Sexual dimorphism in cognition and behaviour: the role of X-linked genes

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Abstract

Chimpanzees and humans last shared a common ancestor between 5 and 7 million years ago; 99% of the two species’ DNA is identical. Yet, since the paths of primate evolution diverged, there have been remarkable developments in the behavioural and cognitive attainments of our species, which ultimately reflect subtle differences in gene structure and function. These modifications have occurred despite evolutionary constraints upon the diversity of genetic influences, on the development and function of neural tissue. Significant species differences can be observed both at the levels of function (gene expression) and structure (amino acid sequence). Protein evolution is driving an accelerating increase in brain complexity and size. Playing centre stage, in terms of the proportion of genes involved in brain development and cognitive function, is the X chromosome. Recently, it has become clear that a long-standing theory, implicating X-linked genes in a sexually antagonistic evolutionary role, is probably correct. Genes on the sex chromosomes can directly influence sexual dimorphism in cognition and behaviour, independent of the action of sex steroids. Mechanisms by which sex-chromosomal effects, due to X-linked genes, influence neural development or function are reviewed. These include the biased expression of genes subject to X-inactivation, haploinsufficiency (in males) for non-inactivated genes with no Y homology, sex-specific brain functions and genomic imprinting of X-linked loci. Evidence supporting each of these mechanisms is available from both human and animal models. Recently, the first candidate genes have been discovered.

Evolution and the sex chromosomes

Chimpanzees and humans last shared a common ancestor between 5 and 7 million years ago. In crude terms, 99% of the two species’ DNA is identical. Yet, since our paths of evolution diverged, that 1% of genetic difference has changed humans substantially, in terms of our behavioural and cognitive traits – presumably driven to a large extent by positive Darwinian selection. In this article, the evidence for evolutionary changes in terms of gene structure and gene expression in brain will be discussed, followed by a review of the specific characteristics of the X chromosome and its potential role in the development of sexually dimorphic characteristics.

We can now compare chimpanzee and human genomes, both in expression patterns of associated genes (transcriptomes) and in DNA structure (genomes). Evolutionary pressures that distinguish us from our nearest primate cousins can be summarized both in terms of transcriptome and genome, by comparing variation between individuals within a species (human or chimpanzee in this instance) and variation between species. In order to identify evidence for positive selection, a measure of divergence to diversity is calculated. Comparisons are made to a common ancestor of both chimps and humans, the so-called out-group method. In practice, the out-group chosen usually comprises macaque monkeys, which last shared a common ancestor with us at least 20 million years ago (2). A high ratio of gene expression divergence between species, to gene expression diversity within species, could imply positive selection. Evolutionary constraints upon gene expression are generally greater for genes that are ubiquitously expressed, than for those with restricted expression in one tissue (e.g. brain). Ubiquitously expressed genes differ less among individuals both within and between species, than do genes expressed in single tissues (1). Khaitovich et al. (1) found substantial expression divergence for X-linked genes in testes comparing humans to chimpanzees (but low diversity within species). The above finding supports the theory that X-linked loci can evolve rapidly and
influence sexual development, if there is an evolutionary advantage to males (3). Gene expression patterns tend to differ less between humans and chimpanzees in the brain than in most other tissues measured (1) presumably because of the greater impact of constraints. However, it is possible to estimate the relative magnitude of rate of change in gene expression, comparing chimpanzees and humans over the past 5 million years. This rate of change has been greater in the human lineage, particularly in brain. Expression divergence between species has mainly involved upregulation. Compared with the out-group macaque, up to 92% of human genes are upregulated in the brain (a much higher proportion than in other tissues), many of which are involved in neuronal functions, synaptic activity and metabolism (4).

Evolutionary pressure on gene structure, in terms of alterations in the DNA sequence, can be estimated by comparing synonymous nucleotide substitutions with non-synonymous substitutions. High ratios could indicate positive selection for a functionally different gene. Genes that are expressed in brain (whether ubiquitous or not) have relatively few structural variants within and between species, a similar picture to that observed for gene expression (1). There have nevertheless been potentially functionally significant changes in DNA sequence for genes expressed in brain: the average rate of protein evolution for nervous system genes is much higher in primates than in rodents (measuring both from an independent common ancestor; for rodents, the common ancestor of rat and mouse) (5). When we compare the changes in gene structure since our last common ancestor, we find accelerated evolution for genes involved in brain development and cognitive function in humans, compared with chimpanzees (6), reflected in relatively more dramatic changes in brain complexity and size.

