

# Using genetic findings in autism for the development of new pharmaceutical compounds

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## Abstract

**Rationale** The main reason for the current lack of effective treatments for the core symptoms of autism is our limited understanding of the biological mechanisms underlying this heterogeneous group of disorders. A primary value of genetic research is enhancing our insight into the biology of autism through the study of identified autism risk genes.

**Objectives** In the current review we discuss (1) the genes and loci that are associated with autism, (2) how these provide us with essential cues as to what neurobiological mechanisms may

be involved, and (3) how these mechanisms may be used as targets for novel treatments. Next, we provide an overview of currently ongoing clinical trials registered at [clinicaltrials.gov](http://clinicaltrials.gov) with a variety of compounds. Finally, we review current approaches used to translate knowledge derived from gene discovery into novel pharmaceutical compounds and discuss their pitfalls and problems.

**Conclusions** An increasing number of genetic variants associated with autism have been identified. This will generate new ideas about the biological mechanisms involved in autism, which in turn may provide new leads for the development of novel pharmaceutical compounds. To optimize this pipeline of drug discovery, large-scale international collaborations are needed for gene discovery, functional validation of risk genes, and improvement of clinical outcome measures and clinical trial methodology in autism.

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## Introduction

While both Kanner and Asperger, in their first descriptions of autism and Asperger syndrome suggested the role of “inborn” or heritable factors (Frith 1991; Kanner 1968), only since the mid-1990s has twin data provided unequivocal evidence for a substantial role of heritable factors in the causation of autism (Bailey et al. 1995). It took at least another decade before the first specific genetic variants were identified, with robust and replicable proof of association with autism (Freitag 2007; Devlin and Scherer 2012). It is now becoming increasingly clear that the complex genetic architecture of autism is the principle culprit for the delay between establishing the heritability of autism and the actual identification of causal and contributing genetic variants. In addition to its genetic

heterogeneity, autism, although a well-recognizable clinical syndrome, is also marked by a high degree of phenotypic heterogeneity. This implies that autistic patients differ from each other with regard to the nature and severity of symptoms in the domains of social interaction, communication, and repetitive behaviors. Therefore, while the heritability of autism is high (Bailey et al. 1995), the proportion of patients in whom causal or contributing genetic variants are identified, is limited. To date, this gap between the expected and observed variance explained by genetic variation is still wide, commonly referred to as “the missing heritability” (Manolio et al. 2009). Autism is a group of clinically and etiologically heterogeneous disorders that have a large number of symptoms in common. These symptoms are grouped into two domains: (a) abnormal social interaction and verbal and nonverbal communication, and (b) stereotyped and rigid patterns of behavior, and unusual sensory reactions (American Psychiatric Association 2013). The clinical variation is broad ranging from patients with mild symptoms and relatively spared language to patients with severe symptoms often accompanied by intellectual disability and epilepsy. Between 0.5 and 10 % of individuals with autism show unusual abilities, ranging from splinter skills such as the memorization of trivia to the extraordinarily rare talents of prodigious autistic savants. Patients with autism have also increased rates of somatic diseases, such as problems of the digestive tract (Buie et al. 2010) and immunological abnormalities (Gesundheit et al. 2013).

The present article will focus on probably the most important aim of genetic research in autism, i.e., to generate unique biological information about the molecular mechanisms involved. In turn, this can be used to formulate entirely novel hypotheses about the relevant gene–protein pathways and subsequently about the development of new pharmaceutical compounds.

To date, medication is available to manage troublesome and disruptive behaviors frequently associated with autism such as irritability, self-injury, anxiety, aggressive behaviors, hyperactivity, impulsivity, and inattention (Dove et al. 2012). However, there is no effective medication to improve the core deficits of autism, i.e., impairments of social interaction and verbal and nonverbal communication. By far, the most important reason for the lack of effective medication for autism is our limited understanding of its molecular and neurobiological base. Hence, drug development has lacked well defined molecular targets and consequently was limited to a phenotype-based approach where phenotype-modifying characteristics of potential compounds are tested, often without a priori insight of the biological mechanisms involved (Waldman and Terzic 2013). This is unlike other areas in medicine, where new pharmaceutical interventions have been developed on the basis of known disease mechanisms. For example, medications that lower blood pressure have typically been designed to act on certain pathways involved in the

pathophysiology of hypertension, such as renal salt and water absorption and vascular contractility. Treatments for diabetes mellitus are aimed at improving insulin release from the pancreas and sensitivity of the muscle and fat tissues to insulin action. Disease mechanisms however are unknown for autism, and in this context, for any other mental disorder. In this respect, genetic research may prove to be most valuable, mainly because the identification of autism-associated genetic variants can provide leads towards disease mechanisms involved, and biological targets for drug development.

However, we should be aware that gene discovery is one thing, but that, once a risk gene has been identified, subsequent studies are required to elucidate the timing and localization of expression as well as the function of the protein it encodes. Next, the effects should be studied of a functional change of the identified gene on the relevant biological pathways and how this contributes to our understanding of the etiology of autism. Translating knowledge about disrupted gene–protein pathways into personalized molecular therapies undoubtedly represents the major long-term goal of genetic investigations in autism. Personalized medicine refers to the selection and tailoring of a specific treatment for an individual patient, based on genetic and/or other biomarkers profiles, including transcriptomics, proteomics, and metabolomics (see Ruggeri et al., this issue). Importantly, to fulfill this ambition, strong international collaboration is a necessity in order to link gene discovery to biological knowledge, provide rationale for potential drug targets, and pave the road for efficient (pre)clinical trials.

Currently available psychotropic medications are based, except for few instances, on affecting the activity of the major classic neurotransmitters dopamine, noradrenaline, and serotonin through binding to presynaptic or postsynaptic receptor systems, inhibition of reuptake through binding to the transporters, or inhibition of enzymatic breakdown. However, the actions on these major neurotransmitter systems are not translated into therapeutic changes of the core symptoms of autism, indicating that these neurotransmitter systems do not affect the central pathophysiological mechanisms of the social and communicative symptoms of autism (Buitelaar 2003). Hence, new effective medication probably will tap into alternative, novel mechanisms, such as interacting with abnormal intracellular pathways. This challenging move from traditional psychopharmacology to novel, more personalized molecular therapies has begun in very recent years, primarily stimulated by two developments.

First, the discovery of genetic disorders with neurodevelopmental phenotypes, whose pathophysiology could be elucidated sufficiently to allow the design of targeted molecular treatments (see Section 4). Importantly, these lines of investigation were always triggered by the identification of the genes affected by mutations, triplet repeat expansions or copy number variations (CNVs), and ultimately producing the clinical phenotype;

Second, the correction of neuropathological and behavioral abnormalities in rodent models of these disorders by genetic or pharmacological strategies, not only during critical periods in prenatal neurodevelopment but also in the adult animal (Ehninger et al. 2008a, b; Guy et al. 2007; Tropea et al. 2009; van Woerden et al. 2007), for review see Ehninger et al. (2008a). The latter results support the hypothesis that autism is a disorder of synaptic functioning and that these functional abnormalities in synaptic connectivity may be reversible or at least modifiable (Ecker et al. 2013). This spurs hope in personalized molecular treatments yielding functional recovery of patients with autism well beyond the first few years of life.

