Published in final edited form as:
*Per Med*. 2008 November ; 5(6): 599–607. doi:10.2217/17410541.5.6.599.

# Pharmacology and genetics of autism: implications for diagnosis and treatment

**Zoran Brkanac**[1,†], **Wendy H Raskind**[1,2], and **Bryan H King**[1]

[1]Department of Psychiatry and Behavioral Sciences and Seattle Children's Hospital, Box 35-6560, University of Washington, Seattle, WA 98195-6560, USA

[2]Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA 98195, USA

## Abstract

Autism has the highest estimated heritability (>90%) among behaviorally defined neuropsychiatric disorders. Rapidly advancing genomic technologies and large international collaborations have increased our understanding of the molecular genetic causes of autism. Pharmacogenomic approaches are currently being applied in two single-gene disorders, fragile X syndrome and Rett syndrome, which capture many aspects of the autistic phenotype. This review describes the current state of the genetics of autism and suggests how to extend pharmacological principles pioneered in fragile X and Rett to the broader group of patients with autism.

### Keywords

autism; disease-modifying treatments; fragile X; genetics; molecular pathways; Rett syndrome

Autistic disorder is the prototypical neurodevelopmental disorder from the group of pervasive developmental disorders (PDD). This group of clinical syndromes includes autistic disorder, Asperger's disorder, PDD not otherwise specified (PDD NOS), childhood disintegrative disorder and Rett disorder. All PDDs have childhood age of onset and are characterized by varied degrees of impairments in communication, social interactions, and restricted repetitive and stereotyped patterns of behavior, interest and activities [1]. These impairments are frequently accompanied by cognitive–intellectual deficits. Within the PDD group, disorders are differentiated either by difference in impairment in one of the domains or on the basis of a distinctive developmental course. Autistic disorder requires impairments in all three domains: communication, social interaction and repetitiveness. Asperger's disorder is distinguished by the lack of communication impairments and better-preserved intellectual functioning. PDD NOS is diagnosed in cases when diagnostic criteria for autistic or Asperger's disorders are not met. We will refer to these three disorders as autism spectrum disorders (ASD).

The current gold standard for diagnosing ASD is based on clinical history and patient observation performed by trained clinicians with the use of structured diagnostic tools such as the Autism Diagnostic Interview – revised (ADI-R) [2] and the Autism Diagnostic Observation Schedule (ADOS) [3]. For childhood disintegrative disorder and Rett disorder, the hallmark of diagnosis is disruption of apparently normal development. In Rett syndrome this



†Author for correspondence: Tel.: +1 206 543 9573, Fax: +1 206 616 7366, zbrkanac@u.washington.edu.

developmental disruption is now being simultaneously broadened and characterized in detail since the discovery of causal mutations in *MECP2* Owing to variation in diagnostic criteria for autism and differences in their implementation and in study designs, epidemiological estimates of the prevalence of ASD range from 2.5 to 72.6 in 10,000 individuals, with a best estimate of 60 in 10,000 individuals [4]. It is also possible that ASD is becoming more common.

Current pharmacological approaches for the treatment of autism are based on pharmaceuticals whose effectiveness for ameliorating behavioral symptoms with a high impact on individual functioning is inferred from experience with other disorders. Examples are schizophrenia and attention deficit hyper activity disorder (ADHD), where pharmaceutical interventions can reduce symptoms such as aggression, irritability and hyperactivity. In order to assess safety and efficacy of pharmacological interventions for behavioral targets in autism, a systematic evaluation of selected agents was initiated through the National Institute for Mental Health and Research Units on Pediatric Psychopharmacology [5]. As a result of these studies, risperidone became the first drug to receive US FDA approval for use in treatment of an autism-associated behavior: irritability [6,7]. Effectiveness of methyl phenidate for children with autism and ADHD symptoms has also been confirmed [8,9]. These large, multicenter studies demonstrated the feasibility and importance of conducting placebo-controlled pharmacological studies in autism and have substantially added to the evidence base. Pharmacological and behavioral strategies in the treatment of autism have recently been reviewed elsewhere [10,11]. In this review, we will first describe the current understanding of the genetics of autism. Next, we will review the progress in genetic and pharmacological approaches that are being pioneered in Rett syndrome and fragile X (fraX), other diseases with known genetic bases and high incidence of autism, and draw parallels with ASD with respect to treatment approaches.

## Genetics of autism

Autism is considered to be among the most heritable neuropsychiatric disorders. Twin studies in epidemiologically based samples have found a higher concordance for monozygotic twins (69–95%) than for dizygotic twins (0–24%) and estimated a heritability of greater than 90% for autism [12–14]. In these studies, the concordance rates were higher when ASD was considered in addition to the strict diagnosis of autism. Despite the high heritability estimates, identification of the genes responsible for the disorders remains elusive. Results of family studies used to elucidate the mechanism of transmission have not been consistent with single-gene models. One of the challenges in assessing the models of transmission has been the substantial variability in the phenotypic expression.

