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CHAPTER 16

Genetic Influences and Autism

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When Kanner (1943) first described autism, he suggested that it resulted from an inborn defect of presumably constitutional origin. Nevertheless, over the next three decades, the possible role of genetic factors tended to be dismissed. In part, this was because the zeitgeist at that time was one of expecting environmental causes for all forms of psychopathology. This was the era of supposed "refrigerator" parents of autistic children and of "schizophrenogenic" mothers (see Rutter, 1999b). However, reviews by geneticists were equally dismissive (Hanson & Gottesman, 1976). The emphasis tended to be placed on the lack of vertical transmission (i.e., the rarity with which children with autism had parents with autism), the very low rate of autism in siblings, and the lack of identified chromosome anomalies associated with autism (Rutter, 1967).

QUANTITATIVE GENETICS

Twin Studies

An awareness that the logic of these arguments was faulty (Rutter, 1968) led Folstein and Rutter (1977a, 1977b) to undertake the first small scale ($N = 21$) twin study of autism. The earlier reasoning on genetic influences was false because follow-up studies had shown that few autistic people developed love relationships, and that it was very rare for them to have children, and hence vertical transmission would not be expected; also relative to the rate of autism in the general population (about 2 to 4 cases per 10,000 as defined at that time), the rate of autism in siblings (then estimated to be about 2%) was very high; furthermore, the cytogenetic techniques available in the 1960s were quite primitive so that the failure to show anomalies was essentially noncontributory.

There were two crucial findings from this first twin study. First, despite the small numbers, there was a significant monozygotic-dizygotic (MZ-DZ) difference in concordance. The fact that the population base rate of autism was so low implied a strong underlying genetic liability. Second, concordance within MZ pairs included a range of cognitive and social deficits and not just the seriously handicapping condition of autism itself. This implied that the genetic liability extended beyond autism proper. It also raised questions about the diagnostic boundaries of autism and led to an appreciation of a need to consider the likelihood of a broader phenotype of autism or of lesser variants of the same condition.

During the late 1980s and early 1990s, genetic research into autism advanced through further twin studies and genetic-family studies. Both the Scandinavian Twin Study (Steffenburg et al., 1989) and a further British twin study (Bailey et al., 1995) confirmed the great strength of genetic influences on the underlying liability for autism. The British study included four key design features. First, there was total population screening of the cases, with all clinics and special schools in the country contacted

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and all twin registers examined. Second, systematic standardized methods of diagnosis using both parental interviews (Le Couteur et al., 1989) and observation of the child (Lord et al., 1989) were employed. Third, there was thorough screening for medical conditions and chromosomal abnormalities in order to focus on the study of genetic factors in idiopathic autism. Fourth, blood groups were used to test for zygosity. That was important because the marked behavioral differences associated with autism sometimes led parents and professionals to infer that the twins were nonidentical, whereas in fact they were identical.

Four main findings were particularly crucial:

1. The huge disparity in concordance rates for autism between monozygotic (MZ; $N = 25$) and dizygotic (DZ; $N = 20$) pairs (60% versus 5%—if the rate in siblings as well as DZ twins is used to estimate the figure, twins and DZ twins being genetically comparable) confirmed the earlier findings on the strength of the genetic influence. Quantitative analyses indicated a heritability in excess of 90%.
2. The exceedingly low rate of concordance in DZ pairs compared with that in MZ pairs pointed to the likelihood of epistatic effects involving synergistic interaction among several genes. The pattern was not compatible with a single gene Mendelian disorder. The fall-off rate from MZ to DZ twins, together with that from first-degree to second-degree relatives, was used by Pickles and colleagues (1995) to estimate the number of genes that were likely to be involved. The logic of this analysis is based on the fact that whereas MZ twins share 100% of their genes, and 100% of all combinations of their genes, the situation is quite different in DZ twins and siblings. On average, they share 50% of their segregating genes but this means that they will share only a quarter of two gene combinations, and an eighth of three gene combinations. The findings from the analysis by Pickles and colleagues (2000) suggested that three or four genes were most probable, but that any number between 2 and 10 genes was a possibility (depending on the relative strength of the effect of any one of these genes). However, Risch et al. (1999) suggested that the number of susceptibility genes might be much higher than that. Uncertainties also remain on the likelihood that the susceptibility genes will involve relatively common allelic variations or rather rare variants reflecting a disease mutation (Pritchard, 2001).
3. The finding that the genetic liability for autism extended to include a broader phenotype was confirmed. Some 90% of MZ pairs were concordant for mixtures of social and cognitive deficits that were qualitatively similar to those found in traditional autism, but milder in degree (i.e., the broader phenotype). This applied, however, to only about 1 in 10 DZ pairs. Focusing on the 10 MZ and 20 DZ pairs' discordant for autism or autism-spectrum disorders, it was shown that there was a similar contrasting concordance for this broader phenotype, the difference being statistically significant (Bailey et al., 1995; Le Couteur et al., 1996). A follow-up of the original Folstein and Rutter sample also showed that this broader phenotype is associated with important deficits in social functioning that continued into adult life.
4. An examination of 16 MZ pairs concordant for autism or autism spectrum disorders showed that there was enormous clinical heterogeneity even when pairs shared exactly the same segregating genetic alleles. Surprisingly, individuals within MZ pairs were no more alike in IQ or symptomatology than were pairs selected at random from different twin pairs (Le Couteur et al., 1996).

Family Studies

Family-genetic studies were important in order to determine the rate of autism in siblings and in parents, to check family patterns of transmission in case there were single-gene Mendelian variants (this cannot be assessed from twin studies), and to better delineate the breadth and pattern of the possible broader phenotype. The Maudsley Hospital Study and the Johns Hopkins Study, initially planned together, provided the first systematic findings on sizable samples using standardized mea-

sures. Subsequent studies have added to the findings in important ways.

The Maudsley Hospital Family Study used measurement methods directly comparable with those in the British Twin Study and similarly excluded families in which the autism was associated with some known medical condition that was likely to be causal. The families of 99 individuals with autism were compared systematically with 36 families of individuals with Down syndrome using exactly the same methods of measurement (Bolton et al., 1994). There was direct assessment of all first-degree relatives, and systematic standardized reports on more distant relatives. The rate of autism in the siblings of autistic individuals was found to be 3%, with an additional 3% showing some form of, more broadly conceptualized, autism spectrum disorder. No cases of autism or autism spectrum disorder were found in the siblings of individuals with Down syndrome. As in the twin study, a broader phenotype of autism, comprising mixed patterns of cognitive and social deficits and repetitive stereotyped interest patterns, were even more frequent. Depending on how stringent a definition was used, the comparative rates of the broader phenotype as compared with Down syndrome families were about 12% versus 2%, or 20% versus 3%. The findings provided striking evidence that the broader phenotype might be much more common in the general population than previously considered.

Because some data on families (August, Stewart, & Tsai, 1981) had suggested that autism that was accompanied by profound mental retardation might be somewhat different from the rest of autism in which nonverbal IQs were above 50, the Maudsley Hospital group undertook a further family study to determine whether this might be the case (Pickles et al., 2000; Starr et al., 2001). However, in contrast with the earlier suggestion, the rate of autism and of the broader phenotype in the relatives was not significantly different from that found in the first family study. The only possible lead was the uncertain indication that cognitive problems in the relatives might be somewhat more common when autism was associated with profound retardation. What did emerge, from the combination of the two studies, was the finding that the linear association between severity of autism and the level of family loading seemed to apply only to cases of autism in which there was some useful speech (Pickles et al., 2000). The implication is that when autism is associated with a very severe lack of language skills, it might be genetically different in some way. However, this remains only as a possibility to be further tested, rather than as an established fact.

The Johns Hopkins study was particularly important because of its evidence on the probable importance of pragmatic language problems (Landa, Wzorek, Piven, Folstein, & Isaacs, 1991; Landa et al., 1992), of social abnormalities (Piven et al., 1990, 1991), and of unusual personality features (Piven et al., 1994). The early findings had particularly emphasized the familial loading of language delay but, although this seemed to be part of the overall picture in some cases, the later findings from all studies have suggested the probably greater importance of social deficits (Bailey, Palferman, Heavey, & Le Couteur, 1998; Folstein & Piven, 1991; Piven, Palmer, Jacobi, Childress, & Arndt, 1997; Rutter, Bailey, Simonoff, & Pickles, 1997).

