AE), consisting of 1031 multiplex families with 1553 affected offspring from: AGRE (801 families) ^a + NIMH (341 families)	Affymetrix	ASD	ADI-R
Salyakina <i>et al.</i> ¹⁴	Combined sample: discovery sample from two US clinical sites (124 families) + AGRE validation sample (110 families)	Illumina	ASP	ADI-R + DSM-IV
Hussman <i>et al.</i> ¹⁵	Combined sample: sample from two US clinical sites (597 families) + AGRE sample (696 families)	Illumina	Autism	ADI-R + DSM-IV
Anney <i>et al.</i> ¹⁶	AGP Consortium discovery sample (AE): Autism (809 families) + ASD (1369 families)	Illumina	Autism + ASD	ADOS + ADI-R

Abbreviations: ACC, Autism Case Control; ADOS, Autism Diagnostic Observation Schedule; ADI-R, Autism Diagnostic Interview-Revised; AE, all ethnicities; AGP, Autism Genome Project; AGRE, Autism Genetic Resource Exchange; ASD, autism spectrum disorder; ASP, Asperger's syndrome; CAP, Collaborative Autism Project; CHOP, Children's Hospital of Philadelphia; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders-fourth edition; GWAS, genome-wide association study; NIMH, National Institute for Mental Health.

^aThere is considerable overlap between these two clinical samples.^{11,13}

data from the five GWASs^{11–15} yielded a total of 200 ASD candidate genes (Supplementary Table 1). Corroborating evidence for a role of these genes in ASD aetiology (see Materials and methods for criteria), was found through rare variant studies. We found that 11 of the 200 candidate genes from the GWASs (5.5%) were reported to contain mutations in people with ASDs (Supplementary Table 1), including findings from 5 recently published ASD exome sequencing studies.^{30–34} In addition, 93 of the 200 GWAS candidate genes (46.5%) have been observed in CNVs in people with ASDs (Supplementary Table 1). Furthermore, we found that 100 (50%) of the GWAS-identified candidate genes were targets of ASD-implicated microRNAs (see Materials and methods, Supplementary Table 1 and Supplementary Table 2). Lastly, 28 of the 200 GWAS candidate genes (14%) are additionally implicated in ASD aetiology through other genetic evidence, including data from candidate gene association studies, gene expression studies, gene/protein function studies and genetic animal studies (Supplementary Table 1). Through literature study, we also added 33 strong ASD candidate genes, including *CNTNAP2*,^{35,36} *MET*^{37,38} and *NRXN1*,^{39,40} implicated in the disorder through at least two independent lines of strong genetic evidence other than GWASs (Supplementary Table 3).

Bioinformatics analysis. For the 200 top-ranked ASD GWAS genes, the Ingenuity pathway software revealed a significant enrichment (multiple testing corrected $P = 1.64 \times 10^{-4}$) of the gene category 'neurological disease', with 81 of the 200 genes falling into this category. Moreover, 8 of the 9 other significant categories in the top 10 of nervous system-related gene categories could be directly linked to neurite outgrowth or synaptic function (Supplementary Table 4).

Literature analysis and identification of protein networks. On the basis of the findings from the Ingenuity

analysis and our systematic literature analysis (see Materials and methods), we found that the proteins encoded by 150 of the 233 above mentioned ASD candidate genes (64%) fit into three distinct signaling networks that correspond very well to the three aforementioned biological themes for ASDs. The first network is involved in regulating the production and metabolism of the steroid hormones testosterone and estradiol in testicular Leydig cells (Figure 1), whereas the second network relates to directed neurite outgrowth, and integrates molecular signaling cascades in the extracellular matrix, cell membrane, cytoplasm and nucleus of developing neurons (Figure 2a) with signaling cascades that modulate the cytoskeleton in and the extracellular matrix that surrounds the growth cone of these neurons (Figure 2b).

The third network regulates and modulates (glutamatergic) neurotransmission across the synapse (Figure 3). A detailed description of the evidence linking all the genes and encoded proteins in the networks shown in Figures 1–3 is provided in the Supplementary Information. Importantly, signaling cascades within all three networks are modulated and regulated by the proteins encoded by 10 ASD-implicated AKAP genes (Supplementary Table 5).

Replication and validation. As indicated in the Materials and methods section, the sixth ASD GWAS by Anney *et al.*¹⁶ was used for validation and replication purposes, as it was entirely independent of the five other published ASD GWASs.^{11–15} A total of 15 genes from GWASs 1–5 (7.5%) were directly replicated—they were implicated through SNPs that meet the selection criteria of this study in 2 of the 6 published GWASs—and 13 of these genes were found in GWAS 6 (Supplementary Table 6). In addition, the proteins encoded by 111 of the 333 top-ranked (protein coding) genes from GWAS 6 (33%) could be placed in one or more of the three identified networks (Supplementary Table 7), which provides a strong validation of the involvement of these