Observed patterns of transmission have led to an oligogenic, common-disease common-variant (CDCV) hypothesis, that assumes that common variations in multiple genes contribute in an interactive manner to confer autism susceptibility [15,16]. The estimate of the number of contributing genes varied depending on the study and analytical assumptions made. A study of twins and families using a latent recurrence-risk model concluded that three epistatic loci (range: 2–10 loci) of equal effects provide the best fit to the data [17]. An analysis of sibpairs estimated the number of the genes involved to be 15 or more based on observed allele sharing between concordant and discordant siblings [16,18]. Use of the same data set and Monte Carlo simulation of the multilocus model concluded that a model with 100 loci best fits the data [16]. Although the estimates of the number of genes involved varied, the oligogenic hypothesis was the basis for sample ascertainment and analysis for most genetic linkage studies.

An alternative to the CDCV hypothesis is the proposal that genetic susceptibility is conferred by rare deleterious mutations in any of a large number of genes [16]. In this scenario, the observed phenotypic variability of autism could be due to mutations in a multiplicity of genes. Phenotypic variation within families could be a consequence of reduced penetrance of some

of the genes involved. In addition, a substantial number of cases could result from *de novo* events. *De novo* events are known to be a common cause of large cytogenetic abnormalities, and recently smaller pathogenic microdeletions, micro duplications and mutations have been found in a number of cases, supporting the role for multiple rare variants.

## Linkage studies

Multiple genome-wide linkage studies have been performed using a variety of ascertainment and analytical strategies. However, interpretation of the results is complicated by the panoply of locations throughout the genome for which evidence in favor of linkage to an autism phenotype has been reported [19–22]. In general, there has not been a high level of agreement between studies, but several regions, including ones on chromosomes 2q, 7q and 17q, have provided suggestive evidence for linkage in multiple studies. The lack of replication in genome-wide studies is probably a consequence of several factors, including high levels of genetic locus and allele heterogeneity, small effect size attributable to any particular gene, lack of diagnostic specificity and, as recently demonstrated, presence of *de-novo* alterations, such as copy-number variations (CNVs), even in familial samples. These factors can all adversely affect the power of even large studies to detect linkage. Nevertheless, fine-mapping studies to corroborate and narrow the identified regions are underway and candidate genes in the regions are being evaluated, both for sequence changes and with further genomic and functional analyses. Recently, the first study designed to detect recessive mutations through homozygosity mapping in consanguineous pedigrees has been published [23]. Consistent with the *a priori* assumption of high levels of genetic heterogeneity, positive evidence for linkage was found in non-overlapping loci in 12 out of 78 consanguineous pedigrees (15.4%). For linkage regions in five of these families (6.4%), large inherited homozygous deletions that are likely to be causative disruptions were identified, further supporting the strength of linkage studies for gene identification.

## Chromosomal alterations

Structural chromosomal alterations, such as rearrangements, duplications and deletions, have been identified in 3–5% of autism subjects. The most frequent changes are duplications of 15q11–13 and deletions of 2q37, 7q31, 22q11.2 and 22q13.3 [22,24]. Application of novel array-based genotyping technologies has resulted in renewed interest in the role of genomic alterations in the etiology of ASD. In a sample of 1469 ASD families, collected through the Autism Genome Project Consortium and genotyped using the Affymetrix (CA, USA) 10K v2 SNP array, possible detrimental copy-number abnormalities (CNA) were shared by the affected individuals in 7.6–11.5% of families, depending on the analytical approach [25]. Using a form of comparative genomic hybridization (CGH) in a sample of 195 individuals from families with single and multiple cases of autism, mostly derived from the Autism Genetic Resource Exchange (AGRE), *de novo* CNAs were observed in 14 out of 195 individuals (~7.2%) [26]. Most of these CNAs were unique to the observed families.

The most recently published studies have focused on the discovery of recurrent *de novo* variants. By CGH a recurrent 500 kb 16p11.2 microdeletion was identified in two of 180 autism probands from AGRE [27,28]. In an additional sample of 532 probands, two more deletions were found, for a combined frequency of 0.6%. In a larger study the same recurrent micro deletion was then identified in five of 1441 AGRE case subjects, five of 515 children from a clinical sample with developmental delay or ASD, and three of 299 ASD subjects from Iceland, for a combined frequency of 0.57% in ASD [29]. The same study identified a reciprocal duplication of the 16p11.2 region in a statistically higher frequency in AGRE and Icelandic case samples than in control samples. Another large study of 427 ASD families has confirmed the relatively high frequency of approximately 1% of 16p11.2 deletion/duplication in autism,

and has found that approximately 7% of randomly selected idiopathic cases harbor *de novo* genomic CNA rearrangements [30].