In this article, we will first review the genetic variants associated with autism that have turned out to be promising for further functional validation and translation study. Parameters used to select such promising genes for review in this article were (1) statistical association of the gene with autism based on genetic studies, and convergence of the gene with other genes in a shared (putative) biological mechanism, and (2) recently completed and ongoing clinical trials with compounds that were developed on the basis of biological knowledge inferred from the association of these genes with autism. Next, we will describe more in detail the various steps and techniques involved in the process of validation and translation to the development of promising novel compounds in the treatment of autism and will discuss pitfalls and problems of these translational steps. We end with a plea for the necessity of international collaborative networks to allow both cross-disciplinary targeted studies as well as efficient orchestration of (pre)clinical trials of potential compounds.

## Methods

Relevant publications were retrieved via Pubmed using query terms such as “autism” and “autist\*” or “neurodevelopment” combined with “genetics” or “therapy” or “pharmacologic,” and subsequent searches were undertaken using combination of these terms with identified gene or protein names mentioned in the retrieved papers (e.g., “oxytocin” and “autism”). In parallel, we searched clinicaltrials.gov (august 2013) for studies on autism (419 studies), which we restricted to interventional studies (320) and subsequently to interventional studies involving drugs, which generated 274 results. Given our focus on the link between identified genetic variants in autism and the development of novel pharmaceutical compounds, we retained from our Pubmed search only those genes that have contributed to the theoretical rationale upon which pharmaceutical compounds are tested in any of these 274 drug-intervention trials registered in clinicaltrials.gov. This strategy yielded 36 drug intervention trials that are listed in Table 1. Finally, references cited in identified articles were used for further retrieval of relevant papers.

## Myriad of genetic etiologies, but limited number of shared biological pathways

Our current understanding of the genetic underpinnings of autism implicates a highly complex architecture. The observed familial clustering and the increased concordance in monozygotic twins indicate a high heritability of this neurodevelopmental disorder. Common genetic variants are likely relevant (Klei et al. 2012) although genome wide association studies thus far have been less successful in identifying significant associated loci in comparison to schizophrenia (Devlin et al. 2011; Vorstman et al. 2013). The role of rare variants, including CNVs (Pinto et al. 2010) and single nucleotide variants (SNVs), (Sanders et al. 2012) has been firmly established in a host of studies during the past several years (Geschwind 2011). Both classes of common and rare variants have incited two different architectonic models for autism that often have been opposed against each other; the common-disease-common-variant and the common-disease-rare-variant model. In reality however, it appears that both models are not mutually exclusive (Visscher et al. 2012) and a spectrum of genetic variants is likely to exist ranging from rare to common with variable effect sizes.

The high number of genetic variants that have been identified over the last years as either causative or contributing to autism indicates a substantial genetic heterogeneity of the disorder. Indeed, it has been suggested that autism is in reality the shared phenotypic expression of a myriad of different disorders (Waterhouse 2008). Recently, an overview of over 100 genetic loci with evidence in support of association with autism was published (Betancur 2011), while based on the findings of recent sequencing studies the total number of risk genes for autism was estimated to be well over 1,000 (Sanders et al. 2012; O’Roak et al. 2012a). Does this, theoretically, imply that the cure for autism will require the development of a unique pharmacological agent for every single genetic cause of autism that has been identified so far?

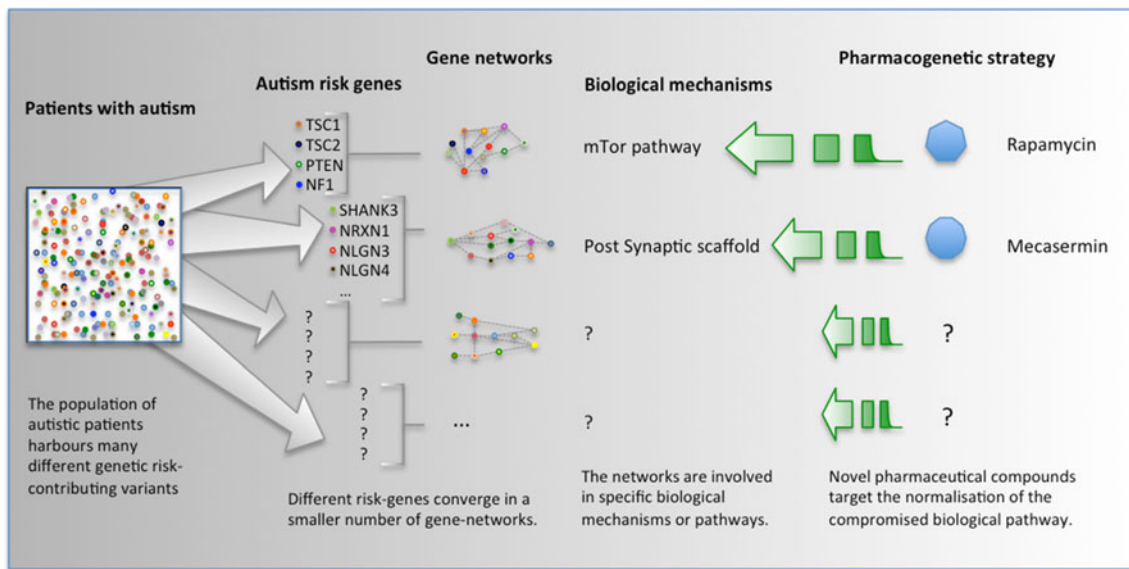
Fortunately, biological functions are virtually always the resultant of the concerted action of multiple genes. Thus, many different genes seem to converge into one a relatively limited number of biological pathways (Berg and Geschwind 2012; Poelmans et al. 2013). Consequently, this limited number of abnormal biological pathways leading to autism significantly increases the chances of finding new effective molecules. Thus, two autistic patients may have genetic variants affecting entirely different genes, while sharing the same compromised biological pathway. Both patients could then, in theory, benefit from the same therapeutic agent (see Fig. 1). In addition, it is possible that the phenotypic heterogeneity of autism may be reduced when subgroups within the general autism spectrum disorder (ASD) population are considered, based on shared biological etiology (Bruining et al. 2010)

**Table 1** Personalized molecular therapy for neurodevelopmental disorders: pathophysiological underpinnings, drug names [and alternative drug names], and NIH-approved clinical trials, as available at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) by NCT identifier

