Heterogeneity and the genetics of autism

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The objective of this review is to summarize recent data on the genetics of autism, highlight the evidence for genetic heterogeneity and extend the implications of these findings for the identification of susceptibility genes in this disorder. Family studies have shown that autism runs in families and twin studies indicate that the basis of that familial aggregation is genetic. As a result the prospects for the identification of susceptibility genes using either linkage or association studies are quite good. However, recent evidence is accumulating suggesting that the disorder is genetically heterogeneous; higher functioning individuals with autism may arise from separate genetic mechanisms that lower functioning ones. If true, this will make the detection of linkage and association much more difficult.

Cette étude vise à résumer les données récentes sur les aspects génétiques de l'autisme, à mettre en évidence les données probantes relatives à l'hétérogénéité génétique et à étendre les répercussions de ces constatations à l'identification des gènes de susceptibilité de ce trouble. Des études familiales ont démontré que l'autisme sévit dans des familles et des études sur des jumeaux indiquent que l'agrégation familiale a un fondement génétique. C'est pourquoi les perspectives d'identification des gènes de susceptibilité au moyen d'études de liens ou d'association sont très bonnes. Un nombre croissant de données probantes récentes indiquent toutefois que le trouble est hétérogène sur le plan génétique. Des personnes atteintes d'autisme qui fonctionnent à un niveau plus élevé peuvent être issues de mécanismes génétiques distincts de ceux des sujets plus atteints. Si cela s'avère, le lien et l'association seront beaucoup plus difficiles à détecter.

Introduction

In 1976 an authoritative review[1] was published that concluded, based on the available evidence, that genetic factors played little role in the etiology of early-onset psychoses or infantile autism. The next year, the first systematic twin study of autism appeared,[2] demonstrating that the concordance rate for autism among monozygous twins was very much higher than among dyzygous twins. This provided the first compelling evidence that genetic factors did play an important role in the etiology of autism. In the next 20 years, the evidence that there exist susceptibility genes for autism has grown stronger, and recently several large-scale mapping studies have been launched to identify these genes. Compared with schizophrenia and bipolar disorder, genetic research in autism is relatively new, but progress has been rapid. However, there remain several unresolved issues in the genetic epidemiology of the disorder. The issue most likely to lead to significant obstacles in identifying susceptibility genes is that of genetic heterogeneity — the possibility that 2 or more

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independent genetic mechanisms might lead to the disorder. The objective of this paper is to present recent data indicating that genetic heterogeneity may be present in autism and then to illustrate the implications of this for linkage studies.

Epidemiology and classification

Autism is one of a group of pervasive developmental disorders (PDD) characterized by impairments in reciprocal social interaction, impairments in verbal and nonverbal communication, and a pattern of repetitive, stereotypical behaviours, activities or interests.[3] The disorder is "pervasive" insofar as these 3 impairments affect all aspects of a child's development and it is "developmental" in that the behaviours change as the child gets older. Nevertheless, these 3 components remain constant, although they are expressed in altered phenotype depending on the mental age of the child.

Autism is the best known example of a pervasive developmental disorder, but through the years other subtypes of autistic-like children have been identified as well. Both the DSM-IV[3] and ICD-10[4] have attempted to catalogue PDD subtypes with the greatest evidence of diagnostic validity. These include Asperger's syndrome, Rett syndrome, disintegrative disorder, and PDD not otherwise specified (PDDNOS) or atypical autism.[3] Asperger's syndrome refers to children with many PDD traits but who demonstrate an absence of clinically significant cognitive and language delay. Both Rett syndrome and disintegrative disorders are quite rare and are characterized by a specific natural history consisting of relatively normal development up to a certain age and then deterioration with the appearance of autistic features. Rett syndrome appears almost exclusively in girls and is associated with specific physical changes.[5] Disintegrative disorder is much more common in boys.[6] Atypical autism or PDDNOS refers to children who have an age of onset later than 36 months or who fall below the threshold for autism with respect to the number of symptoms or the number of domains met. The diagnostic boundaries between these PDD subtypes and autism remain unclear, and the extent to which the subtypes differ with respect to etiologic variables and outcome remains unresolved.[7]

