NeuroMolecular Medicine

ISSN0895-8696/06/08:451–460/$30.00 (Online) 1559-1174
doi: 10.1385/NMM:8:4:451

REVIEW ARTICLE

The Genetics of Autism Spectrum Disorders

Dorothy E. Grice[1] and Joseph D. Buxbaum*[*,2]

[1]Department of Psychiatry, UMDNJ/New Jersey Medical School, Newark, NJ 07103; and [2]Laboratory of Molecular Neuropsychiatry, Departments of Psychiatry, Neuroscience, and Geriatrics and Adult Development, Mount Sinai School of Medicine, New York, NY 10029

Received February 27, 2006; Accepted March 6, 2006

Abstract

Epidemiological twin studies demonstrate that autism spectrum disorders (ASDs) represent genetic disorders. Subsequent analyses indicate that the causes of ASDs include less common single-gene mutations and chromosomal abnormalities, as well as ASDs caused by multiple interacting genes of weak effect. Genome-wide linkage analysis has identified several susceptibility loci for the ASDs, and positional and functional candidate genes have been identified that appear to represent susceptibility genes for the ASDs. Analysis of additional larger samples and the use of genome-wide association and high-throughput variant detection will lead to the identification of further genes for ASDs.

doi: 10.1385/NMM:8:4:451

Index Entries: Association; autism spectrum disorders; genetics; linkage; pervasive developmental disorders; susceptibility locus.

Introduction

Autism, also referred to as autistic disorder, is a developmental neuropsychiatric disorder that was first described in 1943 by Leo Kanner. Autism is a clinical diagnosis, and currently the *Diagnostic and Statistical Manual, 4th edition* (DSM-IV; American Psychiatric Association, 1994) criteria used for autism and other autism spectrum disorders (ASDs) are categorized to be part of pervasive developmental disorders. The clinical criteria required to meet the diagnosis of autism, according to the DSM-IV, encompass three areas—namely: impairments in social interaction and communication as well as repetitive and stereotypic patterns of behavior or restricted interests—with an onset by the age of 3.

Impairments in social interaction and presence of repetitive or stereotypic behaviors (but without impairment in communication) are defined according to the DSM-IV as Asperger syndrome. The DSM-IV also identifies a diagnosis for pervasive developmental disorders when there are impairments

*Author to whom all correspondence and reprint requests should be addressed. E-mail: joseph.buxbaum@mssm.edu

in all three domains, but only in one domain do the criteria reach the threshold for autism. Finally, DSM-IV includes Rett syndrome (RS) and childhood disintegration syndrome. For the purposes of this review, autism, Asperger syndrome, and pervasive developmental disorders not otherwise specified will be identified as autism spectrum disorders (ASDs). Recent research in family members suggests that ASDs can extend into a broader autism phenotype, in which individuals have some features of ASDs, but without reaching a threshold of clinical impairment (Piven et al., 1997; Pickles et al., 2000; Dawson et al., 2002; Bailey and Parr, 2003).

It is also important to distinguish "idiopathic" ASDs, of no known specific cause, and "secondary" ASDs, in which a known environmental agent, chromosome abnormality, or single-gene disorder can be identified as causative. Approximately 5–10% of individuals with autism can be more specifically diagnosed with secondary autism, and the remaining 90–95% have idiopathic autism. In the current review, the rare examples of ASDs resulting from environmental agents, including *in utero* exposures to rubella (German measles), valproic acid, and thalidomide, are not discussed further. The authors instead focus on the compelling evidence for genetic causes of both idiopathic and secondary autism.

Epidemiological Twin Studies of Autism and Autism Spectrum Disorders

To understand the causes of ASDs, it is instructive to look back at careful epidemiological twin studies done as long as 30 yr ago, preceding some of the current controversies about the causes of ASDs. This is very important because twin studies carried out in an epidemiologically appropriate manner can determine whether a given disease is resulting from environmental, genetic, or mixed causes. Epidemiological twin studies are very time-consuming and difficult because when appropriately done, they can involve the complete ascertainment of all individuals within a particular defined geographic region. This type of study design can, for example, involve a door-to-door search to identify all twin pairs, in which at least one twin is affected by the disorder being studied. Such approaches are critical for determining genetic vs environmental causes. Simply relying on cases that come to the attention of the doctor or researcher will bias a sample inappropriately, and can result, for example, in an overascertainment of twins, in which both are affected by the disorder of interest. Although epidemiological twin studies have become increasingly difficult to carry out as the world's population becomes more mobile, and, for example, as parents with more than one affected child may be more likely to move to areas with improved or more accessible resources.

