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REVIEW

Imprinting, the X-Chromosome, and the Male Brain: Explaining Sex Differences in the Liability to Autism

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ABSTRACT

Males are at least four times more likely to develop autism than females. Among relatives with a broader autistic phenotype, males predominate too. Autism is a highly heritable disorder, yet genome scans have not revealed any predisposing loci on the sex chromosomes. A nongenetic explanation for male vulnerability, such as exposure to prenatal androgens, is unlikely for a variety of reasons. A novel genetic mechanism that resolves many of the outstanding difficulties is outlined here. The imprinted-X liability threshold model hypothesizes that the threshold for phenotypic expression of many autistic characteristics is influenced by an imprinted X-linked gene(s) that is protective in nature. Imprinted genes are known to play an important role in normal fetal and behavioral development. The gene is expressed only on the X-chromosome that is inherited from the father and raises the threshold for phenotypic expression. It is normally silenced when transmitted maternally. Because only females have a paternal X-chromosome, the threshold for phenotypic expression is higher in them than in males. Evidence for the existence of the genetic locus was found in a study of females with X-monosomy (Turner's syndrome) in which females had either a single paternal or maternal X-chromosome. Identifying the sites of action of this X-linked gene could lead to the discovery of autosomal loci that confer more directly a predisposition to autism. **(*Pediatr Res* 47: 9–16, 2000)**

Abbreviations

WT1, Wilms' tumor 1
HTR2A, serotonin-2A (5-HT_{2A}) receptor
PDD, pervasive developmental disorder
HFA, high-functioning autism

Sex differences in the prevalence of autism have been described in both singleton and multiplex families (1–4). Boys with the phenotype outnumber girls by at least 4 to 1. Epidemiologic studies carried out over the past two decades have also consistently demonstrated strong familiality and a much higher prevalence in siblings, for whom the recurrence rate is 3–5%. This could represent a 50- to 100-fold increase in risk compared with individuals in the general population, depending on the true prevalence of the disorder (5, 6). Psychosocial environmental factors only rarely appear to be influential in bringing about phenotypic expression (7). Accordingly, a genetic mechanism is thought to be responsible for the strong familiality. This is further suggested by the high concordance rate of autism among monozygotic twins [*e.g.* 60%; (8)]. There is little or no concordance for the full phenotype in dizygotic twins. The main aim of this paper is to consider the various mechanisms that could explain the striking difference in prevalence, by sex, in the disorder. At first sight this should also have a genetic explanation, but the nature of the genetic mechanism involved has proved difficult to elucidate. Although the issue has been relatively little discussed in the scientific literature, an understanding of why there is such vulnerability of males to autism and related conditions such as Asperger's syndrome could provide valuable information about their etiology.

The familiality of autism is even more striking when less specific phenotypic characteristics are considered. The broader phenotype (9) resembles autism to some degree, but the range and severity of symptoms are insufficient for a formal diagnosis. A proportion of first and second degree relatives are usually found to show abnormalities in one or more of the three areas of impairment typical of autistic disorders: cognitive,

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social, and repetitive stereotyped behaviors (10, 11). The broader phenotype is highly heritable: 92% of MZ pairs in a recent twin study (8) were concordant for a broader spectrum of related cognitive or social abnormalities compared with just 10% of DZ pairs (9). The degree of risk to family members is not influenced by the IQ of the autistic proband. Autistic features (whether defined narrowly or by broader criteria) have similar prevalence among the first-degree relatives of both high and low IQ probands (12).

What do we know about the nature of the genetic risk of developing the disorder? Risch (13) has estimated that for any disorder caused by a single major gene, the risk to relatives should fall by roughly one half as genetic distance increases. Thus, siblings share 50% of their genes with the proband by chance. Uncles and aunts share 25%, first cousins 12.5%, and so on. Family study data are inconsistent with a single major gene and indicate instead a model involving a small number of epistatic loci. But they do not explain the pattern of sex differences in base rates of autism; nor do they explain differences in familial phenotypic expression by sex (14). All that can be concluded from the results of studies to date is that the genetic predisposition to autism also confers susceptibility to the broadly or narrowly defined lesser variant and that a small number of autosomal genes would seem to be responsible.