The family study by Szatmari and colleagues (2000) differed from the others in its strategy of comparing biological and nonbiological relatives (the latter being represented by stepparents and adoptive families). The finding that social, communicative, and repetitive behaviors were all more common in biological relatives supported the inference that the familial loading reflected a genetically mediated liability. They also found that the rate of broader phenotype in relatives was higher in multiplex than simplex families and was greater when the proband with autism had an IQ above 60.

As well as language deficits in the relatives of individuals with autism, the early findings had suggested that there might also be a familial loading for reading and spelling difficulties. However, that now seems not to be the case. Fombonne, Bolton, Prior, Jordan, and Rutter (1997) undertook a detailed analysis of cognitive patterns in relatives in the Maudsley Family Study. The findings showed that neither low IQ nor specific problems in reading or spelling showed an increased loading in the families of individuals with autism if these

problems were not accompanied by other manifestations of the broader phenotype. In other words, although reading and spelling difficulties could constitute an important part of the broader phenotype, these specific cognitive deficits, when they occurred in isolation, did not seem to be indicators of a genetic liability to autism. Also, family data suggest that phonological processing deficits (in contrast to pragmatic deficits) are not part of the broad autism phenotype (Bishop et al., 2004). Similarly, language impairments do constitute an important part of the broader phenotype but ordinarily they do not appear to be genetically connected to autism if they occur without either social deficits or circumscribed interest patterns. Interestingly, both Fombonne et al. and Piven and Palmer (1997) found that the relatives of individuals with autism tended to show a cognitive pattern with verbal skills that were superior to visuospatial skills. This is the opposite of what is ordinarily found in autism. This unexpected finding requires confirmation from other studies but, if confirmed, other research will be required to determine what it means.

Over the years, more and more clinical features, particularly including affective disorder and social anxiety (DeLong & Nohria, 1994; Smalley, McCracken, & Tanguay, 1995), came to be added to possible variations of the broader phenotype. The key question was whether these affective features reflected the same genetic liability that underlies autism, or whether it reflected some other genetic or environmental mechanism. Bolton, Pickles, Murphy, and Rutter (1998) confirmed that the rates of clinically significant affective disorder were increased in the relatives of individuals with autism and Murphy et al. (2000), as in the Johns Hopkins study (Piven et al., 1994), found that the relatives of individuals with autism showed an increase in the traits of shyness and aloofness and also the traits of anxiety and oversensitivity. However, the meanings of these two sets of traits were somewhat different. The evidence suggested that shyness and aloofness were manifestations of the broader phenotype whereas anxiety and oversensitivity were related to anxiety or depressive disorders, rather than to the broader phenotype of autism. Depression was associated with depression in other family members but it was not associated, at either the individual or the family level, with cognitive or social deficits. The cause of the increased rate of depression in the families of individuals with autism remains unclear but it does not seem to reflect a genetic liability to autism.

Retrospective studies of individuals with schizophrenia at one time led to the claim that, although not diagnosed at the time in childhood, schizophrenia had been preceded by an autism spectrum disorder. However, prospective studies of individuals with autism have not found that autistic individuals have an increased rate of schizophrenia in adult life (Volkmar & Cohen, 1991). Family studies, similarly, have shown no increase in the familial loading for schizophrenia in the relatives of individuals with an autism spectrum disorder (Bolton et al., 1998). It is possible, however, that obsessive-compulsive disorder, which is somewhat more frequent in the relatives of individuals with autism, may index an underlying genetic liability to autism (Bolton et al., 1998).

The evidence on the reality, and relative frequency, of the broader phenotype of autism has been well demonstrated in numerous studies and the concept is no longer controversial. Nevertheless, the precise boundaries of the broader phenotype have yet to be established and clear-cut criteria for differentiating the broader phenotype of autism from the many other varieties of social deficit have yet to be determined. Similarly, although it is apparent that the broader phenotype occurs in at least 10% to 20% of the first-degree relatives of individuals with autism, it is not yet known whether it constitutes a common, but still a qualitatively distinct, category, or whether, instead, it constitutes a continuously distributed dimension.

There are two crucial differences, too, between the broader phenotype and autism as traditionally diagnosed. Unlike autism, the broader phenotype is not associated with mental retardation, and it is not associated with epilepsy. As yet, we do not know why that is so. Questions arise as to whether the broader phenotype represents a lesser "dose" of genetic liability, a different pattern of susceptibility genes, or some kind of "two-hit" mechanism in which an additional risk factor is required to take individuals over the threshold from the

broader phenotype into a more seriously handicapping disorder.

CHROMOSOMAL ABNORMALITIES AND GENETICALLY DETERMINED MEDICAL CONDITIONS

During the 1970s and 1980s, there was a range of medical studies of autism demonstrating positive findings (Rutter, 1999a). Gillberg (1992) claimed that 37% of cases of autism were associated with a diagnosable medical condition and, on this basis, argued that a wide range of intrusive medical investigations (including lumbar puncture, EEG, brain imaging, and metabolic studies) should be undertaken as a routine (Gillberg, 1990; Gillberg & Coleman, 1996). However, Rutter and colleagues (Rutter, Bailey, Bolton, & Le Couteur, 1994), putting together the evidence from several studies, concluded that the rate was probably more like 10%. Nevertheless, this figure is certainly sufficiently high to mean that all individuals suspected of having an autism spectrum disorder should have a careful medical assessment. The question of whether they should have invasive investigations is a rather separate matter. The key issue is how often the investigations lead to a diagnosis that cannot be obtained more straightforwardly through clinical history and examination. The answer seems to be that it is rare for the tests to reveal undiagnosed medical conditions; many of the supposedly abnormal laboratory findings have no unambiguous clinical implications; and the clinical value seems so slight as not to justify the distress inevitably caused to young children if such investigations are routinely undertaken. Rather, the recommendation is that a thorough clinical history and examination should be routine and that the decision as to whether to undertake systematic laboratory investigations should be decided on the basis of the clinical findings. Further investigations will be mandatory in some cases but they should not be undertaken routinely in a mindless fashion that ignores the need for clinical decision making.

Similar issues have arisen with respect to chromosomal abnormalities. Thus, the early reports on the fragile X anomaly led to claims that this was a very common cause of autism (Gillberg & Wahlstrom, 1985). Moreover, initial findings were often presented as providing a "minimum figure," failing to appreciate the dangers of relying on findings based on small samples. The ratio of false positives to true positives in a small sample is necessarily much greater than that in a large sample and the size of a difference between groups is no guide to the true strength of the association. To achieve statistical significance, the difference in a small sample is bound to be a large one, and the true difference will almost certainly be very much smaller (see Cohen, Cohen, & Brook, 1995; Pocock, 1983). With respect to the fragile X anomaly, there was the additional concern that, during the 1980s, this had to be diagnosed using problematic cell culture methods. The fragile X anomaly was initially often diagnosed on the basis of only 1% to 3% of X chromosomes showing a fragile site. Once DNA techniques were available, it became clear that this was an unwarranted inference (Gurling, Bolton, Vincent, Melmer, & Rutter, 1997). Available estimates indicate that fragile X anomalies are present in less than 5% of individuals with autism (Bailey et al., 1993; Dykens & Volkmar, 1997). This is still a meaningful association, the rate of which is well above that expected on the basis of the rates of both in the general population. In other words, it seems reasonable to assume that the fragile X anomaly can play a causal role in the etiology of autism, even though it does so only in a small proportion of cases. Nevertheless, it should be noted that although the fragile X anomaly constitutes an uncommon cause of autism, social abnormalities (of a kind that includes, but is not confined to autism) are frequently present in individuals with the fragile X anomaly (Reiss & Dant, 2003).

There are individual case reports of associations between autism and anomalies of one sort or another on almost all chromosomes (Gillberg, 1998; Yu et al., 2002); most of these are based on single cases, but a few have been replicated, and the meaning of many of the anomalies is quite unknown. Nevertheless, there is consistent evidence of chromosome 15 anomalies associated with autism (Buxbaum et al., 2002; Folstein & Rosen-Sheidley, 2001; Kim et al., 2002; Nurmi et al., 2003; Shao et al., 2003). Most involve interstitial duplications of maternal origin; the parental effect

suggests the involvement of genomic imprinting. The mechanisms underlying the association between chromosome 15 anomalies and autism remain unclear but the reality of the association appears well established.

