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Variable Expression of the Autism Broader Phenotype: Findings from Extended Pedigrees

A. Pickles
MRC Child Psychiatry Unit and the Institute of Psychiatry, King's College, London, U.K

E. Starr
University of Windsor, Ontario, Canada

S. Kazak
Orta Dogu Teknik Universitesi, Ankara, Turkey

P. Bolton
University of Cambridge, U.K.

K. Papanikolaou
"Aghia Sophia" Children's Hospital, Athens, Greece

A. Bailey, R. Goodman, and M. Rutter
MRC Child Psychiatry Unit and the Institute of Psychiatry, King's College, London, U.K.

Factors influencing the rate, form, and severity of phenotypic expression among relatives of autistic probands are examined. Family history data on 3095 first- and second-degree relatives and cousins from 149 families with a child with autism and 36 families with a child with Down syndrome are studied. The results provide further evidence of an increased risk among autism relatives for the broadly defined autism phenotype. Of proband characteristics, severity of autism and obstetric optimality were confirmed as being related to familial loading for probands with speech. There was little variation in loading among probands lacking speech. The type of phenotypic profile reported in relatives appeared little influenced by characteristics of the relative or the proband, except for variation by degree of relative, parental status of relative, and perhaps proband's birth optimality score. Phenotypic rates among parents suggested reduced fitness for the severest and more communication-related forms of expression but not for the more mild and social forms of expression. Patterns of expression within the families did not support a simple X-linked nor an imprinted X-linked mode of inheritance. The basis for sex differences in rates of expression is discussed.

Keywords: Autism, broader phenotype, familial loading, phenotypic profile, X-linkage, imprinted X-linkage, sex difference.

Autism is a disorder characterized by deviant communication, impaired reciprocal social interaction, and restricted and repetitive behaviours. It is more common in boys than girls (4:1). It is first manifest in early childhood and it is rare for autistic individuals to marry and have children. However, the potential importance of a genetic aetiology became evident when it was realized that the prevalence of 2–3% for autism among siblings of autistic probands was about 75–100 times that in the general population (Smalley, Asarnow, & Spencer, 1988). Twin studies also showed much higher concordance rates among monozygotic twins compared with dizygotic twins (Bailey et al., 1995; Folstein & Rutter, 1977a, b). These same studies showed that in addition to relatives developing autism, many others showed fewer or more mild abnormalities in one or more of the three areas of impairment (communication, social, and repetitive/stereotyped behaviour) characteristic of autism, only a few of which met criteria for Asperger's syndrome. Data from these and other studies (e.g. Le Couteur et al., 1996; Pickles, Bolton, MacDonald, Sim, & Rutter, 1995; Spiker et al., 1994; Szatmari et al., 1995) have been reviewed by Bailey, Le Couter, Palferman, and Heavey (1998) and Szatmari, Jones, Zwaigenbaum, and MacLean (1998).

It was to investigate this lesser variant, its constituent elements, boundaries, and mechanism of inheritance, that the family genetic study of autism reported here was undertaken at the MRC Child Psychiatry Unit, London (Bolton et al., 1994), with a parallel project of similar design at Johns Hopkins School of Medicine (Piven et al., 1990). To both simplify diagnostic issues, and to ensure that any identified familial loading was specific to autism rather than mental handicap, the London study chose as probands children with IQs above 30. This study has been subsequently extended (Starr et al., 2000) to probands with lower IQs. We report here on findings from both London samples combined.

Of course, particularly in the case of the lesser variant, the increased risk to relatives may arise, at least in part,

Requests for reprints to: Professor Andrew Pickles, School of Epidemiology and Health Science, University of Manchester, Manchester M13 9PT, U.K.
(E-mail: andrew.pickles@man.ac.uk).

through familial environmental exposure. It would not be surprising if the experience of growing up in a family with an autistic child led to psychological stresses and difficulties in family functioning that might increase risks of developmental and psychiatric problems. Both family genetic studies therefore included comparison groups of families with a Down syndrome child. These families were chosen because they presented a family environment similarly characterized by the presence of a disabled child but the child's disorder reflected no increased or decreased genetic risk for communication, social, or behavioural impairment.

We have previously described some of the findings for first-degree relatives in the London study for higher (Bolton et al., 1994) and lower (Starr et al., 2000) IQ probands. In summary, family history interview data provided further evidence for a genetic aetiology for the lesser variant, with an odds ratio of around 7.8 for sibs of autistic probands as compared with sibs of Down probands for deficits in at least one of the three characteristic areas of impairment. The odds ratio remained high (5.7) even after the exclusion of relatives with autism, atypical autism, and Asperger syndrome. Within the families of autistic probands, familial loading was weakly associated with a measure of proband's Performance IQ but strongly associated with a measure of proband's verbal ability. Within the group of probands with speech, strong associations were also found with a measure of proband symptom severity and with obstetric optimality, associations not found among families of probands without speech. The association with obstetric optimality was interpreted as supporting the view that the latter was a consequence of abnormality in the foetus rather than an environmental risk factor for autism (Bolton et al., 1997). The pattern of variation in risk by degree of relative was shown to be consistent with the operation of several epistatic genes. Multiple genes and genetic heterogeneity suggest phenotypic variation as likely, but the Le Couteur et al. (1996) study of monozygotic twins found that the extent of variable expression even among relatives with identical genes was considerable. Identifying systematic and genetically related variation thus requires a large sample size. The combination of our two family studies provides this large sample in which we can systematically investigate how the characteristics of probands and relatives are related to the rate, type, and severity of the phenotype expressed.

One key question concerns the basis of the marked sex differences seen not only in autism, but in the more mild forms of phenotypic expression, and perhaps also in social communication in the general population. X-linkage as a possible explanation has received little support from the current molecular genetic linkage studies (International Molecular Genetics Study of Autism Consortium, 1998) but these have tended to focus on narrowly defined autism-autism or autism-PDD affected sib pairs to minimize the risk of phenocopies. Such samples are, however, costly to ascertain and such sib pairs alone would not immediately identify the effects of an imprinted X-locus of the sort suggested by Skuse et al. (1997) as possibly underlying both autism and more general sex differences in social communication. The use of more extended designs involving the broader phenotype (Piven, Palmer, Jacobi, Childress, & Arndt, 1997) is thus likely to be essential in fully understanding the aetiology of autism.