The earlier twin studies done in the 1970s and 1980s, were groundbreaking. These studies were carried out by renowned researchers with no *a priori* hypothesis about the cause of ASDs, and they made some startling discoveries (Folstein and Rutter, 1977a, 1977b; Steffenburg et al., 1989; Bailey et al., 1995). The most important discovery was the realization that the concordance rate for monozygotic (MZ) and dizygotic (DZ) twins differed substantially.

The concordance rate is a statistical measurement of the presence of a given trait in a pair of related individuals. For twin studies in ASD, this can be thought of as the probability that if one twin has an ASD, the co-twin will have an ASD as well. The important distinction between MZ and DZ twins arises because MZ twins are genetically identical, whereas DZ twins are genetically equivalent to siblings (sharing, on average, 50% of their DNA). However, MZ and DZ twins are assumed to equally share the prenatal, perinatal, and postnatal environments with their co-twins. If MZ and DZ twins are equally concordant for a particular trait, then that trait is very likely to be determined by environmental factors. As a trivial example, in an examination of the concordance rate for the first language of MZ and DZ twins, if the first twin identified speaks English as a first language, the likelihood that the co-twin will also speak English as a first language will not depend on whether twins are MZ or DZ. The first language of the twins is entirely determined by environmental causes. If, however, the trait being studied is entirely genetic, then the MZ and the DZ twins will have very different concordance rates. For example, if the trait being considered is a fully penetrant autosomal-dominant genetic disorder (i.e., in which one copy of a mutation is sufficient to cause the disorder), if one MZ twin has the disorder, it is an essential certainty that the co-twin will have the disorder as well. However, in the case of DZ twins, if one twin has the disorder, there is a 50% chance that the co-twin will be concordant, as the mutant gene

might not be shared. In this case, the ratio of concordance rates (i.e., 100/50%) between the MZ and DZ twins is not 1:1 (as it would have been with the language example) but rather 2:1. In the case of a fully penetrant recessive genetic disorder, if an MZ twin has the disorder, it is an essential certainty that the co-twin will also have the disorder. If, however, the twins are DZ, because this is a recessive disorder, the probability that the co-twin will have the disorder is 25%. In this case, the ratio of concordance between MZ and DZ twins is 4:1, a ratio indicative of a recessive disorder. For disorders involving more than one genetic locus, the ratio of the concordance rates between MZ and DZ twins becomes higher. In the epidemiological twin studies from the 1970s and 1980s, the concordance rate for autism when comparing with MZ and DZ twins, was approx 10:1. This was immediate evidence that autism and associated ASDs are genetic disorders that likely involve more than one genetic locus. This led to a reframing of the etiological relationship between narrowly defined autism and other ASDs and the relationship between ASDs and perinatal complications. Furthermore, these twin studies directed research efforts at identifying the genes underlying ASDs.

Penetrance in Narrowly Defined ASDs

It is noteworthy that the concordance rates for MZ twins in the previously mentioned epidemiological twin studies did not reach 100%. Penetrance is a measure of the frequency for a specific phenotype, given a particular genotype. An example of reduced penetrance is when a genetic variant does not always cause a trait to emerge. Reduced penetrance can come from many causes, and one example (Rett syndrome [RS]) is noted next. (It is more informative to look at the ratio of MZ to DZ concordance rates because penetrance is removed from the equation. For example, even if a mutation causes an autosomal-dominant disorder with 50% penetrance, the ratio of concordance for MZ to DZ twins is 50:25%, which is the familiar 2:1 for dominant disorders.) MZ concordance rates that are less than 100% are sometimes incorrectly interpreted as being evidence for an environmental cause of a genetic disorder. In fact, a concordance rate among identical twins that does not reach 100% can be due to many factors, and is not an evidence of any specific effect. Some of the mechanisms by which concordance can be less than 100% include stochastic (random) variations, mitochondrial effects, methylation changes, or environmental causes. Until now, we do not know why MZ co-twins in ASDs can differ in their phenotypes to some modest degree and might, therefore, be identified as discordant by specific criteria. However, it is important to note that there has been little or no evidence for an environmental component in ASDs (Folstein and Rosen-Sheidley, 2001; Veenstra-Vanderweele et al., 2004). To give an example of just how important some of these other factors can be, one can look at the role of stochastic variation in RS (Naidu et al., 2003; Ham et al., 2005). In RS, which, like ASDs, is a pervasive developmental disorder, the gene that accounts for most cases of RS has been identified as *MeCP2*. However, it is possible that in two individuals (related or not) with the same MeCP2 mutation, one of them can have very profound RS (including ASD-like symptoms, mental retardation, and the loss of purposeful use of hands, all with a regressive onset), whereas the other individual may have very modest clinical phenotype. While initially this appears improbable, it is now understood that this is resulting from the process of random X-chromosome inactivation, in which one of the two copies of the X chromosomes is randomly inactivated early *in utero* development in females (Chow et al., 2005; Segawa and Nomura, 2005). If the X chromosome with the normal copy of the *MeCP2* gene is inactivated, the impairment will be severe. However, if the X chromosome with the mutant copy of the *MeCP2* gene is inactivated early in development, the individual may even have no discernable phenotype. Note that random X-chromosome inactivation, which involves two X chromosomes, only occurs in females since they have two X chromosomes, but not in males as they have one X chromosome and one Y chromosome.