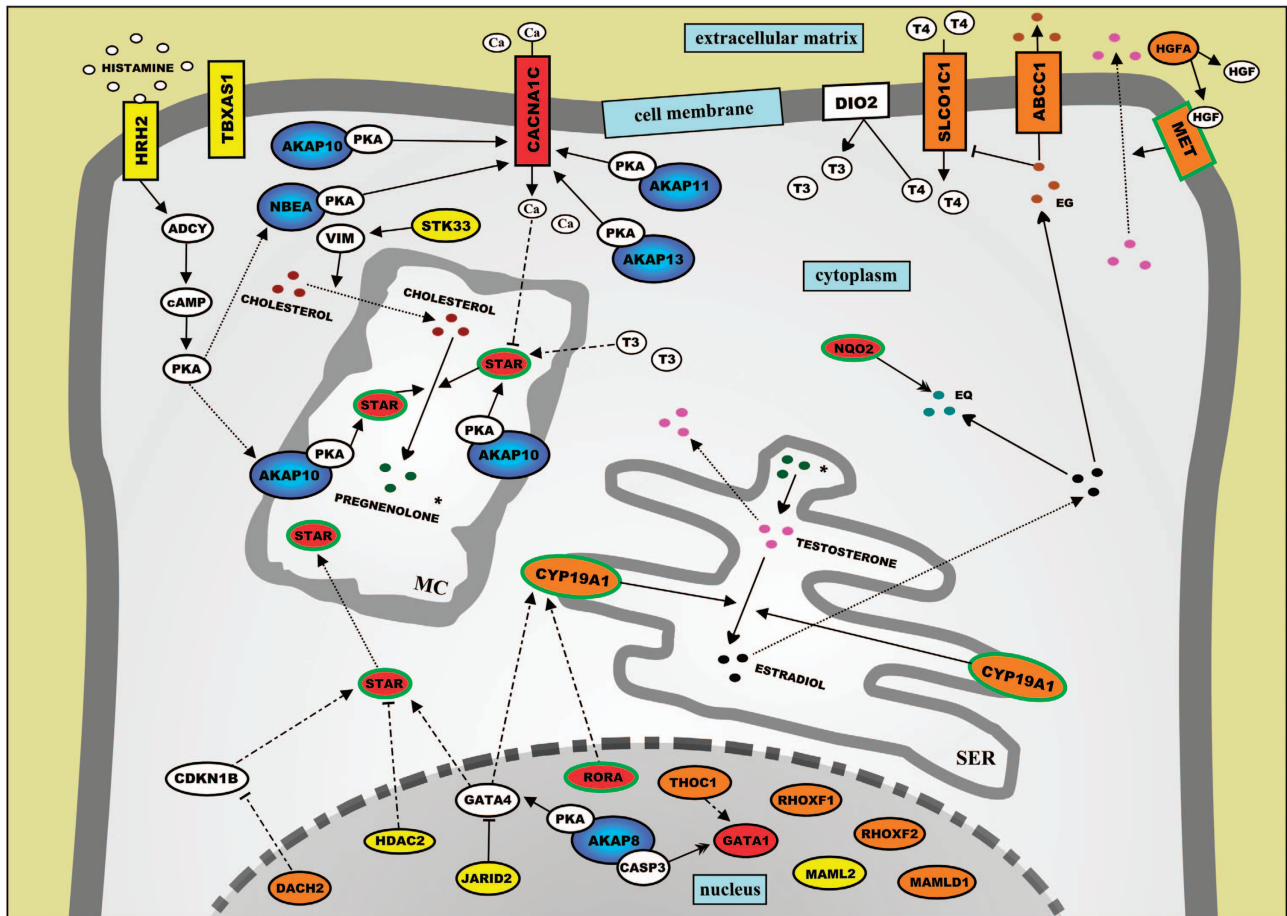


Figure 1 Schematic representation of a protein network that is implicated in autism spectrum disorders (ASDs) through regulating the production and metabolism of the steroid hormones testosterone and estradiol in testicular Leydig cells. The proteins encoded by genes implicated in ASDs through common genetic variants—single-nucleotide polymorphisms (SNPs) from five published genome-wide association studies (GWASs) (Supplementary Table 1) and/or ASD candidate gene association studies—are indicated in yellow. The proteins encoded by genes implicated in ASD aetiology through rare genetic variants—one or more mutations and/or copy number variations (CNVs) affecting the gene—are indicated in red, whereas the proteins encoded by genes implicated in ASDs through both common and rare genetic variants are indicated in orange. In addition, all A-kinase anchor proteins (AKAPs) are dark blue and the proteins encoded by genes that have been implicated in ASD aetiology through ‘other’ genetic evidence—including gene expression studies, gene/protein function studies and genetic animal studies—have a green border. In the Supplementary Information, the network is described in detail, and the current knowledge about the function of the network proteins is presented.

networks in ASD aetiology. In this respect, it is also very intriguing that an intronic SNP in *NQO2*, a gene that had already been implicated in ASD aetiology (Supplementary Table 3) and that encodes a protein with an important function in the identified steroidogenesis and neurite outgrowth networks (see Figures 1 and 2a, and Supplementary Information), yields genome-wide significant association ($P=2 \times 10^{-15}$) with ASD in GWAS 6 (Supplementary Table 7).