Thus, on the face of it, complex genetic influences would seem to account both for vulnerability to autism itself and for a liability to develop the lesser variants associated with it. Liability appears to be more common among male than among female relatives of probands. Recent studies by Pickles *et al.* (15) and Szatmari *et al.* (4) have both found an excess of affected male relatives of both male and female autistic children. It did not matter whether the proband was male or female; in both cases, there was a significant (and proportionately similar) excess of male affected relatives. Could the sex difference in liability to develop autistic features that fall well short of autism itself also have a genetic explanation? Under a multifactorial liability threshold model, an excess of affected relatives would be expected in the extended families of female probands (the less often affected sex) because a relatively greater degree of genetic risk is necessary to produce phenotypic features in them (16). Remarkably, no evidence has been adduced to support this prediction. For example, in the Pickles *et al.* (15) study, the proportion of first- and second-degree relatives with a broader phenotype, including cousins on both sides of the family, was similar. It was approximately 8% for the relatives of both male and female probands. Also, the male to female ratio among all affected relatives was similar. It was 2.1:1 for male probands and 1.5:1 for female probands. Equivalent findings are reported by Szatmari *et al.* (4).

A GENETIC CONUNDRUM

Accordingly, we need to understand the basis of the marked sex differences seen not only in autism but also in the milder forms of phenotypic expression. If a genetic explanation is responsible, sex linkage is the obvious answer. Yet no X-linked locus has been found by linkage studies (17, 18). Neither a sex-linked nor a simple sex-limited additive multifactorial threshold model is a likely explanation for male vulnerability to the full phenotype and the excess of affected male relatives (8, 19–21). Because no conventional genetic explanation accounts for the distortion in the sex ratio, Pickles *et al.* (15) and Szatmari *et al.* (14) suggested there must be a mechanism that acts independent of genetic liability. In other words, risk could be greater in males because of nongenetic or epigenetic factors (22). One possibility is a maternal effect, a mechanism mediated by the intrauterine environment equivalent to that of phenylketonuria (23). However, no evidence of such a mechanism has yet been found, and it would, in any case, have to interact with some other sex-specific factor to produce the distortion in risk by sex of offspring. An examination of the risks of phenotype concordance in dizygotic twin pairs compared with full siblings might also shed light on this matter. In the Bailey *et al.* (8) study, 10% of dizygotic twin pairs were concordant for the broader spectrum of autism. The equivalent figure for full siblings from Bolton *et al.* (12) was almost identical at 8.6%. This argues against a significant maternal intrauterine effect, which would be expected to have a stronger influence on DZ twins than siblings.

Alternatively, expression of the genes predisposing to autism and its lesser variant or the neurobiologic consequence of their expression could be influenced by exposure to male sex hormones (24, 15). This idea has been around for a long time (25). It is generally believed that sexual dimorphism in the vertebrate brain is generated by the epigenetic action of gonadal hormones (26). The conventional view is that androgens organize male-type brain circuitry regardless of the genetic sex (27). Androgen, after crossing the blood-brain barrier, may be aromatized to estradiol-17β, the steroid thought to be responsible for the establishment of a male brain (28). The organizing effect of androgenization is believed to be irreversible and to occur during a critical period or a time-limited window in development. The sex chromosomes must play a critical role in the determination of sexual phenotype either directly or indirectly, but autosomal loci also contribute to sexual dimorphism (29). Although they are important for gonadal formation, it is not known whether such autosomal genes are expressed in the brain or whether they affect behavioral regulation.

What evidence for such an endocrinologic mechanism, resulting in male vulnerability to autism, has been found? Presumably, if androgens were responsible, they must act via points of developmental sensitivity such as cognitive brain systems that are androgen-sensitive (30). The existence of such systems would be reflected in phenotypic traits that are sexually dimorphic. Enhanced male-brain characteristics should thus be found among autistic males compared with normal males (24). This is supported by evidence up to a point. For example, certain visuospatial abilities are normally superior in males to females (31). Prenatal androgen exposure is believed to be responsible for that sex difference (27). Males with HFA outperform normal males on some visuospatial tasks (32), suggesting, on the face of it, that androgen exposure does indeed potentiate the effects of a genetic predisposition. However, this hypothesis fails to account for the superior performance (compared with normal individuals) of autistic females and female relatives of autistic probands on those self-same

abilities (33, 34). In other words, there is evidence not only that autistic females are superior to normal females on sexually dimorphic tasks that usually confer male advantage, but also that mothers of autistic probands are at least as good as normal males at such tasks and their husbands are even better (33). These findings, which have been replicated (F. Happe, personal communication), seem to indicate that androgen exposure is neither sufficient nor necessary for the enhancement of visuospatial abilities in autistic individuals and their relatives, therefore calling into question the validity of the androgen-exposure hypothesis of male vulnerability.