It has been commonly assumed that autism is a rare disorder, occurring in about 4 in 10 000 children.[8] However, more recent evidence suggests that the disorder is more common than that. Earlier reports were characterized by measurement and sampling biases that tended to miss higher functioning or younger PDD children. Perhaps the most comprehensive epidemiologic study was conducted by Bryson et al[9] in Nova Scotia; they reported that the prevalence of autism is 10 in 10 000 children. Atypical forms of autism appear to be at least as common as autism, if not twice as common.[8] There is only 1 epidemiologic study of the prevalence of Asperger's syndrome.[10] This report found widely differing rates of between 20 and 60 in 10 000 children, depending on the diagnostic criteria used. If the prevalence rates for the different PDD subtypes are combined, it would not be unreasonable to suggest that the prevalence of PDD is as much as 50 in 10 000 children, or 0.5%. This estimate suggests that PDD is not an uncommon disorder and confirms a view held by clinicians and frontline workers who increasing see such children in day care and preschool settings.

Evidence that autism has a genetic etiology

The first line of evidence that autism has a genetic etiology comes from family studies that estimate the risk of autism among siblings of autistic probands. These family studies have been recently reviewed (P. Szatmari, M.B. Jones, J.E. MacLean, L. Tuff, G. Bartolucci, G. Mahoney, et al: unpublished data, 1998), and if the results are combined, the overall sibling risk is 2.2% (95% confidence interval [CI] 1.1% to 3.3%). Therefore, the relative risk (i.e., sibling risk divided by the general population rate) is approximately 20, suggesting a degree of familial aggregation that is twice the rate for schizophrenia and bipolar illness, which is 10,[11,12] although it is lower than the 50 to 100 usually quoted.[13] There are 4 studies that have demonstrated that siblings of autistic probands have an increased risk of nonautistic forms of PDD.[14–17] The combined sibling risk for nonautistic PDD based on these 4 studies is 3.6% (95% CI 1.6% to 5.6%). Thus, the risk for autism and any other form of PDD in siblings of autistic probands may be as high as 6% (2.2%+3.6%). If the general population rate of PDD is taken to be 0.5%, the relative risk to siblings is roughly 10.

Four studies have also looked at the risk of autism to second- and third-degree relatives.[15,18–20] Combining the data from these studies gives a risk of 0.18% (95% CI 0.03% to 0.33 %) for second-degree relatives and 0.12% (95% CI 0.01% to 0.023%) for third-degree relatives. It is striking that these risk estimates to extended relatives

are very much lower than the risk for siblings, suggesting that multiple interacting genes must be involved in etiology.[19]

These family studies indicate that the disorder is familial in nature, not that the mechanism of that familial aggregation is genetic. Twin and adoption studies are needed to disentangle the mechanism of familial aggregation. Unfortunately, there are no adoption studies in autism, but 4 twin studies have been published.[2,21-23] Three of these[2,21,22] used systematic sampling of twins and reported monozygous pairwise concordance rates from a low of 36% to a high of 91% and a dyzygous pairwise concordance rate of 0%, suggesting that the disorder is strongly genetic. The fact that the dyzygous pairwise concordance rate is lower than the sibling rate is most likely an artifact of small numbers.

There are 2 important implications from these twin studies. First, the difference between the monozygous and dyzygous concordance rates can be used to estimate heritability (i.e., the proportion of the variance that is accounted for by genetic factors). Given these figures, it is possible to estimate that the heritability for autism is greater than 90%. This represents a substantial proportion of the variance (greater than recent estimates for schizophrenia and bipolar illness[11,12]) and suggests that genetic factors are extremely important in the etiology of autism. The second important implication from the monozygous–dyzygous differences is that the mode of transmission must be non-Mendelian. The drop in concordance rate from monozygous to dyzygous twins is greater than a factor of 4, which would be expected, given an autosomal recessive mode of inheritance. These data are consistent with the fall in risk observed in first-, second- and third-degree relatives, again suggesting that multiple interacting genes are involved in etiology.