Concordance for Autism Among Monozygotic Twins

It is also important to appreciate that lack of concordance between MZ twins with ASDs can be subtle. The epidemiological twin studies in ASDs identified concordant or discordant twins, typically using narrow research definitions. In the epidemiological twin studies, it was noted that with a broader definition, which would be more akin to what a clinician,

health care provider, teacher, and so on, might consider as ASDs, the concordances rated for MZ twins was much higher (Folstein and Rutter, 1977b; Bailey et al., 1995). Furthermore, when the "discordant" MZ twins in the 1977 study were recontacted as adults in a follow-up study 18 yr later when all initial respondents were at least young adults, it was evident that the discordant, nonautistic twin typically had severe deficits (Bailey et al., 1995). Four of these discordant twins had no lifelong confiding relationships: quite likely by modern criteria and modern definitions they would be identified as having an ASD. In the one case, in which the discordant nonaffected twin had a confiding relationship and in fact was married, the "affected" MZ co-twin, i.e., the twin who was given a research diagnosis of autism, was extremely high functioning (A. Bailey, personal communication). It appears, therefore, that more severely affected MZ twins are almost certainly going to have a severely affected co-twin, whereas mildly affected MZ twins, may have a mildly or even apparently unaffected co-twin. Using the looser definitions of affectedness to include what would now be called ASDs, the MZ concordance rates in the epidemiological twin studies are more than 90%, again providing very strong evidence for a genetic etiology of ASDs.

Defining ASDs

Accurately defining and diagnosing autism and ASDs is critically important for all research studies and great effort is placed on this in all research studies. Most commonly, researchers prefer to use both an informant report (typically, the Autism Diagnostic Interview-Revised [ADI-R]; Lord et al., 1994) and an observational scale (typically, the Autism Diagnosis Observation Schedule-Generic [ADOS-G]; Lord et al., 2000) for diagnosis, after carefully excluding other conditions that can also present with ASD-like symptoms (including, e.g., fragile X syndrome and tuberous sclerosis). Research-oriented diagnoses are different than diagnoses provided by educators, clinicians, and other health care workers. In many research studies, autism is defined only narrowly to avoid complicating the study. The epidemiological twin studies described previously first used such a narrow definition. When comparing the twins, one of whom had narrowly defined autism, the researchers determined that whereas the co-twin did not necessarily have narrowly defined autism he or she typically did have an ASD. As a result, these researchers recognized that autism and the other ASDs they were seeing were part of a continuum and were genetically related. Thus, by careful diagnoses and assessment, an important aspect of the ASDs was identified.

Within the health care provider and education community, specific trends can be identified (Eagle, 2004; Rutter, 2005). First, individuals with higher functioning ASDs, who might not have been given any diagnosis in the past, might now be identified as having an ASD diagnosis. Second, people with lower functioning ASDs, who might have been given a nonspecific diagnosis of mental retardation, might now be identified as having an ASD diagnosis. More accurate diagnosis has also allowed for more specific and focused clinical interventions and features. Looking at ASDs from the perspective of genetics, it is exceedingly unlikely that the disorders studied in the epidemiological twin studies could show a large increase in prevalence. In such a brief window of time, the most parsimonious understanding of the data is that improvements in detection of ASDs and changes in ASD diagnosis could likely account for the reported increased prevalence of the ASDs.

Genetics, Birth Complications, and ASDs

It has been noted by some researchers and clinical groups that there is a higher than expected number of twins with ASDs and this has been used to argue that birth complications commonly associated with twin pregnancies might contribute to the development of ASDs. For example, significantly higher than expected numbers of twins are observed in families that have been collected as a result of having two or more nuclear family members with ASDs (Greenberg et al., 2001; Betancur et al., 2002). However, one of the interesting effects of the strong genetic liability to a disorder is that there will be more twins in data sets such as these, because in genetic disorders, when one twin has the disorder, it is likely that the co-twin will be concordant for the disorder. If one is studying families with two or more individuals with ASDs, there will, therefore, be an overrepresentation of MZ twins (Hallmayer et al., 2002). This observation re-enforces the important maxim that correlation does not imply causation. Perinatal complications also have been associated with ASDs. It is tempting to conclude

that the complications might have caused, or contributed to, ASDs, but again, one might confuse correlation with causation. In fact, studies have shown that healthy siblings of individuals with ASDs also have a higher rate of birth complications (e.g., Bailey et al., 1995; Glasson et al., 2004), so perhaps a more likely interpretation is that the genetic liability that can lead to ASDs can also contribute to perinatal complications, independent of the ASD diagnosis (Bailey et al., 1995; Glasson et al., 2004).