Second, there is no evidence that females exposed to exceptionally high levels of androgens are more likely to develop autism or autistic-like behaviors (27). However, genetic liability to autism is presumably rather rare, and it could be argued that it would be unusual for such females to be at genetic risk.

Third, an androgen-exposure hypothesis fails adequately to address the observation that female autistic probands do not have an excess of affected first- and second-degree male relatives, as would be predicted by a polygenic threshold of risk model (16). If this model were correct, the relatives of female autistic probands would presumably be subject to a greater degree of genetic risk than the first- and second-degree male relatives of male probands. Male (androgen exposed) relatives of female probands should be at exceptional risk due to androgenic potentiation of their genetic liability. It follows that there should be an increase in the ratio of male:female broader phenotypes in the first- and second-degree relatives of autistic females compared with the relatives of male probands. No such distortion has been described.

An alternative test of the hypothesis that affected females are indeed at higher genetic risk would be to examine the gender of siblings of autistic females who show phenotypic characteristics. Because of "stoppage" (the increased probability that parents who have had an autistic child decide not to have further children) and the rarity of affected females, few data on this subject are available (4, 14). The bias introduced by stoppage means that interpretation of sex ratios should select only families in which there are children born after the autistic proband. There are two possible predictions, depending on the genetic mechanism proposed. First, under a simple polygenic threshold model, the male siblings of an affected female should be at higher risk than her sisters, and the risk should be greater than in families containing only an affected male. Second, a plausible alternative position is that there is genetic heterogeneity in autism and that, in some rare instances, there could be several X-linked genes (on both X-chromosomes) that interact to increase risk in females. If this were so, we should expect the siblings of female autistic children to show an excess of affected females (rather than males). Examining published data from multiplex families provides little support for either the former or the latter prediction (35, 36, 20). Finally, there is no evidence of altered postnatal androgen secretion in individuals with autism compared with normal males (37).

The reason for sexual dimorphism in autism and PDD remains obscure. No simple genetic explanation accounts for the data. Mechanisms based upon putative hormonal influences that confer male vulnerability (or female invulnerability) are also unsatisfactory. Yet there is an alternative possible explanation, and it is based upon well-established genetic principles. The idea was outlined in a study (38) that provided indirect evidence for the existence of an imprinted genetic locus on the X-chromosome. This imprinted locus appears to influence the development of the skills that are necessary for normal fluent social communication. Such skills are seriously impaired in childhood autism as well as in other neurodevelopmental disorders with a male excess, such as attention-deficit hyperactivity disorder and Asperger's syndrome (39).

GENOMIC IMPRINTING

A mechanism based upon X-linked imprinting could account for sexual dimorphism in any phenotypic characteristic, independent of the influence of sex hormones on brain development or functioning (40). The term genomic (gametic) imprinting refers to the differential marking of maternally and paternally inherited alleles of specific genes or chromosome regions during gametogenesis, leading after fertilization to differential expression during development (41). The imprint is placed during spermatogenesis or oogenesis and allows the cell to discern the parental origin of each allele. The allele from one parent is silenced, so that normal development is dependent solely on the function of the allele from the other parent. This imprint is normally erased at some time between generations. Female mammals have a maternally derived X-chromosome (X^m) and a paternally derived X-chromosome (X^p) in each cell, but, in males, the single X is invariably maternal in origin (X^m). Until very recently, no imprinted gene had been described on the X-chromosome in humans, but Naumova *et al.* (42) have reported evidence for such a locus at Xp11.4-p21.1, which is involved in the survival of human male embryos.

We found evidence of an X-linked imprinted locus by comparing classes of females with Turner's syndrome. In this chromosomal disorder, all or a substantial part of one X-chromosome is missing due to nondisjunction or chromosome loss during gametogenesis or early cleavage of the zygote. In 70% of monosomic (45,X) Turner's syndrome, the single X-chromosome is maternal in origin (43); in the remainder, it is paternal in origin. The single X-chromosome in X-monosomy is never inactivated. We karyotyped a series of monosomic (45,X) females (42) and determined the parental origin of the normal X-chromosome by comparing proband and parental DNA polymorphisms located on distal Xp. We have now studied 110 45,X females of whom 31 were $45,X^p$ and 79 were $45,X^m$ with ages from 6 to 25 y. Impaired social competence and adjustment are frequent in Turner's syndrome (44), but a minority has good social skills (45). Intelligence is usually normal in monosomic (45,X) cases, although many have specific learning difficulties (46). We found clear evidence that females with a single paternal X-chromosome possessed superior social-communicative skills compared with those whose single X was maternal in origin (38).