Potential statistical bias. An important potential bias in the analysis of the top findings from GWASs is the fact that large genes, which are often brain expressed, may be more likely found as associated with a phenotype in a GWAS as a result of chance, as more SNPs are present in these large genes.⁴¹ Indeed, the genes found among the top findings of ASD GWASs 1–5 were considerably larger than the average gene size of the human genome, with the 200 (unique) ASD candidate genes having an average size of 221 kb

(Supplementary Table 8) versus an average gene size in the human genome of 27 kb.⁴²

In order to test whether the GWAS genes included in this study were likely to constitute false positive findings, we looked for confirmation from other types of genetic studies and we performed a comparative analysis of the top ranked GWAS findings from unrelated disorders. First, a total of 111 of the 200 candidate genes from GWASs 1–5 were also implicated in ASDs through rare genetic variants—that is, mutations and/or CNVs affecting the gene in people with ASDs—and/or other genetic evidence, which includes SNPs/common genetic variants from classic candidate gene association studies, gene expression and function studies and genetic animal studies (Supplementary Table 1).

Most importantly, 42 of the 47 (unique) large genes (size > 250 kb) from GWASs 1–5 (89%) and all of the 26 very large genes (size > 500 kb) were implicated in ASD aetiology through at least one additional line of genetic evidence (Supplementary Tables 1 and 8). Second, arguing that if large

genes are more likely found to be associated with a phenotype of a GWAS because of chance, this would be expected to be the case for GWASs of nonpsychiatric disorders as well. Therefore, we compared the results of our Ingenuity analyses of the 20 top ASD candidate genes from GWASs 1–5 with those for the top 20 candidate genes from four published GWASs for diabetes mellitus type I and Crohn's disease (Supplementary Tables 9 and 10), two polygenic disorders that are assumed not to primarily originate in the brain. This comparison showed that the neurological disease category was significantly enriched in the ASD GWAS findings (with 9 of the 20 top genes falling into this category), only contained a single gene in diabetes type I and was not enriched at all in Crohn's disease (Supplementary Table 10).

Discussion

In this study, we used both bioinformatics and extensive manual literature mining to integrate the top-ranked findings of six published GWASs of ASDs into three biological processes. In addition, we have comprehensively addressed the subject of possible gene size-based bias.

Importantly, we found that many of the genes encoding proteins in the three networks have been implicated in ASD aetiology through both common genetic variants (that is, SNPs from GWASs and classic candidate gene association studies) and rare genetic variants (that is, one or more mutations and/or CNVs affecting the gene in people with ASDs). All these proteins are indicated in orange in Figures 1–3, and this finding suggests that the often made distinction between rare and common genetic ASD variants (see above) is somewhat artificial: to a considerable extent, multifactorial and oligogenic forms of ASDs—caused by common and rare genetic variants, respectively—seem to share aetiological pathways.

On the matter of potential gene size-based bias, and based on the corroborating evidence from other sources as well as the analysis of unrelated nonpsychiatric disorders, we would like to submit that the (vast) majority of the ASD candidate genes from the GWASs were not identified because of gene size bias and therefore represent true associations. The corroborating evidence for many of the large(*r*) GWAS genes comes from mutation, CNV, microRNA and other genetic data that are not subjected to potential gene size bias, as well as from the replication and validation through an entirely independent additional GWAS (GWAS 6). We have come to our conclusion about the absence of gene size-based bias despite the fact that our chosen statistical cutoff for association ($P < 1 \times 10^{-4}$) is essentially an arbitrary threshold. It is below the commonly used threshold for genome-wide significant association ($P < 5 \times 10^{-8}$),^{11–16} but was chosen because it is often used to designate 'suggestive' association (for example, in four published ASD GWASs^{11–13,16}) and because it provided us with a manageable number of genes for subsequent analyses.