With respect to medical conditions, the association with autism is most firmly established in the case of tuberous sclerosis (Smalley, 1998). One percent to 4% of individuals with autism have tuberous sclerosis and the rate may be as high as 8% to 14% among the subgroup of those with a seizure disorder. Tuberous sclerosis is a wholly genetic Mendelian disorder associated with a gene on either chromosome 9 or chromosome 16. In about two-thirds of cases the mutations arise de novo, rather than being inherited. Opinions differ on whether an abnormal tuberous sclerosis gene directly influences the development of autism, or whether the association with autism comes about because a susceptibility gene for autism lies in close proximity to a tuberous sclerosis gene, or because the brain abnormalities resulting from tuberous sclerosis predispose toward autism. The last possibility is suggested by the evidence that the occurrence of autism is, in part, a function of epilepsy and low IQ, and in part a function of the size and location of the tubers occurring in the brain (Bolton & Griffiths, 1997; Bolton, Park, Higgins, Griffiths, & Pickles, 2002; Lauritsen & Ewald, 2001; Weber, Egelhoff, McKellop, & Franz, 2000).

It has also been claimed that autism is associated with neurofibromatosis (Gillberg & Forsell, 1984) but the reality and strength of the association remains somewhat uncertain (Folstein & Rosen-Sheidley, 2001). Early reports had suggested that autism might be associated with phenylketonuria, but it is not clear whether this was an important association because the findings on autism were not based on standardized measurement (Folstein & Rutter, 1988). In any case, it is no longer a relevant issue in most developed countries because newborn screening has meant that untreated phenylketonuria is extremely rare.

MOLECULAR GENETICS

Over the past dozen years or so, there have been important technical advances that have facilitated the search for susceptibility genes for psychiatric disorders, as well as for other multifactorial conditions (Maestrini, Marlow, Weeks, & Monaco, 1998; Rutter, 2000; Rutter, Silberg, O'Connor, & Simonoff, 1999). Most particularly, the combination of the development of robotic techniques and the availability of a very large number of polymorphic micro-satellite and single nucleotide genetic markers has made a total scan of the genome a practical possibility. In essence, there are two main approaches that may be followed—namely, linkage and association strategies. They work on a somewhat different principle and they involve a different mix of advantages and disadvantages.

Linkage studies examine co-inheritance—meaning inheritance within families in which there is a linkage between the gene locus being studied and the condition being investigated. Traditionally, this approach was mainly applied to very large families involving many individuals who were affected with the condition being studied. However, the approach required specification of the mode of inheritance involved and that is not known in the case of autism, any more than it is with most psychiatric conditions. Also, as a consequence of the relatively low frequency of autism, the collection of sizable samples of extended families with many affected members is not a practical proposition. A further disadvantage of the traditional family approach was that it was unclear how to deal with cases in which the diagnostic status was uncertain. Because of these features, most research groups have switched to the study of affected sib pairs, or affected relative pairs (Rutter, Silberg, et al., 1999). The strategy requires the collection of samples in which there are two or more affected individuals in the same family and it is necessary only to concentrate on those for whom the diagnosis is unambiguous. The analysis then determines whether the co-occurrence of particular gene loci coincides with the diagnosis to an extent that exceeds chance expectations. With this approach, it is not necessary to specify in advance where the locus is expected to be. Rather, there can be a scanning of the whole genome, using markers that are sufficiently close to one another to ensure that if there is a relevant susceptibility gene locus it will be picked up.

The strategy (which can be applied to large pedigrees as well as affected relative pairs; Davis, Schroeder, Goldin, & Weeks, 1996) has the huge advantage of involving a minimum of untestable assumptions but it has two considerable disadvantages. First, it will detect gene loci only when there are relatively strong effects. Second, even when a likely gene locus has been identified, the area of the chromosome within which the gene should be found in a complex genetic disorder is very large. This means that the area will include a very large number of possible genes. Accordingly, it will usually be necessary to combine linkage studies with association strategies.

Association studies are based on the quite different strategy of using linkage disequilibrium to search for differences between cases and controls in allelic patterns. They have the advantage of being better able than linkage strategies to detect very small genetic effects (Risch & Merikangas, 1996). The concern with association strategies derives from the fact that stratification bias may arise because cases and controls differ in their allelic patterns as a result of their ethnic origins, rather than for any reason to do with the disorder being studied. Opinions differ as to how big a problem this is if major ethnic differences have been taken into account (Ardlie, Lunetta, & Seielstad, 2002; Cardon & Palmer, 2003). Nevertheless, it is desirable to control for stratification biases and this is possible through the transmission disequilibrium test (TDT), which also tests for both association and linkage (Malhotra & Goldman, 1999; Spielman & Ewens, 1996). Ordinarily, it requires DNA samples on trios—meaning an affected child and both parents. A further limitation of association strategies, at least up to now, has been the fact that they rely on the availability of candidate genes, which are singularly lacking in the case of autism. It has been claimed that the technique of DNA pooling (in other words, combining DNA samples across cases and similarly across controls) provides a possible way forward (Barcellos et al., 1997; Daniels et al., 1998). There are three possible difficulties with the use of DNA pooling. First, the number of markers required would be very much greater than in linkage studies because the association strategies can produce positive findings only in relation to a susceptibility gene that is very close to the markers used, or is the trait marker itself. Second, there are statistical problems in determining the significance of case control differences when a very large number of gene markers have to be tested. Third, the pooling of all cases means that the groups are made up on the basis of the assumption that the susceptibility gene concerns the disorder, rather than the components of the disorder (see the discussion that follows).

Single Gene Major Mutations: The Case of Rett Syndrome

Rett syndrome is a progressive neurodevelopmental disorder with an incidence of about 1 in 10,000 in girls (Hagberg, 1985; Hagberg, Aircardi, Dias, & Ramos, 1983). Classically, the girls develop normally until 6 to 18 months of age, then gradually lose speech and purposeful hand use, developing microcephaly, seizures, social impairment, ataxia, intermittent hyperventilation, and stereotypic hand movements. Witt-Engerström and Gillberg (1987) noted that the majority of cases of Rett syndrome were initially suspected of having autism because of the social impairment. Gillberg (1989) went on to argue that the apparent symptomatic similarities might mirror common pathophysiological abnormalities at the brainstem level. However, although Rett syndrome may, in the past, have been misdiagnosed as autism, the social features of Rett syndrome and autism are somewhat different (Olsson & Rett, 1985, 1987). In 1999, Amir and colleagues showed, using a systematic gene screening method, that a mutation in the MECP2 gene was the cause of many cases of Rett syndrome. This has now been confirmed by numerous other investigators. Most crucially, it has been shown that mouse models of the MECP2 mutation causes a neurobehavioral syndrome that is closely similar to that found in humans (Guy, Hendrich, Holmes, Martin, & Bird, 2001; Shahbazian et al., 2002). The evidence is clear-cut that the genetic mutation constitutes a single gene disorder with the genetic abnormality providing a sufficient explanation for the clinical syndrome, without the need to invoke any other genetic or environmental factors.

However, as is usual with single gene disorders, it has been found that there are several different MECP2 mutations that have generally similar phenotypic consequences (Amir et al., 2000). Also, as is commonly the case with single gene mutations, it has been found that the clinical picture is more varied than was at first considered to be the case (Shahbazian et al., 2002). Because of this, several research groups have studied samples of patients with autism in order to determine whether mutations in the MECP2 gene might be present. Two of the studies (Beyer et al., 2002; Vourc'h et al., 2001) concluded that mutations in the coding region of MECP2 did not play a major role in autism susceptibility. However, a third study (Carney et al., 2003) reported that 2 out of 69 cases of autism did show the MECP2 mutation. Apparently, the affected individuals did not show an overall syndrome that was similar to Rett syndrome (although they did have some Rett syndrome features). The balance of evidence suggests that the MECP2 mutation invariably leads to a serious neurodevelopmental disorder, that in the great majority of cases this approximates to the clinical picture of Rett syndrome, but occasionally (but not often) the clinical picture may be somewhat different and, perhaps, uncommonly it may take the form of autism. It seems unlikely, however, that the MECP2 mutation has any broader significance in relation to autism. However, Zoghbi (2003) has argued that both autism and Rett syndrome could turn out to be similar disorders of synaptic modulation or maintenance.