Samples, Measures, and Methods

Samples

Details of sample selection can be found in Bolton et al. (1994) and Starr et al. (2000). In brief, probands were selected from a pool of clinic patients at the Maudsley Hospital, after preliminary exclusions to avoid ethnic heterogeneity and concomitant medical disorder (Rutter, Bailey, Bolton, & Le Couteur, 1994), including fragile-X. In the first study, the sample selection was restricted to probands of IQ > 30 and also stratified to increase the relative proportion of female probands. Data were available on 99 families from this study. In the second study data were available from 47 families with probands in the low-functioning category (performance IQ < 50, Vineland Adaptive Behavior < 40), and a further 3 families excluded from that study as being higher functioning. One family with two autistic children, one lower and one higher functioning, were included in both studies. Each relative from this family was randomly assigned to one or other study before the samples were combined for the analyses reported here, with the exception of the two probands who each appeared systematically once, each as the sibling of the other.

The control families containing probands with Down syndrome were selected from a pool of 199 (Gath & Gumley, 1986) to broadly match the autistic probands of the first study with respect to age, sex, parental social class, birth order, and maternal age. The final comparison sample consisted of 36 families.

The eligible relatives for the analyses of this paper were defined as full sibs, parents, half sibs, grandparents, aunts, uncles, nephews, nieces, and cousins all aged 8 years or more at the time of interview.

Assessment of Autistic Probands

The proband measurement protocol included the measures listed below. Complete data on all measures were not available due to occasional lack of proband cooperation, parental permission for only part of the protocol, or inability to recall relevant information.

Diagnosis. Diagnoses were checked using the Autistic Diagnostic Interview (Le Couteur et al., 1989, 1996) with the parent or principal caretaker and by using the Autism Diagnostic Observational Schedule in either its standard or Pre-Lingual form (DiLavore, Lord, & Rutter, 1995; Lord et al., 1989). These instruments provide algorithms for the diagnosis of autism. The patterns of item scores obtained confirmed that all probands met ICD-10 (World Health Organization, 1992) and DSM-IIIR (American Psychiatric Association, 1987) criteria for autism.

Test scores of cognitive ability. Performance IQ was assessed in the first study using, as appropriate, tests from among the Wechsler Scales (Wechsler, 1974, 1981), the Merrill-Palmer (Stutsman, 1948), and Raven's Matrices (Raven, Court, & Raven, 1982). In the second study the Merrill-Palmer was the principal test, the Leiter International Performance Scales (Levine, 1986) being used for two probands. Eight low-ability autistic probands were untestable and random scores between 15 and 30 were imputed. Scores were left missing for a further two autistic probands for whom testing could not be arranged. In view of the very low verbal abilities expected of probands in the second study, no measure of verbal IQ was attempted. The analyses reported use Performance IQ only.

Minor congenital anomalies. A neurodevelopmental examination included the minor congenital anomalies of the Waldrop and Halverson Scale (1971), a total score being obtained by the simple sum over the set of binary items. Items were scored using what we considered the most appropriate normative data, but inadequacies remain for some items (e.g. interpupiliary distance in adults).

Obstetric optimality. Mothers were questioned about the details of the proband's pregnancy and delivery using the Obstetric Enquiry Schedule (OES), a semistructured, in-

Table 1
*Descriptive Statistics for Autism Probands (*N = *149)*

Measure	Mean	*SD*	Frequency			Range
			Low	Med	High	
ICD 10 symptoms[a]	18.1	3.1	40	62	43	11–25
Obstetric Optimality score[b]	2.6	1.7	43	61	38	0–8
Waldrop Congenital Anomaly score[c]	2.9	2.0	18	69	46	0–9
Performance IQ[d]	54.7	25.8	53	55	39	15–127

[a] ICD-10 symptoms were incomplete for 4 probands.
[b] Unavailable for 7 probands (Low = 0/1, Med = 2/3, High $\geqslant$ 4).
[c] Unavailable for 16 probands (Low = 0, Medium = 1/3, High $\geqslant$ 4).
[d] Unavailable for 2 probands (Low = 0/39, Medium = 40/69, High $\geqslant$ 70).

vestigator-based interview covering items corresponding to those of the Rochester Research Obstetrical Scale (Zax, Sameroff, & Babigan, 1977) and Gillberg and Gillberg (1983). This measure was unavailable for seven probands.

Epilepsy. A standardized interview schedule was used to assess clinical features of any seizure disorder in probands. This measure was available for all probands.

Among the 149 probands with autism, 30% were female, 25% had epilepsy, and 45% lacked useful speech. Summary data for the measures described above are shown in Table 1.

Assessment of Relatives

Family History Interview. Parents were interviewed using the Family History Interview (FHI), an investigator-based interview about the presence of any developmental disorders of speech, reading, and spelling, abnormalities in socio-emotional development, and psychiatric disorders in all first- and second-degree relatives and cousins on both mother's and father's sides of the family. The schedule was designed to measure, in both childhood and adulthood, the tendency for relatives to exhibit abnormalities in three areas of functioning that characterize autism; namely deviant communication, impaired reciprocal social interaction, and restricted or repetitive patterns of interests, activities, and behaviours. For all possibly affected relatives, case vignettes were written and rated by at least four investigators blind to proband type, with rating inconsistencies being resolved by consensus. The individual items and their combination into the phenotypic measures analyzed in this paper have been presented previously (Bolton et al., 1994; Fombonne, Bolton, Prior, Jordan, & Rutter, 1997). We distinguish four levels of phenotypic severity: unaffected; the mild variant of the broader phenotype for a deficit in one of the three key areas; the severe variant of the broader phenotype for a deficit in two areas; and those affected in three areas—including but not limited to those relatives with an ICD-10 diagnosis of autism. Fombonne et al. (1997) reported on the validity of measures in the communication domain as reflected by psychometry undertaken around the time of interview.