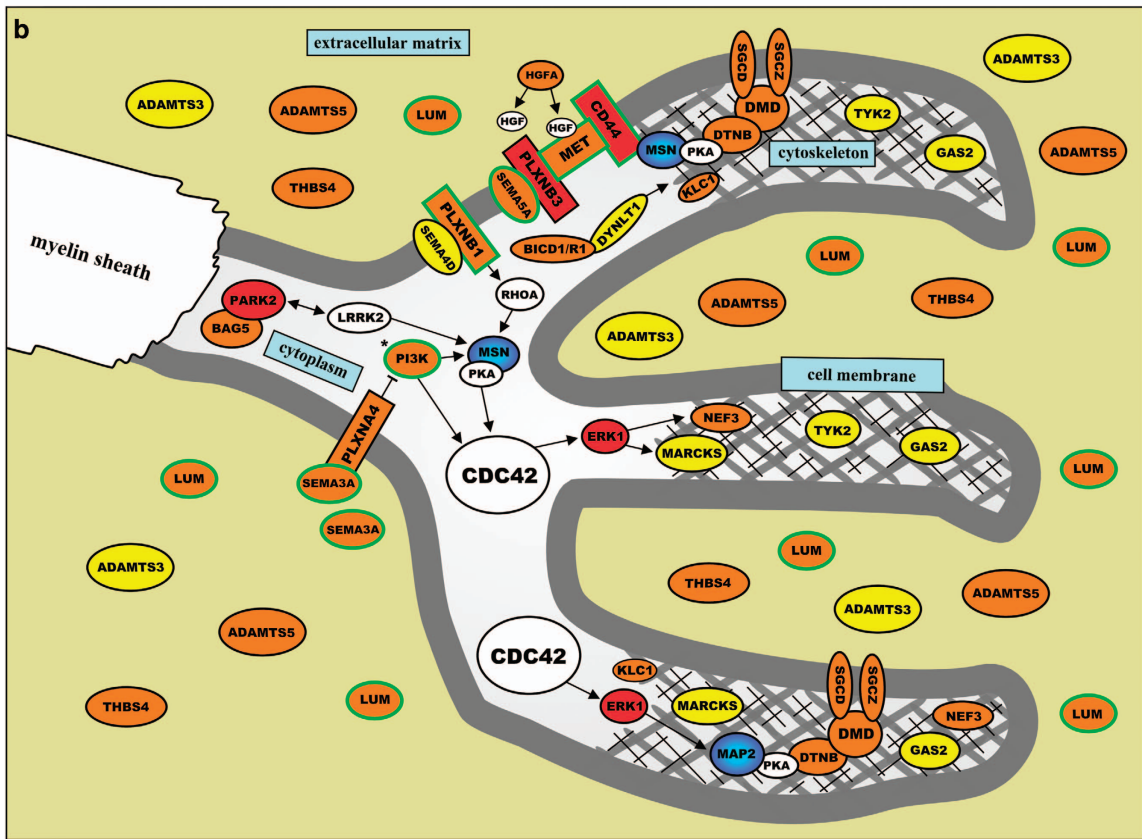
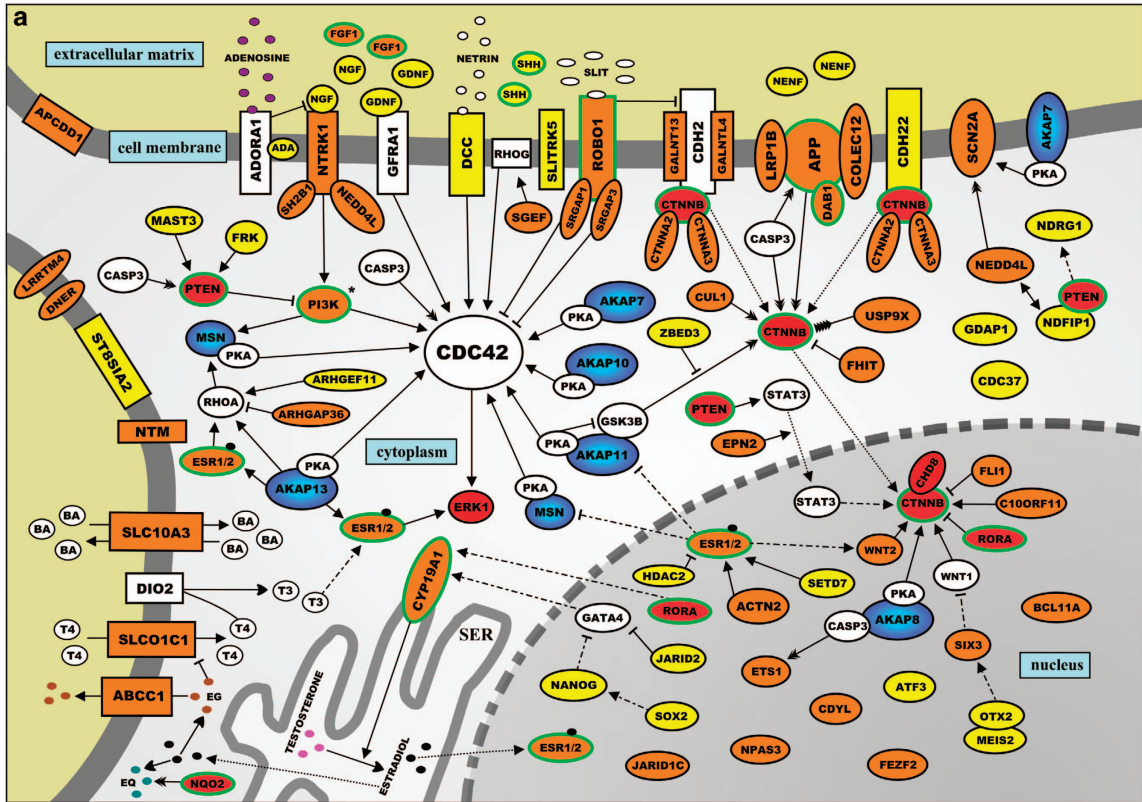
Using a threshold below the genome-wide significance level (in conjunction with validation through other genetic data) is also supported by polygenic analyses showing that many signals from GWASs, although not reaching genome-wide

significance individually, contribute to a given trait or disease.^{43,44} That being said, we explicitly do not wish to exclude the possibility that a number of GWAS-identified ASD candidate genes are spurious findings.