Genome-Wide Screens of Sib Pair Samples

The discovery of the MECP2 mutation was important, but the evidence that autism is a multifactorial disorder and that multiple susceptibility genes are likely to be operative implies that it should be expected that, in most instances, these genes would be normal, common, allelic variations rather than rare pathological mutations, although the latter might also contribute to the liability (Pritchard, 2001; Rutter, 2004). Genome-wide scans of sib pair samples in order to detect susceptibility gene loci have been published by eight different groups (Auranen et al., 2002, 2003; Barrett et al., 1999; Buxbaum et al., 2001; Collaborative Linkage Study of Autism [CLSA], 1999; International Molecular Genetic Study of Autism Consortium [IMGSAC], 1998, 2001b; Liu et al., 2001; Philippe et al., 1999; Risch et al., 1999; Shao, Raiford, et al., 2002; Shao, Wolpert, et al., 2002; Yonan et al., 2003), of which the first was the International Molecular Genetic Study of Autism Consortium (1998, 2001b). The findings have been reviewed by Folstein and Rosen-Sheidley (2001), Gutknecht (2001), and Lamb, Moore, Bailey, and Monaco (2000; Lamb, Parr, Bailey, & Monaco, 2002). A locus on chromosome 7q has been found in four of the studies (IMGSAC, 1998, 2001a), the collaborative linkage study of autism—CLSA (1999; Ashley-Koch et al., 1999; Philippe et al., 1999; Shao, Wolpert, et al., 2002), and confirmed in a meta-analysis (Badner & Gershon, 2002). It is undoubtedly encouraging that several different groups have come up with comparable findings regarding a locus on chromosome 7. However, the peak of linkage is quite wide and the precise location has not been identical in all the studies. This is not necessarily a concern because simulation studies have shown that there is a large variation in peak location relative to actual disease-gene location (Roberts, MacLean, Neale, Eaves, & Kendler, 1999), the strength of the finding varies across studies. The postulated locus on chromosome 7q includes several key candidate genes. Thus, it is close to the location of the FOXP2 gene, which has been shown to be responsible for an unusual familial speech and language disorder (Lai, Fisher, Hurst, Vargha-Khadem, & Monaco, 2001). It was suggested that autism and severe language impairment might derive from the same gene in this area (Folstein & Mankoski, 2000) but the available evidence suggests that this is not likely to be the case (Newbury et al., 2002). The human reelin gene also maps to an area on 7q that is close to the peak of linkage found in relation to autism. The reelin protein plays an important role in neuronal migration during brain development and, hence, constitutes a plausible candidate gene in respect of autism. However, the evidence to date suggests that it probably does not play a major

role in autism etiology (Bonora et al., 2003), although it is too early to conclude that it plays no role.

The strongest evidence for linkage in the International Molecular Genetic Study of Autism Consortium findings (IMGSAC, 2001b) was a susceptibility region on chromosome 2q. This was also the case in the findings of the Buxbaum and colleagues (2001) group, and of the Shao and colleagues group (Shao, Raiford, et al., 2002; Shao, Wolpert, et al., 2002). Again, the region of chromosome 2q includes a number of interesting genes that are potential candidates for a role in the etiology of autism.

Other positive linkage findings have been reported for chromosome 16p (IMGSAC, 2001b; Liu et al., 2001), chromosome 1p (Risch et al., 1999), chromosome 3 (Auranen et al., 2002, 2003; Shao, Raiford, et al., 2002; Shao, Wolpert, et al., 2002), chromosome 13q (CLSA, 1999), 5q (Liu et al., 2001), 5p (Yonan et al., 2003), 17q (IMGSAC, 2001b; Yonan et al., 2003), 19p and 19q (Liu et al., 2001), and Xq (Shao, Raiford, et al., 2002; Shao, Wolpert, et al., 2002).

Candidate Gene Strategies

The literature contains several positive findings with respect to candidate genes (Folstein & Rosen-Sheidley, 2001). They had been promising, for a variety of good reasons—including their role in neurotransmitters (such as serotonin, dopamine, and glutamate), because of connections with chromosome anomalies associated with autism, or because of their proximity to the locations derived from the genome screen linkage findings. However, the findings so far are contradictory and inconclusive with many failures to replicate. Although it is too early to rule out any of the candidate gene possibilities, the evidence that they do have an actual role in the liability to autism is so far unconvincing. A possible exception to the generally negative story may be provided by the report that mutations in two X-linked genes coding the neuroligands NLGN3 and LNNLGN4 are associated with autism spectrum disorders (Jamain, Betancur, et al., 2002). However, this finding has yet to be replicated.

Recently, two further claims have been made with regard to possible susceptibility genes for autism. Gharani et al. (2004), using a family-based association method with the Autism Genetic Resource Exchange (AGRE), found a significant association with two intronic markers of a cerebella patterning gene located on chromosome 7 (but no association with the flanking exons). Ramoz et al. (2004), using a partially overlapping data set, found association with common intronic polymorphisms in a gene located on chromosome 2 that is involved with mitochondrial aspartate/glutamate function. Both findings require replication before conclusions can be drawn, but it is of interest that the postulated genetic variants are common and that they are concerned with regulating functions rather than the production of polypeptides as such.

One of the striking findings with respect to autism concerns the marked male preponderance. Not surprisingly, therefore, attention has focused on the X chromosome in order to determine whether X linkage might be responsible. However, with the exception of Shao and colleagues (Shao, Raiford, et al., 2002; Shao, Wolpert, et al., 2002), the findings have been generally negative (Hallmayer et al., 1996; Yirmiya et al., 2002) and the evidence of father to son transmission of autism (which could not involve the X chromosome) indicates that it is unlikely that X linkage is responsible for the overall male preponderance (Hallmayer et al., 1996). Findings for the Y chromosome have also been negative (Jamain, Quach, et al., 2002). Skuse and his colleagues (Skuse, 2000; Skuse et al., 1997) put forward a somewhat different hypothesis. They found that in subjects with Turner syndrome (who have an XO karyotype), social interaction difficulties were much more prevalent in those who inherited the X chromosome from the mother as compared with those who inherited it from the father. It was argued that there might be an imprinted locus on the X chromosome that might serve to make males more susceptible because their X chromosome would have to have been inherited from the mother. By contrast, females would have an X chromosome from each parent, that from the father possibly providing some protection from a genetic liability to autism that derived from some other (presumably autosomal) locus. The hypothesis is intriguing and it has a certain amount of support

within Skuse's Turner syndrome data set (Skuse, 2003). However, despite the fact that the hypothesis was first put forward over half a dozen years ago, no replications have been reported, apart from a single case report (Donnelly et al., 2000), which inevitably raises questions about the original findings.

EPIDEMIOLOGICAL FINDINGS

One of the most striking features of the findings on the rate of autism spectrum disorders in the general population is the enormous increase that has taken place over the past half century (Fombonne, 1999, 2003; Rutter, in press). The first study by Lotter (1966) suggested a rate of about four cases per 10,000. This contrasts starkly with the estimates from the best recent studies of between 30 and 60 cases per 10,000 (Baird et al., 2000; Chakrabarti & Fombonne, 2001). It is clear that this increase is largely a function of better ascertainment combined with a considerable broadening of the diagnostic criteria. Thus, for example, in recent studies, a high proportion of the children with autism spectrum disorders had a normal level of measured intelligence, whereas that applied to only a small minority in the early studies. However, whether or not this constitutes the whole story remains quite uncertain (Bock & Goode, 2003). It is possible that there has been a real rise in the incidence of autism and the available data neither confirm nor disconfirm the suggestion. If there has been a true rise, it would certainly have to involve the operation of environmental risk factors of some kind.