**Sexual selection**

Characteristics that are selectively favoured in one sex, but selected against in the other sex, are known as ‘sexually antagonistic’. If selection acts on existing genetic variation within a population, a sexually dimorphic trait will evolve from the initial monomorphic state. Genes advantaging males will increase the chances of their passing on those genes to the next generation (8). The evolution of the X chromosome is intrinsically linked to the evolution of the Y chromosome (9), and Y-linked genes are obligatorily sexually dimorphic. Could X- or Y-linked genes directly influence the development and function of neural tissues in a sexually dimorphic way, independent of the action of sex steroids? Evidence was initially found in the Zebra finch. This songbird is sexually dimorphic in the production of vocalizations (males sing). The genetic sex of the brain directs masculinization independent of gonadal influences (10). One cannot induce the female to sing by giving male sex hormone. Either one can cause the males to desist from singing by castration early in life, or by blocking sex steroid receptors. Art Arnold (11) has been the main proponent of a view that sex chromosomes directly influence sexual differentiation in mammals as well as birds (which have a completely different sex-chromosome system).

Sexually dimorphic influences on human cognition and behaviour may affect the phenotypic expression of ‘disorders’ and ‘traits’. ‘Disorders’ are sporadic/heritable abnormalities due to non-functional or otherwise mutated genes. ‘Traits’ represent normal variation in sexually dimorphic characteristics. X-linked disorders, such as fragile X syndrome or Rett syndrome, are sexually dimorphic in their expression but they represent extreme cases – dysfunction of a critical gene, even though expression of that gene might be neither dominant nor recessive in the conventional Mendelian sense (12). X-linked behavioural traits, quantitative variants, include male aggression and parental behaviour (13). Sexually dimorphic cognitive traits include spatial orientation; in rodents, male spatial learning advantages observed in the radial or water maze are caused by male–female differences in strategy selection. Females (rats and humans) navigate preferentially using landmarks, but males rely on a broader set of spatial representations (14). These traits are probably influenced by a Y-linked locus (15), although an X-linked locus may play a contributory role (16). During evolution, could X-linked genes for specific cognitive abilities, and a female preference for males who demonstrate those traits, have become closely linked, and hence jointly inherited (17)? Owing to the obligatory expression of all X-linked genes in males, any X-linked trait that is advantageous to males (or to females) would spread rapidly in the population. ‘If higher cognitive abilities were a critical step in our own evolution, it makes sense that you might find those functions on the X-chromosome’ (Willard, quoted by Erica Check (7)).

**Cognition, behaviour and the X chromosome**

It is estimated that there are 807 protein-coding genes on the X chromosome (Ensembl v37), together with many others coding for RNAs. In 2006, Online Mendelian Inheritance in Man recorded 1329 entries for ‘mental retardation’. Of these, 342 (25.7%) mapped to the X chromosome, suggesting X-linked genes play a disproportionate role in the development of human intelligence. Why should there be such a concentration on this particular chromosome (18)? One possible
Mechanisms of sexual dimorphism involving X-linked genes

Mechanisms of sexually dimorphic gene expression in the brain include the following: first, genes subject to random X-inactivation in females will be fully expressed from the male’s single X chromosome, but their region of expression in females will depend on a mosaic pattern of X-inactivation. Secondly, some X-linked genes may be differentially expressed in male and female brains. Thirdly, many X-linked genes escape inactivation either partially or completely, lacking a functional homologue on the Y chromosome, consequently they are potentially haploinsufficient in males. Fourthly, X-linked genes may be subject to genomic imprinting, expressed only from the allele inherited from either mother or from father.

X-inactivation and sexual dimorphism

During X chromosome inactivation, one of the pairs of homologous X chromosomes present in the same nucleus is (partially) silenced (33). In mammalian cells, there is only one active X, irrespective of aneuploides. Inactivation proceeds in stages. First, there is a counting process, where the number of X chromosomes relative to autosomes is determined. Secondly, a choice is made about which X remains active. Silencing of the other X chromosome is initiated and spreads from the X-inactivation centre. A range of heterochromatic features maintains the inactive state through subsequent cell divisions, even when the inactive chromosome is transferred into another species (e.g. human X into hybrid mouse cells). X-inactivation is a random process occurring very early on in development, at the blastocyst stage. Clones of cells deriving from the progenitor cells maintain the same pattern of X-inactivation; we do not know how extensive these clones are in brain, or whether they are selected according to any particular pattern depending on the parent of origin.