Statistical Methods

Ordinary, multivariate, and ordinal logistic regression models were estimated using the survey analysis commands of STATA (StataCorp, 1999). These methods allow analysis of the phenotype of relatives using logistic models for (i) a simple binary measure of phenotypic expression, (ii) a set of three (i.e. multivariate) binary responses reflecting the profile of phenotypic expression across the communication, social, and stereotyped behaviour areas, and (ii) an ordered categorical measure representing four levels of severity of expression (0, 1, 2, or 3 areas affected). Models of profile (type ii) and severity (type iii) allow for the testing of specificity of effect. For example, female relatives might, as compared to male relatives, show less expression in all three phenotypic areas. This would be identified by a significant main effect for females within a type (ii) model. However, female relatives might express a phenotype in which specifically social abnormalities predominated as compared to male relatives. This would be identified by a type (ii) model that required a term for a female by area of expression interaction. Within type (iii) models of severity of expression, if the effect of being a female relative was to decrease all levels of expression (mild, middling, and severe) in a proportionate fashion (in the sense of the proportional odds model of Peterson and Harrell, 1990), this would be identified by a main effect for female. But where, for example, the comparative reduction of expression among female relatives was restricted to lowering rates of the more severe expression only, then a female by level interaction would be required. As a consequence of the small frequencies in the most severe phenotypic category, for the purposes of these tests we grouped the top two severity categories (2 or 3 areas affected). In rather different ways, both these tests of interaction terms explore the extent to which phenotypic expression in autism is a single simple dimension. Practical details for the fitting of models of this kind are available elsewhere (Maughan, Collishaw, & Pickles, 1998).

In addition the survey methods take account of the fact that the relatives, being sampled by family, represent a clustered sample for which the usual assumptions of independent random sampling may not be appropriate (see, for example, Binder, 1983; Graubard & Korn, 1993).

Tables and graphical displays of relatively raw data have been used to support and help interpret the results of model fitting. We used local regression smooths (Cleveland, 1979) to examine evidence of possible threshold effects or other non-linearities. If one considers a scatter plot of outcome Y against a predictor variable X, then local regression involves estimating the regression line for the points that fall within a restricted "window" defined on the X-axis, with the regression being repeated many times as the window is moved progressively across the whole range of X. The resulting regression line is bendy, reflecting the variation in the strength of the relationship between the two variables. The method is also robust, in the sense that the effect of any particularly influential point contributes to the estimated line only in its local vicinity.

In all cases reported confidence intervals (CI) are for the 95% significance level.

Results

Basic Rates

Among the total of 3301 relatives known to be eligible, 206 were excluded from analysis because the informants were not sufficiently familiar with them to be able to provide adequate data. Table 2 presents the distribution of the remaining 3095 relatives, by study and proband type. Overall 178/2360 (7.5%) of the relatives of autistic probands were classified as falling within the broader phenotype category and 20/735 (2.7%) of the relatives of Down probands. Taking the rate in the Down sample as

typical of the general population rate gave relative risks of 3.88 (CI 1.72–8.73, $p = .001$) for first-degree relatives, 2.41 (CI 0.85–6.76, $p = .1$) for second-degree relatives, and 2.50 (CI 1.11–5.64, $p = .03$) for cousins.

Variation in the Rate and Severity of Phenotypic Expression in Relatives

Table 3 gives the basic frequencies and rates where each relative was classified according to whether they were affected in 1, 2, or 3 areas. Proportional odds logistic models were fitted to this ordinal phenotypic measure for relatives from both the autism and the Down samples. Main effects for the sex of the relative, parental relative type, degree of relatedness, and study type (old study versus new) were examined, as were interactions with level of expression to test for nonproportionality. Women were identified as showing significantly lower levels of expression ($p = .001$) but the tendency for this reduction in rates to be higher at more severe levels of expression was of marginal significance (interaction $p = .1$). Rates by degree of relative showed a significant interaction effect ($p < .004$), the fall-off in reported rates by degree of relative being greater for higher levels of severity of expression. There was a similar trend for parental relatives (parents and grandparents) to be only mildly affected (interaction $p = .1$). When predictors were examined together in the ordinal logistic model both this interaction effect and that with degree of relative were significant ($p = .006$ & $p < .001$ respectively) but not that for sex of relative ($p = .1$).

The same model also indicated that the effect of proband type (autism vs. Down) was uniform (interaction $p = .3$) across levels of severity, and that the old and new autism samples showed no clear differences in rate of expression (interaction $p = .3$, main effect $p = .2$, data not shown).

Figure 1 summarizes the relationship between proband characteristics and severity of expression among the relatives of the 149 probands with autism. For selected characteristics Fig. 2 presents running regression smooths (transformed from the logit scale) of these relationships, which allow an assessment of the robustness, nonlinearity, and specificity of these relationships. All test statistics are those from ordinal regression models using the proband categories of Fig. 1 unless otherwise specified.