Prerequisites to linkage and association analysis

Given the strong heritability of the disorder, linkage and association studies are an obvious next step in identifying susceptibility genes. Linkage analysis has been extraordinarily successful in identifying susceptibility genes for "Mendelian" disorders, that is, those genetic conditions that clearly follow specific modes of transmission (autosomal dominant, recessive and X-linked). There are several reasons that parametric forms of linkage analysis has been so successful, but they do require that the genetic mode of transmission be specified beforehand.[24] This methodology has been much less successful in complex genetic disorders where a 1-to-1 relation between phenotype and genotype does not exist. Affected sib-pair and affected relative methods have been devised that do not need such information, but rely instead of the degree of allele sharing among affected relatives.[25] The problem with these methods is that the sample size requirements are much larger than for the lod score methods.[25,26] Regardless, there are 3 prerequisites for both methods of linkage analysis. First, there must be an accurate definition of the phenotype; that is, one must decide who is actually affected in a pedigree in order to know who to genotype. Second, genetically homogenous families must be studied; that is, the subset of families caused by the hypothesized disease mutation must be isolated from phenotypically similar families in which the disorder is caused by other mechanisms. Third, one must study disorders caused by a small number of genes of moderate to strong effect because both parametric and nonparametric methods have very poor power to detect linkage when a large number of interacting genes are involved. Family-based association studies are able to isolate genes that contribute a smaller portion of the variance[25] but the strength of the genetic factors and the recombination fraction (or distance between marker and disease locus) become key determinants of success.[27,28]

In other words, the power to detect susceptibility genes using either linkage or association studies critically depends on the relative risk associated with that genetic locus.[29] Accurate definition of the phenotype, genetic homogeneity, and the number of genes involved are all important precisely because these factors ensure that the relative risk associated with a specific genetic locus will be high and not diluted either by measurement error, by including the wrong types of families or by studying disorders caused by multiple interacting genes. Other important factors include accuracy in the assignment of genotype, the recombination fraction and the degree of heterozygosity at the marker locus. However, it is relative risk that largely determines the power to detect linkage given a particular sample size.[29] The relative risk of 20, referred to earlier, is the combined effect of all genetic loci interacting together. It does not translate into the relative risk associated with a specific locus. The relative risk at a particular locus will be lower than 20 if the genetic mechanism is additive or multiplicative, if the sample is genet-

ically heterogeneous, or if there is considerable error in the assignment of phenotype.

Heterogeneity in autism and PDD

The PDDs demonstrate considerable *clinical* heterogeneity. Not only are there several different subtypes of PDD, distinguished by symptom patterns or natural history,[3] there is also considerable variation in outcome, cognitive functioning, verbal abilities and social skills within each subtype. It is unclear whether this variation is associated with variation in etiology, however. About 10% of cases of autism are associated with a specific neurological disease such as tuberous sclerosis or neurofibromatosis.[30] It is true that these central nervous system (CNS) disorders and other more nonspecific signs of organicity, such as epilepsy and EEG abnormalities, are more common in lower functioning probands with autism (or those with a worse outcome) than higher functioning ones. What is unclear is whether, in the other 90% of idiopathic cases, higher and lower functioning children with autism have a different etiology.

There is also some evidence that PDD is heterogeneous by sex. Boys outnumber girls by about 3 to 1,[9] but it is intriguing to note that this sex ratio varies by IQ; that is, among children with autism whose IQ is under 35, the relative proportions of males and females are roughly equal.[31,32] A female affected with autism or PDD tends to be more cognitively but less socially impaired than a male.[33,34]

The extent to which this heterogeneity by sex or level of functioning is linked to differences at the level of the genotype is, however, unclear. The best way to identify such genetic, as opposed to clinical, heterogeneity is to see whether there is significant variation in lod scores or allele sharing by families. However, in the absence of positive genetic linkage results, such tests cannot be conducted. There are clinical ways of testing for genetic heterogeneity, but these only indicate the possibility of such heterogeneity. Families can then be stratified on these clinical variables and linkage analysis restricted to certain subsets of families. A common method is to see whether familial aggregation of clinical features exists within families; that is, do certain signs or symptoms run true within families. For example, in both Alzheimer's disease and breast cancer, early onset in a proband is associated with early onset in an affected relative as well. This finding led to the suggestion that early- and late-onset forms of these diseases are different, a result that has been confirmed with linkage analysis.[35,36] An alternative clinical way to test for genetic heterogeneity is to see whether the risk to relatives varies as a function of certain proband characteristics. Again, using Alzheimer's disease and breast cancer as an example, early onset in the proband is associated with a greater risk of disease in a relative than late onset in the proband. What is impressive about this finding is that the same clinical variable (age of onset) is a marker of heterogeneity in both methods and identifies a more homogeneous subset of families. These families are more "Mendelian" in their mode of transmission and easier to diagnose. Thus the power to detect linkage is much greater than if early- and late-onset families are combined.