Skewing from the expected 50/50 ratio may occur simply by chance, extremely skewed inactivation patterns can result from the mutations of the X-inactivation centre, or from large deletions of part of the X chromosome. Potentially, any characteristic associated with an X-linked polymorphism, which is subject to random X-inactivation would be expressed differently in dichorionic monozygotic (MZ) twin girls, than in MZ boys because even ‘identical’ female twins are not identical in terms of their random pattern of X-inactivation. Theoretically, we would expect greater diversity of phenotype in any trait linked to an inactivated allele, in females. Accordingly, we should be able to identify cognitive or behavioural traits that are influenced by X-linked genes (subject to inactivation) by looking at within-MZ pair correlations for
sexual dimorphism. Examining data from a large twin study to test this hypothesis, Loat et al. (34) found that prosocial behaviour, verbal skills and peer relationships were influenced in the predicted direction (MZ female pairs less similar than MZ male pairs), although the effect size was very small. The hypothesis also predicts female dizygotic (DZ) pairs should be more similar than male DZ pairs for equivalent traits, because males receive only one of their mother's two X chromosomes. Assuming each son receives a different X chromosome, with functionally distinct alleles, such allelic differences will be exaggerated to their full extent. On the other hand, females would theoretically have a more mixed picture, which is complicated by allelic heterogeneity and X-inactivation. Loat et al. (34) found that this prediction was supported in terms of the phenotypes prosocial behaviour and verbal skills, supporting their hypothesis that X-linked genes do influence these traits directly. Incidentally, they did not find greater male variance for these X-linked traits, implying the X-inactivation 'male extremes' hypothesis may not be valid, at least in terms of prosocial behaviour and verbal abilities.

**Genetic sex: models of sexual dimorphism**

To work out the potential impact of the genetic sex of cells, independent of gonadal phenotype, on brain development and function we must develop model systems. The preferred model is the mouse, in which it has been proved possible to manipulate the sex-determining region of the Y chromosome in ingenious ways (35). Females that have a Y chromosome but lack Sry (the sex-determining gene necessary for the development of the testis) can be produced. Males can be produced with two X chromosomes plus a functionally distinct allele, such allelic differences will be exaggerated to their full extent. On the other hand, females would theoretically have a more mixed picture, which is complicated by allelic heterogeneity and X-inactivation. Loat et al. (34) found that this prediction was supported in terms of the phenotypes prosocial behaviour and verbal skills, supporting their hypothesis that X-linked genes do influence these traits directly. Incidentally, they did not find greater male variance for these X-linked traits, implying the X-inactivation 'male extremes' hypothesis may not be valid, at least in terms of prosocial behaviour and verbal abilities.

**Genes escaping X-inactivation and sexual dimorphism**

Pairing between the X and Y chromosomes in the 2.7 Mb pseudoautosomal region (PAR1) at the tip of the short arm (Xp) is obligatory in male meiosis, and there is a second 330 kb pseudoautosomal region at the tip of Xq (PAR2). Genes in the PAR1 region are identical on both chromosomes, and escape inactivation, as do most genes in PAR2. Many X-linked genes outside these regions escape inactivation too (40). This is surprising because, beyond the PAR, only 25 genes have functional homology with the Y chromosome (41). If many X-linked genes with no Y chromosome equivalent escape X-inactivation, a potential mechanism exists for sexually dimorphic expression; XY males could be haploinsufficient relative to XX females. Carrel and Willard (40) discovered only about 65% of X-linked genes are completely inactivated in normal XX females. A further 20% are inactivated in some, but not all cells, and 15% consistently escape inactivation. The implication of this remarkable research is that females have considerable heterogeneity in gene expression. At least 15% of X-linked genes are likely to be expressed at higher levels in females than males, but an additional
10% show heterogeneous X-inactivation. Could this mechanism have evolved to engender greater diversity in the female phenotype, than in males who will necessarily express only a single copy of such genes?