### Sequencing candidate genes

In addition to fraX and Rett syndromes, the direct sequencing approach has identified several other strong single gene candidates for autism. Neuroligin 4 (*NLGN4* OMIM#300427) is located on chromosome Xp22.33 in a region where deletions were found in several patients with autism [31]. Frameshift mutations in *NLGN4* were found in two Swedish ASD siblings [32] and in ten affected male family members (three with ASD) from a French family with an X-linked mental retardation [33]. Additionally, four putative missense mutations were identified in Portuguese and US ASD patients [34], and the deletion of exons 4, 5 and 6 was found in a proband with autism, tic disorder and mental retardation, his brother with mild cognitive deficits and Tourette syndrome and their mother with learning disability as well as depression and anxiety [35]. *NLGN4* homologs, *NLGN3* (OMIM#300336) and *NLGN4Y* (OMIM#400028) have also been screened and missense mutations in these genes have been associated with autism [32,36]. Two other genes from the neuroligin pathway have been evaluated. Neurexin 1 (*NRXN1* OMIM #600565) was found to have putative missense variants [37,38], hemizygous deletions [25] and gene disruptions due to translocations [37] in patients with autism. Mutation and microdeletion screening of the *SHANK3* (OMIM #606230) gene identified seven families in which *SHANK3* alterations were associated with ASD [39,40]. The *PTEN* gene (OMIM #601728) that causes a family of rare related autosomal dominant hamartoma-tumor syndromes is also implicated in autism. *PTEN* deletions and point mutations have been described in nine probands with autism and pronounced macrocephaly [41–44]. In addition, a truncating mutation in *CNTNAP2* was found to cause an autosomal recessive cortical dysplasia – focal epilepsy syndrome accompanied with mental retardation, ADHD and autism [45]. Subsequent analyses have found a set of common and rare *CNTNAP2* variants that are associated with ASD [46–48].

## Fragile X syndrome

Fragile X (OMIM #300624) is the most common genetic cause of intellectual disability, and the prevalence of autism in fraX ranges from 15 to 35%, mostly depending on the method used for diagnostic evaluation [49]. Conversely, fraX is currently one of the most common identified causes of autism, accounting for 2% of cases [50]. In addition to disturbances in communication, social interaction and stereotypic behaviors that are characteristic of autism, common behaviors observed in fraX include attention disturbances, hyperactivity, anxiety, sensory hypersensitivity and depression. Individuals with fraX also have a specific cognitive profile with weaknesses in attention/executive function and visual–spatial skills, and strengths in verbal skills [51]. FraX is caused by an expansion of an untranslated CGG repeat tract that inactivates transcription of fraX mental retardation 1 gene (*FMR1* OMIM#309550), and as a consequence no fraX mental retardation protein (FMRP) is made. *FMRP* is an RNA binding protein that is involved in regulation of protein translation. The assumed mechanism of disease is an increase in translation of proteins that are normally down-regulated by FMRP's repressor activity [52]. Expansion of the triple nucleotide repeat is the main cause of the disorder. Other mutations in the gene such as deletions and point mutations have been described [53]; however, their relative contributions to disease in this population have not been systematically evaluated.

Based on the observation of dendritic spine abnormalities, altered synaptic plasticity and increased cortical excitability in *Fmr1* knockout (KO) mice, it was proposed that overactive metabo tropic glutamate receptor signaling contributes to some of the brain structural changes and neurological symptoms of fraX [54]. A mouse model that combines *Fmr1* inactivation with a 50% reduction in metabotropic glutamate receptor 5 (*GRM5;* OMIM#604102) expression confirmed this hypothesis, as it showed that decreased activity of the glutamate

pathway rescues several anatomic and behavioral consequences of fraX [55]. In a similar mouse model, after it was observed that inhibition of p21-activated kinase results in synaptic features that are the opposite of *Fmr1* KO mice, it was found that inhibition of p21-activated kinase rescues the cellular and behavioral symptoms of *Fmr1* KO mice [56]. Genome-wide expression analysis of fraX lymphoblastoid cell lines identified genes with changed expression levels, and these genes may reveal additional cellular pathways that are disturbed in fraX [57]. Results from genetic approaches that implicated glutamate have been validated with pharmacological approaches to reduce GRM5 activity in fraX. The GRM5 antagonist 2-methyl-6-(phenylethyl)-pyridine (MPEP) was shown to rescue neuronal morphology, learning and courtship behavior deficits in drosophila [58], and in a mouse model of fraX, MPEP suppressed audiogenic seizures and normalized open field test performance [59]. These findings implicating glutamate in animal models are currently being extended to clinical trials of GRM5 antagonists. The finding of altered neuronal excitability has sparked an interest in inhibitory pathways and the role of the GABA(A) receptor [60], that has led to investigations of GABA agonists in the treatment of fraX. Other observations in mouse models of fraX and in fraX patients, such as reduced α-amino-3-hydroxy-5-methyl-4-isox-azolepropionic acid receptors [61] and deregulated hypothalamic–pituitary–adrenal axis [62], stimulated investigations of the usefulness of ampakine activators and cortisol antagonists in amelioration of the phenotype.