Observation underlying this strategy	Putative pathophysiology	Drug	Therapeutic target	Clinical trials by NCT number
Rett syndrome; implication of MeCP2	Abnormal regulation of gene expression, impairing neurite sprouting and synaptogenesis	(1–3) IGF1 [Mecasermin, Increlex]	Enhance neuritic sprouting and synaptogenesis	01253317, 01777542
22q13 deletion (Phelan-McDermid Syndrome) and SNVs affecting SHANK3	Disrupted scaffolding of the post-synaptic elements, leading to reduced dendritic spines and synaptogenesis			01525901
Fragile X syndrome; implication of FMR1	Increased translation in dendritic spines	MPEP Fenobam STX107 AFQ056 [Mavoglurant]	mGLUR5 antagonism	None 01806415 01325740, 00965432 01357239, 01253629, 01482143, 01348087, 01433354, 00718341
		RO4917523		01750957, 01015430, 01517698
		STX209 [Arbaclofen]	GABA-B receptor agonism	00788073, 01282268, 01555333 (terminated), 01325220
		CX516 [Ampalex]	Positive allosteric modulation of AMPA receptors	00054730
Fragile X syndrome; implication of immune system.	Microglial activation	Minocycline	Microglial inhibition	00409747
Association of immune disorders in idiopathic autism.	Increased expression and activity of MMP9		MMP9 inhibition	01053156, 0858689
Tuberous Sclerosis; implication of TSC1/TSC2	Disinhibition of the mTOR pathway	Rapamycin [Sirolimus]	mTOR inhibition	00457808 01289912, 01070316, 01730209, 01713946
Autism with macrocephaly; implication of PTEN		Everolimus [RAD001, Afinitor]		None
Neurofibromatosis; implication of NF1	Disinhibition of RAS activity and mTOR pathway	Lovastatin	Ras activity inhibition	00352599
Genetic variants in OXTR	Inadequate action of oxytocin	Oxytocin	Enhance oxytocin tonus	01337687, 01788072, 01624194, 01308749, 01183221, 1256060
CNVs affecting 15q11-13 implicating GABRB3, GABRA5, and GABRG3	Excitatory effect GABAergic neurons due to abnormally elevated intracellular Chloride	Bumetanide	Reinforcement of GABAergic inhibition via reduction of intracellular chloride levels	01078714

*MPEP*, 2-methyl-6-(phenylethynyl)pyridine





**Fig. 1** Diagram representing the link between genetic studies in autism and the targeted development of novel pharmaceutical compounds. Autism is thought to be genetically heterogeneous; studies are identifying a growing number of genes involved in the etiology of this disorder. Based on biological information, such as function and expression profile, these

At present, research findings are consistent with this model. For instance, *TSC1*, *TSC2* (Prather and de Vries 2004), *PTEN* (Zhou and Parada 2012), and *NF1* (Walsh et al. 2013) are different genes independently associated with autism, but they are biologically closely related as partners in the mTOR pathway (Ehninger 2013). Likewise, previous studies have shown the involvement of *CHD7* (Hartshorne et al. 2005; Johansson et al. 2006) and *GRIN2B* (Yoo et al. 2012) in autism. In addition, a recent multiplex sequencing study revealed recurrent mutations in *GRIN2B*, *CHD8*, *DYRK1A*, *TBR1*, and *TBL1XR* (O’Roak et al. 2012b). Interestingly, these genes were all found to interact in the B-catenin-chromatin-remodeling protein network (O’Roak et al. 2012a). These and other examples indicate that while the *genetic* heterogeneity of autism is likely to be important, the *biological* heterogeneity of autism may be much more limited, providing shared biological pathways as a point of departure for the development of pharmaceutical interventions.

### From genes to biology

Biological insights obtained through the study of known genetic disorders associated with autism

For a number of neurodevelopmental disorders that express the autism phenotype, causative genetic factors have been established (Vorstman and Ophoff 2013). Such disorders currently under trial with personalized therapies include Rett syndrome, Fragile X syndrome, tuberous sclerosis, and autism associated with macrocephaly, caused by *PTEN* mutations (Table 1). In the next sections, we will briefly review genetic

“autism genes” are linked together in gene networks, which in turn are involved in biological mechanisms. Hence, the effect of genetic variants becomes apparent in the efficacy of specific biological mechanisms. Pharmacogenetic strategies consist of the correction or compensation of the deficient biological mechanism

findings and ongoing clinical trials in a number of these conditions

### Rett syndrome

Approximately 70 % of girls affected with Rett syndrome carry disruptive mutations in the *MECP2* gene on Xq28. Their clinical phenotype displays, after an initial period of normal development, growth arrest at 6–18 months resulting in microcephaly, regression of acquired verbal and social skills, mental retardation, typical “hand wringing” stereotypies, seizures, scoliosis, dyspraxia, and other symptoms. The dysregulation produced by the inactivation of methyl-CpG-binding protein 2 (MECP2) is complicated; as a transcription factor, it regulates the expression of multiple target genes and may also modulate RNA splicing processes (Na et al. 2013). Despite the complexity of these epigenetic effects, the most representative functional outcomes, i.e., impaired dendritic branching, reduced numbers of dendritic spines, and decreased synaptic contacts, represent a plausible treatment (Calfa et al. 2011).

In the mouse models of these genetic mutations, treatment with insulin-like growth factor I (1–3)IGF-1, through its stimulatory effect on neuronal cell survival and synaptic maturation, produces a partial, yet substantial recovery in dendritic branch length and synaptic counts, with significant improvement or even normalization of behavioral deficits present in untreated *Mecp2* knock-out (KO) mice (Tropea et al. 2009). Based on this evidence and on the approved use of this drug in the pediatric population to treat body growth deficits due to insufficient production of IGF1, therapeutic trials with (1–3)IGF1

(also known as Mecasermin or Increlex) are currently under way (see Table 1).

### *Fragile X*

Perhaps, the most representative for our current efforts toward developing personalized molecular therapies in autism is Fragile X syndrome (Kremer et al. 1991). The lack of protein encoded by *FMR1* (FMRP) consequent to the triplet repeat expansion present at the Fragile X locus disrupts the concerted translation of FMRP-bound mRNAs, especially relevant to dendritic spine function (Ashley et al. 1993; Eberhart et al. 1996). One of the main consequences of this disruption consists in excessive protein synthesis driven by stimulation of metabotropic glutamate receptors (mGluR1/5), resulting in the downregulation of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors through internalization from the cell surface (Huber et al. 2002; Bear et al. 2004). Blocking mGluR5 transmission using either genetic (Dolen et al. 2007) or pharmacological means reverses many abnormal neurochemical, neuroanatomical, and behavioral features present in FMRP-deficient mice (Levenga et al. 2011; de Vrij et al. 2008; Yan et al. 2005; Su et al. 2011). Several newer compounds with variable antagonistic effects on mGluRs are currently under trial, including Fenobam, STX107, STX209 also known as Arbaclofen (a gamma-aminobutyric acid (GABA)-B receptor agonist), AFQ056 also known as Mavoglurant, and RO4917523 (Table 1). Of note, the STX209 trial was recently terminated because of insufficient evidence for treatment effects on clinical endpoints.