Identifying the Genes for ASDs

ASDs represent complex genetic disorders (Veenstra-Vanderweele et al., 2004). This means both that different genes can cause the disorder and that in some, and likely in most cases of ASDs, it is the action of multiple genes acting together that leads to the disorder. When talking about genetic contributions to the ASDs, one can distinguish between genetic factors, including chromosomal abnormalities and specific genetic mutations, which by themselves appear to cause ASDs (with or without other associated conditions), and susceptibility loci, which are neither necessary nor sufficient by themselves to cause ASDs. It is currently thought that most ASDs are caused by the interaction of several susceptibility loci and the minority of ASDs is caused by causal genetic variations.

Causal Genetic Variations

When considering the precise genetic causes of ASD, it is important to note that a significant portion (>5%) of ASDs are resulting from chromosomal abnormalities and specific gene mutations, that involve multiple genes (recently reviewed by Veenstra-Vanderweele et al., 2004; Cohen et al., 2005). Specific chromosomal abnormalities that are associated with ASDs, and even with a primary diagnosis of ASDs, include, most commonly, chromosomal deletions or duplications in the 15q11-g13 region (also called the Prader-Will/Angelman syndrome [PW/AS] region; Dykens et al., 2004), as well as Down syndrome, Smith-Magenis syndrome, 22q13 deletion syndrome (recently attributed to haploinsufficiency of a single gene, *SHANK3*/*PROSAP2*; Bonaglia et al., 2001; Wilson et al., 2003), and other more rare conditions and syndromes (Cohen et al., 2005). Several genes previously associated with X-linked or autosomal mental retardation (reviewed by Ropers and Hamel, 2005), have been found in children who have an ASD diagnosis. These include the RS *MeCP2* gene, the fragile X-syndrome *FMR1* gene, the tuberous sclerosis *TSC* genes, the Smith-Lemli-Opitz syndrome *DHCR7* gene, the neuroligin genes (*NLGN3* and *NLGN4*), *ATR-X*, neurotrypsin, and other genes as well (*see* Veenstra-Vanderweele et al., 2004). As more genes associated with mental retardation are screened in ASDs it seems likely that some will also be identified in individuals with ASDs. These genes appear to be sufficient by themselves to lead to an ASD phenotype. But they only appear to account for a minority of cases, with the majority of ASDs being caused by the combined effect of multiple susceptibility loci, each of weak to modest effect.

Susceptibility Loci

Many genes have been postulated to increase susceptibility to ASDs, and there have been more than 90 association studies published with various genes of interest (summarized by Wassink et al., 2004). Of these, only a small number have shown nominally significant associations (*see* Vorstman et al., 2006). Given the large number of genes that have been tested, an important question that emerges is which of these apparently associated genes are "true" susceptibility loci (i.e., not false-positives). A related question is which of the apparently not associated genes have been definitely excluded (i.e., not false-negatives). Some apparently positive association studies may result from a phenomenon called stratification. In such cases, the observed association is a false-positive due to a failure to properly match cases and controls such that ethnic or other differences between cases and controls leads to a false-positive association. Even controlling for stratification, a positive finding can of course still arise by chance. For this reason, replication of a finding in independent samples is considered a key aspect of modern genetic analyses. But, it should be noted that, on theoretical grounds, it has been shown that even for a "true" positive, well-designed follow-up studies might not detect a confirmatory association. Given this, we have chosen to focus on those genes that have been positive in two or more independent studies, at this point the list is quite short and includes such genes as the γ-aminobutyric acid (GABA) receptor subunits on chromosome 15, the serotonin transporter SLC6A4,

and the aspartate–glutamate carrier (AGC1, also known as SLC25A12). The authors briefly summarize these studies.