Table 2
Frequencies and Percentages for Types of Relative by Sample

Relationship to proband	Autism						Downs	
	Old study (Bolton et al., 1994)		New study (Starr et al., 2000)		Combined			
	N	%	*N*	%	*N*	%	*N*	%
Grandparent	364	22.6	163	21.8	527	22.3	139	18.9
Parent	193	12.0	92	12.3	285	12.1	72	9.8
Full sib	137	8.5	52	7.0	189	8.0	64	8.7
Half sib	9	0.6	21	2.8	30	1.3	1	0.1
Uncle/aunt	355	22.0	188	25.2	543	23.0	166	22.6
Nephew/niece	8	0.5	4	0.5	12	0.5	16	2.2
First cousin	547	33.9	227	30.4	774	32.8	277	37.7
Total	1613	100.0	747	100.0	2360	100.0	735	100.0

Table 3
Severity and Type of Expression by Characteristics of Relatives

	Relatives of Downs probands		Relatives of autism probands		Sex of relative		Degree of relative			Parent status of relative: Combined autism	
	%	*N*	%	*N*	Male %	Female %	1st %	2nd %	Cousins %	Parent/ G.parent %	Other %
Severity of expression											
Affected in 1 area	2.4	18	6.0	142	6.3	4.0	8.5	5.0	3.4	6.0	4.8
Affected in 2 areas	0.3	2	1.1	25	1.3	0.4	2.8	0.5	0.3	0.9	0.9
Affected in 3 areas	0.0	0	0.5	11	0.5	0.2	1.5	0.1	0.0	0.0	0.5
Type of expression											
Communication	1.6	12	3.8	90	4.4	2.1	6.9	2.4	2.5	1.8	4.1
Social	1.1	8	3.9	93	4.3	2.2	6.7	3.1	1.4	4.4	2.7
Repetitive	0.3	2	1.7	40	1.6	1.1	4.6	0.9	0.1	1.6	1.3

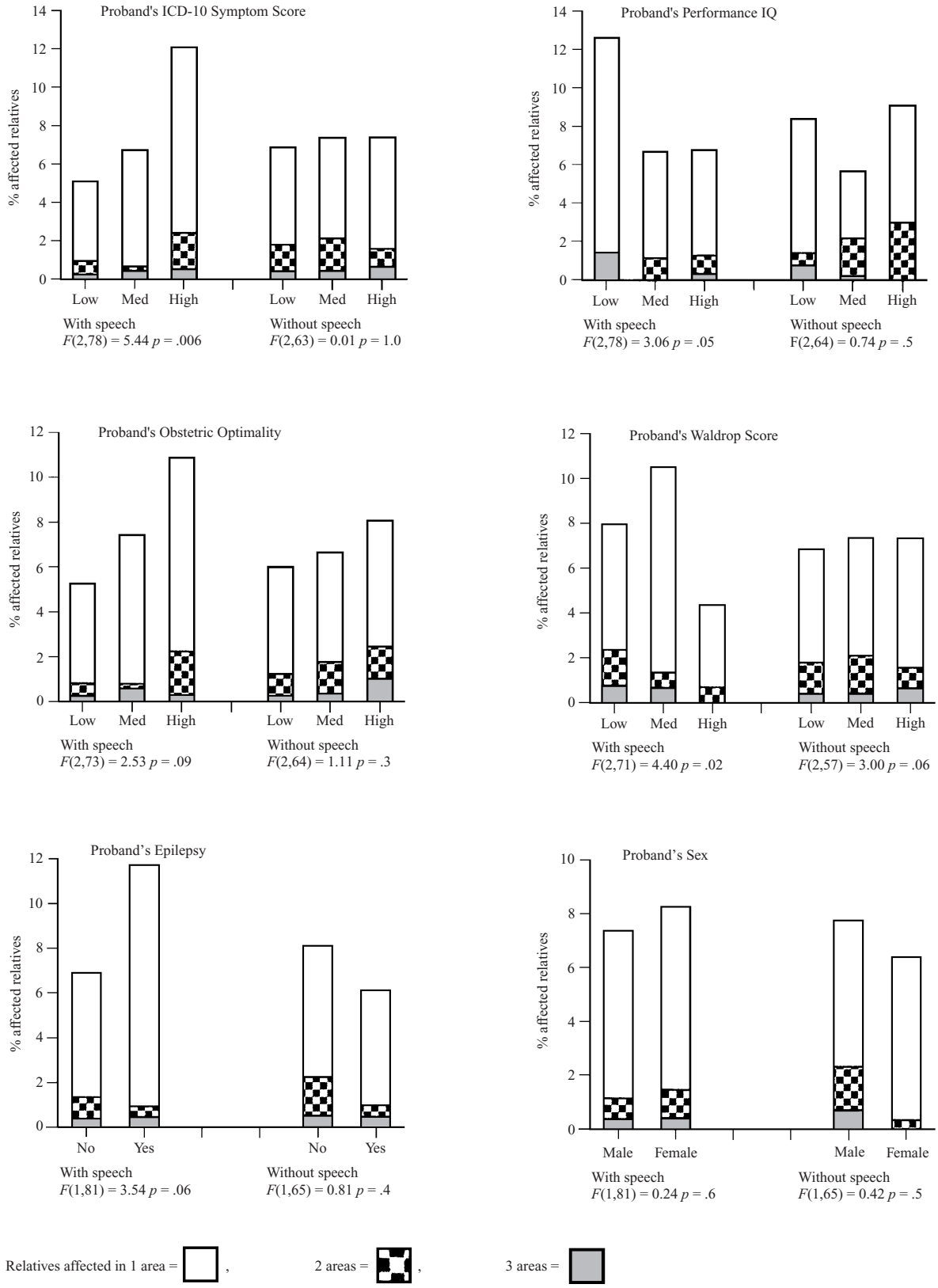

Figure 1. Rate and extent of affected status of relatives of probands with autism, by proband's level of language and other characteristics.

For probands with speech the ICD-10 autism symptom score was strongly associated with increased expression among relatives ($p < .006$) and this effect appeared to be uniform across all levels of severity of expression among those relatives (interaction $p = .6$). For probands without speech, the symptom score appeared unrelated to expression in relatives (uniform effect $p = 1.0$, interaction $p = .8$). Figure 2 suggested these relationships were linear and a corresponding ordinal linear logistic model (with symptom score as a continuous variable) identified the difference in slopes for probands with and without speech as marginally significant ($p = .08$).