## Rett syndrome

In 1999, mutations in the *MECP2* gene were identified as the cause of Rett syndrome (OMIM #312750) [63]. The encoded protein is widely expressed in human tissues, selectively binds to methylated DNA and mediates transcriptional repression. Rett syndrome was initially described in girls only, and most of the cases had a strictly defined core phenotype. It was thought to be an X-linked dominant disorder that was a prenatal lethal trait in males. Since the discovery of the gene and implementation of molecular methods for diagnosis, the phenotypic expression of Rett syndrome has been extended to males and broadened in females. The spectrum of severity in females includes cases with intrauterine developmental aberrations, individuals without initial normal development, ‘classic’ Rett syndrome, individuals with late loss of developmental skills, individuals who have preserved speech, individuals with autistic spectrum phenotypes , and apparently normal female carriers [64]. The phenotypic spectrum in males is also wide, ranging from prenatal mortality, to severe neonatal encephalopathy and severe to mild nonspecific mental retardation [65]. In addition, increased *MECP2* gene copy-number is a cause of neurodevelopmental delay in males [66,67]. The addition of genetic testing to clinical evaluations of patients with Rett syndrome phenotype identified patients who do not have detectable changes in the *MECP2* gene. Subsequently, mutations in additional genes that cause Rett syndrome variants were found. Mutations in *CDKL5* (OMIM#300203) are responsible for the severe early-onset seizure variant [68–70], and mutations in *FOXG1* (OMIM#164874) were recently identified in a congenital variant [71]. The wide range of symptom severity in both males and females is attributed to allelic heterogeneity of mutations in *MECP2*, as well as to random versus nonrandom X-chromosome inactivation patterns in females. The sequence alterations include nonsense and missense mutations, microdeletions, and micro-duplications [72]. It appears that mutations that are predicted to affect protein function more severely, such as those that lead to inability of the protein to bind to DNA, cause more severe phenotypes. Screening of males with mental retardation and patients with autism has identified a small number of cases with missense mutations [73,74].

Animal models have contributed to our understanding of the role of *MECP2* in CNS development. The highest level of *MECP2* expression is in mature neurons. Loss of MECP2 protein leads to decreased size of neuronal cell bodies and reduction in dendritic spines numbers and arborization. The phenotypic sequel is microcephaly without gross brain abnormalities. A broad search for targets of MECP2 transcriptional repression inactivation did not find a large

number of genes that are consistently abnormally expressed [75]. Targeted studies have found that several CNS proteins and neurotransmitters with known importance for neuronal function are regulated by MECP2. They include neurotransmitters, glucocorticoid-regulated genes and brain-derived neurotropic growth factor (*BDNF*). Mouse models with partial deletion of the *Mecp2* gene manifest a phenotype that closely resembles Rett syndrome, and offer an excellent tool for further studies of pathophysiology and potential treatments. It was demonstrated that postnatal activation of *Mecp2* reversed the neurological and behavioral defects [76,77]. Since BDNF is regulated by MECP2, it offers another avenue for treatment of the Rett phenotype. In studies of Mecp2-mutant mice crossed with *Bdnf* KO or overexpressing mice, the Mecp2-mutant phenotype was worsened by *Bdnf* deficiency and ameliorated by Bdnf increase [78]. This finding underscores the feasibility of developing therapies aimed at preventing or reversing abnormalities caused by MECP2 deficiency. Based on the observation that glutamate receptors are increased in the brains of young Rett syndrome patients, clinical trials of dextromethorphan, an NMDA glutamate receptor blocker, are currently underway.

## Molecular pathways

New discoveries in the genetics of autism are enabling different approaches to diagnosis and treatment. The complexity of ASD behavioral and biological phenotypes has frustrated research into its etiology and many causes have been postulated. The current understanding of monogenic diseases such as fraX, Rett syndrome, and the subset of ASDs caused by mutations in neuroligins, *NRXN1, SHANK3, PTEN* and *CNTNAP2* supports a notion of high biological complexity, even for the single-gene subtypes of ASD. However, some monogenic forms of autism could involve the same molecular pathways and thus respond to the same treatment approaches. One possible pathway might include *FMR1*, neuroligins, *NRXN1* and *SHANK3*. In fraX the absence of the FMRP results in excessive neuronal protein synthesis and affects neuronal network performances mediated through metabotropic glutamate receptors. It is proposed that in autism neuronal networks are disturbed, and some networks might represent a common pathway for larger subsets of affected individuals. A common pathway might converge through metabotropic glutamate signaling. Rare mutations in neuroligins, *NRXN1* and *SHANK3* are responsible for some cases of ASD. Neurexins are presynaptic ligands of the neuroligins and together they play a role in excitatory glutamatergic and inhibitory GABAergic synapses [79,80]. SHANK3 binds to neuroligins [81] and thus might be involved in glutamatergic synapse formation. Normalizing the disruption of excitatory and inhibitory networks through pharmacological modulation of glutamatergic signaling might be beneficial, not only in fraX as currently demonstrated in animal models, but also in genetically defined subtypes of autism, such as the ones with disruptions in neuroligins, *NRXN1* or *SHANK3*, which might be affecting glutamatergic signaling. Pharmacological studies in animal models of neuroligin, *NRXN1* or *SHANK3* subtypes of autism could confirm such hypotheses.

## Future perspective & conclusion

The emerging understanding of the role of genetics in ASD will undoubtedly affect diagnostic and treatment approaches. Until recently autism was associated with a recognized cause in at most 10% of individuals, most commonly those with microscopically visible chromosomal abnormalities or clearly defined syndromes such as fraX. The application of novel genomic technologies is increasingly revealing additional single-gene and submicroscopic genomic disorders, including chromosomal rearrangements, microdeletions and microduplications. As higher resolution genome scanning methods and novel sequencing technologies become more widely available, detection of novel genetic alterations with large effects on the phenotype will only increase. The discovery of novel rare variants presents challenges in regards to proving that the variant is causative to the disease. In order to strengthen the confidence in the findings, and obtain statistically significant results, evaluation of large cohorts of cases and controls will