GABA Receptors

There are multiple studies of GABA receptors in ASDs. One reason for this is the association of chromosomal abnormalities in the PW/AS region with ASDs. The genes in the PW/AS region include several GABA receptor subunits. In addition, GABA receptors are the major inhibitory transmitter receptors in the brain, have a central role in anxiety, and disruption of these receptors can lead to epilepsy, a common, but not diagnostic, feature of ASDs. For the GABRB3 subunit, which maps to the PW/AS region, there was a positive association (Cook et al., 1998) with some follow-up positive and negative studies (reviewed by Buxbaum et al., 2002). One analysis of some of the negative studies suggest that they might reflect more of an underpowered analysis (Buxbaum et al., 2002), as replication studies may need to be several times larger than an original study to have a high probability of replicating a true-positive (Goring et al., 2001; Lohmueller et al., 2003). Failure to account for gene–gene interactions might also lead to a failure to replicate true-positive findings. Methods are now being developed to appropriately analyze genes in concert. For example, a recent study examined interactions between 14 GABA receptor genes and identified significant gene–gene interactions between GABRA4 and GABRB1 in ASDs (Ma et al., 2005).

The Serotonin Transporter, SLC6A4

There have been more than 10 studies of the SLC6A4 transporter in ASD (Devlin et al., 2005). The interest in serotonin in ASDs arises because of the robust and reproducible observation that of blood serotonin levels are elevated in about 30% of individuals with ASD. Additional evidence for a role for serotonin in ASDs comes from the beneficial effects of selective serotonin reuptake inhibitors for certain core symptoms in ASD. Multiple groups have investigated a functional promoter variant in SLC6A4, termed HTTLPR, which is known to modulate the expression of the gene. There are two predominant forms of the HTTLPR variant, a short and a long form, and one would anticipate that in studies showing association of HTTLPR with ASD, the same variant (long or short) would contribute to risk. The curious result from all of the studies of HTTLPR in ASDs studies is that, in addition to some studies being positive and some studies being negative, there are differences in what is the risk variant among the positive studies. In fact, among the positive studies, the studies are almost equally divided between overtransmission of either the long or short variant (reviewed by Devlin et al., 2005). It is, therefore, possible that the association of this gene is not with ASD *per se* but rather with some aspect of ASD (such as high serotonin; Coutinho et al., 2004). It is also possible that it is not just the HTTLPR variant that contributes to a risk of ASD but rather other variants of the gene that also contribute to risk. The recent identification of a new subvariant of the long form of HTTLPR (Hu et al., 2005), indicates that further detailed studies will need to be carried out. Furthermore, a reanalysis of one of the studies implicating the SLC6A4 locus in ASDs indicates that there might be serious difficulties (and hence errors) in the genotyping of HTTLPR (Yonan et al., 2006), and, as a result, the positive studies may need to be re-evaluated.

The recent identification of rare variants of the *SLC6A4* gene suggests that even within this single gene, significant heterogeneity exists and it might contribute to the complex nature of the association studies. One recent study suggested that there is allelic heterogeneity in the *SLC6A4* gene that is directly related to ASD (Sutcliffe et al., 2005). Thus even in individuals who have susceptibility variants in the SLC6A4 locus, there may be different variants within the gene that may contribute to each case. In cases of allelic heterogeneity, traditional methods of association (in which specific variants are analyzed in large cohorts) may not be appropriate, and the alternative of looking specifically in each family for rare, or "private," mutations would be the preferred means to go forward.

Engrailed 2

The homeobox transcription factor engrailed (*EN*)2 is involved in cerebellar development and disruption of this gene in mice can generate abnormalities reminiscent of some cerebellar abnormalities in ASD. The EN2 gene maps to a region of chromosome 7 that has been implicated by linkage to contain an ASD susceptibility locus. A case–control association study indicated that *EN2* was associated

with autism (Petit et al., 1995). A recent follow-up study that made use of a family-based design also identified a significant association (Gharani et al., 2004). In a follow-up study by the same group, but using independent samples, there was further evidence for association with ASDs (Benayed et al., 2005). As is the case for all the other genes discussed here, there have been studies (Zhong et al., 2003), which does not see any evidence of association of *EN2* with ASDs.

AGC1/SLC25A12

SLC25A12 is a gene found on human chromosome 2, in an interval that has shown linkage to ASDs in multiple studies. This gene is involved in mitochondrial function and is responsible for maintaining the levels of ATP (a critical source of energy) in the cell. Recently, it was demonstrated that genetic variants in *SLC25A12* were associated with ASD (Ramoz et al., 2004). The association for these same genetic variants was recently confirmed in an independent sample (Segurado et al., 2005). The genotype-relative risk (a measure of how much the risk for developing ASDs increases in individuals harboring these variants) was estimated to be between 2 and 5 in both of these studies. There has, however, been one published study in which no association between *SLC25A12* and ASDs was observed (Blasi et al., 2006). Recently, a mouse knockout of *SLC25A12* was derived from the Omni Bank (Lexicon Genetics), and subsequently characterized (Jalil et al., 2005), implicating the *AGC1/SLC25A12* gene in myelination. Dysmyelination causes decreased neuronal connectivity, a phenomenon that might account for some of the behavioral observations in ASD (Courchesne and Pierce, 2005).