A social communication checklist was devised to summarize the main features of their behavior (Table 1). By social communication, we mean the set of cognitive skills that is necessary to engage in normal reciprocal social interaction with

Table 1. *Social Communication Checklist*

Complete the following section by circling 0 if the statement is not at all true of your child, 1 if it is quite or sometimes true of your child, and 2 if it is very or often true of your child:
Lacking an awareness of other people's feelings
Does not realize when others are upset or angry
Is oblivious to the effect of his/her behavior on other members of the family
Behavior often disrupts normal family life
Very demanding of people's time
Difficult to reason with when upset
Does not seem to understand social skills *e.g.,* interrupts conversation
Does not pick up on body language
Unaware of acceptable social behavior
Unknowingly offends people with behavior
Does not respond to commands
Has difficulty following commands unless they are carefully worded

Internal consistency for set of 12 questions: Standardized item alpha 0.94

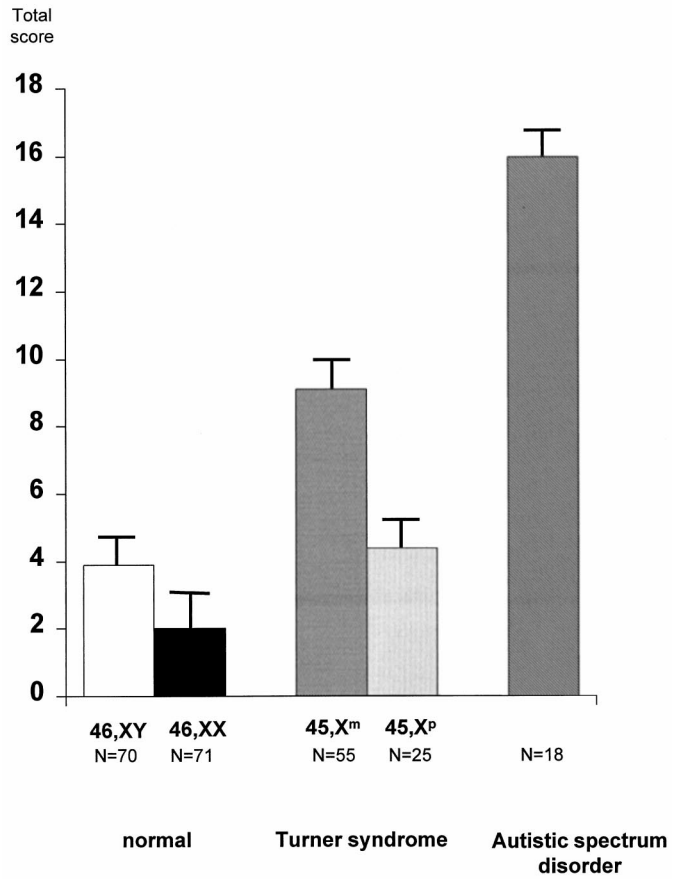

Figure 1. Subscale scores (mean + SE) of questionnaire on social-communicative impairment. Comparison between normal and Turner's syndrome subjects with autistic children (6–18 y). The Social Communication Checklist (Table 1) was completed by parents. In a survey of 175 Turner's syndrome subjects for whom we obtained parental ratings on two occasions, a mean of 2.7 y apart, the intraclass correlation coefficient was 0.81 ($p < 0.01$). Scores correlate with the self-rated social problem subscale of the YSR (67) 0.58 ($p < 0.002$), with the teacher rating on the Teacher's Report Form (68) 0.54 ($p < 0.001$), and with the parent-rated Child Behavior Checklist (69) 0.69 ($p < 0.001$). Range of scores was 0–23 in the Turner's sample, 0–24 in the PDD group, and 0–21 in the normal sample (maximum 24). The validity of the scale was evaluated with a sample of 101 patients attending a neurodevelopmental disorders clinic and 101 age- and sex-matched population controls (6–15 y of age). Using relative operating characteristic (ROC) analysis (70), the optimal cutting point between clinical and normal subjects was 12/13 in this sample (50% prevalence), at which sensitivity was 0.86 and specificity was 0.94. The total area under the ROC curve was 0.94, which is indicative of excellent discriminant validity. Thirty-one percent of the total population of Turner's subjects ($n = 73/221$) scored above this threshold.