At least one randomized, double-blind, placebo-controlled study of Fragile X patients treated with AFQ056 provided encouraging results, but only in patients with complete *FMR1* promoter methylation, and with the caveat of resulting from a post-hoc, rather than from a pre-defined endpoint analysis (Jacquemont et al. 2011). Therefore, further clinical trials are required to establish whether mGluR5 antagonism can exert relevant positive effects on core autism symptoms. Nonetheless, in theory, effects of mGluR5 antagonism may extend beyond Fragile X. For Black and Tan BRachyury mice, an inbred strain whose phenotype encompasses the more closely resembling human autism deficits (Moy et al. 2007), 2-methyl-6-(phenylethynyl)pyridine, an antagonist of mGluR5, also improved stereotypic behaviors (Silverman et al. 2010) and ampakine, a positive modulator of the AMPA receptor, enhanced social interaction (Silverman et al. 2013)

### *The protein mammalian target of rapamycin mTOR pathway (mTOR)*

Another pathway wherein different genes involved in autism converge is the mTOR pathway. The central hub in this pathway is the protein mammalian target of rapamycin which forms

a complex with other proteins (mTOR complex 1, mTORC1). Rapamycin is a natural macrolide produced by bacteria; it was originally developed as an antifungal agent but became known for its immunosuppressant and antiproliferative actions. The mTOR pathway physiologically links nutrient availability to body growth by regulating protein translation rates and hence cell proliferation (Ma and Blenis 2009). This pathway is negatively modulated by four tumor suppressor genes all known to yield autism phenotypes when inactivated: the *TSC1/TSC2* heterodimeric complex, *PTEN*, and *NF1*.

Dominant mutations of *TSC1* or *TSC2* are responsible for tuberous sclerosis, characterized by abnormal neuronal cell growth leading to the formation of “tuber-like” nodules in the brain (but glial brain tumors are also frequent) and sebaceous adenomas in the skin. Interestingly, the degree of cognitive impairment and the presence of autistic traits often associated with tuberous sclerosis in humans and in rodent models do not correlate with tuber number (Numis et al. 2011), as much as with dysregulated mTOR signaling. Similarly, somatic *PTEN* mutations have been identified in a variety of tumors, while germline mutations can result in various conditions (Cowden, Bannayan-Riley-Ruvalcaba syndrome, Proteus, and Proteus-like syndromes), as well as in cognitive impairment and/or autistic disorder accompanied by prominent macrocephaly and by macrosomy (Eng 2003; Goffin et al. 2001; Butler et al. 2005). Remarkably, postnatal rapamycin administration to TSC mice models leads to a reversal of cognitive deficits including spatial learning and context discrimination, paralleled by improvements in neuroanatomical deficits including reduction of brain size (Ehninger et al. 2008b). Recently, everolimus, a rapamycin derivative, was demonstrated to achieve primary endpoints in a double-blind, placebo-controlled trial of TSC patients on efficacy for reduction of subependymal giant cell astrocytomas (Franz et al. 2013). This seminal phase 3 clinical trial demonstrates the response of subependymal giant cell astrocytomas to everolimus. Even though in this trial no autism or behavioral endpoints were measured, it provides an encouraging example of how detailed knowledge of genetic and molecular abnormalities can pave the way to target specific pathways leading to improved neurological outcomes in TSC patients. However, key secondary endpoint such as reduction in seizure frequency was not significantly altered, and thus unfortunately, no conclusions could be drawn with regard to other neuropsychiatric endpoints. Similar improvements can be achieved in *Pten* KO mouse both at the cellular, cytoarchitectonic, and behavioral levels by administration of rapamycin (Zhou and Parada 2009).

Intriguingly, the functional effects of the loss of FMRP in Fragile X does not only include excessive mGluR1/5 stimulation (discussed above) but also dysregulation of mTORC1 (Sharma et al. 2010; Qin et al. 2005; Hoeffler et al. 2012). The p70 ribosomal S6 kinase 1 (*S6K1*) is a common downstream effector of mTORC1 signaling and plays a direct role in

regulating translation. Recently, double KO mice were generated where *S6k1* was deleted from *Fmr1* KO background (Bhattacharya et al. 2012). Deletion of *S6k1* corrected exaggerated protein synthesis in the hippocampus of the Fragile X model mice and normalized enhanced mGluR-mediated long-term depression (LTD). LTD of excitatory synaptic strength is an important mode of action of mGluRs (Luscher and Huber 2010). In addition, the *S6k1* deletion improved several behavioral abnormalities, including social anxiety, impaired cognition, and behavioral inflexibility, and prevented immature dendritic spine morphology. Moreover, in these double KO mice, there was a reversal of peripheral Fragile X pathologies not addressed by mGluR5-targeted pharmaceuticals, including reduction in weight gain, and macro-orchidism.

Neurofibromatosis is caused by mutations affecting *NF1*, causing a dysfunction of neurofibromin. As a result, Ras activity is abnormally enhanced and the normal inhibitory effect of the *TSC1/TSC2* complex of mTOR is reduced (Gipson and Johnston 2012). Cognitive deficits observed in *Nf1* KO mice were normalized upon lovastatin treatment, a potent inhibitor of Ras activity (Li et al. 2005). A trial with this compound in neurofibromatosis is underway (Table 1).

Identifying novel targets within the mTOR pathway for pharmacological intervention is critical and could allow differentiated therapies for autism. Given the regulatory function of the mTOR pathway on protein synthesis, it is not surprising that mTORC1 inhibition leads to multiple pleiotropic actions including the stimulation of autophagy. Therefore, the challenge is to develop pharmacological interventions that interact with this pathway in a sufficiently specific manner. For example, specific inhibitors of p70 ribosomal S6 kinase 1 (S6K1) or pharmacological inhibition of eIF4E activity (a translational activator in mTOR pathway) could result in specific inhibition of the translational dysfunction observed in both mice models and patients with autism. Another putative molecular target in the mTOR pathway is the GTPase Rheb (Ras homologue enriched in brain), which functions as a switch between the heterodimeric complex of TSC1 or TSC2 and mTOR. Inactivation of Rheb by TSC1 or TSC2 prevents overactivation of mTOR.

A final chapter regards immune-related therapies in autism and in Fragile X (Table 1). These will not be dealt in detail here because although likely dependent upon mutations or CNVs affecting immune genes, these genetic abnormalities have not been clearly defined to date. Nonetheless, the abnormal activation of the immune system in autism and particularly of its innate components (Vargas et al. 2005; Garbett et al. 2008) as well as the excessive expression and activity of matrix metalloprotease 9 (*MMP9*) in Fragile X (Siller and Broadie 2012) have also led newly proposed molecular treatment strategies which are currently under scrutiny (Table 1)

Genetic variants, including copy number variants and common variants associated with autism, can further our biological understanding

In addition to the rare genetic disorders that indicate the relevance of mGluRs and the mTOR pathway in autism, there are more clues that can be obtained from genetic studies. Common and less rare genetic variants, including copy number variants, associated with an increased risk of autism that may reveal other avenues where genetics can translate to therapies include variants in *OXTR*, *GABR*, and *SHANK3*.

Oxytocin is a nine-amino acid peptide synthesized in the hypothalamus. Apart from regulating lactation and uterine contraction, oxytocin acts as a neuromodulator in the central nervous system (Lucht et al. 2009; Yamasue et al. 2009). Both animal experiments and clinical research have confirmed the role oxytocin plays in social and repetitive behaviors (Green and Hollander 2010). Therefore, the oxytocin system might be potentially involved in the pathogenesis of autism, and the human oxytocin receptor (*OXTR*) gene is regarded as a promising candidate gene to study.