Nguyen and Disteche (19) used array expression profiles to study global transcriptional output from the mammalian X chromosome compared with the rest of the genome. Since only one X chromosome is present in males, and in females much of the second X is inactivated, there should, in theory, be a doubling of the product output of each such X-linked gene in order to match autosomal gene dosage. Dosage-dependent differences in chromosome-specific gene expression would be detected by comparing the mean global expression of X-linked genes to that of autosomal genes. If no compensation did occur, the X-autosome expression ratio would be 0.5, otherwise it would be unity. The calculated ratio is indeed close to unity in adult somatic tissues. Targeting specifically the 15% of genes that escape X-inactivation in females with two X chromosomes, Nguyen and Disteche (19) found the average female to male expression ratio to be 1.11 in humans (relatively few X-linked genes escape X-inactivation in mice: evolution seems to have influenced a peculiarly human arrangement). Nevertheless, there was a wide variation in the observed female-to-male ratio for the 27 genes investigated (0.1–2.94). This implies sexually dimorphic expression of some such genes can occur, and may have functional consequences. However, there were in general rather low levels of expression from ‘escape’ genes on the inactive X chromosome, so gross dosage may not be much affected. One of the most interesting findings concerned X-linked gene expression in brain. Exceptionally highly expressed X-linked genes (where the transcriptional output is more than doubled) are relatively common in human brain, but unusual in other somatic tissues. A similar observation can be made in the brain tissues of other mammalian species, including chimpanzee, gorilla and macaque, independent of gender. Genes that are expressed both in brain and in somatic tissues are, if biased one way or the other, substantially more likely to be over-expressed in brain. How this upregulation is achieved is, at present, unknown.

**Mouse models of X monosomy**

If there are adequate mechanisms to upregulate the single X chromosome in males, it might be thought that syndromes of X monosomy would be associated with relatively few phenotypic consequences. In contrast to the human, mice have proportionately far fewer genes that escape X-inactivation (42). For many years, X-monosomic mice were considered not a good model for Turner syndrome (human X monosomy) because they are fertile, and do not have gross phenotypic anomalies in terms of growth or cognitive abilities. On the other hand, there are subtle differences in their behaviour compared with XX mice, and these have been the subjects of recent investigation. 39,X mice can be generated by the fertilization of a normal gamete by a sex-chromosome null gamete, and therefore are free from the problem of mosaicism – which might potentially influence the correct interpretation of X-monosomic data in humans (43). 39,X mice show greater fear reactivity than 40,XX mice (44); they spend less time on the open arm of the elevated plus maze, a standard method for measuring anxiety in mouse models. Importantly, careful controls show that excessive anxiety associated with X monosomy is not influenced by the stage of the oestrus cycle, locomotor activity, response to novelty or the parental origin of the single X chromosome. What candidate genes might be responsible? This might seem fairly straightforward to investigate because of the paucity of mouse X-linked genes escaping inactivation and therefore being potentially haploinsufficient. One prime suspect was the steroid sulphatase gene Sts, the product of which is known to interact with gamma-aminobutyric acid (GABA)A receptors. The GABA system plays a critical role in the pathophysiology of anxiety (45) and Sts enzymatic activity correlates with aggression (46). Unfortunately, that hypothesis was not supported (44); in a partial X-deletion mouse model, designed to ensure that both Sts expression and the expression levels of associated GABA A subunits were normal, excessive fear reactivity persisted. Therefore, it must be related to haploinsufficiency for a different (as yet unidentified) X-linked gene that escapes inactivation in mice.

**Human X monosomy**

The clinically defined condition of Turner syndrome is due to the partial or complete loss of one of the sex chromosomes, either the second X chromosome or the Y chromosome (47). A contributory factor is oestrogen (and androgen) insufficiency. Since non-inactivated (hence potentially haploinsufficient) genes contribute to the development and maintenance of ovarian tissues (48), there is early degeneration of the ovaries. Turner syndrome is associated with short stature, due largely to haploinsufficiency for the SHOX gene, expressed from PAR1 (49), plus other variable physical feature. Females with Turner syndrome nearly always have normal verbal intelligence, although about 80% are deficient in terms of visuospatial abilities, such as the ability to complete a jigsaw puzzle (50). Arithmetical abilities are often seriously impaired too (51). Dosage-sensitive X-linked genes appear to be involved in numerical cognitive skills and spatial intelligence (52).