others. This was completed by parents of our Turner's sample, by parents of age-matched normal male and female comparisons, and by parents of children with PDD. Higher scores imply poorer social communication and, hence, poorer social adjustment. Figure 1 shows the results for subjects 6 to 15 y of age. These confirm that there are significant differences between $45,X^m$ and $45,X^p$ females in the predicted direction ($p < 0.0001$). Normal boys also obtained significantly higher scores on the questionnaire than normal girls ($p < 0.001$), although this difference is most marked in the 6–11-y age group (47). Children with autism spectrum disorders (including autism, Asperger's syndrome, and other PDD) score exceptionally highly on this scale (mean 16.1, SD 5.9, $n = 18$). That is not surprising, given that social communication deficits are central to the diagnosis of those conditions. However, it is important to note that high scores are not peculiar to PDD. In our own series, children meeting criteria for a diagnosis of attention-deficit hyperactivity disorder (48) obtained an even higher mean score of 19.8 (SD 4.5, $n = 27$). These data suggest that the actions of the locus may not be to increase liability to autism as such but rather to increase male vulnerability to social communication impairments in a range of neurodevelopmental disorders, which preferentially affect males. Further research is needed to elucidate this point.

The imprinting mechanism is deceptively simple. The genetic locus is silenced when it is transmitted by a mother and is switched on when transmitted by a father. A schematic illustration is given in Figure 2. A mother passes a silent copy of the locus to both her sons and daughters, but fathers will only pass on an expressed copy to their daughters. This illustrates the situation in which the expressed locus is on X^p. An imprinted X-linked locus could result in sexual dimorphism in phenotypic characteristics by various mechanisms. The nature of the dimorphism would depend not only on the parental allele from which the imprinted gene is expressed. It would also depend on whether or not the allele in question is subject to X-inactivation (49) and whether or not there is a gene homologue (which is not imprinted) on the Y-chromosome (40). In this example, we assume there is no such homologue.

AN IMPRINTED-X LIABILITY THRESHOLD MODEL

Male vulnerability to autism could be explained by an imprinted-X liability threshold model of risk. According to this model, genetic vulnerability is due primarily to the effects of autosomal loci. Genetic heterogeneity as an explanation of the differing familial patterns of autistic disorder is irrelevant to the model; vulnerability is no more likely to be inherited from paternal than maternal relatives even though phenotypic characteristics will be found more often among male relatives. Females, by virtue of their possessing an imprinted protective locus that is expressed from the paternally derived X-chromosome, have a higher threshold for expression of phenotypic