NONGENETIC RISK FACTORS

Monozygotic Twinning as a Risk Factor

There tends to be an assumption that the nongenetic factors involved in the etiology of autism must involve specific environmental risks. However, that is not necessarily the case (Jensen, 1997; Molenaar, Boomsma, & Dolan, 1993). Greenberg and colleagues (Greenberg, Hodge, Sowinski, & Nicoll, 2001), and also Betancur and colleagues (Betancur, Leboyer, & Gillberg, 2002) reported an apparent excess of twins among affected sibling pairs with autism (but see Hodge, Greenberg, Betancur, & Gillberg, 2002; Visscher, 2002). If this finding were valid, it would suggest that being a twin constitutes a risk factor for autism. If that were the case, it might either reflect obstetric complications or the effect of developmental perturbations. Congenital anomalies have been found to be more common in individuals with autism and these probably index the ways in which development, which is probabilistically rather that deterministically programmed, may go awry (Vogel & Motulsky, 1997). Thus, congenital anomalies are more common in twins than in singletons and are more common in children born to older mothers (Myrianthopoulos & Melnick, 1977; Rutter et al., 1990). Thus, it could be that developmental perturbations enhance the adverse effects of a genetic liability to autism. However, it should be noted that congenital anomalies show an increased rate in a wide range of psychiatric disorders. Also, some skepticism is necessary with respect to the supposed finding that the rate of twinning is increased in autism. Ascertainment biases are likely to have played a major role and it is noteworthy that the most systematically ascertained twin sample of Bailey and colleagues (1995) did not include a significant excess of monozygotic twins; nor did Hallmayer and colleagues' (2002) Australian twin sample. It may be concluded that more evidence is needed on the postulated increased risk for autism associated with being a twin but, on the evidence available to date, it is not likely that being a monozygotic twin is a major risk factor.

Obstetric Complications

Numerous studies have shown a significant association between autism and obstetric complications (Tsai, 1987). Three very different hypotheses may be put forward to account for the finding: (1) It could be a secondary consequence of birth order effects; (2) it could reflect brain damage brought about by obstetric complications, however caused; or (3) it could reflect an epiphenomenon in which the obstetric complications derive from the presence of a genetically abnormal fetus. These possibilities were systematically examined by Bolton and colleagues (1997), who concluded that it was unlikely that the association reflected en-

vironmentally caused brain damage (because, among other things, the main excess of complications were mild, rather than severe), and that, rather, either the obstetric adversities represented an epiphenomenon or derived from some shared risk factors.

Measles-Mumps-Rubella Vaccination

In 1998, on the basis of totally inadequate evidence, Wakefield and colleagues suggested that autism might be caused by adverse effects stemming from measles-mumps-rubella (MMR) vaccination. The suggestion arose because of an observed temporal association between the timing of the MMR vaccination and the timing of the first manifestations of autism. Such an association would, of course, be expected by chance alone because the first manifestations are usually evident at about the age when it is recommended that MMR be given. Subsequently, it was suggested that the MMR vaccination was responsible for the major rise in the incidence of diagnosed autism that has occurred over time. The hypothesis has been examined in a number of different ways, all of which have produced findings that run counter to the hypothesis (Rutter, in press). First, the close temporal association has not been confirmed (Farrington, Miller, & Taylor, 2001; Taylor et al., 1999) and the rise in the rate of autism does not follow the pattern that would be expected on the basis of the MMR effect. Thus, the beginning of the rise began before the introduction of MMR; there was no stepwise increase in the rate of autism following the introduction of MMR; and the rate did not plateau during the period when MMR vaccination rates were both high and stable (Dales, Hammer, & Smith, 2001; Hillman, Kanafani, Takahashi, & Miles, 2000; Kaye, Melero-Montes, & Jick, 2001). Most crucially, too, the rate of autism in Japan continued to rise after MMR ceased to be used (Honda, Shimizu, & Rutter, in press). Case-control comparisons have been similarly negative (K. M. Madsen et al., 2002). It had been further suggested that the autism associated with MMR usually involved developmental regression and was accompanied by bowel abnormalities (Wakefield et al., 1998). However, the evidence runs counter to these suggestions (Fombonne & Chakrabarti, 2001; Taylor et al., 2002; Uchiyama, Kurosawa, & Inaba, submitted). It may be concluded that it is quite implausible that MMR is generally associated with a substantially increased risk for autism. It is not possible to rule out the possibility that there may be occasional idiosyncratic responses to MMR that involve autism, but there is no good evidence that this happens.

Somewhat similar concerns have been expressed with respect to the possibility that Thimerosal, a vaccine preservative that contains ethyl mercury, might cause autism (Bernard, Enayati, Redwood, Roger, & Binstock, 2001). The question has biological plausibility in that it is known that mercury, in high dosage, can cause neurodevelopmental sequelae (Clarkson, 1997; Stratton, Gable, & McCormick, 2001). The opportunity to test this hypothesis arose in Denmark, where, from 1970 onward, the only Thimerosal-containing vaccine was the whole cell pertussis vaccine. Between April 1992, and January 1997, the same vaccine was used but without Thimerosal, and the vaccine was then replaced by an acellular pertussis vaccine. Data from the Danish Psychiatric Central Register was used to compare the rate of autism and autism-spectrum disorders in individuals who received only Thimerosal-free vaccinations and those containing Thimerosal. The Danish civil registration system allowed identification of the vaccinations used in each child and the number of doses given (thereby allowing calculation of the total Thimerosal dosage received). No difference in the rate of autism spectrum disorders was found between the groups that differed with respect to receipt of Thimerosal (Hviid, Stellfeld, Wohlfahrt, & Melbye, 2003). The causal hypothesis could also be tested by looking at time trends in the incidence of autism among children between 2 and 10 years of age, both before and after removal of Thimerosal from vaccines (K. E. Madsen et al., 2003). The findings showed that the discontinuation of Thimerosal-containing vaccines in 1992 was followed by an increase in the incidence of autism and not the predicted decrease (see Rutter, in press). The natural experiment provided by the removal of the postulated risk factor (Thimerosal) provided a good opportunity to

test the causal hypothesis, with findings that were completely negative. The two-phase retrospective cohort study by Verstraeten et al. (2003), undertaken using a very large health records database in the United States similarly found no significant increased risk for autism associated with Thimerosal usage. There is no reason to suppose that Thimerosal is likely to be a general risk factor for autism spectrum disorders and certainly it cannot account for the rise in the rate of diagnosed autism in Denmark, as found also in other countries. As with MMR, the data do not allow testing of the different hypothesis of a rare, unusual, idiosyncratic response to Thimerosal in individual children, although there is no available evidence to indicate that such a response actually occurs. Moreover, the available evidence suggests that vaccines containing Thiomersal do not seem to raise blood concentration of mercury above safety levels (Pichichero, Cernichiari, Lopreiato, & Treanor, 2002), although this conclusion must be tentative in view of the paucity of evidence on what is a safe level.

Other Environmental Factors

Case reports and small scale studies have suggested a range of possible other environmental risk factors for autism (see Folstein & Rosen-Sheidley, 2001; Medical Research Council [MRC], 2001; Nelson, 1991; Rodier & Hyman, 1998). These include maternal hypothyroidisim, congenital hypothyroidism, maternal thalidomide use, maternal valproic acid use, maternal cocaine or alcohol use, and congenital cytomegalovirus infection. It is quite possible that these factors play at least a contributory causal role in individual cases but it seems unlikely that they constitute commonly operating risk factors for autism.

Probably the best evidence is that concerned with a possible causal link between congenital rubella and autism (Chess, 1977; Chess, Kern, & Fernandez, 1971). The findings derive from a systematically studied large sample of children with congenital rubella and the observations have been supported by other investigators. However, it is noteworthy that the follow-up showed that the course of apparent autism in these children tended, on the whole, to be rather different from that associated with idiopathic autism. In particular, although the children remained markedly handicapped, the autistic features diminished as they grew older. In any case, the findings are of very limited contemporary relevance in view of the rarity of congenital rubella following vaccination programs.

Phenocopies

Over the past decade or so, evidence has accumulated on the existence of what appear to be phenocopies—meaning clinical features that look somewhat like autism but are not due to the same genetic liability. Thus, atypical syndromes of autism have been found to be associated with congenital blindness (Brown, Hobson, & Lee, 1997), with profound institutional privation (Rutter, Anderson-Wood, et al., 1999), and with a mixed bag of medical conditions all with profound mental retardation (Rutter et al., 1994). In each case, the clinical pictures are somewhat atypical and the implication is that the syndromes do not involve the same genetic liability that applies to ordinary idiopathic autism. However, although it seems highly likely that these atypical syndromes are distinct from the more usual varieties of idiopathic autism, that remains an inference rather than an established fact.