Next Steps in the Genetics in ASDs

As noted previously, secondary ASDs resulting from a chromosomal abnormality, or single-gene mutation, account for a 5–10% proportion of ASDs. In addition, it is likely that both known and as-yet unidentified genes for mental retardation might prove to be etiological for some of what we currently identify as idiopathic ASDs. However, there is likely significant genetic heterogeneity even within idiopathic autism. To find ASD genes, one will need to use clinical, imaging, and other data to define more homogeneous subgroups. One approach is to distinguish between what has been termed as "complex autism" and "essential autism" (Miles and Hillman, 2000; Miles et al., 2005). In this summary, complex autism was defined by the presence of dysmorphic features and/or microcephaly in an individual with an ASD diagnosis. Approximately 20–30% of children with idiopathic autism have complex autism. Essential autism was defined by the absence of macrocephaly and the absence of dysmorphic features. Approximately 70% of children with idiopathic autism have essential autism. Children with essential autism are more likely to be male, have a higher sibling recurrence risk and greater family history of autism. In these studies, all identifiable genetic conditions were confined to the complex autism group. The lack of identifiable genetic conditions in the essential group, and the important differences in recurrence risk between the two groups, indicate that such an approach will be useful in further genetic studies. Large-scale resequencing of ASD samples for rare variants, and large-scale searches for genomic changes might be focused on the complex group, whereas linkage analysis and genome-wide association studies would be more appropriately focused on the essential autism group.

Individuals with complex autism will include cases of autism arising from *de novo* genetic variants. This will in part account for the lower sibling recurrence risk. In contrast, groups with essential autism, with the higher sibling recurrence risk, will include the individuals with autism caused by multiple interacting genes. As it is very likely that it will be several—or even many—interacting genes that contribute to a given incidence of essential autism or related disorder, the siblings of the index case, who will, on average, share half of their genes, will share a significant number of the ASD genes. This is why the recurrence risk for ASDs and for broader autism phenotype will be so high in siblings of individuals with essential autism. In fact, in a 3-yr search for siblings of individuals with autism that had absolutely no symptoms, after screening more than 300 families, only a tiny number (approx 30) could be found (Susan Santangelo, personal communication). Therefore, it should be of no surprise that there are many examples of families with more than one child with an ASD. One interpretation as to the presence and prevalence of multiple susceptibility genes of weak effect is that some of these common variants may be beneficial by themselves and it is only when

too many appear together that there is a deleterious effect.

Discussion

There is good evidence that indicates that ASDs are genetic disorders. Whereas 5–10% of ASDs result from chromosomal abnormalities and causal mutations, a larger proportion are a result of the interaction of multiple susceptibility loci. The exact nature of the genes that are implicated in ASDs, for example, whether they are common variants or rare variants, will impact on the feasibility of defining specific genetic assays as predictors for increased risk for ASDs, and the exact form the assays would take. There is empirical evidence that an ASD can in some cases respond to intensive behavioral interventions (Francis, 2005), and so identifying individuals with greater risks of ASDs at an earlier age is likely to have important clinical and practical implications. Even if one can only identify people with several fold increased risk compared to others, there still might be good reason to introduce cognitive–behavioral interventions, because even with a moderately elevated risk, there would ultimately be a lesser cost than no interventions, given the potential consequences of ASD. It will require the simultaneous analysis of a multiple genes before effective tools for the assessment of ASD risk can be developed. Identifying these multiple genes will necessitate pooling of data sets for both the identification and the subsequent replication of ASD susceptibility genes. As ASD genes are identified, pathway analyses may lead to the identification of further genes. Moreover, as the genes are identified, animal models, such as those described earlier, can be developed. These models can clarify the function of the gene products in vivo, and may be useful at some future date to evaluate novel pharmaceutical interventions.

References

American Psychiatric Association. (1994) *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.* Washington DC: Author.

Bailey A. and Parr J. (2003) Implications of the broader phenotype for concepts of autism. *Novartis Found Symp.* **251,** 26–35; discussion 6–47, 109–11, 281–97.

Bailey A., Le Couteur A., Gottesman I., et al. (1995) Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol. Med.* **25,** 63–77.

Benayed R., Gharani N., Rossman I., et al. (2005) Support for the Homeobox Transcription Factor Gene ENGRAILED 2 as an Autism Spectrum Disorder Susceptibility Locus. *Am. J. Hum. Genet.* **77,** 851–868.