The three ASD networks we describe fit very well with the three biological themes that have already emerged from diverse types of ASD studies (see above), which adds further weight to our findings. The identified networks have important implications for advancing our understanding of the molecular basis of ASDs. First, the testicular steroidogenesis network provides additional proof for both the excess of males in ASDs (see above) and the 'extreme male brain' theory of ASDs. This theory integrates several lines of evidence and states that ASD behaviors are associated with increased fetal testosterone levels and are therefore more common in males, as well as in females with disorders characterized by an overproduction of testosterone.^{45,46} Furthermore, our findings relating to the involvement of disturbed neurite outgrowth and synaptic function in the genetic aetiology of ASDs fit very well with literature reports describing aberrant structural and functional brain connectivity in people with ASDs.⁴⁷ Hence, ASDs can be seen as disorders of 'neuronal communication' that, at the level of individual neurons, could be caused by disturbances or alterations in the efficiency, direction and/or timing of neurite outgrowth (during brain development) and/or synaptic function (throughout adult life). This is further corroborated by a study reporting enrichment of several neurite outgrowth-related and ASD-implicated genes in a set of genes that were correlated with structural connectivity in the adult rat brain through gene expression patterns during brain development.⁴⁸

Despite being important contributing factors, impairments of neurite outgrowth and synaptic function are not specific to the aetiology of ASDs, and our research group has previously reported that (impaired) neurite outgrowth is also important in the genetic aetiology of dyslexia⁴⁹ and attention deficit hyperactivity disorder,⁵⁰ two neurodevelopmental disorders that show high comorbidity and are genetically correlated with each other⁵¹ and with ASDs.^{52–54} Impaired synaptic function is also implicated in the aetiology of schizophrenia,⁵⁵ a neurodevelopmental disorder that manifests in late adolescence or early adulthood and shares genetic factors with ASDs.⁵⁶ Nevertheless, we believe that the clinical specificity of these neurodevelopmental disorders could be at least partially explained by the different functional consequences of disturbed or abnormal neurite outgrowth and/or synaptic function in the most affected brain region(s) for each disorder. The cerebellum and, more specifically, cerebellar Purkinje cells have been consistently found to be among the most affected brain regions in ASDs, with the disorders often linked to an actual loss of these cells.^{57,58}

As several of the ASD-implicated microRNAs show dysregulated expression in post-mortem cerebellar cortex of people with ASDs,¹⁷ and many of the proteins encoded by identified ASD candidate genes—including the ASD-implicated *AKAP5*, *AKAP9* and *AKAP11* (see below)—are highly expressed and/or have a specific function in cerebellar Purkinje cells (see Supplementary Tables 1,3,4 and 6), cerebellar dysfunction of the identified genetic networks may be very important in the aetiology of ASDs.



As already indicated above, the proteins encoded by 10 AKAP genes (*AKAP5*, *AKAP7*, *AKAP8*, *AKAP9*, *AKAP10*, *AKAP11*, *AKAP13*, *MAP2*, *MSN* and *NBEA*) constitute a common functional theme within the identified networks. The AKAPs constitute a growing protein family of currently more than 50 members that share an ability to bind protein kinase A (PKA) to its substrates, that is, the proteins that are phosphorylated and hence regulated in their activity by PKA. AKAPs are found in many subcellular locations and expressed in many tissues, including testis and brain. Apart from anchoring PKA, AKAPs can also directly bind and/or regulate many other proteins, including kinases other than PKA and phosphatases.⁵⁹ As shown and described in Figures 1–3 and the Supplementary Information, ASD-implicated AKAPs are involved in signaling cascades regulating Leydig cell-based steroidogenesis, neurite outgrowth of developing neurons and glutamatergic synaptic transmission. Within the neurite outgrowth network, AKAPs also regulate the two main signaling ‘hubs’, by regulating both the activity of CDC42 and (the transcription factor function of) CTNNB. Furthermore, in a study using weighted gene coexpression analysis in human neuronal progenitor cells, it was reported that *AKAP7* and *AKAP11* are coexpressed in a ‘coexpression module’ containing genes—including previously implicated ASD candidate genes—that are likely to be involved in the onset of differentiation as well as the cessation of proliferation of these cells.⁶⁰ Lin *et al.*⁶¹ reported that *MAP2* and *NBEA*, two AKAP genes that have been strongly implicated in ASD aetiology (Supplementary Table 4), as well as a large number of (candidate) genes from the identified networks, including the two AKAP-regulated network ‘hubs’ *CDC42* and *CTNNB*, undergo significant changes in their expression during the transition from pluripotent stem cells to early differentiating neurons. All these data provide further evidence for the functional involvement of the identified protein networks as well as specific ASD-implicated AKAPs in early brain development.