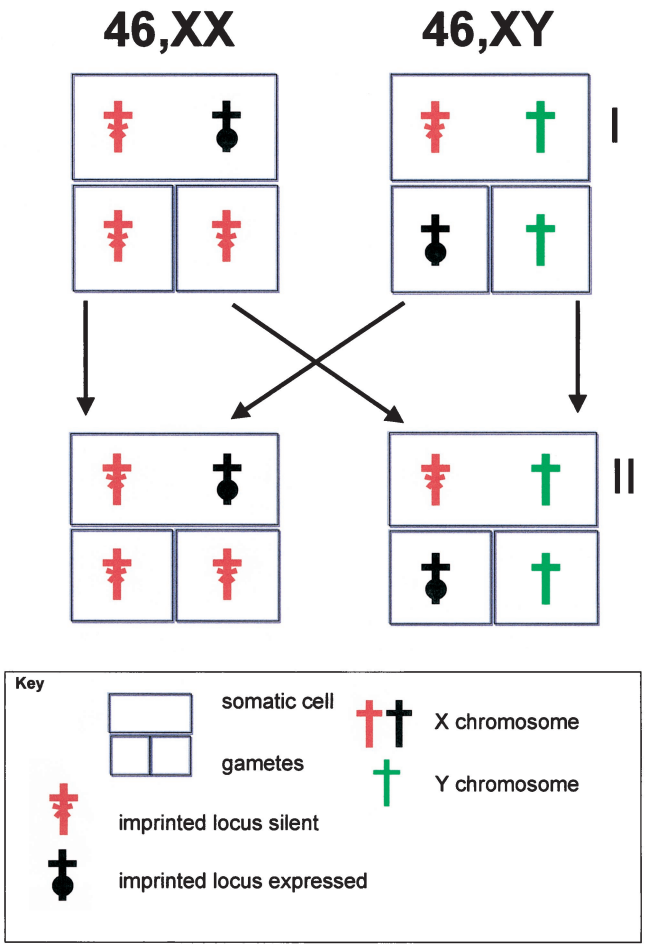

Figure 2. Schematic illustration, showing imprinting of X-chromosome with expression from paternal X in two generations. The putative imprinting mechanism is illustrated. We assume the imprinted locus escapes X-inactivation on the basis of preliminary evidence from the study of females with partially deleted paternal X-chromosomes (38). It is expressed only in somatic cells containing a paternally derived X-chromosome. The imprint, which silences the locus, is set during gamete formation in the female. Hence, in all her gametes, the locus will be silent whether or not that gamete and its X-chromosome is transmitted to her son or daughter. In the male, whose X-chromosome is invariably maternal in origin, the imprinted locus is not expressed in somatic cells. However, gamete formation in the male is associated with removal of the imprint and, hence, an expressed locus is transmitted to daughters. Sons, of course, receive a Y-chromosome from their fathers, and we assume there is unlikely to be a Y-homologue of the imprinted gene (40). Accordingly, the imprinted locus is only expressed when transmitted by a male to a daughter; otherwise it will remain silent in somatic cells (and may do so for generations if passed exclusively down the female line).

features associated with the actions of the imprinted locus than males. The single X-chromosome of males is maternally derived and the protective locus is thereby normally always silenced in men. The imprinted locus is not necessarily functionally polymorphic, although that is the situation for a number of human imprinted genes (50). The failure of studies searching for loci predisposing to autism to detect any X-linked gene of significance (17, 18) may be due in part to this fact and also to the difficulty of detecting parent of origin effects with conventional methods (51).

KEY HYPOTHESES AND PREDICTIONS

A number of specific predictions, some of which are supported by published data, can be made if the proposed model is accepted. The first hypothesis is that social communication skills in which females are normally superior to males (52) are due to the actions of an imprinted gene(s), which is expressed from X^p. A predisposition to autism serves to impair those skills to a uniform degree in males and females. This is illustrated schematically in Figure 3. Figure 3 illustrates a liability threshold model for autism in which ability is continuously and normally distributed in a particular cognitive domain. For example, it could be the ability accurately to identify emotions from facial expressions. This is a skill we know is not only sexually dimorphic in some respects (53), but which is impaired in autistic individuals (54).

We assume that full expression of the autistic phenotype is associated with impairment in a range of such domains, which are usually correlated with one another. A suspicion that an ability is influenced by the imprinted locus arises from the observation that $45,X^m$ Turner's females are less skilled in the domain by the value (a) than $45,X^p$ females (Fig. 3*i*). A genetic predisposition to develop autism in females is indicated by the shift in the distribution of ability (b) in Figure 3*ii*. In Figure 3*iii*, we see the situation for males, whose mean ability in the cognitive domain in question is lower than in normal females by the quantity (a), because they do not possess the expressed