Indeed, family-based and population-based association tests, SNPs and haplotypes in *OXTR* have been reported to confer risk for autism in different ethnic groups, with reported effect sizes up to 1.4 (odds ratio) (Liu et al. 2010; Li et al. 2012; Lerer et al. 2008; Jacob et al. 2007; Wu et al. 2005), although the association has not been consistently replicated (Tansey et al. 2010), as predicted for a complex disorder with heterogeneous underpinnings like autism. Furthermore, a recent study identified significant increases in the DNA methylation status of *OXTR* in peripheral blood cells and temporal cortex, as well as decreased expression of *OXTR* mRNA in the temporal cortex of autism cases, suggesting that epigenetic dysregulation may be involved in the pathogenesis of autism (Gregory et al. 2009). Given the genetic and functional evidence in support of the involvement the oxytocin pathway in autism, potential therapeutic effects of oxytocin administration is explored in several studies (Tachibana et al. 2013; Anagnostou et al. 2012) (Table 1).

GABA is the chief inhibitory neurotransmitter in the brain, acting by binding to a GABA receptor. The receptor is a multimeric transmembrane receptor that consists of five subunits arranged around a central pore. The GABA receptor subunits are homologous, but are both structurally and functionally diverse (Menold et al. 2001). Of the GABA receptor subunit genes, *GABRB3*, *GABRA5*, and *GABRG3* are localized to chromosome 15q11–q13, one of the most complex regions in the genome involved with genome instability, gene expression, imprinting, and recombination (Martin et al. 2000).

Several lines of evidence strongly suggest that this region is implicated in autism. Both deletions and duplications of 15q11–q13 are associated with autism (Vorstman and Ophoff 2013) and a significant peak encompassing *GABRG3* was detected in more than one linkage study (Cook et al. 1998; Kim et al. 2006; Yoo

et al. 2009; Buxbaum et al. 2002). In addition, results of several studies indicate association of markers in this region with autism (Menold et al. 2001; McCauley et al. 2004; Ma et al. 2005). However, these findings are not replicated consistently (Martin et al. 2000; Maestrini et al. 1999; Curran et al. 2005). Interestingly, the mouse model for human 15q11–q13 duplication demonstrated autistic features (Takumi 2010; Nakatani et al. 2009). Based on the hypothesis that GABAergic signaling may be altered in autistic patients, with the persistence of “immature” GABA receptor subunit compositions yielding excitatory, rather than inhibitory, effects, bumetanide, a diuretic that reinforces GABAergic inhibition through the reduction of intracellular chloride ion levels, is being examined with promising initial results (Lemonnier et al. 2012).

Human studies exploring the rate of 22q13 deletions estimate the frequency of 22q13 deletion in 0.5–1 % of in large samples with autism (Geschwind 2009). The convergence of early case reports on cytogenetic abnormalities in autistic patients indicated 22q13 as a potential region of interest (Vorstman et al. 2006) and genetic studies have reported the identification of several mutations/rare variants in the *SHANK3* gene in autistic patients (Wilson et al. 2003; Moessner et al. 2007; Gauthier et al. 2009). The clinical phenotype of 22q13 deletion/Phelan-McDermid syndrome includes hypotonia, neurodevelopmental delay including absent to severely delayed speech, autistic behavior, and minor facial dysmorphisms (Phelan and McDermid 2012) and is thought to be caused by a haploinsufficiency of *SHANK3*.

*SHANK3* is a large synaptic scaffolding protein with multiple protein–protein interaction domains important in glutamatergic synapses including the cortical–striatal synapses. Interestingly, abnormalities similar to those observed in the mouse model of the *MECP2* inactivation, i.e., dendritic abnormalities, are also observed in *Shank3* KO mice, rodent models of 22q13 deletion, both due to inactivation of the *Shank3* gene (Bozdagi et al. 2010; Yang et al. 2012). The protein encoded by *SHANK3* is critical to the actin-dependent formation of post-synaptic dendritic spine formation in glutamatergic excitatory synapses (Durand et al. 2012). Dendritic spines are the major site of communication for excitatory synapses in the nervous system. Dynamic changes in the configuration of actin filaments meshwork lead to the forming and reshaping of spines that are essential for synaptic plasticity (Matus et al. 1982; Kiraly et al. 2010; Matus 2000) The postsynaptic membrane on which the ionotropic (AMPA and NMDA) and metabotropic (mGluR) glutamate receptors reside is anchored in the post-synaptic density (PSD). In the organization of the PSD, the scaffolding proteins (in particular PSD95 and the *SHANK* proteins) play a central role and link the PSD to the filamentous actin network that extends further down into the spine cytoplasm (Verpelli et al. 2012; Boeckers 2006). This organization allows variations in synaptic activity to be transmitted via the PSD in order to regulate dendritic spine morphology by altering actin filament configuration. In addition

to *SHANK3*, many other autism-related genes converge in this “synaptic-plasticity” pathway, including *NRXN1* (Bucan et al. 2009; Kim et al. 2008; Marshall et al. 2008; Glessner et al. 2009) (presynapse), *NLGN3*, *NLGN4* (Jamain et al. 2003), *TRKB* (Correia et al. 2010) (post-synaptic membrane), *DLGAP2*, *SYNGAP1* (Pinto et al. 2010), *SHANK1* and *SHANK2* (Berkel et al. 2010; Leblond et al. 2012) (PSD and scaffold genes), *DIAPH3* (Vorstman et al. 2011), and *BAIAP2* (Guy et al. 2007) (actin filament regulation). The results of these and other studies indicate that dysfunction of excitatory synapses may be one of the pathways into which multiple different genetic variants identified in autistic patients converge (Bourgeron 2009).

### From biology to novel pharmaceutical interventions

Documenting that a rare or common genetic variant is significantly associated with autism is nothing more than the beginning of a long and complicated process towards the development of an eventually new medication. For most autism genes, there is no conceptual knowledge about the molecular and cellular mechanisms involved and about how these may lead to abnormal cognition and behavior. To gain insight into these mechanisms, a multi-level (molecule, molecular network, synapse, neural network, behavior) and multi-systems (in vitro and in vivo) approach has to be adopted. However, there is no easy answer to the question how we should prioritize genes for such an in-depth mechanistic study despite efforts such as the TDR Targets database (Aguero et al. 2008). Criteria that should be considered in the prioritization are (1) How strong is the association between the gene variant and autism, what is the effect size of the gene, and how strong is the evidence? (2) What is the biological function of the gene, which protein(s) are coded by the genes, and does the disease gene variant lead to different levels or sorts of these proteins? and (3) Bioinformatic analysis of the gene–protein networks involved as well as evidence for expression of the genes in brain regions relevant for autism seem to be important.