In addition, there is ample genetic evidence for the involvement of the 10 AKAPs in the aetiology of ASDs (Supplementary Table 5). First, six AKAP genes (*AKAP7*, *AKAP10*, *AKAP11*, *MAP2*, *MSN* and *NBEA*) are implicated to have a role in these disorders through SNP associations that have been detected in the published GWASs^{11,16} and/or a recent candidate gene association study.⁶² In this respect,

rs5918959, a SNP located 66 kb downstream of the *moesin* (*MSN*) gene, showed a genome-wide significant *P*-value of 1.22×10^{-10} for association with ASDs in the combined discovery samples from the GWAS by Wang *et al.*¹¹ Furthermore, eight AKAP genes (*AKAP5*, *AKAP8*, *AKAP9*, *AKAP10*, *AKAP13*, *MAP2*, *MSN* and *NBEA*) have been found in CNVs in people with ASDs^{63–73} and *NBEA* was found to be disrupted by a chromosomal translocation in a male with autism.⁷³ *AKAP9* is also present in a locus linked to autism.⁷⁴

Moreover, five AKAP genes are targets of ASD-implicated microRNAs (*AKAP5*, *AKAP8*, *AKAP11*, *AKAP13* and *NBEA*) (Supplementary Tables 2 and 5) or expressed differentially in lymphoblastoid cells of people with ASDs (*AKAP7*).²⁰ Expression studies also implicate *MSN* and *MAP2* with increased⁷⁵ and decreased⁷⁶ expression, respectively, found in the post-mortem brain of autistic people. In addition, exposure to low levels of polychlorinated biphenyls in rats leads to increased cerebellar expression of *Akap11* (ref. 77) and autism-like behavior.⁷⁸ Recently, it was also reported that *Nbea*^{+/-} mice—that is, mice that are haploinsufficient for the *Nbea* gene—exhibit several ASD-like features, including changes in social behaviors as well as spatial learning and memory.⁷⁹

An eleventh AKAP gene is implicated in ASDs, in that rs10038113, a SNP yielding genetic association with ASDs in the GWASs by Wang *et al.*¹¹ ($P=7.40 \times 10^{-8}$) and Ma *et al.*¹² ($P=3.40 \times 10^{-6}$), maps only 7 kb upstream of the *moesin pseudogene 1* (*MSNP1*) gene (<http://www.ensembl.org>). Although Wang *et al.*¹¹ speculate that *CDH10* and/or *CDH9*—two cadherin genes that are located 1257 kb upstream and 978 kb downstream of rs10038113, respectively—may be implicated in this association, *MSNP1* is the only gene in a linkage disequilibrium block of ~100 kb in size that contains rs10038113 as well as several SNPs yielding genome-wide significant associations with ASDs for the combined discovery and replication samples in the GWAS they published.¹¹ Interestingly, rs10038113 and rs1896731, a SNP present in the same linkage disequilibrium block and mapping 11 kb upstream of *MSNP1*, were recently also found to be nominally associated with autistic traits,⁸⁰ whereas rs4307059, a SNP from the linkage disequilibrium block that is located 56 kb downstream of *MSNP1*, associates with ASD-like social communication difficulties in the general population.⁸¹ Moreover and most importantly, *MSNP1* shows a very

Figure 2 (a) Schematic representation of a protein network that is implicated in autism spectrum disorders (ASDs) and leads to neurite outgrowth through molecular signaling cascades located in the extracellular matrix/compartments, cell membrane, cytoplasm and nucleus of developing neurons. The proteins encoded by genes implicated in ASDs through common genetic variants—single-nucleotide polymorphisms (SNPs) from five published genome-wide association studies (GWASs) (Supplementary Table 1) and/or ASD candidate gene association studies—are indicated in yellow. The proteins encoded by genes implicated in ASD aetiology through rare genetic variants—one or more mutations and/or copy number variations (CNVs) affecting the gene—are indicated in red, whereas the proteins encoded by genes implicated in ASDs through both common and rare genetic variants are indicated in orange. In addition, all A-kinase anchor proteins (AKAPs) are dark blue and the proteins encoded by genes that have been implicated in ASD aetiology through ‘other’ genetic evidence—including gene expression studies, gene/protein function studies and genetic animal studies—have a green border. In the Supplementary Information, the network is described in detail, and the current knowledge about the function of the network proteins is presented. (b) Schematic representation of a protein network that is implicated in ASDs and leads to neurite outgrowth through molecular signaling cascades located in the growth cone and surrounding extracellular matrix/compartments of developing neurons. The proteins encoded by genes implicated in ASDs through common genetic variants—SNPs from five published GWASs (Supplementary Table 1) and/or ASD candidate gene association studies—are indicated in yellow. The proteins encoded by genes implicated in ASD aetiology through rare genetic variants—one or more mutations and/or copy number variations (CNVs) affecting the gene—are indicated in red, whereas the proteins encoded by genes implicated in ASDs through both common and rare genetic variants are indicated in orange. In addition, all AKAPs are dark blue and the proteins encoded by genes that have been implicated in ASD aetiology through ‘other’ genetic evidence—including gene expression studies, gene/protein function studies and genetic animal studies—have a green border. In the Supplementary Information, the network is described in detail, and the current knowledge about the function of the network proteins is presented.