be needed. As some of the variants are associated not just with strictly defined autism but also with autism spectrum behaviors, detailed phenotypic evaluation of cases and controls will be necessary. If the variant is very rare, even large samples might not be sufficient to demonstrate statistical significance of the findings. Another way to strengthen the case for the causality of the variant is through family studies. Family studies offer the opportunity to evaluate the segregation patterns and phenotypic effects of the variant related to gender differences and developmental stage, as was recently shown for a mutation in *NLGN4* [35]. In this way, our understanding of genotype effects on autism spectrum phenotypes and other psychiatric and behavioral phenotypes will be enhanced. As the fraction of ASD for which a specific etiology can be identified increases, one can envision that molecular screening may become part of the newborn screening panel in the not-so-distant future. Recent work on animal models of fraX and Rett syndromes indicates that some of the manifestations of the disease can be prevented or reversed, emphasizing the need for early diagnosis. The level of heterogeneity of autism identified to date implies that individual subtypes will be rare. Therefore, collaborations through treatment and evaluation center networks will be necessary in order to ascertain sufficient numbers of cases with the same genetic etiology.

Early screening for autism through structured behavioral observation is becoming part of standard clinical practice. Only a subset of patients is currently evaluated by medical geneticists who can coordinate the appropriate cytogenetic and molecular genetic tests based on clinical indications. With identification of an increasing number of genes that are involved in autism, molecular genetic evaluation will become increasingly more important as a standard screening tool together with behavioral assessment. The rapid pace of improvements in mutation detection, coupled with encouraging results for possible treatments makes that goal feasible and justifiable. As screening increases, both in research laboratory and clinical settings, it is equally important to delineate the impact of mutations on the phenotype. As a general rule, the initial description of a disorder is biased by clinical ascertainment of the most severe cases [82]. The phenotypic spectrum for disease associated with any given gene may broaden considerably as additional patients are identified through more widely available molecular testing. Identification of genetic subtypes will also facilitate development of biomarkers that may be useful for evaluation of disease progression and effectiveness of treatments. As exemplified by Rett syndrome, for ASD there is no discernible phenotype in the first year of life. Presymptomatic molecular diagnosis can offer an opportunity to initiate treatment early to lessen the eventual severity or delay or prevent disease onset, as was recently demonstrated with animal models for Rett syndrome. Similar strategies could be envisioned for other genetic subtypes of autism.

**Executive summary**

### Genetics of autism

- There is strong evidence for a genetic basis of autism, with inherited and *de novo* genetic causes.
- Linkage studies have shown a high level of disagreement but have identified several regions with evidence for linkage in multiple samples.
- Cytogenetically visible chromosomal alterations are identified in 3–5% of subjects with autism.
- Microdeletions/microduplications as defined by current technology are increasingly recognized as causal approximately an additional 10% of cases.
- Candidate genes with high penetrance have been identified – neuroligins, *NRXN1*, *SHANK3*, *PTEN* and *CNTNAP2*.

### Fragile X & Rett syndromes

- Fragile X (fraX) and Rett syndromes capture many aspects of the autism spectrum disorders (ASD) phenotype and represent excellent models for the disease.
- Animal models of fraX and Rett syndromes have demonstrated that postnatal interventions to prevent development of disease phenotype are possible.
- Pharmacological trials in fraX and Rett patients with therapies that directly target the genetically-induced physiological deficits are underway.

### Molecular pathways & future perspective

- Disruption of glutamatergic signaling as demonstrated in fraX, might also be contributing to the phenotype in a subset of persons with autism and mutations in neuroligins, *NRXN1* or *SHANK3*.
- Molecular genetic diagnostic characterization will become an integral part of clinical evaluation and consequent therapeutic trials of ASD.

## Acknowledgments

**Financial & competing interests disclosure**

BH King is a consultant and has received research support from Neuropharm, Seaside Therapeutics, Biomarin, Autism Speaks, and the National Institutes of Health. Z Brkanac and WH Raskind have received research support from the National Institutes of Health. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

## Bibliography

Papers of special note have been highlighted as:

▪ of interest

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Washington DC, USA: American Psychiatric Press; 1994.
2. Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview – Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J. Autism Dev. Disord 1994;24:659–685. [PubMed: 7814313]
3. Lord C, Risi S, Lambrecht L, et al. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J. Autism Dev. Disord 2000;30:205–223. [PubMed: 11055457]
4. Fombonne, E. Epidemiological studies of pervasive developmental disorders. In: Volkmar, F.; Paul, R.; Klin, A.; Cohen, D., editors. Handbook of Autism and Pervasive Developmental Disorders. Hoboken, NJ, USA: Wiley; 2005. p. 42-69.
5. King BH, Bostic JQ. An update on pharmacologic treatments for autism spectrum disorders. Child Adolesc. Psychiatr. Clin. N. Am 2006;15:161–175. [PubMed: 16321729]
6. McCracken JT, McGough J, Shah B, et al. Risperidone in children with autism and serious behavioral problems. N. Engl. J. Med 2002;347:314–321. [PubMed: 12151468]
7. Aman MG, Hollway JA, McDougle CJ, et al. Cognitive effects of risperidone in children with autism and irritable behavior. J. Child Adolesc. Psychopharmacol 2008;18:227–236. [PubMed: 18582177]
8. Aman MG, Arnold LE, Ramadan Y, et al. Randomized, controlled, crossover trial of methylphenidate in pervasive developmental disorders with hyperactivity. Arch. Gen. Psychiatry 2005;62:1266–1274. [PubMed: 16275814]