For Performance IQ, the ordinal logistic models suggested little evidence for effects varying with level of

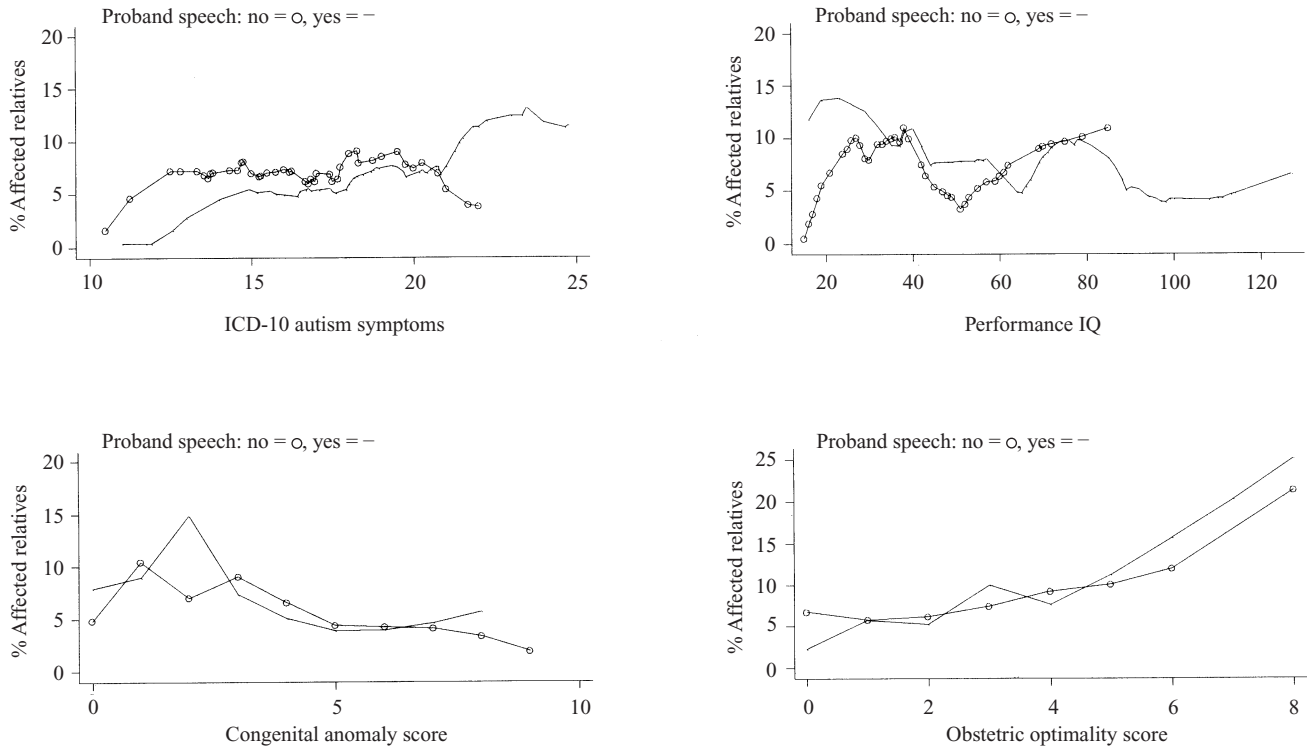

Figure 2. Running smooth plots showing variation in the rate of the broad phenotype among relatives by proband's level of language and other characteristics.

severity (interaction $p = .7$ for probands with speech, $p = .1$ for those without). A marginally significant main (uniform) effect of increased expression among relatives of probands of lower Performance IQ was found for probands with speech ($p = .05$), but not for those without ($p = .5$). Figure 2 suggested any trend to be limited to the IQ range below 60. Interaction tests did not show the effects for probands with and without speech to be significantly different ($p = .7$).

Although less pronounced among probands without speech, the association between obstetric optimality and familial loading was not significantly different between the groups and when combined was significant at $p = .03$. The association with the congenital anomaly score was also more pronounced and significant for probands with speech than for those without ($p = .02$ vs. $p = .06$) but again this difference was not significant ($p = .5$).

There was a trend for the presence of epilepsy in the proband to be associated with higher rates of expression in relatives of probands with speech ($p = .06$), but not those without ($p = .4$), a difference of effect significant at the $p = .06$ level.

No association between the sex of the proband with rates of expression among relatives was suggested.

Table 4 shows results of multivariate models for probands with and without speech of models that included proband characteristics as scores (as shown in Fig. 2) and also included the influential characteristics of relatives identified in the previous section. Possible heterogeneity seemed confirmed with several characteristics of probands with speech having striking and significant associations with familial loading, but this was not the case for probands without speech. In fact, multivariate tests of the terms, involving the interaction of proband speech and each of the other predictors within a joint model fitted to all the data combined, did not find these apparently distinctive patterns of association to be significantly different [heterogeneity of effects of proband characteristics $F(6,118) = 1.63$, $p = .1$; of relative characteristics $F(4,120) = 1.91$, $p = .1$].

The Evidence for X-linkage

The higher rate of autism among males than females might also be expected to apply to the broader phenotype. The male to female ratio among all affected relatives was 2.0 for male probands (81:40) and 1.5 for female probands (34:23).

Under a multifactorial liability threshold model higher familial loading would be expected for female probands (the less often affected sex). As shown in Fig. 1, the overall rate for female probands was 7.6%, almost identical to the rate of 7.5% for male probands.

For a simple X-linked locus male probands inherit from their mothers and so for male probands elevated rates should be seen on the mother's side specifically. Contrary to this pattern, the rate on the mother's side was 5.3%; lower than the 7.3% observed on the father's side. The corresponding figures for female probands were 6.6% and 7.2% respectively.