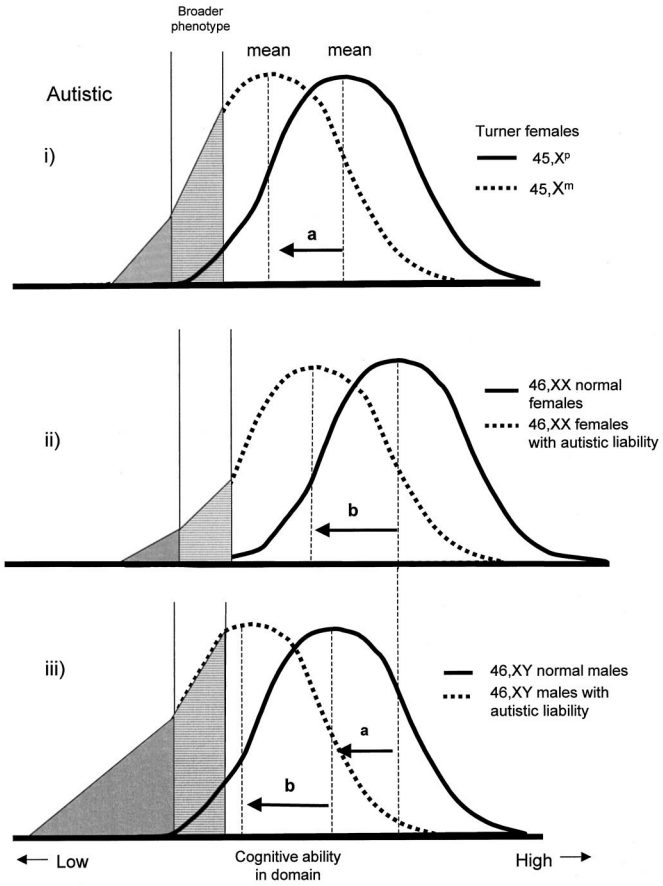

Figure 3. Imprinted-X liability threshold model for cognitive domain influenced by actions of X-linked gene expressed only from paternal X-chromosome.

locus X^p. Now consider the situation if a male possesses an equivalent genetic liability (b) to the autistic phenotype as the female in Figure 3*ii*. Because his mean ability in the domain is already lower, this leads to a correspondingly greater shift of the distribution to the left. Thus, a relatively higher proportion of affected males will be seen with both the broader and the classic autistic phenotype than the proportion of females with an equivalent genetic predisposition. Note that the mean ability in the domain illustrated in Figure 3*i* is lower in both groups of Turner's females than normal females (3*ii*). The relative positions of mean values for 45,X, 46,XX, and 46,XY individuals in respect of scores on the Social Communication Checklist approximate those shown, according to our preliminary findings. The overall impairment in Turner's syndrome (relative to normal females) is likely to be due to nonspecific effects of haploinsufficiency of X-linked genes upon brain development and functioning (55).

Phenotypic features in the relatives of autistic probands could reflect a wide range of autistic characteristics. The threshold for their phenotypic expression will follow the same principles illustrated in Figure 3. Just which features will be observed will depend on whether or not there is any specificity between the genetic predisposition (*i.e.* the exact configuration of genes responsible for the autistic proband) and the cognitive and other deficits associated with autism. This is not known, although Szatmari *et al.* (56) reported a high phenotypic correlation among siblings with autism, suggesting that, within families, there is probably some consistency. Whether or not there is genetic heterogeneity in the etiology of autism, expression of phenotypic features in female relatives who are carrying the predisposing genetic liability to autism will be most marked in domains in which the imprinted locus is *not* influential. In general, these will be aspects of behavior or cognition, related to the autistic phenotype, in which $45,X^m$ females do not differ from $45,X^p$ females. By extension, they would be domains in which there is normally no gender difference in prevalence or ability or domains of cognitive ability in which there is normally male superiority, such as performance on the embedded figures task (57).

Autism is associated in approximately 75% of cases with mental retardation (7). The reason for this is unknown, but it could indicate a lowering of the threshold for phenotypic expression in the presence of low IQ. If that were the case, we would expect to find fewer relatives with a broader phenotype among the families of retarded autistic individuals than among the families of those with higher functioning. We would incidentally also expect to find an association between low IQ and autistic features among relatives of probands. There are few comparisons of this sort, but, overall the data support the prediction. In particular, relatives of probands with higher IQ are at greater risk than those of probands with lower IQ (4).

If females are normally protected from developing autism by the actions of a genetic locus on the paternal X-chromosome, the most common reason for female autism is likely to be a lowering of the threshold for phenotypic expression by virtue of impaired general (especially verbal) intelligence. Accordingly, we should expect the male:female ratio among autistics with mental retardation to be lower than it is among those with high IQ (HFA). This is reportedly the case (58, 2). The model also predicts that autistic females will have, in general, lower IQs than autistic males (36).

If the imprinted-X liability threshold model is true, we should find a genetic explanation for the lowered threshold of protection among higher functioning females with autism. There may be imprinting variation at the X-linked locus due to metabolically modified imprint setting during gametogenesis (see Fig. 2). Note that this would be an epigenetic rather than a DNA sequence variation explanation for polymorphism. Accordingly, some females may be conferred lesser protection for nongenetic reasons. Functional polymorphic imprinting has been described for the human genes encoding IGF-II, WT1, and the human 5-HT_{2A} receptor gene *HTR2A* (50).