9. Posey DJ, Aman MG, McCracken JT, et al. Positive effects of methylphenidate on inattention and hyperactivity in pervasive developmental disorders: an analysis of secondary measures. Biol. Psychiatry 2007;61:538–544. [PubMed: 17276750]
10. Rogers SJ, Vismara LA. Evidence-based comprehensive treatments for early autism. J. Clin. Child Adolesc. Psychol 2008;37:8–38. [PubMed: 18444052]
11. Parikh MS, Kolevzon A, Hollander E. Psychopharmacology of aggression in children and adolescents with autism: a critical review of efficacy and tolerability. J. Child Adolesc. Psychopharmacol 2008;18:157–178. [PubMed: 18439113]
12. Bailey A, Le Couteur A, Gottesman I, et al. Autism as a strongly genetic disorder: evidence from a British twin study. Psychol. Med 1995;25:63–77. [PubMed: 7792363]
13. Folstein S, Rutter M. Infantile autism: a genetic study of 21 twin pairs. J. Child Psychol. Psychiatry 1977;18:297–321.321 [PubMed: 562353] First twin study establishing evidence for genetic etiology and high heritability.
14. Steffenburg S, Gillberg C, Hellgren L, et al. A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. J. Child Psychol. Psychiatry 1989;30:405–416. [PubMed: 2745591]
15. Pritchard J, Cox N. The allelic architecture of human disease genes: common disease – common variant… or not? Hum. Mol. Genet 2002;11:2417–2423. [PubMed: 12351577]
16. Pritchard JK. Are rare variants responsible for susceptibility to complex diseases? Am. J. Hum. Genet 2001;69:124–137. [PubMed: 11404818]
17. Pickles A, Bolton P, Macdonald H, et al. Latent-class analysis of recurrence risks for complex phenotypes with selection and measurement error: a twin and family history study of autism. Am. J. Hum. Genet 1995;57:717–726.726 [PubMed: 7668301] Infuential study of transmission patterns of autism presenting support for multiple-locus model of inheritance.
18. Risch N, Spiker D, Lotspeich L, et al. A genomic screen of autism: evidence for a multilocus etiology. Am. J. Hum. Genet 1999;65:493–507. [PubMed: 10417292]
19. Yang MS, Gill M. A review of gene linkage, association and expression studies in autism and an assessment of convergent evidence. Int. J. Dev. Neurosci 2007;25:69–85. [PubMed: 17236739]
20. Gupta AR, State MW. Recent advances in the genetics of autism. Biol. Psychiatry 2007;61:429–437. [PubMed: 16996486]
21. Veenstra-Vanderweele J, Cook EH Jr. Molecular genetics of autism spectrum disorder. Mol. Psychiatry 2004;9:819–832. [PubMed: 15197396]
22. Freitag CM. The genetics of autistic disorders and its clinical relevance: a review of the literature. Mol. Psychiatry 2007;12:2–22. [PubMed: 17033636]
23. Morrow AM, Yoo YS, Flavell SW, et al. Identifying autism loci and genes by tracing recent shared ancestry. Science 2008;321:218–223. [PubMed: 18621663]
24. Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L. Identification of novel autism candidate regions through ana lysis of reported cytogenetic abnormalities associated with autism. Mol. Psychiatry 2006;11:18–28.
25. Szatmari P, Paterson AD, Zwaigenbaum L, et al. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nat. Genet 2007;39:319–328.328 [PubMed: 17322880] First of the studies harnessing new genotyping technologies to establish the importance of copy-number variations (CNVs) in autism.
26. Sebat J, Lakshmi B, Malhotra D, et al. Strong association of *de novo* copy number mutations with autism. Science 2007;316:445–449. [PubMed: 17363630]
27. Kumar RA, KaraMohamed S, Sudi J, et al. Recurrent 16p11.2 microdeletions in autism. Hum. Mol. Genet 2008;17:628–638. [PubMed: 18156158]
28. Christian SL, Brune CW, Sudi J, et al. Novel submicroscopic chromosomal abnormalities detected in autism spectrum disorder. Biol. Psychiatry 2008;63(12):1111–1117. [PubMed: 18374305]
29. Weiss LA, Shen Y, Korn JM, et al. Association between microdeletion and microduplication at 16p11.2 and autism. N. Engl. J. Med 2008;358:667–675. [PubMed: 18184952]
30. Marshall CR, Noor A, Vincent JB, et al. Structural variation of chromosomes in autism spectrum disorder. Am. J. Hum. Genet 2008;82:477–488. [PubMed: 18252227]