Goodman, Skuse, and Pembury (2000) extend this logic to the case of an imprinted X-locus, arguing that at such a locus females are not identical by descent with their mother, father, or maternal grandmother, but have a 50:50 chance of being identical by descent with their paternal grandmother. Thus for female probands higher rates should be expected for paternal grandmothers than maternal grandmothers. Our data give rates of 0/41 and 3/43 respectively. A similar argument suggests that for female cousins of female probands, higher rates would be expected for the daughters of uncles on the paternal side than for other female cousin types. We obtain 1/25 and

Table 4
Relative and Autism Proband Characteristics and Rate of Phenotypic Expression among Relatives: Results from Proportional Odds Logistic Regression (N = *2010 relatives from 124 probands*)

	Families of probands with speech (N = 66 families, 1021 relatives)		Families of probands without speech (N = 58 families, 989 relatives)	
	Odds ratio	p	Odds ratio	p
Characteristics of relatives				
Female	−0.22	.4	−1.10	.002
Parent	−0.84	.01	−0.24	.5
Degree 2	−1.21	< .001	−1.06	< .001
Degree 3	−1.95	< .001	−1.77	< .001
Characteristics of probands				
ICD-10 symptoms	0.14	.008	−0.01	.9
Performance IQ/100	−1.41	.02	0.18	.8
Obstetric optimality	0.15	.04	0.13	.3
Congenital anomalies	−0.26	< .001	−0.08	.2
Epilepsy	0.32	.2	−0.03	.9
Female	−0.06	.8	−0.24	.5

4/88 respectively. Thus, though numbers are small, neither the contrast involving grandmothers nor that for cousins is in the direction that would be expected from an imprinted X-linked locus.

Variation in the Type or Profile of Phenotypic Expression

The data shown in the lower half of Table 3 indicate that rates of all three phenotypic components are higher among relatives of autistic probands, with the risk ratio and specificity appearing to be highest for the repetitive behaviours measure and lowest for the communication measure. However, multivariate logistic regressions found no significant measure by group interaction, $F(2,184) = 0.53$, $p = .6$, providing little evidence that those affected among the Down sample, and which from a genetic perspective are likely phenocopies, had a distinctive profile.

Among the relatives of the autistic probands there was no evidence of differences in profile between the old and the new studies [measure by study interaction $F(2,148) = 0.01$, $p = 1.0$, data not shown]. Table 3 shows that although female relatives had lower rates of expression for all aspects of the phenotype, there was little evidence for distinctive male and female profiles [measure by sex interaction $F(2,148) = 0.80$, $p = .5$]. However, compared with other relative types, those who had raised children (parents/grandparents) showed less expression in the communication domain, similar stereotyped expression, and higher levels of social expression compared to nonparent relative types, a difference in pattern that was significant, $F(2,148) = 11.23$, $p < .001$. Some variation in the profile of expression by degree of relative was evident, $F(4,148) = 3.82$, $p = .006$, with second-degrees being reported as showing relatively more social expression, and third-degrees showing much less stereotyped expression, than first-degree relatives (this may well reflect patterns of reporting errors).

Although simple profile differences by presence or absence of useful speech in the proband were not found [measure by proband's speech interaction $F(2,148) = 0.06$, $p = .9$], the running smooths of Fig. 3 suggested some heterogeneity in the effects of proband's characteristics on the phenotypic profile of relatives. An omnibus test of the three-way interaction terms for measure, proband's speech, and each of the seven predictors of familial expression (the degree, parental status, and sex of relative, and the proband's autism symptom, Performance IQ, birth optimality, and Waldrop scores) suggested significant heterogeneity, $F(14,110) = 2.26$, $p = .009$. However, when explored individually little clear pattern could be identified.

Controlling for the parent and degree of relative effects described above, there was no suggestion that the ICD-10 symptom score for probands with speech was preferentially related to any one component of the broad phenotype [measure by ICD-10 score interaction $F(2,144) = 0.96$, $p = .4$]. Similarly, for nonspeaking probands, the absence of a relationship to overall expression reported above appeared to be shared by all three measures, $F(2,144) = 1.05$, $p = .4$.

For Performance IQ, Fig. 3 suggests that, among relatives of higher-functioning probands, there was a declining rate of the communication component and a slight increase in the social component, but this specificity of effect was not significant [measure by Performance IQ interaction $F(2,146) = 0.22$, $p = .8$]. For probands without speech, neither the figure nor the statistical test indicated any specificity in the relationship, $F(2,146) = 0.51$, $p = .6$.

For obstetric optimality among probands with speech, Fig. 3 suggested specificity for the communication measure, an effect that was significant [measure by optimality interaction $F(2,141) = 3.45$, $p = .03$]. For probands without speech neither the figure nor the test suggested any specificity, $F(2,141) = 0.37$, $p = .7$.

There was no evidence that the Waldrop congenital anomaly score had any specific association with the form of expression [measure by Waldrop interaction $F(2,132) = 0.86$, $p = .4$ for probands with speech, $F(2,132) = 1.09$, $p = .3$ for probands without speech]. Similarly there was no strong evidence for variation in the phenotypic profile according to proband's sex [measure by sex interaction $F(2,148) = 0.07$, $p = .9$ for probands with speech and $F(2,148) = 1.65$, $p = .2$ for probands without speech] nor proband epilepsy [measure by epilepsy interaction $F(2,148) = 0.50$, $p = .6$ for probands with speech and $F(2,148) = 2.12$, $p = .1$ for probands without speech].

Further insight as to whether the different aspects of the phenotype represent variable expression or the segregation of different component specific genotypes that