A further possible phenocopy concerns the marked social impairments that are associated with many cases of the severe developmental disorders of receptive language (Clegg, Hollis, Mawhood, & Rutter, in press; Howlin, Mawhood, & Rutter, 2000). Although the social impairments found in association with these severe cases of language delay differ from the syndrome of autism in many respects, it is nevertheless striking that the marked difficulties in social functioning persist into mid-adult life and are accompanied by considerable impairment. It is also noteworthy that there was a significant impairment on theory of mind tasks, albeit, not as severe as usually associated with autism.

GENETIC PARTITIONING OF AUTISM

Genetic findings throughout internal medicine have made it clear that, whether dealing with single gene conditions or multifactorial disorders, genetic heterogeneity must be expected. Accordingly, there have been various attempts

to determine whether such heterogeneity can be indexed by clinical features.

Familial Clustering

Familial clustering could provide important clues in this connection. Le Couteur and colleagues (1996) used the strategy of comparing phenotypic variations within and between monozygotic pairs to examine the question. It was argued that variation within each monozygotic pair could not index genetic heterogeneity because both twins must share all of the same genes. By contrast, there is every reason to suppose that different pairs of monozygotic twins will vary genetically as much as any other population of individuals with autism. Variation within real monozygotic pairs was compared with the variation within pairs created statistically by having the pair made up of one twin from one pair and one twin from another, different, pair. The findings were striking, and surprising, in showing that, within the monozygotic pairs that were concordant for autism, there was as much variation in symptom severity and pattern and in cognitive level as that found within these pseudo-pairs that had been created statistically. For example, in one true monozygotic pair there was an IQ difference of over 50 points. It was evident that, even when the susceptibility genes were exactly the same, very wide phenotypic variation was still possible. The findings provided few clues on possible clinical indices of genetic heterogeneity.

Inevitably, the sample size was small and the same issues can be examined on much larger numbers by using affected sib pairs. Although it cannot necessarily be assumed that the susceptibility genes for autism will be the same in two pairs of siblings (whereas that could be assumed with monozygotic twin pairs), it is certainly likely that the genetic heterogeneity within affected sib pairs would be substantially less than that in the population as a whole. Relevant studies have been undertaken by Spiker and colleagues (1994), Silverman and colleagues (2002), and IMGSAC (Bailey, personal communication, 2004). The familial question was also studied by Szatmari and colleagues (1996, 2000). In the IMGSAC study, the strongest indication of familial clustering was for epilepsy; although the sample size was very small, this also seemed a possibility in the Le Couteur and colleagues (1996) monozygotic twins study. However, there needs to be caution in the interpretation of this finding because of the peak age of onset of epilepsy in individuals with autism being late adolescence. This means, inevitably, that many younger children will be misclassified as not having epilepsy when, in reality, they are due to develop epilepsy when older. The similarity in age within pairs will influence clustering for epilepsy much more than it will for features that are manifest from the preschool years onward.

Epilepsy aside, the evidence on familial clustering has not been particularly informative on possible indices of genetic heterogeneity. The IMGSAC study (Bailey, personal communication, 2004) found no clustering for the degree of language delay (but this finding is inevitably influenced by inclusion/exclusion criteria used in the study). Nevertheless, the lack of clustering does cast doubt on any hypothesis that autism and Asperger syndrome are genetically distinct (because the diagnostic criteria usually employed specify a lack of significant language delay for the diagnosis of Asperger syndrome). However, although language delay as such does not seem to show any marked familial clustering, the sib pair studies have shown a significant (although not marked) tendency for sibs in each pair to be more similar on their degree of abnormalities on social reciprocity, communication, and repetitive behavior than are unrelated individuals with autism. Both verbal and performance IQ measures similarly showed some familial aggregation in the IMGSAC study (Bailey, personal communication, 2004), although not in the Spiker et al. (1994) study. The findings on repetitive stereotyped behavior were, however, somewhat different in that familial aggregation was not influenced by IQ or language-related measures whereas the other features were influenced by language levels.

In all the studies, the cases included have had to meet specified diagnostic criteria for autism or for a broader concept of autism spectrum disorder. Frequently, too, there has been exclusion of individuals with profound mental retardation. There are very good practical reasons for molecular genetic studies to adopt rigorous standardized diagnostic criteria for inclusion and exclusion but it is important

to appreciate that this will have implications for findings on familial clustering. Thus, for example, if the genetic liability to autism represents a continuously distributed risk dimension, familial clustering would be more appropriately examined without exclusions. Equally, if autism associated with profound mental retardation is genetically different, familial clustering is likely to show this only if cases of autism across the whole IQ distribution are included. For the moment, it is difficult to go much beyond the rather general conclusion that there is some tendency for affected relatives in the same family to be more alike in their autistic features, and in their cognitive functioning, than unrelated individuals with autism, but neither the pattern nor the extent of clustering gives clear guidance on how autism spectrum disorders might better be subdivided.

Multiplex-Singleton Comparisons: Developmental Regression

Recently, strong claims have been made that use of the MMR vaccine leads to an unusual variety of autism that is especially characterized by developmental regression (Torrente et al., 2002; Wakefield et al., 2000, 2002). As already noted, the epidemiological evidence provides no support for this claim. However, it could still be the case that autism involving developmental regression might be etiologically distinct. As already noted in the findings from the IMGSAC study (Parr et al., poster, 2002), there was no familial loading for regression. Another approach to the question is provided by comparison between multiplex cases and singletons. The rationale is that in families in which there are two affected siblings the autism may have a stronger genetic component than cases in which there is only one affected family member. Parr and colleagues found that the rate of regression in the large IMGSAC sample of multiplex families was closely comparable with that reported in previous studies of singletons. The finding indicates that, in cases where strong genetic influences may be inferred, there is no reduced rate of regression. Thus, the hypothesis that cases of autism with regression represent an environmentally caused subgroup has no empirical support. However, two caveats are necessary. First, cases of developmental regression are almost certainly heterogeneous. At one extreme, there is the frequent phenomenon of children who gain just a few words of vocabulary and then subsequently lose this minimal amount of speech. In such cases, there are inevitable doubts about the reality of the developmental regression. At the other extreme, there are cases of children who gained substantial language and who then lost well-established language skills. The original version of the ADI-R (Lord, Rutter, & Le Couteur, 1994) provided a somewhat uncertain differentiation of patterns of developmental regression. The current version (Le Couteur, Lord, & Rutter, 2003; Rutter, Le Couteur, & Lord, 2003) provides much better assessment but it has been in use for too short a time to produce data on regression in large samples. Second, although comparisons of multiplex cases and singletons have often been used as a way of subdividing groups according to strength of genetic influence, it constitutes a methodologically weak approach (Rutter et al., 1990) particularly with a relatively uncommon disorder such as autism. When most nuclear families are quite small, there are bound to be many singleton cases that would have shown a familial loading if the families had been larger (Eaves, Kendler, & Schulz, 1986). Accordingly, although the conclusion clearly must be that there is no evidence that cases of autism with developmental regression are etiologically distinct, the inference is necessarily a relatively weak one.

Linkage Evidence

For obvious reasons, the starting point for most molecular genetic studies of psychiatric disorders has been the traditional diagnostic concept. However, it is entirely possible that individual genes will provide a susceptibility, not for the syndrome as a whole, but rather for some components of it. Thus, some findings suggested that this might be the case in relation to different components of dyslexia (Grigorenko et al., 1997) although subsequent research findings have raised queries (Fisher et al., 1999, 2002). In relation to autism, some studies have found stronger evidence for linkage in autism relative pairs with evidence of language delay or a family history of language delay (Alarcón et al., 2002; Bradford et al.,

2001; Buxbaum et al., 2001; Shao, Raiford, et al., 2002; Shao, Wolpert, et al., 2002), although this was not found in the IMGSAC study. Alarcón and colleagues (2002) applied nonparametric multipoint linkage analyses to the three main traits derived from the Autism Diagnostic Interview, and concluded that there may be separate quantitative trait loci for language and for stereotyped behavior—both on chromosome 7. At present, the evidence is far too fragmentary for any firm conclusions. Nevertheless, the general strategy of either determining whether the linkage evidence is stronger in relation to particular phenotypically different subgroups of individuals with autism, or looking for evidence of linkage in relation to particular dimensions or subcomponents of autism, remain worthwhile strategies (Folstein, Down, Mankoski, & Tadevosyan, 2003). It would be similarly worthwhile to use cognitive measures as a way of subdividing autism—for example, according to the presence or absence of severe mental retardation, or the presence or absence of unusual special talents or splinter skills.