31. Thomas NS, Sharp AJ, Browne CE, Skuse D, Hardie C, Dennis NR. Xp deletions associated with autism in three females. Hum. Genet 1999;104:43–48.48 [PubMed: 10071191] One of the first applications of the sequencing of the candidate gene approach to identify rare mutations in autism.
32. Jamain S, Quach H, Betancur C, et al. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nat. Genet 2003;34:27–29. [PubMed: 12669065]
33. Laumonnier F, Bonnet-Brilhault F, Gomot M, et al. X-linked mental retardation and autism are associated with a mutation in the *NLGN4* gene, a member of the neuroligin family. Am. J. Hum. Genet 2004;74:552–557. [PubMed: 14963808]
34. Yan J, Oliveira G, Coutinho A, et al. Analysis of the neuroligin 3 and 4 genes in autism and other neuropsychiatric patients. Mol. Psychiatry 2005;10:329–332. [PubMed: 15622415]
35. Lawson-Yuen A, Saldivar JS, Sommer S, Picker J. Familial deletion within *NLGN4* associated with autism and Tourette syndrome. Eur. J. Hum. Genet 2008;16:614–618. [PubMed: 18231125]
36. Yan J, Feng J, Schroer R, et al. Analysis of the neuroligin 4Y gene in patients with autism. Psychiatr. Genet 2008;18:204–207. [PubMed: 18628683]
37. Kim HG, Kishikawa S, Higgins AW, et al. Disruption of neurexin 1 associated with autism spectrum disorder. Am. J. Hum. Genet 2008;82:199–207. [PubMed: 18179900]
38. Feng J, Schroer R, Yan J, et al. High frequency of neurexin 1β signal peptide structural variants in patients with autism. Neurosci. Lett 2006;409:10–13. [PubMed: 17034946]
39. Moessner R, Marshall CR, Sutcliffe JS, et al. Contribution of *SHANK3* mutations to autism spectrum disorder. Am. J. Hum. Genet 2007;81:1289–1297. [PubMed: 17999366]
40. Durand CM, Betancur C, Boeckers TM, et al. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat. Genet 2007;39:25–27. [PubMed: 17173049]
41. Buxbaum JD, Cai G, Chaste P, et al. Mutation screening of the *PTEN* gene in patients with autism spectrum disorders and macrocephaly. Am. J. Med. Genet. B Neuropsychiatr. Genet 2007;144:484–491. [PubMed: 17427195]
42. Herman GE, Henninger N, Ratliff-Schaub K, Pastore M, Fitzgerald S, McBride KL. Genetic testing in autism: how much is enough? Genet. Med 2007;9:268–274. [PubMed: 17505203]
43. Herman GE, Butter E, Enrile B, Pastore M, Prior TW, Sommer A. Increasing knowledge of *PTEN* germline mutations: two additional patients with autism and macrocephaly. Am. J. Med. Genet. A 2007;143:589–593. [PubMed: 17286265]
44. Butler MG, Dasouki MJ, Zhou XP, et al. Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline *PTEN* tumour suppressor gene mutations. J. Med. Genet 2005;42:318–321. [PubMed: 15805158]
45. Strauss KA, Puffenberger EG, Huentelman MJ, et al. Recessive symptomatic focal epilepsy and mutant contactin-associated proteinlike 2. N. Engl. J. Med 2006;354:1370–1377. [PubMed: 16571880]
46. Alarcon M, Abrahams BS, Stone JL, et al. Linkage, association, and gene-expression analyses identify *CNTNAP2* as an autism-susceptibility gene. Am. J. Hum. Genet 2008;82:150–159. [PubMed: 18179893]
47. Arking DE, Cutler DJ, Brune CW, et al. A common genetic variant in the neurexin superfamily member *CNTNAP2* increases familial risk of autism. Am. J. Hum. Genet 2008;82:160–164. [PubMed: 18179894]
48. Bakkaloglu B, O'Roak BJ, Louvi A, et al. Molecular cytogenetic analysis and resequencing of contactin associated protein-like 2 in autism spectrum disorders. Am. J. Hum. Genet 2008;82:165–173. [PubMed: 18179895]
49. Hagerman RJ. Lessons from fragile X regarding neurobiology, autism, and neurodegeneration. J. Dev. Behav. Pediatr 2006;27:63–74. [PubMed: 16511373]
50. Cohen D, Pichard N, Tordjman S, et al. Specific genetic disorders and autism: clinical contribution towards their identification. J. Autism Dev. Disord 2005;35:103–116. [PubMed: 15796126]
51. Reiss AL, Hall SS. Fragile X syndrome: assessment and treatment implications. Child Adolesc. Psychiatr. Clin. N. Am 2007;16:663–675. [PubMed: 17562585]