Betancur C., Leboyer M., and Gillberg C. (2002) Increased rate of twins among affected sibling pairs with autism. *Am. J. Hum. Genet.* **70,** 1381–1383.

Blasi F., Bacchelli E., Carone S., et al. (2006) SLC25A12 and CMYA3 gene variants are not associated with autism in the IMGSAC multiplex family sample. *Eur. J. Hum. Genet.* **14,** 123–126.

Bonaglia M. C., Giorda R., Borgatti R., et al. (2001) Disruption of the ProSAP2 gene in a t(12;22)(q24.1;q13.3) is associated with the 22q13.3 deletion syndrome. *Am. J. Hum. Genet.* **69,** 261–268.

Buxbaum J. D., Silverman J. M., Smith C. J., et al. (2002) Association between a GABRB3 polymorphism and autism. *Mol. Psychiatr.* **7,** 311–316.

Chow J. C., Yen Z., Ziesche S. M., and Brown C. J. (2005) Silencing of the Mammalian X Chromosome. *Annu. Rev. Genomics Hum. Genet.* **6,** 69–92.

Cohen D., Pichard N., Tordjman S., et al. (2005) Specific genetic disorders and autism: clinical contribution towards their identification. *J. Autism. Dev. Disord.* **35,** 103–116.

Cook E. H. Jr., Courchesne R. Y., Cox N. J., et al. (1998) Linkage-disequilibrium mapping of autistic disorder, with 15q11-13 markers. *Am. J. Hum. Genet.* **62,** 1077–1083.

Courchesne E. and Pierce K. (2005) Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. *Curr. Opin. Neurobiol.* **15,** 225–230.

Coutinho A. M., Oliveira G., Morgadinho T., et al. (2004) Variants of the serotonin transporter gene (SLC6A4) significantly contribute to hyperserotonemia in autism. *Mol. Psychiatry* **9,** 264–271.

Dawson G., Webb S., Schellenberg G. D., et al. (2002) Defining the broader phenotype of autism: genetic, brain, and behavioral perspectives. *Dev. Psychopathol.* **14,** 581–611.

Devlin B., Cook E. H. Jr., Coon H., et al. (2005) Autism and the serotonin transporter: the long and short of it. *Mol. Psychiatry* **10,** 1110–1116.

Dykens E. M., Sutcliffe J. S., and Levitt P. (2004) Autism and 15q11-q13 disorders: behavioral, genetic, and