Alternatively, less efficient protection could come about because the imprinted locus is damaged, thereby absent, or silenced because of structural rearrangements of the X-chromosome (59). If this were the case, a number of further predictions could be made. Females with autism (especially those with HFA) should be excessively likely to have structural abnormalities of the paternal X-chromosome. No systematic cytogenetic investigation has yet been made of this matter, although we are undertaking such a study at present in collaboration with the Wessex Regional Genetics Laboratory and the Cambridge Autism Group. By analogy, we should expect to find an excess of autism (or at least the broader phenotype) among females with structurally abnormal (paternal) X-chromosomes or with absent paternal X-chromosomes (X-monosomy in Turner's syndrome). On the other hand, structurally abnormal or absent maternal X-chromosomes should not be associated with increased risk. There are several published accounts of autism in association with structural abnormalities of the X-chromosome (60, 61), although these have not reported the parental origin of the abnormality. The model predicts that females with HFA may show phenotypic features that differ somewhat from those of males. Features that are influenced by the actions of the imprinted locus (*i.e.* in domains in which there is a female advantage in normals) will be less marked in females who have developed the disorder because they are relatively protected in such domains (Fig. 3). There have been several reports that autism, when it occurs in females, is associated with a less severe phenotype in respect to social impairment than when it occurs in males (2, 3). This could reflect the fact that social communication skills are closely linked to the actions of the expressed locus. On the other hand, our own research did not find that behaviors such as restricted interests and stereotyped behaviors were more common among $45,X^m$ than among $45,X^p$ females (of equivalent IQ). For that reason, these features of the autistic phenotype are not likely to be influenced by the actions of the imprinted locus, and they should be found as frequently among autistic females as males. This prediction is also supported by some studies (3).

The imprinted-X liability threshold model also predicts that structurally abnormal paternal (but not maternal) X-chromosomes will be associated with an increased liability to autism. We have now identified 10 Turner's females with autism (meeting ICD-10 criteria) (62) out of a total sample of 221 who

were personally assessed in the course of this investigation (4.5%) (63). All had retained a normal maternal X-chromosome and had either a missing or a structurally abnormal paternal X. Of these 10, four have now been assessed with the Autism Diagnostic Interview (ADI) (1), and they meet full ADI algorithm criteria. The other six were assessed with the Autism Behavior Checklist (64). Proportionately, 10/156 Turner's females with a structurally abnormal or missing paternal X-chromosome were affected, and 0/65 with a retained normal paternal X-chromosome. This difference is statistically significant ($p = 0.028$; the Fisher exact test).

If females who are lacking an expressed copy of the imprinted locus on the X-chromosome are particularly vulnerable to autism, why is their threshold for phenotypic expression not the same as in males (who also lack the protective mechanism)? Our data on Turner's females suggest a rate of disorder that is substantially (>100 times) higher than the population prevalence of 4.8/10 000 from epidemiologic studies (6). If the X-imprinted threshold model is correct, affected Turner's syndrome females may have an excess of relatives with the lesser variant of autism. We have, as yet, no data to confirm or refute this hypothesis, but family studies will be conducted to address the issue. On the other hand, it is possible that there is a more general effect of X-monosomy or a large structural X-chromosomal anomaly causing chromosomal imbalance (65) that provokes the autistic phenotype in females lacking a complete paternal X-chromosome.

Finally, does the postulated existence of an imprinted X-linked locus that protects females from developing autism have any relevance to studies aimed at discovering the genetic basis of the disorder? The answer is definitely in the affirmative. The effects of the imprinted locus are clearly wide-ranging. From what is know about the actions of other imprinted loci in mammals, a surprising number do not code for proteins at all but code for RNA that have been conserved through evolution, thus presumably having important biologic functions (66). Imprinted genes often regulate the actions of other genes elsewhere on the genome. Once the X-linked imprinted locus (or loci) has been identified in molecular terms, it would be theoretically possible to discover its site(s) of action. For instance, the RNA from the imprinted locus may bind to regulatory regions of autosomal genes that influence susceptibility to autism. Identifying the binding sites would provide a route toward discovering the autosomal loci involved. It is not implausible to assume that working out just how this imprinted locus operates will give clues to the whereabouts of sites that confer vulnerability to autism and its variants.

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