52. Verkerk AJ, Pieretti M, Sutcliffe JS, et al. Identification of a gene (*FMR-1*) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 1991;65:905–914. [PubMed: 1710175]
53. Hammond LS, Macias MM, Tarleton JC, Shashidhar Pai G. Fragile X syndrome and deletions in *FMR1*: new case and review of the literature. Am. J. Med. Genet 1997;72:430–434. [PubMed: 9375726]
54. Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. Trends Neurosci 2004;27:370–377. [PubMed: 15219735]
55. Dolen G, Osterweil E, Rao BS, et al. Correction of fragile X syndrome in mice. Neuron 2007;56:955–962.962 [PubMed: 18093519] Demonstrates that in a mouse model of fragile X syndrome, reduction of glutamate mGluR5 receptor expression corrects many aspects of abnormal phenotype.
56. Hayashi ML, Rao BS, Seo JS, et al. Inhibition of p21-activated kinase rescues symptoms of fragile X syndrome in mice. Proc. Natl Acad. Sci. USA 2007;104:11489–11494. [PubMed: 17592139]
57. Bittel DC, Kibiryeva N, Butler MG. Whole genome microarray analysis of gene expression in subjects with fragile X syndrome. Genet. Med 2007;9:464–472. [PubMed: 17666893]
58. McBride SM, Choi CH, Wang Y, et al. Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a Drosophila model of fragile X syndrome. Neuron 2005;45:753–764. [PubMed: 15748850]
59. Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP. Suppression of two major fragile X syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. Neuropharmacology 2005;49:1053–1066. [PubMed: 16054174]
60. D'Hulst C, Kooy RF. The GABAA receptor: a novel target for treatment of fragile X? Trends Neurosci 2007;30:425–431. [PubMed: 17590448]
61. Li J, Pelletier MR, Perez Velazquez JL, Carlen PL. Reduced cortical synaptic plasticity and GluR1 expression associated with fragile X mental retardation protein deficiency. Mol. Cell Neurosci 2002;19:138–151. [PubMed: 11860268]
62. Hessl D, Glaser B, Dyer-Friedman J, et al. Cortisol and behavior in fragile X syndrome. Psychoneuroendocrinology 2002;27:855–872. [PubMed: 12183220]
63. Amir RE, van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl-CpG-binding protein 2. Nat. Genet 1999;23:185–188. [PubMed: 10508514]
64. Erlandson A, Hagberg B. *MECP2* abnormality phenotypes: clinicopathologic area with broad variability. J. Child Neurol 2005;20:727–732. [PubMed: 16225826]
65. Villard L. *MECP2* mutations in males. J. Med. Genet 2007;44:417–423. [PubMed: 17351020]
66. van Esch H, Bauters M, Ignatius J, et al. Duplication of the *MECP2* region is a frequent cause of severe mental retardation and progressive neurological symptoms in males. Am. J. Hum. Genet 2005;77:442–453. [PubMed: 16080119]
67. del Gaudio D, Fang P, Scaglia F, et al. Increased *MECP2* gene copy number as the result of genomic duplication in neurodevelopmentally delayed males. Genet. Med 2006;8:784–792. [PubMed: 17172942]
68. Mari F, Azimonti S, Bertani I, et al. CDKL5 belongs to the same molecular pathway of MeCP2 and it is responsible for the early-onset seizure variant of Rett syndrome. Hum. Mol. Genet 2005;14:1935–1946. [PubMed: 15917271]
69. Scala E, Ariani F, Mari F, et al. CDKL5/ STK9 is mutated in Rett syndrome variant with infantile spasms. J. Med. Genet 2005;42:103–107. [PubMed: 15689447]
70. Weaving LS, Christodoulou J, Williamson SL, et al. Mutations of *CDKL5* cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. Am. J. Hum. Genet 2004;75:1079–1093. [PubMed: 15492925]
71. Ariani F, Hayek G, Rondinella D, et al. *FOXG1* is responsible for the congenital variant of Rett syndrome. Am. J. Hum. Genet 2008;83:89–93. [PubMed: 18571142]
72. Philippe C, Villard L, De Roux N, et al. Spectrum and distribution of *MECP2* mutations in 424 Rett syndrome patients: a molecular update. Eur J. Med. Genet 2006;49:9–18. [PubMed: 16473305]
73. Ben Zeev Ghidon B. Rett syndrome. Child Adolesc. Psychiatr. Clin. N. Am 2007;16:723–743. [PubMed: 17562589]

74. Coutinho AM, Oliveira G, Katz C, et al. *MECP2* coding sequence and 3′UTR variation in 172 unrelated autistic patients. Am. J. Med. Genet. B Neuropsychiatr. Genet 2007;144B:475–483. [PubMed: 17427193]

75. Francke U. Mechanisms of disease: neurogenetics of MeCP2 deficiency. Nat. Clin. Pract. Neurol 2006;2:212–221. [PubMed: 16932552]

76. Guy J, Gan J, Selfridge J, Cobb S, Bird A. Reversal of neurological defects in a mouse model of Rett syndrome. Science 2007;315:1143–1147.1147 [PubMed: 17289941] Report describing a robust phenotypic reversal with reactivation of MeCP2 expression in a mouse model of Rett syndrome.

77. Giacometti E, Luikenhuis S, Beard C, Jaenisch R. Partial rescue of MeCP2 deficiency by postnatal activation of MeCP2. Proc. Natl Acad. Sci. USA 2007;104:1931–1936. [PubMed: 17267601]

78. Chang Q, Khare G, Dani V, Nelson S, Jaenisch R. The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression. Neuron 2006;49:341–348. [PubMed: 16446138]

79. Varoqueaux F, Aramuni G, Rawson RL, et al. Neuroligins determine synapse maturation and function. Neuron 2006;51:741–754. [PubMed: 16982420]

80. Graf ER, Zhang X, Jin SX, Linhoff MW, Craig AM. Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. Cell 2004;119:1013–1026. [PubMed: 15620359]

81. Meyer G, Varoqueaux F, Neeb A, Oschlies M, Brose N. The complexity of PDZ domain-mediated interactions at glutamatergic synapses: a case study on neuroligin. Neuropharmacology 2004;47:724–733. [PubMed: 15458844]

82. McCabe LL, McCabe ER. Expanded newborn screening: implications for genomic medicine. Annu. Rev. Med 2008;59:163–175. [PubMed: 18186702]