Unfortunately, there is little information on genetic heterogeneity in autism or PDD. Spiker et al[37] demonstrated there was no familial aggregation of specific autistic behaviours in 37 families with multiple incidence of autism. Le Couteur et al[38] reported similar results using a sample of monozygous twins, though it was interesting to note that a measure of nonverbal IQ did show less variation within pairs than between random pairs. Perhaps level of functioning, not PDD symptoms, shows familial aggregation, though Le Couteur et al[38] did not comment on this possibility. Ritvo et al[39] reported that the risk of autism for relatives of female probands was 14%, whereas for the relatives of male probands the risk was 7%. However, the sample size was too small to test this difference with sufficient statistical power. Instead, Bolton et al[14] looked at the risk of more common PDD-like traits (or the lesser variant) in siblings and found that the risk was higher if the proband had a very low verbal IQ. Limited as these data are, they do suggest that level of functioning, sex (which is related to level of functioning) or both might be a marker of genetic heterogeneity, but clearly more studies are needed.

Familial aggregation of PDD subtype and level of functioning

The objective of the McMaster Family Study of Autism/PDD was to identify susceptibility genes by first isolating more genetically homogeneous subtypes using both methods described above. We first looked at the familial aggregation of PDD subtypes, symptom severity and level of functioning. The distribution of PDD subtypes in a sample of multiplex families was studied by MacLean et al[40] in 46 multiple incidence families comprised of 50 sibling pairs. Autism, Asperger's syndrome and atypical autism were diagnosed by 3 clinicians

independently reviewing clinical records and making a diagnosis according to DSM-IV criteria. If Asperger's syndrome always appears to run in families where the proband has Asperger's syndrome and if autism only occurs in the siblings of probands with autism, this would suggest that the genes for these 2 PDD subtypes are separate and independent.

The data from the sib-pairs reported by MacLean et al[40] suggest however that PDD subtypes cross family boundaries. For example, of the 32 probands with autism, there were 25 affected siblings with autism, 2 with Asperger's syndrome and 5 with atypical autism. Of the 7 probands with Asperger's syndrome, 6 of the affected siblings had autism. The overall level of agreement for these PDD subtypes was low ($\kappa = 0.22$) and nonsignificant. These data indicate that PDD subtype is not a marker of genetic heterogeneity.

MacLean et al[40] also investigated the extent to which symptom severity and level of functioning show familial aggregation. The intraclass correlation (ICC) was used to measure familial resemblance, which compares the variation within a sibship to variation between sibships. A high ICC suggests that there is less variation within a sibship than between sibships. PDD symptoms were measured using the Autism Diagnostic Interview (ADI), and summary measures of impairments in social reciprocity, in verbal and nonverbal communication and repetitive behaviours were calculated. The ICC for all these measures was low and nonsignificant except for nonverbal communication, which had an ICC of 0.39 ($p < 0.05$).

In contrast to these estimates, the ICC for nonverbal IQ and adaptive behaviour in socialization and communication (as assessed by the Vineland Adaptive Behaviour Scales) was quite high. For example, the ICC for IQ was 0.42, for socialization was 0.40 and for communication was 0.50; all these were significant at $p < 0.001$. Perhaps the reason that the ADI measure of nonverbal communication showed a high ICC was because this summary measure was moderately correlated with IQ (–0.43, $p < 0.05$). Thus, these data suggest that it may be level of functioning that shows familial aggregation, not individual summaries of PDD symptoms.

There are 2 possible explanations for this dissociation in familial resemblance between symptoms and level of functioning. The first is that perhaps current summary measures of autistic symptoms are not sensitive to variations in genotype or that the familial aggregation is obscured by measurement error. Alternately, there may be a separate set of genes for PDD symptoms and for level of functioning, and it is only the second set of genes that show genetic heterogeneity.