The issue however is complicated. Many identified genetic risk variants have small effect sizes and/or are present in only a minority of patients. Nonetheless, it would still be premature to conclude that these genetic variants are not relevant for drug discovery. It might well be that such a variant indexes a biological mechanism that is only relevant in a subgroup of patients, and that for those patients drugs affecting this mechanism may provide therapeutic benefits. Further, there are examples of effective therapies that target a protein coded by risk genes with small effect on disease risk. For example, Ustekinumab is a monoclonal antibody that blocks interleukin 12 and has been licensed for the treatment of psoriasis. Interestingly, the effect size of *IL12B* as a genetic risk factor for psoriasis is quite small with an odds ratio of 1.5 (McInnes et al. 2013). This example well documents why interest in



common genetic variants does not stem from the relative risk conferred by each variant, which is typically small (1.1–1.4 on average), but rather in the underlying pathophysiological mechanisms they outline. Also, non-coding SNPs have been shown to be pathogenic. For example, non-coding SNPs in *NRG1* (Law et al. 2006) and *ERBB4* (Law et al. 2007) were shown to affect the expression of a specific isoform of each gene. This isoform specificity led to specific abnormalities of downstream pathways (Law et al. 2012; Mei and Xiong 2008).

Finally, genes expressed outside the brain may confer risk to autism, among other by affecting immune mechanisms (Careaga and Ashwood 2012; Depino 2013).

The following steps may be part of the translational pipeline (see also Fig. 2).

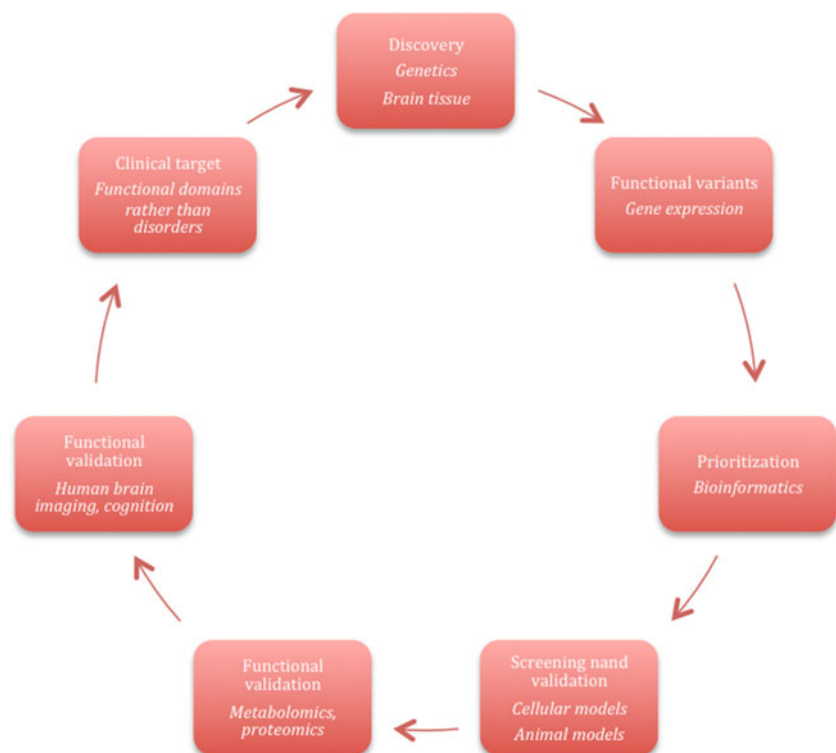
#### Bioinformatics—target identification and validation

It is clear that high-throughput experimental technologies within -omics disciplines lay the data-collecting foundation for pursuing complex biomedical research in autism. However, merely collecting huge amounts of data does not automatically provide new biological insights or clinical applications. For the complete translation of the wealth of data into concrete biological knowledge and clinical practices, it needs to be carefully analyzed (Searls 2000). However, state-of-the-art statistical models and analyses do not yet handle data from high-throughput experiments sufficiently well. In order to overcome this for application in autism, it will require (1) the development of statistical methods that are applicable

in typical situations of modeling high-throughput data, i.e., methods that are robust to model misspecifications, resilient to violations of standard model assumptions, and further can perform well on datasets with many variables/few observations and (2) the utilization of this analysis for the estimation of the performance of treatment outcome prediction models.

Bioinformatics analysis to identify the gene–protein network involved is dependent on the usage of both manual search approaches and automated complex algorithms to further pharmaceutical research. As such, it includes the modeling of molecular interactions, prediction of biological effects of molecules, and identification of potential new drug targets and relevant chemical classes to modulate them. With the increasing amount of available genome sequences, there is a high demand for genome annotation. Genome annotation is the process of attaching biological information to sequences of identified elements on the genome. The structural annotation includes the identification of the gene structure and coding regions, open reading frame prediction, and the localization of regulatory motifs. By functional annotation biochemical and biological functions can be assigned to genes. Automatic annotation tools such as Galaxy are able to perform these steps by computer analysis as a framework for interactive large scale genome analysis which allows the integration of user-defined tools (Goecks et al. 2010). Performing complete genome alignments facilitates the comparison of identified genes, products, and metabolites (Blankenberg et al. 2010). The results can be further employed to construct kinetic models to direct pharmaceutical engineering.

**Fig. 2** Summary of the various steps of a genetic/genomics strategy to drug development. Although the display suggests the steps to occur in a sequential manner, in practice many steps will be taken in parallel



Knowledge about gene relationships and the influence of miRNA on gene regulation may be useful for in-depth analyses of the causes and the development of complex diseases (Soifer et al. 2007). In addition to traditional statistical evaluations of microarray data which aim at the detection of misregulated genes among different probes, a network-based approach encompassing both the genetic network and its regulatory elements may be useful. In this scenario, groups of interacting genes may be critical for the detection of conditional dependencies between individually regulated genes.

Approaches such as Bayesian networks can be used to derive a network of interacting genes (Vignes et al. 2011). Based on mRNA and miRNA array datasets related to autism targets, unknown dependencies can be identified to extend the knowledge of regulatory networks and potential “druggable” targets

*In silico* screening for new pharmaceuticals and use of support vector machines

Novel approaches such as proteochemometrics predict the susceptibility of known and novel compounds on new drug targets (Hopkins 2008). The advantage of proteochemometrics, compared to other prediction methods, is that it is based on the chemical effects by the drugs and the chemical effects of amino acid substitutions in the target molecule. This leads to models which are able to afford predictions for new receptors/enzymes and for new drugs. By combining various types of techniques to model protein interactions which include combinations of molecular dynamics studies of drug–target interactions, proteochemometrics, clinical therapy outcome data, and Bayesian statistics improves the prediction methods. Moreover, validation of the approach by the chemical synthesis of novel compounds which can be tested on a library of appropriate targets in a high-throughput manner (Lipinski and Hopkins 2004). As such, this approach enables the better prediction of target efficacy/safety and to provide methods to produce improved drugs for autism.

Disease-relevant extracellular or intracellular protein–protein interactions occurring at defined cellular sites have great potential as drug targets. The selection of intracellular targets allows for highly specific pharmacological interference with defined cellular functions. Drugs targeting such interactions (if expressed only in a subset of cells) are likely to act with fewer side effects than conventional medication influencing whole cell functions, e.g., by targeting extracellular G-protein coupled receptors with wide receptor expression.

Solved structures of target–protein complexes give fundamental insights into protein function and molecular recognition (Lipinski and Hopkins 2004). The number of structurally solved and diverse target–protein complexes is limited, but can be greatly extended by models generated by homology

modeling and target–protein docking. The interface areas of complexes can be systematically screened for target pockets that are suitable for the binding of small molecules. Binding pockets can be transferred into a pharmacophore that can be further applied for *in silico* screening of compound libraries (Paolini et al. 2006). After a refinement step (compound docking), high-scoring drug candidates can be identified (Terstappen and Reggiani 2001).