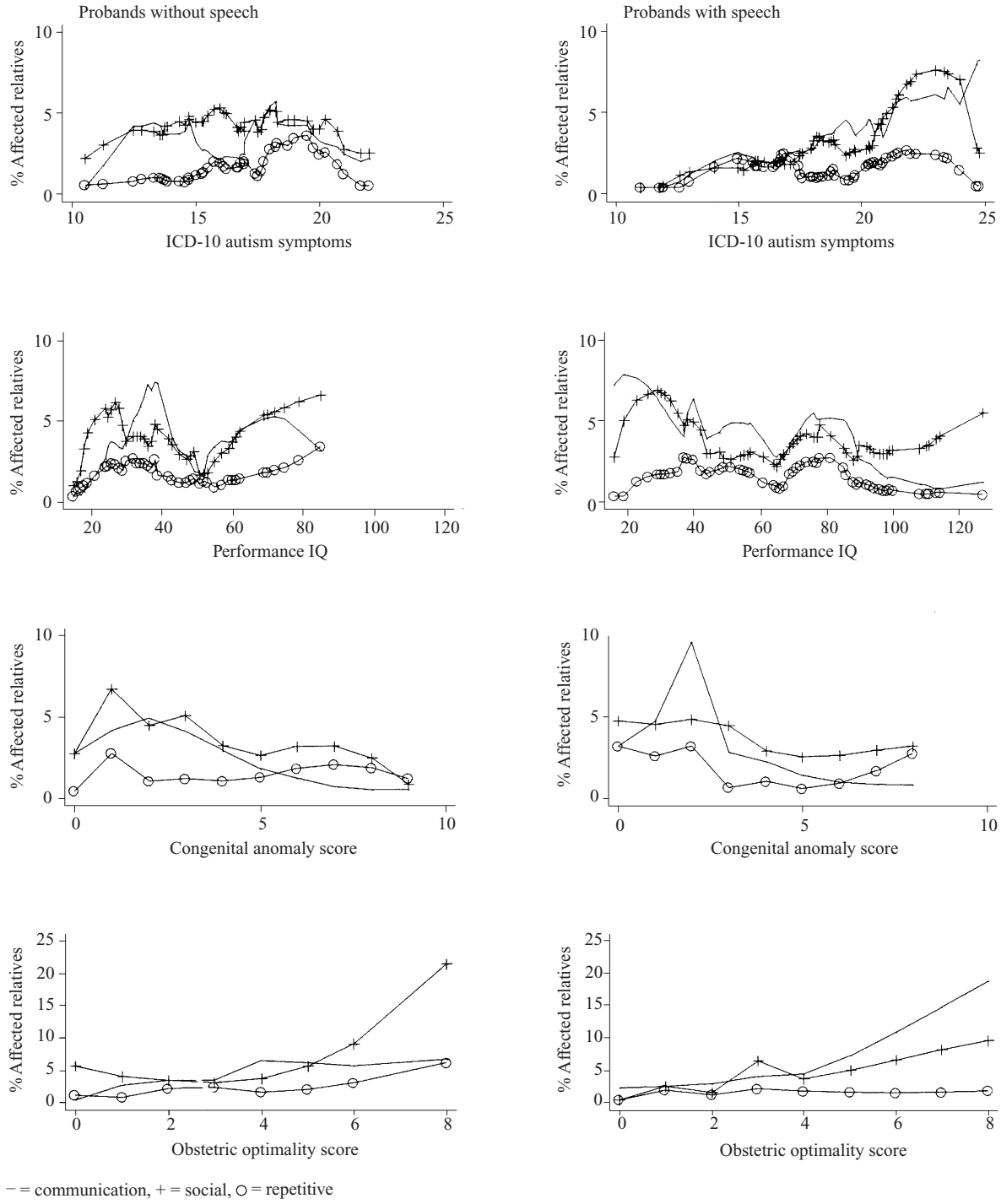

Figure 3. Running smooth plots showing variation in the rate among relatives of each of the three phenotypic components by proband's level of language and other characteristics.

in combination predispose to autism, can be obtained from an examination of the type of expression of affected relatives by side of family. Of the 149 autism probands, 37 had affected relatives on the paternal side only, 27 on the maternal side only, and just 16 had relatives with the broader phenotype on both maternal and paternal sides. Within these 16 families, evidence for independently segregating phenotypic components was slight. Twelve of the 16 families had relatives affected in one or more of the same areas on both maternal and paternal sides of the family (4 communication, 5 social, 1 repetitive, 1 communication and social, 1 social and repetitive). The remaining four families had communication maternal side and social paternal side, repetitive maternal side and social paternal side, and two with social maternal side and communication paternal side.

Discussion

Limitations of the Study

The results presented here are based on two samples that together form very much the largest family study in the field of autism. They rely, however, on the family history method. Though often the only practical method of data collection beyond first-degree relatives, there are inevitable concerns about the quality of data. Andreasen (1986) suggested relatively high specificity but quite low

sensitivity for the family history approach. Ratings of case vignettes from the autism family and twin study showed high reliability (Bolton et al., 1994), but it would not be surprising if sensitivity declined with declining level of an informant's first-hand experience of the subject. Thompson, Orvaschel, Prussof, and Kidd (1982), for example, found that from among the most distant relatives only the most severe cases were identified. However, it is far from clear that the alternative of using subject's report is always to be preferred (Brewin, Veltro, Wing, MacCarthy, & Brugha, 1990), and particularly in the case of personality disorders, with which our measures share some similarity, some argue strongly in favour of the informant approach (Pilgrim & Mann, 1990). Overall, we would prefer an appropriate combination of subject, informant, and, in addition, objective testing (e.g. Bailey, Le Couteur, Palferman, & Heavey, 1998; Baron-Cohen & Hammer, 1997; Hughes, Leboyer, & Bouvard, 1997) for the presence of different aspects of the broader phenotype.

A second possible weakness relates to the uncertainty as to the components defining the putative phenotype. Evidence that some forms of depression and social phobia should also be included (De Long & Dwyer, 1988; Piven et al., 1990, 1991; Smalley, McCracken, & Tanguay, 1995), sharing at least part of the genetic underpinnings of autism, has been presented. Such a redefinition would, for example, substantially equalize base-rates in overall expression among male and female relatives, radically altering rates of a number of genetically informative contrasts. However, the evidence for their inclusion is, as yet, not persuasive (Bolton, Pickles, Murphy, & Rutter, 1998). The potentially associated evidence for elevated whole blood serotonin (5-HT) among some autism probands and their relatives (Cook et al., 1994) requires further investigation.

Although desirable for the purposes of defining the lesser variant in relatives, the use of probands meeting all criteria for diagnosis of autism restricts the range of severity of proband symptomatology over which variation in familial loading and expression could be examined. A comparison with relatives of more mildly affected probands, including those with Asperger syndrome, would have been valuable.