The effect of proband characteristics on risk to relatives

We also conducted a family history study to see whether certain proband characteristics were associated with an increased risk of PDD-like traits in second- and third-degree relatives. The lesser variant consists of individual PDD-like traits that are too mild to qualify for a specific PDD subtype. Examples of these have been operationalized and incorporated in a family history interview. Bolton et al[14] previously reported that the risk of the lesser variant was higher if the proband had a low verbal IQ than if the proband had a higher verbal IQ. We also conducted a family history study to identify second- and third-degree relatives with the lesser variant using parents, a maternal and paternal grandparent and a maternal and paternal uncle or aunt as informants. Multiple informants on both sides of the family were employed to improve the sensitivity of the family history interview. Data were collected by telephone or by questionnaire from relatives of 4 groups of families: multiplex families, families with a single affected male, families with a single affected female, and adopted or step-parent families (where the PDD proband was either adopted or had a step-parent). This allowed us to have a collection of parents who would be knowledgeable about PDD but were not biologically related to the proband.

All 3 components of the lesser variant were more common in biological second- and third-degree relatives of the proband than the nonbiologic relatives. For example, communication impairments occurred in 10.1% of biological parents and 1.8 % of nonbiologic parents. Similar differences were observed for repetitive activities (7.0% v. 2.7%) and social impairments (14.7% v. 7.5%). Thus, the lesser variant does appear to be a familial trait, although the relative risk is very much lower than for autism because these traits are common in the general population.

We then looked at the risk of the lesser variant to biological relatives as a function of proband characteristics: IQ, sex, PDD subtype (autism v. Asperger's syndrome or atypical autism) and genetic loading (that is, whether the proband came from a multiplex or simplex family). Both genetic loading and IQ of the proband influenced the risk of the lesser variant to relatives. When proband IQ was dichotomized at 60 (the approximate median

value) relatives of higher functioning probands had a higher risk of the lesser variant than relatives of lower functioning probands. Genetic loading also had an effect in that relatives of probands from multiplex families had a higher risk than those from simplex families.[41]

The fact that higher functioning probands carry a higher risk is inconsistent with a simple polygenic model, in which the risk of developing autism is simply a function of the number of autism genes a child inherits from both parents. In this context, lower functioning probands would be expected to have a higher "dose" of the autism genotype and so their relatives should be at higher risk of the lesser variant as well. Instead, our data suggest that it was the relatives of higher functioning probands (i.e., those with the lower "dose") that were at increased risk. These findings support the previous data on familial aggregation of level of functioning and indicate that IQ in the proband may be a marker of genetic heterogeneity. The higher functioning subtype is characterized by affected siblings with mild forms of PDD and relatives with the lesser variant. The lower functioning subtype is characterized by severely affected siblings and a paucity of affected relatives. What measure of severity and what threshold should be used to identify these subtypes, though, is unclear.

Implications for linkage analysis

To understand the implications of these findings for identifying susceptibility genes in autism, it is important to review the data on relative risk, because this is the critical determinant of the power to detect linkage. A reasonable estimate of the relative risk for autism to siblings is around 20. A reasonable estimate of the prevalence of other PDD subtypes might be 50 in 10, 000 children. With a sibling risk of 5%, this would mean that the relative risk of PDD to siblings is roughly 10. Components of the lesser variant are only 2 to 3 times more common in siblings of autistic probands than in the general population.[14] What is striking about these estimates is the extent to which the value of the relative risk drops as the phenotype is broadened. This is because the general population rates increase much more rapidly than the risk to siblings. Because it is likely that multiple interacting genes contribute to these relative risk estimates, the relative risk at each locus will be less, depending on its unique contribution. Including these broader phenotypes in linkage analysis will not, as a result, increase power to detect linkage at a specific locus.

There are some clinical data suggesting that the disorder is genetically heterogenous and that the genes for higher functioning autism may be different than those for lower functioning autism. Again, the relative risk estimates provided above do not take this heterogeneity into account and will further reduce the relative risk for PDD at a specific locus. The tables provided by Risch and Merikangas[27] for affected sib-pair and association studies suggest that the sample sizes needed to detect genes of this effect will be quite large, often beyond the resources of individual studies. This may make it very difficult to detect susceptibility genes, particularly if genome scans are used where there is 10 centimorgan distance between markers.