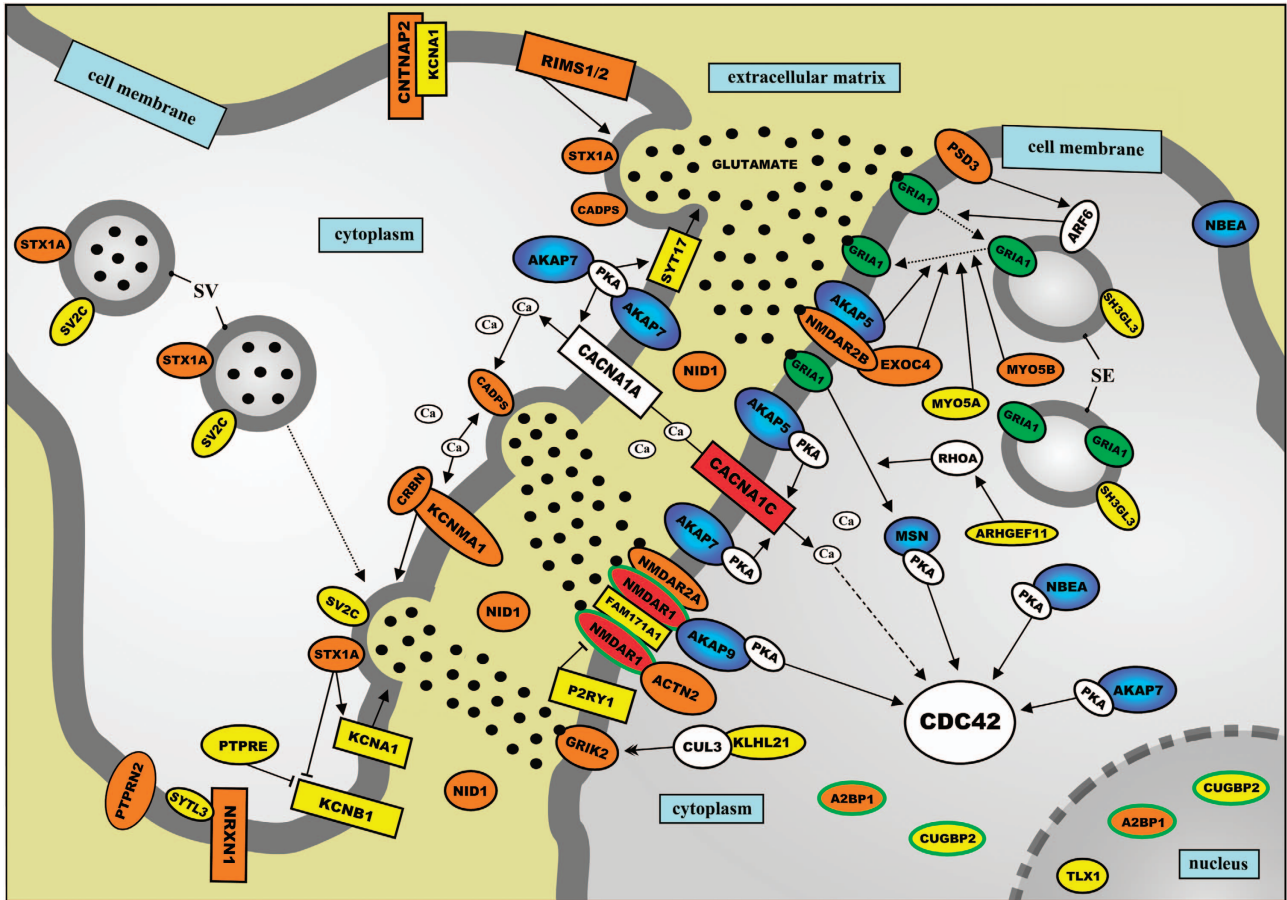


Figure 3 Schematic representation of a protein network that is located in the neuronal synapse and implicated in autism spectrum disorders (ASDs) by its involvement in modulating glutamatergic neurotransmission. The proteins encoded by genes implicated in ASDs through common genetic variants—single-nucleotide polymorphisms (SNPs) from five published genome-wide association studies (GWASs) (Supplementary Table 1) and/or ASD candidate gene association studies—are indicated in yellow. The proteins encoded by genes implicated in ASD aetiology through rare genetic variants—one or more mutations and/or copy number variations (CNVs) affecting the gene—are indicated in red, whereas the proteins encoded by genes implicated in ASDs through both common and rare genetic variants are indicated in orange. In addition, all A-kinase anchor proteins (AKAPs) are dark blue and the proteins encoded by genes that have been implicated in ASD aetiology through ‘other’ genetic evidence—including gene expression studies, gene/protein function studies and genetic animal studies—are green/have a green border. In the Supplementary Information, the network is described in detail, and the current knowledge about the function of the network proteins is presented.