Neurocognitive Endophenotypes

There has been increasing interest in the possibility of using cognitive findings to define endophenotypes that are not synonymous with the diagnostic symptoms pattern but which may constitute the relevant genetically influenced traits (Rutter, 2004). On this basis, Tager-Flusberg and Joseph (2003) presented evidence that there may be two different subtypes in autism—one based on language abilities and one based on IQ discrepancy scores. The language deficit group was found, using magnetic resonance imaging (MRI), to have a reversal of the usual brain asymmetry. The group with a discrepantly high nonverbal IQ was found to have a large head size and large brain volume, and more severe autism symptoms. The subgroupings would seem likely to be useful in genetic analyses.

Biological Findings

Two well-established biological correlates of autism are elevated platelet serotonin (Cook & Leventhal, 1996) and increased head circumference (Fombonne, Rogé, Claverie, Courty, & Fremolle, 1999; Lainhart, Piven, Wzorek, & Landa, 1997; Woodhouse et al., 1996). The potential importance for subdividing cases of autism is provided by the fact that in both cases, although the correlate is a common one, it is far from universal. Accordingly, it would appear worthwhile to examine linkage findings separately according to the presence or absence of either of these features (Veenstra-VanderWeele et al., 2002). The situation is complicated, however, by the fact that in both cases there is evidence that the same biological features may also be elevated in other family members (Cook & Leventhal, 1996).

Minor congenital anomalies or dysmorphic features of one kind or another may well constitute another useful differentiating feature (Miles & Hillman, 2000). It appears that cases of autism without major or minor congenital anomalies have a much higher frequency of affected relatives and also show a much stronger male-female sex ratio.

Epigenetic Mechanisms

Baron-Cohen (2003) has suggested that high levels of prenatal testosterone may lead to an exaggeration of masculine features and that autism might constitute, in effect, an extreme of maleness (Baron-Cohen, 2002; Baron-Cohen, Richler, Bisarya, Gurunathan, & Wheelwright, 2003). The starting point for this suggestion was the marked male preponderance associated with autism. The suggestion that autism constitutes an extreme of maleness remains highly speculative. Autism is far from the only neurodevelopmental condition involving a male preponderance (Rutter, Caspi, & Moffitt, 2003). Attention deficit/hyperactivity disorder, dyslexia, and developmental language disorders all similarly show a male excess. Nevertheless, it is possible that sex hormone levels in the prenatal period affect gene expression in some manner, through epigenetic mechanisms. Although it is not at all likely that high levels of prenatal testosterone cause autism, it is possible that they might have a contributory role in conjunction with genetic risk determined in other ways.

There is a need to consider possible genomic imprinting; hence, possible loci for susceptibility genes need to be considered with respect to differential maternal and paternal

transmission (Reik & Walter, 2001). Also, other epigenetic effects involving DNA methylation may turn out to be important means by which as yet to be identified environmental influences affect gene expression (Jaenisch & Bird, 2003; Robertson & Wolffe, 2000).

FUTURE DIRECTIONS

Quantitative Trait Loci (QTL) Approaches

As already noted, the finding that the broader phenotype of autism probably occurs in as many as one in five first-degree relatives of individuals with autism has raised the possibility that the susceptibility to autism may be based on a continuously distributed trait in the population that extends far beyond the syndrome of autism. Constantino and Todd (2003) reported that autistic features, as measured by their social responsiveness scale, were continuously distributed and were moderately to highly heritable as judged by twin sample findings. Spiker and colleagues (Spiker, Lotspeich, Dimiceli, Myers, & Risch, 2002), showed much the same (using the ADI-R) within a group of multiplex families with autism spectrum disorders. Several of the major autism research groups in the world are currently involved in developing measures of different facets of autism that can provide a differentiation of individuals who do not have the syndrome of autism as such. It is likely that QTL analyses may provide a useful additional strategy in molecular genetic studies. In that connection, as well as studying the distribution of autistic traits in all family members, there is something to be said for studying extremely discordant sib pairs in which one has the full syndrome of autism and the other completely lacks any autistic features (Risch & Zhang, 1995, 1996).

Leads for Candidate Genes

Three main approaches have been followed in selecting possible candidate genes for autism:

1. Attention has been paid to the location of chromosomal abnormalities. As noted earlier, the best evidence concerns the maternal duplications found on chromosome 15q11–13. However, the genome screens undertaken to date have failed to find any linkage in this region (Folstein & Rosen-Sheidley, 2001). Chromosome translocations of various kinds have also been reported on chromosome 7 (see references in Folstein & Rosen-Sheidley, 2001). However, most have not concerned the area in which linkage has been found, and, as with chromosome 15, they have not proved particularly useful as guides to candidate genes. The notion of using chromosome abnormalities as a lead is a reasonable one but it has not had much success to date.
2. The second approach is provided by genes that are concerned with one of the neurotransmitters that might plausibly be involved in autism (see Folstein & Rosen-Sheidley, 2001). Although there have been some positive findings, they have not been replicated and, again, leads have not proved as useful as was hoped.
3. The third approach has been to select candidate genes on the basis of the combination of their position near linkage signals that have been found on genome scans and functions that might plausibly be related to autism. Thus, this has applied to the reelin gene and the FOXP2 gene, among a variety of others (see Folstein & Rosen-Sheidley, 2001). The two genes known to be associated with tuberous sclerosis have also been thought to provide possible pointers.

Unfortunately, so far, none of these approaches has paid off in the case of autism, but the strategies remain potentially worthwhile.

Subdivisions within Autism

The potential value of considering molecular genetic strategies in relation to possibly meaningful subgroups within autism has already been noted in the section dealing with genetic dissection.

Animal Models and Studies of Postmortem Brain Tissues

Just as genetic findings can provide invaluable leads for other forms of biological investigation, so biological findings can provide useful

leads for genetic research. This is most obviously the case with respect to evidence from animal models and from the study of postmortem brain tissues (see Bock & Goode, 2003). Both areas of research, in relation to autism, are in their infancy and no strong leads for candidate genes have yet emerged. However, as there is further progress in these areas, good leads may become available.

CLINICAL IMPLICATIONS

Initially, Kanner (1943) had postulated that autism was likely to be of constitutional origin. In the years that followed, however, many clinicians and researchers came to believe that autism was largely caused by "refrigerator parenting" and other types of maladaptive upbringing (see Rutter, 1999a). This concept of autism was later dropped—partly because of a lack of evidence in support of environmental causation and in part because of the growing evidence in favor of the view of autism as a neurodevelopmental disorder. The finding of strong genetic influences on autism also played a part in the demise of the refrigerator parent notions. But, more particularly, the genetic evidence led to an appreciation that the unusual personality features seen in some parents might represent genetically influenced traits rather than environmental causation of autism.

Clinical Assessment and Medical Investigations

The genetic findings have also been crucial in making a systematic medical assessment of individuals with autism standard practice. Thus, it would now be mandatory to examine all children suspected of autism for possible indications of tuberous sclerosis, followed by the appropriate medical investigations when there were positive findings from clinical examination. Although the fragile X anomaly accounts for only a very small proportion of cases of autism, it is important that the anomaly is identified when present because of its implications for family counseling. Accordingly, DNA methods need to be used in all cases to determine whether the fragile X anomaly is present. Possibly, the same may apply to the MECP2 gene for Rett syndrome, although in view of the conflicting evidence, this remains more uncertain. Chromosome anomalies of one kind or another are found in some 5% to 10% of cases of autism. In most cases, the causal significance of these chromosome anomalies remains quite uncertain. Nevertheless, their potential importance is sufficient for karyotyping of chromosomes to be a routine investigation in all cases of suspected autism. Possibly, too, this should include a more detailed study of the imprinted region of chromosome 15 using Fluorescence In Situ Hybridization—(FISH).