Summary and Assessment of Findings

The results from our analysis of more extended pedigrees are largely consistent with those reported by Bolton et al. (1994) who examined first-degree relatives only. The increased risk for the broader phenotype found among the sibs and parents of autism probands, as compared to control relatives of Down probands, also applied to second-degree relatives and first cousins, although in a substantially attenuated form. Individuals with autism and pervasive developmental disorders represented only a small proportion of all the individuals showing some phenotypic expression.

Evidence for selection effects. The pattern of expression by type of relative is consistent with substantial variation in genetic fitness, with virtually no parents or grandparents having severe expression. However, the rate of expression of the mildest phenotype was not reduced among these relatives, and this may be important for maintaining the predisposing genes within the population. It is of note that parental selection effects appeared to be weakest for the social component of the phenotype, this being even more common among parents and grandparents than unselected relatives like aunts and uncles, a finding in line with those of Piven et al. (1997).

Distinguishing phenocopies. Gaining an ability to distinguish phenocopies from among affected relatives would have been desirable. However, our comparison of the phenotypic profiles of affected relatives of Down and autistic probands found no significant differences. Nonetheless the power of the test is clearly not high, and so the suggestive evidence that the presence of repetitive behaviours might have higher specificity than social or communication abnormalities should not be entirely discounted.

Evidence for genetic heterogeneity. Overall, although proband characteristics such as severity of autism and congenital anomaly score were related to rates and severity of expression, there was little evidence that the phenotypic profile similarly varied. Thus more and less severely affected probands, male and female probands, higher and lower functioning probands, those with epilepsy and those without, and those with higher and those with lower congenital anomaly scores all appeared to have affected relatives with similar phenotypic profiles. This suggests that the type of expression may reflect simple variable expression. The fact that co-twins of monozygotic twins with autism show the whole range of phenotypic expression (Le Couteur et al., 1996) also indicates that much phenotypic variation may not be genetic in origin. Only in the case of obstetric complications was there some suggestion that these were preferentially associated with expression of the communication component among relatives. We have argued elsewhere that the birth complications of an autistic proband are more likely to be a consequence rather than a cause of autism (Bolton et al., 1994, 1997). However, in general the link between poor optimality and cognitive development is well established and what we observe may simply be a reflection of familiality for low optimality.

Speech as an index of heterogeneity. The large study size allowed us to further investigate our previous conclusion that speech provided an index of possible genetic heterogeneity (Bolton et al., 1994). We were unable convincingly to confirm or refute that conclusion. The strong variation of familial loading with such measures as the ICD-10 symptom score for probands with speech appeared absent for probands without speech. Formal tests for such heterogeneity, however, did not consistently identify these differences as significant. Some degree of colinearity among predictors and the low power of tests for heterogeneity are likely to have contributed to this equivocal position. Overall, we would continue to recommend considerable caution in assuming a common aetiology for autism that occurred with and without severe language difficulties.

Basic genetic architecture. The rate of phenotypic expression fell quickly with increasing genetic distance from the proband. When compared to the corresponding relatives in the Down sample, the relative risk was, however, no lower in cousins than second-degree relatives, but this finding is not inconsistent with a smooth monotonic decline given the width of the confidence intervals. The fall-off in rates was steepest for the more severe forms of expression, the phenotype becoming more commonly expressed in its milder forms with increasing genetic distance from the proband. It would be tempting to explain this pattern by postulating that variation in severity was associated with differences in the

combination of epistatic genes of varying penetrance and expression (Pickles et al., 1995); the most penetrant combination, giving autism (usually the proband in our sample), being diluted with increasing genetic distance from the proband to less penetrant combinations. Some caution is required with this interpretation and with the similar suggestion of changes in the phenotypic profile with genetic distance, because the design of the study risks confounding such effects with those due to changing patterns of reporting errors. Nonetheless, the findings are suggestive. Specific genes for specific measures or subtypes of disorder have been suggested for dyslexia (Grigorenko et al., 1997), and a referee suggested that such gene-specific effects are to be expected for the autistic phenotype. (In fact the dyslexia evidence is incomplete, the conclusion being based on differences in linkage *p*-values, one significant and the others not, rather than on a test of a specific linkage for one measure and no linkage for the others). In contrast to Piven et al. (1997), only a minority (16) of our affected families showed expression on both sides of the family, and fewer still showed bilineal patterns consistent with the segregation of area-specific genes. This difference of findings is likely to be related to the smaller male : female sex ratio among the affected individuals identified in the Piven study.

The male preponderance and X-linkage. As expected, a marked excess of males over females was evident among the affected relatives. The lower rate of expression among women would be consistent with their having a higher threshold within a postulated multifactorial trait threshold model as proposed by Jorde et al. (1991). However, there appears to be little evidence for the substantial differences in familial loading, or variation in severity and type of expression in relatives by sex of the proband, which would be an expected consequence of such a model. A trend that was observable from simple tables for male and female affected relatives to have different phenotypic profiles became wholly non-significant within a multivariate analysis.

The absence of a strong relationship of familial loading with sex of proband, in spite of substantial differences in base-rates by sex, and the pattern of the rate of decline with genetic distance (Bailey et al., 1995; Pickles et al., 1995) suggests a simple sex-limited additive genetic multifactorial threshold model as an unlikely candidate. The recurrence risk data may be consistent with a model involving a small number of epistatic loci, but we do not know, as yet, whether the same model can explain the patterns of sex differences in base-rate and variation of phenotypic expression and familial loading. X-linkage, whether in its simple or more complicated form involving maternal imprinting, received no support from our data.

The mechanisms underlying the increased risk to males thus remain obscure, but while preserving the need for caution because of limited statistical power, these data point to a sex difference that is independent of genetic liability and of X-linked predisposing genes. Overall the data clearly point to a role for nongenetic factors and we know that brain development is far from being the mere following of some deterministic DNA encoded plan (Goodman & Alberman, 1996). One possibility, therefore, is that expression of the genes predisposing to autism and its lesser variant, or the neurobiological consequence of their expression, is simply increased in the presence of an X-chromosome, for example being related to levels of sex hormones.

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