high degree of sequence homology to the *MSN* gene⁸² and was recently demonstrated to regulate the expression of *MSN* in the human cerebral cortex through expression of its antisense strand.⁸³

Additional functional evidence points to a role for the *AKAP* genes (and the encoded proteins) in the aetiology of ASDs. *AKAP10*,⁸⁴ *AKAP13*,⁸⁵ and *MSN*⁸⁶ are involved in innate immunity, known to be disturbed in at least a subset of people with ASDs,^{87,88} through regulation of the activity of Toll-like receptors. *AKAP5* and *AKAP8* are both involved in the biosynthesis of the circadian hormone melatonin,⁸⁹ which is markedly disturbed in people with ASDs.⁹⁰ Thus, it is interesting that both anti-inflammatory treatments and melatonin have been proposed as possible new treatments of ASDs.⁹¹ Neuronal *AKAPs* also play an important role in the serotonin-induced release of neurotransmitters.⁹² *AKAP5* is involved in the recycling of postsynaptic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptors (Figure 3) and the regulation of long-term potentiation and depression through glutamate-bound *N*-Methyl-D-aspartate

(NMDA)-type receptors.^{93,94} *AKAP7* regulates signaling cascades downstream of D1-like dopamine receptors.^{95,96} Hence, it is interesting and intriguing that risperidone, one of only two drugs approved by the Food and Drug Administration (FDA) for the treatment of ASDs (<http://www.fda.gov>), functions both as a modulator of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid- and NMDA-type glutamate receptor expression⁹⁷ and regulates the serotonin-induced release of glutamate and dopamine.^{98,99}

Risperidone is also involved in upregulating the brain expression of microtubule-associated protein 2 (*MAP2*)¹⁰⁰—an *AKAP* that is implicated in neurite outgrowth (Figure 2b)—as well as *CTNNB* and *GSK3B*,¹⁰¹ the two proteins that are regulated by *AKAP8* and *AKAP11*, respectively (Figure 2a). *RHOA*, a protein that is positively regulated by *AKAP13* and has an important role in the neurite outgrowth and synaptic networks (Figures 2a, b and 3), also modulates the effects of risperidone treatment for autistic symptoms.¹⁰²

In summary, 8 of the 10 ASD-implicated *AKAPs* that we identified in our analyses—*AKAP5*, *AKAP7*, *AKAP8*,

AKAP10, AKAP11, AKAP13, MAP2 and MSN—regulate two ASD-implicated biological processes (innate immunity and melatonin synthesis) for which new treatments have been proposed and/or are involved in directly or indirectly modulating the downstream effects of risperidone treatment for ASDs. The effects of existing ASD drugs on these and other AKAPs should be investigated in future studies. In this respect, several AKAP-dependent protein–protein interactions and the AKAPs themselves are also increasingly considered and investigated as potential drug targets,⁵⁹ and several molecules that specifically inhibit the binding of PKA to its AKAP-binding site have been patented (http://www.wipo.int/patentscope/search/en/detail.jsf?docId=EP14889042&recNum=2&office=&queryString=EN_ALLTXT%3A%28AKAP%29&prevFilter=&sortOption=Relevance&maxRec=255). One of these molecules is Ht31, a peptide that is derived from AKAP13. Interestingly, administration of Ht31 to rats effectively blocks the ‘masculinization’ of a part of their brain,¹⁰³ which suggests an additional and more direct role of AKAPs in mediating the masculinizing effects of testosterone—or rather, estradiol, which is converted from testosterone in the brain^{104,105} (see Figure 2a)—on brain development. Furthermore, transgenic mice that conditionally express Ht31 in their hippocampus show spatial learning and memory impairments¹⁰⁶ that resemble cognitive deficits in ASDs.^{107,108} For these reasons, we think that the ASD-implicated AKAPs and/or protein–protein interactions dependent on these AKAPs are excellent targets for developing novel ASD drugs.

Conclusions

In this study, we used bioinformatics and literature analyses to investigate and integrate the top-ranked findings of five published GWASs of ASDs. We were able to place the proteins encoded by 117 of the 200 ASD candidate genes from these GWASs and 33 additional strong ASD candidates into three signaling networks regulating steroidogenesis in Leydig cells, neurite outgrowth and glutamatergic synaptic function. Importantly, the sixth published GWAS of ASDs strongly replicated and validated the identified networks. Several members of the AKAP family functionally integrate signaling cascades within and between the three identified networks. Thus, these AKAPs—that are themselves strongly genetically and functionally linked to ASDs—seem ideally suited to be studied further as possible ‘druggable’ targets for the treatment of ASDs.

There is also a strong overlap between rare and common ASD-implicated genetic variants, which implies that multifactorial and oligogenic forms of ASDs partially share aetiologic pathways. Furthermore, we have provided compelling evidence against gene size-based bias within the ASD GWAS data.

Conflict of interest

The authors declare no conflict of interest.

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