Genetic Counseling

Given the strength of genetic influences on autism, it is clearly essential that genetic counseling be available to all families that want it (Simonoff, 1998; Simonoff & Rutter, 2002). The clinical issues involved are quite complex. As with any other form of genetic counseling (Simonoff, 2002), it is essential to make a careful diagnosis of the suspected autism spectrum disorder in the key individual whose problem led to the need for genetic counseling. In addition, however, it is essential to obtain a detailed and thorough family history followed, as necessary, by a detailed individual clinical assessment of the possibly affected family members. The main difficulty in this connection arises from the uncertainties as to exactly what should, and should not, be included in the broader phenotype. This means that, unlike the situation with the genetic counseling needed for Mendelian medical disorders, the counseling needs (at least in the first instance) to be provided by clinicians who are expert in the assessment of both autism spectrum disorders and the broader phenotype pictures with which they are associated.

The second issue is the need to help families understand the difference between the absolute risk and the relative risk of autism spectrum disorders and associated conditions. Thus, sticking with autism spectrum disorders, the available evidence suggests that the absolute rate in siblings is about 6%. In other words, the absolute likelihood of autism being present in a second child in the same family is quite low. This is so despite the fact that the risk relative to the general population is very high—some 20 to 50 times increased, the specific figure

depending a bit on what assumptions are made about the general population base rate. Family members need to be helped to understand how this could happen as a result of autism developing as a consequence of the inheritance of several susceptibility genes, rather than the possession of one gene that leads fairly directly to autism. Thus, the counselor will need to explain how, although siblings share about half their segregating genes on average, the proportion of multiple gene combinations that they share is very much lower than that. In other words, other family members are quite likely to have one or another of the various susceptibility genes associated with autism but they may not have either enough of the susceptibility genes or the necessary pattern of susceptibility genes that leads to the syndrome as a whole. Two considerations complicate the advice that may be given. First, although the rate of autism in siblings is about 6% overall, the recurrence rate of autism (meaning the rate of autism in a second sibling following occurrence of autism in a previous sibling) may be somewhat higher—possibly about 8%. Second, the risk that another child in the same family will have some variety of the broader phenotype is very much higher than the expectation for the syndrome of autism. It is difficult to quantify in the absence of firm knowledge on the boundaries of the broader phenotype but it is likely to be in the region of 20% rather than 6%. The available evidence suggests that these broader phenotype abnormalities are functionally important (that is to say, they lead to difficulties in the children with them) but they are much less handicapping than autism as such.

These figures all refer to the average expectations in relation to idiopathic autism. Clearly, it is highly desirable to be able to individualize the expectations to provide much greater precision. This is straightforward enough in terms of the obvious prior need to rule out specific conditions such as the fragile X anomaly or tuberous sclerosis. However, it is much more difficult to individualize the expectations in cases of idiopathic autism. It is likely that if there are already two siblings in the family with autism, the recurrence risk in relation to a third child is likely to be well above 8% but the evidence is lacking to be more precise than that. On commonsense grounds, too, it must be presumed that if there is a particularly heavy familial loading for either autism as such or for varieties of the broader phenotype, the recurrence risk is likely to be greater. However, the difficulties of being at all precise over the increase in risks stems from the inevitable unreliability of familial loading figures that are based on a relatively small number of relatives (Rutter et al., 1990) and uncertainties that derive from the difficulties in specifying just which social, communicative, and behavioral abnormalities in relatives are part of the broader phenotype of autism, rather than due to something else (Bailey et al., 1998). Conversely, if the autism is associated with some reasonably clear-cut environmental risk factor, it might be thought that the recurrence risk of autism should be lower than average in the general population. The difficulty here is in knowing what is a true environmental cause and effect. Thus, for example, if the autism is associated with particularly severe obstetric abnormalities that have been associated with neonatal problems, it would seem reasonable to infer the probability of some environmentally mediated causal influence. By contrast, however, the mere presence of obstetric complications or low birthweight or premature gestation would not be sufficient on its own.

The guidelines with respect to genetic counseling are, first, that the counselor should provide the family with as honest and well informed an account as is possible in the present state of knowledge. This should include as clear a statement as possible about what is reasonably definite with respect to what is said and what is much more uncertain (indicating why and how the uncertainty arises). Second, the counselor's job is to provide the family with the information that is necessary for them to come to their own decision on whatever issue is being considered. It is not acceptable for the counselor to attempt to push the family in one direction or another. Third, counseling must pay due attention to the ethical issues involved. For example, it is not at all uncommon for the parents of an autistic child to want advice on the risks that an unaffected sibling will have a child with autism. Unless the unaffected sibling (i.e., the parent or potential parent of the grandchild) is part of the consultation, it would be improper for advice to be given. Of course, it is entirely

proper for grandparents to be concerned about the risks for one of their grandchildren but advice should not be given without the agreement and full involvement of the actual parents.

It is very common for families to ask whether or not there is some genetic test that could be done that would help particularize the recurrence risks that are involved. With the exception of the testing for the fragile X anomaly and the MECP2 gene (and testing for chromosome abnormalities), there are no applicable genetic tests. However, even when some susceptibility genes for idiopathic autism are found, there will still be marked limitations in what can be achieved by genetic screening. The point is that with a multifactorial disorder, the finding that someone has a susceptibility gene does not translate easily into a person-specific risk. That is because, unlike the situation with a Mendelian single gene disorder, the risks are probabilistic and may well vary according to other background genetic factors, as well as being possibly contingent on the co-occurrence of some important environmental risk factors. The findings on the APOE4 gene and Alzheimer's disease well illustrate the problem. The risk for Alzheimer's disease if someone is homozygous for the APOE4 allele is quite strong but it constitutes neither a necessary nor a sufficient cause (Liddell, Williams, & Owen, 2002). There are individuals with the APOE4 who will not develop Alzheimer's disease no matter how long they live and there are many individuals without the APOE4 gene who will nevertheless develop Alzheimer's disease. Also, for reasons that remain ill understood, the risk varies across ethnic groups (Kalaria, 2003). Whether or not the same will apply with susceptibility genes for autism remains quite unknown but it is important to be realistic that, even when susceptibility genes have been found, there will be considerable difficulties in translating the findings into a person-specific risk. That is particularly so when, at present, we know so little about environmental risk factors.

Neural Basis for Autism

The real potential value of genetic research in autism lies in the probability that it will provide invaluable leads for biological studies that will succeed eventually in identifying the neural basis of autism. Identification of the susceptibility genes will not, of course, do that on its own. Genes code for proteins and not for psychiatric disorders or behaviors (Rutter, 2004). Many areas of science will be needed in delineating the indirect pathways leading from susceptibility genes through effects on proteins and protein products, through physiological and neurochemical processes, and ultimately to the proximal pathway that leads to the syndrome of autism (Rutter, 2000). This wide-ranging program of research will need to consider how and why there is a transition in some individuals from the broader phenotype to the more seriously handicapping disorder of autism and why autism and autism spectrum disorders are so much more frequent in males than in females (Rutter, Caspi, et al., 2003). If the susceptibility genes are concerned, not with autism as such, but with continuously distributed risk characteristics or subcomponents of autism, there will be the further need to sort out why and how they come together to constitute the syndrome as a whole (Bock & Goode, 2003).

Prevention and Treatment

The ultimate goal, of course, is that this knowledge on the neural basis of autism, together with a parallel understanding of the mode of operation of identified environmental risk factors, will enable the development of new methods of prevention and intervention that will be much more effective than anything that we have available today. To what extent knowledge on the pathophysiology of autism will in fact lead to effective methods of prevention or intervention will, inevitably, depend on just what that pathophysiology comprises. Nevertheless, at the moment, it remains a puzzle that there is every reason to suppose that autism constitutes a systemwide disorder rather than being the result of some localized brain lesion, but yet neurochemical investigations have been so inconclusive and the results of pharmacological treatments so extremely disappointing.

CONCLUSION

At present, the clinical payoff from genetic research has been quite modest and it remains to

be seen just what it will deliver, but there is every reason to suppose that susceptibility genes for autism will be identified during the next decade (probably much earlier than that) and that ultimately the biological understanding, which should follow from the studies to which this identification will give rise, will transform clinical practice in the field of autism spectrum disorders in ways that should be beneficial for children and their families.

Cross-References

Issues of diagnosis are discussed in Chapters 1 and 3 through 6, epidemiology is discussed in Chapter 2, other neurobiological issues are discussed in Chapters 17 through 20.

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