The development of proteochemometrics for the analysis of chemical compounds with wide drug target groups including those with -omic and regulatory elements targets has been an area of exciting innovation. Proteochemometric models for analysis and prediction of drug metabolism, interaction of inhibitors with target enzymes, and proteome wide models for the interaction of chemical compounds with the proteome are all currently in development (Fox 2010; Gustafsson et al. 2010). The challenge for statistical method development to improve modeling of omics data for proteochemometrics lies in the creation of approaches that perform well on datasets with many variables and few observations.

The prediction of drug functional activity can be enabled by the use of support vector machines (Burbidge et al. 2001). These machine learning approaches encompass a variety of methods which exist to classify and predict biological properties of chemical compounds, e.g., principal component analyses, partial least squares, artificial neural networks, evolutionary algorithms, and support vector machines (SVMs) (Warmuth et al. 2003). SVMs are models for non-linear classification and regression. They find a hyperplane with the maximum margin separating samples of two classes of a training set. If samples are not separable linearly, they will be mapped to a high-dimensional “feature” space to find a hyperplane separating classes linearly in that space. To form a set of meaningful descriptors for classification, different characteristics of chemical compounds like size, shape, surface, ring counts, etc. have to be computed. The utilization of chemical databases provide classified, comprehensive datasets for the calculation of descriptor sets enables the creation of training- and test-sets and prediction models for compounds whose functional role are not known, yet.

Predictive toxicology

While the development of suitable pharmaceuticals to enable efficacy at autism core symptoms is important, it is equally important to ensure the safety of these ligands in reducing potentially severe side effects. Knowledge of genetic targets and processes from bioinformatics studies can inform regarding the risk associated with the chosen target but also enable the selection of a battery of “toxic” genetic targets for predictive toxicological screening (Fielden et al. 2002). As such computational methods, tools and predictive models could aid in chemical hazard identification and drug safety

assessment. Using virtual screening and the use of quantitative structure–activity relationship models, risk estimates for various types of toxicity (such as mutagenicity, carcinogenicity, etc.) could be produced for ligands under investigation for use in autism.

#### Examine functional consequences of genetic variants

Currently, there are no established methods to characterize the involved proteins and study the functional consequence of ASD-associated genetic variants, including disturbed transcription factor binding. Typically, genetic variants are thought to either directly affect the function of the gene, or affect *cis*-regulatory elements of genes that are in close proximity. However, it was also shown that due to the three-dimensional organization of the genome, enhancers can interact with far distanced promoters (Sanyal et al. 2012). Therefore, it is crucial to integrate information on the three-dimensional organization of the genome into the functional analysis of genetic variants associated with ASD. This requires a powerful combination of genomics and proteomics technologies, such as unbiased, high-throughput compatible, quantitative mass-spectrometry-based proteomics. This will allow determining the composition and stoichiometry of bound protein complexes and yielding valuable information about altered molecular mechanisms.

#### Examine cellular and neuronal mechanisms associated with autism risk gene variants

A promising technique is to use genetically manipulated rodent primary neurons and neurons derived from human induced pluripotent stem cells (iPSC) to get insight into the mechanisms of disease associated with mutations that are also associated with ASD. By directing iPSCs into disease-relevant cell types, i.e., neurons, one will be able conducting biopsy-like experiments on living tissue from patients with ASD, with the added capacity to study the initial development and progression of pathology. Manipulation of rodent primary neurons is widely used for the functional study of synaptic proteins and has revealed important insight about the synaptic role of selected ASD-associated proteins. The use of human-derived neurons obtained via iPSC (iNeurons) was only recently established and has high promise for studying the effects of human mutations in the appropriate genetic context and in physiologically relevant cells (Marchetto et al. 2010). This has even been extended to the *in vitro* development of a three-dimensional organoid culture system derived from iPSCs. This system includes various discrete brain regions such as the cerebral cortex with different mature cortical neuron subtypes (Lancaster et al. 2013). This setup can be used for high-throughput screening of small-molecule modifiers of cellular and synaptic properties of “autism” neurons.

In this context, it is also important to examine the function of autism genes by measuring synaptic properties and neuronal network properties (long and short range) under controlled and standardized conditions *in vitro*. The global neuronal network firing behavior of primary cultures of rodent cortical neurons can be examined on multi-compartment multi-electrode arrays. These studies on development and synaptic properties of neurons will allow searching for cellular pathways that might explain abnormal brain function.

Finally, the neuronal consequences of specific gene variants in genes *in vivo* can be evaluated using rodent models. See Kas et al. (same issue, 2013, *in press*) for a review of utility, validity, and promise of animal models of autism.

- a. Mouse models. Large collection of tailor-made genetically modified mice available that carry (conditional) mutations in previously identified autism genes. These autism mouse models should be tested for cognitive functions for example by using touch-screen assays (Nithianantharajah et al. 2013). In addition, neuroimaging protocols can be used to test the hypothesis that autism symptoms can be attributed to disrupted connectivity between different regions of the brain in rodents (matching the human data sets).
- b. Rat models. In comparison to mice, the behavioral repertoire of rats is more extensive, particularly in the social domain, which represents one of the core phenotypes of autism. Genetic defects associated with such specific autism patterns may therefore be modeled in rats in which these genetic defects can be introduced.

#### Translation and validation

Once the biological and functional impact of genetic variants has been established, the relevance of these findings need to be translated and functionally validated in patients with ASD in cognitive and neuroimaging paradigms. Just a couple of genes (*NRXN1*, *CNTNAP2*, *OXTR*, *AVPR1A*) have been studied in imaging genetics paradigms and shown to be associated with alterations in circuits that mediate socio-emotional, visuo-spatial, and language processing in ASD (Ameis and Szatmari 2012). This asks for a systematic examination of cognitive and neural effects of ASD genes in large clinical cohorts and databases that include MRI and cognition data. Also large-scale international collaborations within for example the ENIGMA (enhancing neuroimaging genetics through meta-analysis) consortium ([enigma.ionu.ucla.edu](http://enigma.ionu.ucla.edu)).

Although these various steps of the translational pipeline have been described as to be taken sequentially (see also Fig. 2), in practice many steps will occur in parallel. If any, the biological plausibility of the biological mechanisms linked to the gene variant together with successful functional validation appear to be as critical issues as to decide upon further

intensification of the translational process and investments of budget and manpower.