Currently, the most popular strategy to identify susceptibility genes in complex disorders is to recruit very large sample sizes and perform genome scans with very dense marker maps. The issue is whether this strategy is the most cost-effective given the state of the field. Two promising areas have recently been identified as important in the genetic etiology of autism: the serotonin transporter gene[42] and a region on chromosome 7.[43] However, the first finding has not been replicated[44] and the significant lod score result for the second was found in only a subset of families that did not appear to differ ethnically. We are still in the very early stages of looking for susceptibility genes in autism. Should resources be put into even larger sample sizes and more sophisticated automated technology or, instead, into obtaining multiple clinical measures to both reduce measurement error and identify more genetically homogeneous subgroups? The data needed to decide between these alternatives are unfortunately not available. Clearly, there is much more work that needs to be done at the simple clinical genetic epidemiologic level. After all, it was 20 years ago that autism was considered to be a nongenetic disorder. Progress requires patience, skepticism and an ear for clinical subtleties.

References

1. Hanson DR, Gottesman I. The genetics, if any, of infantile autism and childhood schizophrenia. *J Autism Dev Disord* 1976; 6:209-34.
2. Folstein S, Rutter M. Infantile autism: a genetic study of 21 twin pairs. *J Child Psychol Psychiatry* 1977;18:297-321.
3. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders, IV edition (DSM-IV).* Washington (DC): American Psychiatric Association; 1994.
4. *International classification of diseases, 10th edition.* Geneva: World Health Organization, 1992.