However, one of the most striking associated behavioural phenotypic features concerns social adjustment. Many females with Turner syndrome in childhood and adulthood have limited number of friends and
may become socially isolated (53). There is a substantially increased risk of autism (at least 200 times) (54). Profound face and emotion recognition deficits affect a minority and they cannot easily determine the direction of other’s eye gaze and line of sight (55). The nature and severity of these social cognitive deficits is similar to that seen in cases of bilateral amygdalectomy or Urbach–Wiethe disease. Consequently, haploinsufficiency for one or more X-linked genes could have a specific impact on development of the amygdala and its connections with cortical centres involved in the processing of social cognitions, the ‘social brain’ (56). We have attempted to identify the genes responsible, using a deletion-mapping technique. By this means, we have discovered that one or more candidates lie on the proximal short arm, near the centromere, at Xp 11.4 (57). A cluster of genes that escapes X-inactivation can be found at the same location (40). Haploinsufficiency for X-linked genes in Turner syndrome influences brain structure. Deletion of the critical region of the X chromosome at Xp11.4, whether terminal or interstitial, results in an enlarged amygdala and increases in grey matter volume in the orbitofrontal cortex bilaterally, close to a region implicated in emotional learning (57). The increase in amygdala size is even greater than the relative difference normally found between males and females (58) implying that haploinsufficiency for one or more dosage sensitive genes might contribute to sexual dimorphism in this structure. Replication, of the volumetric differences in the amygdala of Turner subjects, has been found by some investigators (59) but not by all (60). Other structural brain changes in X monosomy include the parieto-occipital region (possibly related to the visuospatial and arithmetic difficulties), the cerebellum and the basal ganglia and the superior temporal gyrus (60). The superior temporal gyrus has efferent connections with the parietal lobes and the prefrontal cortex, both of which are structurally anomalous in XO females; so sexual dimorphism in the cortical thickness of certain regions could reflect the actions of X-linked genes (61).

**X-linked imprinting and sexual dimorphism**

The theory that X-linked imprinting could be a mechanism for sexual dimorphism arose from the observation that females with X-monosomic Turner syndrome differed in their cognitive and behavioural phenotypes according to the parental origin of their single X chromosome (62). Imprinted genes are inherited in duplicate, but only one is expressed; this might be either the maternal or the paternal allele. Imprinting of autosomal genes should not bring about any bias in expression of the phenotype by sex, but because of the asymmetrical inheritance of the X chromosome (males invariably inherit only the maternal X) sexual dimorphism could accompany X-linked imprinting. Sexually dimorphic gene expression could occur whether the expressed allele was paternal (inherited only by females) or maternal – provided the latter was subject to random X-inactivation (63), in which case average female expression would be half-male expression. Skuse et al. (62) found that X-monosomic females with a single paternal X were, in general, better socially adjusted than those with a single maternal X. These differences were reflected in normal male:female behaviour, on the same measures, implying X-linked imprinting could contribute to human sexual dimorphism in social cognitive competence.

**X-linked imprinting in X-monosomic mice**

The identification of genes that could be imprinted on the X chromosome and contribute to sexual dimorphism required the generation of a mouse model. 39,X mice were tested for behavioural inhibition. 45,Xm (maternal X-only) Turner syndrome females are less competent than either 45,Xp (paternal X-only) or 46,XX females at a simple task (64), which requires the inhibition of a prepotent response (62). Males are also less competent at the task than normal females. The Y-maze, a visual, non-spatial, serial reversal-learning paradigm, was used in which mice are trained to go down one of the two goal arms to collect a foodstuff reinforcer. 39,Xm mice showed impaired reversal learning. There were no significant differences in performance between the 39,Xp and 40,XX mice. Male–female performance differences in the task were in the predicted direction. The Xlr3 gene is the candidate (64, 65); it is imprinted and maternally expressed. Studies of normal male and female mice indicate the gene is paternally silenced and subject to X-inactivation. Therefore, expression is sexually dimorphic (approximately twice as great in males as females).

**X-linked imprinting in X-monosomic humans**

Structural brain differences in Turner syndrome females reflect the parental origin of their single X chromosome. X-linked imprinting influences the volume of the superior temporal gyrus (66), as well as occipital white matter and cerebellar grey matter (67). 45,Xm women have larger right hippocampal volume than 45,Xp subjects (60), a finding that could be linked to the observation that 45,Xp females have poorer visual memory than 45,Xm females, despite their better social adjustment (68). 45,Xm females also possess significantly smaller volumes of caudate nucleus and thalamus than 45,Xp subjects. Whilst there is consistent evidence concerning the sexual dimorphism of the superior temporal gyrus (61), white and grey matter differences by gender in other structures
potentially influenced by X-linked imprinted genes are less clearly defined.

Conclusions

X-linked genes are under strong evolutionary pressure, and are evolving more rapidly in humans than in other species. They are preferentially expressed in the brain, and are likely to contribute to increasing cortical complexity and size. Sexual dimorphism in the actions of X-linked genes is to be expected. At least four different mechanisms could lead to differential expression of X-linked genes in males and females, and evidence is available to support each one of them. The first candidate genes have recently been discovered.

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