Thus far, for not any mental disorder the translational pipeline has closed successfully the loop from a genetic/genomics starting point up to a convincing positive clinical phase III trial or even brought a new medicine to the market. Many studies however are under way, see Harrison (2013) for examples in schizophrenia. Ultimately clinically successful cases can be found in internal medicine where belimumab was recently approved by the FDA as a new medicine for lupus erythematoses (SLE). The story began in 1996, when a gene was found that was structurally homologous to tumor necrosis factor alpha (*TNF $\alpha$* ), a key proinflammatory cytokine. Subsequently, the gene's product was identified and found to induce B cell proliferation (Moore et al. 1999). Accordingly, the gene and its resultant protein were named B lymphocyte stimulator (*BLYS*). Then, belimumab, a human antibody that binds to BLYS and thereby prevents it from activating various receptors and slows B cell proliferation, was discovered. There were, in retrospect, several critical factors in the further development of the compound. The first was the extreme sensitivity of the assay to even weak activities. The second was the development of mouse models of SLE that had Blys levels tenfold above normal, and the demonstration that inhibition of *Blys* reduced disease symptoms and improved survival in the mouse models. The third was the observation that BLYS levels in patients with SLE changed over time along with the waxing and waning of disease activity. The fourth was the creation of a new clinical end point that better reflected the complex clinical reality of SLE as a multi-system disease (Furie et al. 2009). Finally, it ended with a positive phase III trial (Navarra et al. 2011).

## Conclusion

The current article provides a review of identified genetic variants associated with autism, which have already generated or are likely to generate important new leads for the development of novel pharmaceutical compounds based on the biological knowledge directly derived from these genetic findings. Consequently, this review has focused on clinical trials of which the underlying rationale is based on biological knowledge derived from the observation of genes associated to autism. This does not preclude the potential of other drug intervention trials that were not reviewed in this paper because their rationale was not so much based on a pharmacogenetic strategy.

To uphold the relevance of genetic findings to drug development in autism, we reviewed several autism risk genes converging in a limited number of genetic pathways providing novel insights in the underlying biology. For instance, variation in *TSC1* and *TSC2*, as well as in *PTEN* and *NFI*, can lead

to insufficient inhibition of the mTOR pathway. This biological insight has been a crucial for the rationale of testing therapeutic benefits of an mTOR inhibitor, rapamycin. Similarly, evidence suggests that functional variation in *SHANK3*, *SHANK1*, *SHANK2*, *NRXN1*, *NLGN3*, *NLGN4*, and potentially other autism-related genes can disrupt the biological process of post-synaptic scaffolding, providing a logical argument to test compounds that can enhance synaptogenesis.

Taken together, these observations form a compelling argument that the identification of genetic risk variants and their study to enhance the insight into the neurobiological mechanisms underlying autism should remain an important priority of the field. Currently, the etiology underlying the autisms is still unknown in most patients. However, instances where investigators have been successful at elucidating the underlying genetic cause and how this translates into abnormal neurodevelopment have reached in relatively few years the stage of experimental treatments. While it is plausible that only some of these treatments will eventually reach the clinic, they represent paradigms of personalized molecular therapy: its foundations are deep-rooted in human genetics uncovering the disease gene or CNV, its functional underpinnings grow out of genetically engineered cellular and animal models, and its preliminary support comes from pharmacological studies employing these animal models. This step-wise approach holds promise to change radically in these coming years the clinical approach to neurodevelopmental disorders.

Given the diversity of pathways involved in autistic phenotypes on the one hand and the costs of drug discovery and development on the other hand, translating knowledge of disrupted gene pathways into personalized molecular therapies is not just a question of feasibility but essentially also a matter of major investments and risk. It therefore poses a key challenge in terms of a search for the most common setting in which a specific target is at the cross-road of various upstream affected gene products that are identified in linkage studies. That specific target needs then to be “druggable,” i.e., a possible major challenge in itself. Nevertheless, that scenario is conceivably less impossible than we may think at first glance. As outlined above, many different affected genes converge on a relatively limited number of biological mechanisms, which in theory could normalize, correct, or stabilize various functional pathways downstream.

Advances have been made in recent years in conditions where autism is caused by a known genetic defect, such as Rett Syndrome and Fragile X. These two disorders, although evidence is not yet fully conclusive, are at least to be considered as promising illustrations of how a pharmacogenetic strategy can be fruitfully explored. Both are examples of a baseline shift of synaptic activity, but in opposite directions, and as such, they may represent two etiological models underlying the autistic phenotype. Their different neuronal



activity has direct impact on synapse formation and synaptic density. FMRP is required for suppression of synapse number and in the absence of FMRP such as in Fragile X, an excess of (immature) synapses are found on neurons. In contrast, animal models of Rett syndrome (*Mecp2* KO mice) have altered synaptic transmission and plasticity, and in particular, the loss of glutamatergic synapses is thought to explain their anomalies, i.e., an absence of spines. Nonetheless, changes in synaptic spine density, morphology, and function in these animal models are complex and specific to particular brain regions. Aberrant formation of dendritic spines is correlated with increased (Fragile X) or decreased (Rett) quantity of excitatory (glutamatergic) signaling in the brain. The observation both increase as well as decrease in spine size and/or density can result in an abnormal brain function, which highlights the importance of maintaining an appropriate balance between excitation and inhibition (E/I). Assuming that most autism patients are somewhere on the spectrum of a dysfunctional E/I balance, one could speculate that targeting mechanisms restoring this balance may be an appealing treatment strategy that could benefit to the majority of ASD patients with a limited number of drug approaches. When considering spine density, it should be noted that the quality of synaptic transmission can be altered by factors such as glutamatergic modulators or spine morphology, whereas quantity is altered through differences in the E/I balance.

Speeding up drug discovery through genetics/genomics strategies requires the continuation and even intensification of gene discovery within large scale international collaborations such as the Psychiatric GWAS consortium ([pgc.unc.edu](http://pgc.unc.edu)) and the Autism Genome Project. This work should include GWAS studies and exome and whole genome sequencing studies to identify rare variants, as well as epigenetic genome-wide profiling. Other conditions that should be optimized are the annotation of the genome and the availability of post-mortem brain tissue of patients with autism. Expanding and integrating analyses to multiple levels of biological information in addition to genomics (methylomics, transcriptomics, proteomics, and metabolomics) will significantly enhance the probabilities of successfully characterizing “responders” from “non-responders,” as no single drug can conceivably be beneficial to all autistic patients. Finally, assuming that effective compounds for the treatment of autism will become available in the near future, a parallel issue that needs to be addressed is the challenge of clinical drug development, or rather, the *absence* of any experience in this respect, for autism. Unlike depression or schizophrenia, to date there are no drugs on the market that treat core symptoms of autism, implying that clinical drug development in this field will need to be defined entirely from the start in the next few years. A fundamental requirement to this end is an autism network to provide a platform for professionals—clinicians and researchers—to facilitate and enhance scientific collaboration

and facilitate consensus on various essential principles such as translational, clinical and regulatory end-points, etc. Currently, such a network does not exist in Europe, nor any structure that could serve as the basis for one. To address this need, a consortium was created within the context of the innovative medicines initiative of the European Union. This consortium (EU-AIMS) has defined goals to build on recent advances to further align and support the field as well as to build a unified platform for both pre-clinical and clinical researchers. Therefore, the primary aims of this consortium are (1) to increase the understanding of the underlying neurobiology of autism, including the identification of potential new drug targets, (2) to set new standards in research and clinical development to aid the drug discovery process, and (3) to identify and develop expert clinical sites across Europe to run clinical studies and trials and so create an European Network for Research in Autism that provides an interactive platform for autism professionals (Murphy and Spooren 2012).

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