5. Tsai LY. Is Rett syndrome a subtype of pervasive developmental disorder? *J Autism Dev Disord* 1992;22:551-61.
6. Volkmar FR. Childhood disintegrative disorder: issues for DSM-IV. *J Autism Dev Disord* 1992;22:625-42.
7. Szatmari P.The validity of autistic spectrum disorders: a literature review. *J Autism Dev Disord* 1992;22:583-600.
8. Fombonne E. Epidemiological surveys of autism. In: Volkmar FCUP. *Monograph on autism.* New York: Cambridge University Press; 1998.
9. Bryson SE, Smith IM, Eastwood D. Obstetrical suboptimality in autistic children. *J Am Acad Child Adolesc* Psychiatry 1988; 27:418-22.
10. Ehlers S, Gillberg C. The epidemiology of Asperger syndrome: a total population study. *J Child Psychol Psychiatry* 1993;34:1327-50.
11. MacKinnon DF, Jamison KR, DePaulo JR. Genetics of manic depressive illness. *Ann Rev Neurosci* 1997;20:355-373.
12. Murphy KC, Cardno AG, McGuffin P. The molecular genetics of schizophrenia. *J Mol Neurosci* 1996;7:147-57.
13. Smalley SL, Collins F. Brief report: genetic, prenatal, and immunologic factors. *J Autism Dev Disord* 1996;26:195-8.
14. Bolton P, MacDonald H, Pickles A, Rios P, Goode S, Crowson M, et al. A case-control family history study of autism. *J Child Psychol Psychiatry* 1994;35:877-900.
15. DeLong RG, Dwyer JT. Correlation of family history with specific autistic subgroups: Asperger's syndrome and bipolar affective disease. *J Autism Dev Disord* 1988;18:593- 600.
16. Gillberg C, Gillberg IC, Steffenberg S. Siblings and parents of children with autism: a controlled population-based study. *Dev Med Child Neurol* 1992;34:389-98.
17. Szatmari P, Jones MB, Tuff L, Bartolucci G, Fisman S, Mahoney W. Lack of cognitive impairment in first-degree relatives of children with pervasive developmental disorders. *J Am Acad Child Adolesc Psychiatry* 1993;32:1264-73.
18. Jorde LB, Hasstedt SJ, Ritvo ER, Mason-Brothers A, Freeman BJ, Pingree C, et al. Complex segregation analysis of autism. *Am J Hum Genet* 1991 49:932-8.
19. Pickles A, Bolton P, Macdonald H, Bailey A, Le Couteur A, Sim C 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;57:717-26.
20. Szatmari P, Jones MB, Fisman S, Tuff L, Bartolucci G, Mahoney WJ, et al. Parents and collateral relatives of children with pervasive developmental disorders: a family history study. *Am J Med Genet* 1995;60:282-9.
21. Steffenburg S, Gillberg C, Hellgren L, Andersson L, Gillberg I, Jakobsson G et al.A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. *J Child Psychol Psychiatry* 1989; 30:405-16.
22. Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, et al. Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med* 1995;25:63- 77.
23. Ritvo ER, Freeman BJ, Mason-Brothers A, Mo A, Ritvo AM. Concordance for the syndrome of autism in 40 pairs of affected twins. *Am J Psychiatry* 1985;142:74-7.
24. Ott J. *Analysis of human genetic linkage, revised edition.* Baltimore (MD): Johns Hopkins University Press; 1991.
25. Faraone SV, Tsuang MT. *Methods in psychiatric genetics.* In: Tsuang MT, Tohen M, Zahner GEP, editors. Textbook in psychiatric epidemiology. New York: Wiley-Liss; 1995. p. 81-134.
26. Khoury MJ, Beatty TH, Cohen BH. *Fundamentals of genetic epidemiology.* New York: Oxford University Press; 1993.
27. Risch H, Merikangas KR. The future of genetic studies of complex human diseases. *Science* 1996;173:1516-7.
28. Schaid DJ, Sommer SS. Genotype relative risks: methods for design and analysis of candidate-gene association studies. *Am J Hum Genet* 1993;53:1114-26.
29. Risch N. Genetic linkage and complex diseases, with special reference to psychiatric disorders. *Genet Epidemiol* 1990;7:3-45.
30. Rutter M, Bailey A, Bolton P, Le Couteur A. Autism and known medical conditions: myth and substance. *J Child Psychol Psychiatry* 1994;35:311-22.
31. Tsai LY, Beisler JM. The development of sex differences in infantile autism. *Br J Psychiatry* 1983;42:373-8.
32. Lord C, Schopler E. Differences in sex ratios in autism as a function of measured intelligence. *J Autism Dev Disord* 1985; 15:185-193.
33. Volkmar FR, Szatmari P, Sparrow SS. Sex differences in pervasive developmental disorders. *J Autism Dev Disord* 1993;23: 579-91.
34. McLennan JD, Lord C, Schopler E. Sex differences in higher functioning people with autism. *J Autism Dev Disord* 1993;23: 217-27.
35. Narod SA, Ford D, Devilee P, Barkardottir RB, Lynch HT, Smith SA, et al. An evaluation of genetic heterogeneity in 145 breast-ovarian cancer families. *Am J Hum Genet* 1995:56:254-64.
36. St. George-Hyslop P, Haines J, Farrer LA, Polinsky R, Van Broeckhoven C, Goate A, et al. Genetic linkage studies suggest that Alzheimer's disease is not a single homogeneous disorder. *Nature* 1990;347:194-7.
37. Spiker D, Lotspeich L, Kraemer HC, Hallmayer J, McMahon W, Petersen PB, et al. Genetics of autism: characteristics of affected and unaffected children from 37 multiplex families. *Am J Med Genet* 1994;54:27-35.
38. Le Couteur A, Bailey A, Goode S, Pickles A, Robertson S, Gottesman I, et al. A broader phenotype of autism: the clinical spectrum in twins. *J Child Psychol Psychiatry* 1996 37:785-801.
39. Ritvo ER, Jorde LB, Mason-Brothers A, Freeman BJ, Pingree C, Jones MB, et al. The UCLA-University of Utah epidemiologic survey of autism: recurrence risk estimates and genetic counselling. *Am J Psychiatry* 1989;146:1032-6.
40. MacLean JE, Szatmari P, Jones MB, Bryson SE, Mahoney WJ, Bartolucci G, et al. Familial factors influence the severity of pervasive developmental disorders; evidence for possible genetic heterogeneity. *J Am Acad Child Adolesc Psychiatry.* In press.
41. Piven J, Palmer P, Jacobi D, Childress D, Arndt S. Broader autism phenotype: evidence from a family history study of multiple-incidence autism families. *Am J Psychiatry* 1997;154: 185-90.
42. Cook EH, Courchesne R, Lord C, Cox NJ, Yan S, Lincoln A, et al. Evidence of linkage between the serotonin transporster and autistic disorder. *Mol Psychiatry* 1997;2:247-50.
43. International Molecular Genetic Study of Autism Consortium. A full genome screen for autism with evidence for linkage to a region on chromosome 7q. *Hum Mol Genet* 1998;7(:3):571-8.
44. Klauck SM, Poutstka F, Benner A, Lesch KP, Poustka A. Serotonin transporter (5-HTT) gene variants associated with autism? *Hum Mol Genet* 1997;6(13):2233-8.