pathophysiological issues. *Ment. Retard. Dev. Disabil. Res. Rev.* **10,** 284–291.
Eagle R. S. (2004) Commentary: Further commentary on the debate regarding increase in autism in California. *J. Autism. Dev. Disord.* **34,** 87–88.
Folstein S. E. and Rosen-Sheidley B. (2001) Genetics of autism: complex aetiology for a heterogeneous disorder. *Nat. Rev. Genet.* **2,** 943–955.
Folstein S. and Rutter M. (1977a) Genetic influences and infantile autism. *Nature* **265,** 726–728.
Folstein S. and Rutter M. (1977b) Infantile autism: a genetic study of 21 twin pairs. *J. Child. Psychol. Psychiatry* **18,** 297–321.
Francis K. (2005) Autism interventions: a critical update. *Dev. Med. Child. Neurol.* **47,** 493–499.
Gharani N., Benayed R., Mancuso V., Brzustowicz L. M., and Millonig J. H. (2004) Association of the homeobox transcription factor, ENGRAILED 2, 3, with autism spectrum disorder. *Mol. Psychiatry* **9,** 474–484.
Glasson E. J., Bower C., Petterson B., de Klerk N., Chaney G., and Hallmayer J. F. (2004) Perinatal factors and the development of autism: a population study. *Arch. Gen. Psychiatry* **61,** 618–627.
Goring H. H., Terwilliger J. D., and Blangero J. (2001) Large upward bias in estimation of locus-specific effects from genomewide scans. *Am. J. Hum. Genet.* **69,** 1357–1369.
Greenberg D. A., Hodge S. E., Sowinski J., and Nicoll D. (2001) Excess of twins among affected sibling pairs with autism: implications for the etiology of autism. *Am. J. Hum. Genet.* **69,** 1062–1067.
Hallmayer J., Glasson E. J., Bower C., et al. (2002) On the twin risk in autism. *Am. J. Hum. Genet.* **71,** 941–946.
Ham A. L., Kumar A., Deeter R., and Schanen N. C. (2005) Does genotype predict phenotype in Rett syndrome? *J. Child. Neurol.* **20,** 768–778.
Hu X., Oroszi G., Chun J., Smith T. L., Goldman D., and Schuckit M. A. (2005) An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. *Alcohol Clin. Exp. Res.* **29,** 8–16.
Jalil M. A., Begum L., Contreras L., et al. (2005) Reduced N-acetylaspartate levels in mice lacking aralar, a brain- and muscle-type mitochondrial aspartate-glutamate carrier. *J. Biol. Chem.* **280,** 31,333–31,339.
Lohmueller K. E., Pearce C. L., Pike M., Lander E. S., and Hirschhorn J. N. (2003) Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat. Genet.* **33,** 177–182.
Lord C., Rutter M., and Le Couteur A. (1994) Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J. Autism. Dev. Disord.* **24,** 659–685.
Lord C., Risi S., Lambrecht L., et al. (2000) The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J. Autism. Dev. Disord.* **30,** 205–223.
Ma D. Q., Whitehead P. L., Menold M. M., et al. (2005) Identification of significant association and gene–gene interaction of GABA receptor subunit genes in autism. *Am. J. Hum. Genet.* **77,** 377–388.
Miles J. H. and Hillman R. E. (2000) Value of a clinical morphology examination in autism. *Am. J. Med. Genet.* **91,** 245–253.
Miles J. H., Takahashi T. N., Bagby S., et al. (2005) Essential versus complex autism: definition of fundamental prognostic subtypes. *Am. J. Med. Genet. A* **135,** 171–180.
Naidu S., Bibat G., Kratz L., et al. (2003) Clinical variability in Rett syndrome. *J. Child. Neurol.* **18,** 662–668.
Petit E., Herault J., and Martineau J. (1995) Association study with two markers of a human homeogene in infantile autism. *J. Med. Genet.* **32,** 269–274.
Pickles A., Starr E., Kazak S., et al. (2000) Variable expression of the autism broader phenotype: findings from extended pedigrees. *J. Child. Psychol. Psychiatry* **41,** 491–502.
Piven J., Palmer P., Jacobi D., Childress D., and Arndt S. (1997) Broader autism phenotype: evidence from a family history study of multiple-incidence autism families. *Am. J. Psychiatry* **154,** 185–190.
Ramoz N., Reichert J. G., Smith C. J., et al. (2004) Linkage and association of the mitochondrial aspartate/glutamate carrier SLC25A12 gene with autism. *Am. J. Psychiatry* **161,** 662–669.
Ropers H. H. and Hamel B. C. (2005) X-linked mental retardation. *Nat. Rev. Genet.* **6,** 46–57.
Rutter M. (2005) Aetiology of autism: findings and questions. *J. Intellect. Disabil. Res.* **49,** 231–238.
Segawa M. and Nomura Y. (2005) Rett syndrome. *Curr. Opin. Neurol.* **18,** 97–104.
Segurado R., Conroy J., Meally E., Fitzgerald M., Gill M., and Gallagher L. (2005) Confirmation of Association Between Autism and the Mitochondrial Aspartate/Glutamate Carrier SLC25A12 Gene on Chromosome 2q31. *Am. J. Psychiatry* **162,** 2182–2184.
Steffenburg S., Gillberg C., Hellgren L., et al. (1989) A twin study of autism in Denmark, Finland, Iceland,

Norway and Sweden. *J. Child. Psychol. Psychiatry* **30,** 405–416.

Sutcliffe J. S., Delahanty R. J., and Prasad H. C. (2005) Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. *Am. J. Hum. Genet.* **77,** 265–279.

Veenstra-Vanderweele J., Christian S. L., and Cook E. H. Jr. (2004) Autism as a paradigmatic complex genetic disorder. *Annu. Rev. Genomics Hum. Genet.* **5,** 379–405.

Vorstman J. A., Staal W. G., van Daalen E., van Engeland H., Hochstenbach P. F., and Franke L. (2006) Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol. Psychiatry* **11,** 1, 18–28.

Wassink T. H., Brzustowicz L. M., Bartlett C. W., and Szatmari P. (2004) The search for autism disease genes. *Ment. Retard. Dev. Disabil. Res. Rev.* **10,** 272–283.

Wilson H. L., Wong A. C., and Shaw S. R. (2003) Molecular characterisation of the 22q13 deletion syndrome supports the role of haploinsufficiency of SHANK3/PROSAP2 in the major neurological symptoms. *J. Med. Genet.* **40,** 575–584.

Yonan A. L., Palmer A. A., and Gilliam T. C. (2006) Hardy-Weinberg disequilibrium identified genotyping error of the serotonin transporter (SLC6A4) promoter polymorphism. *Psychiatr. Genet.* **16,** 31–34.

Zhong H., Serajee F. J., Nabi R., and Huq A. H. (2003) No association between the EN2 gene and autistic disorder. *J. Med. Genet.* **40,** e4.