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Autism-lessons from the X chromosome

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Recognized cases of autism spectrum disorders are on the rise. It is unclear whether this increase is attributable to secular trends in biological susceptibility, or to a change in diagnostic practices and recognition. One hint concerning etiological influences is the universally reported male excess (in the range of 4:1 to 10:1). Evidence suggests that genetic influences from the X chromosome play a crucial role in engendering this male vulnerability. In this review, we discuss three categories of genetic disease that highlight the importance of X-linked genes in the manifestation of an autistic phenotype: aneuploides (Turner syndrome and Klinefelter syndrome), trinucleotide expansions (Fragile X syndrome) and nucleotide mutations (Rett Syndrome, Neuroligins 3 & 4, and SLC6A8). The lessons from these diseases include an understanding of autistic features as a broad phenotype rather than as a single clinical entity, the role of multiple genes either alone or in concert with the manifestation of autistic features, and the role of epigenetic factors such as imprinting and X-inactivation in the expression of disease severity. Better understanding of the clinical phenotypes of social cognition and the molecular neurogenetics of X-linked gene disorders will certainly provide additional tools for understanding autism in the years to come.

Keywords: autism; X chromosome; social cognition; genetics

INTRODUCTION

Autism spectrum disorders are characterized by impaired language, impoverished social interaction and repetitive activities and behavior. There is tremendous variability between affected individuals even within the same family. In addition, family members can display mild autistic traits that do not reach diagnostic threshold (Hughes *et al.*, 1997; Hughes *et al.*, 1999; Landa *et al.*, 1992; Murphy *et al.*, 2000; Piven and Palmer, 1999; Piven *et al.*, 1994, 1997; Wolff *et al.*, 1988). This is referred to as the 'broad autism phenotype'. The study of this wide spectrum of phenotypes is contributing to our understanding of autism as a syndrome with multiple non-genetic and genetic causes (Muhle *et al.*, 2004).

The causal role of genes for the broad autism phenotype is supported by a monozygotic twin concordance rate between 60% and 90% and a dizygotic concordance rate of approximately 5% (Bailey *et al.*, 1995; Le Couteur *et al.*, 1996). Identified medical conditions (i.e. prenatal infections), cytogenetic abnormalities (i.e. 15q duplication) and single gene defects (i.e. RELN and UBE3A) account for <10% of cases. The remaining or 'idiopathic' cases potentially result from multiple gene interactions. As the number of recognized cases of autism continues to escalate, the search for etiology with whole genome screens, cytogenetic studies and probing of candidate genes identified from linkage analysis studies continues at a rapid pace.

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Clinically recognized cases of autism and autistic spectrum disorders are on the rise. The historic prevalence of autism at 4/10 000 has been superceded by estimates ranging from 1–6/1000 individuals (Chakrabarti and Fombonne, 2005; Fombonne, 1999). It is unclear whether this increased prevalence is attributable to secular trends in biological susceptibility, or to a change in diagnostic practices and recognition. Understanding the underpinnings of this potentially devastating childhood disorder is of paramount importance to affected individuals, their families and their communities. An important hint concerning etiological influences is the universally reported male excess (in the range 4:1 to 10:1) (Skuse, 2000). Increasing evidence suggests that genetic influences from the X chromosome play a crucial role in engendering this male vulnerability.

In this review, we discuss three categories of genetic disease that highlight the importance of X-linked genes in the manifestation of an autistic phenotype: aneuploides, trinucleotide expansions and nucleotide mutations (Table 1) We review the sex-chromosome aneuploides: Turner syndrome (TS) and Klinefelter syndrome. We consider the trinucleotide expansion disorder, Fragile X syndrome (FXS). Finally, we review the emerging evidence that mutations in the neuroligin genes 3 & 4, and the creatine transporter gene, SLC6A8, may be associated with autistic spectrum disorders. One hopes that the lessons learned from these progressively well-defined disorders will help in understanding and treating idiopathic autism and other diseases of social cognition in the future.

SEX CHROMOSOME ANEUPLOIDES

Some patients with autistic phenotypes have abnormalities in chromosomal structure or in the total complement

Table 1 Estimate of prevalence for X-linked disorders with autistic features

X-linked disorders	Prevalence information
Turner syndrome (XO)	• 5/10 000 live female births (Gravholt, 2005) • Up to 5% with autism, >25% with ASD[16]
Klinefelter syndrome (XXY)	• 10–40/10 000 in general population (Bojesen *et al.*, 2003; Lanfranco *et al.*, 2004)
Fragile X syndrome	• 2.5/10 000 males • Up to 30% of those diagnosed with Fragile X will meet criteria for ASD (Hagerman *et al.*, 2005) • Only 2% of children diagnosed with autism will have the FMR1 full mutation (Reddy, 2005)
Rett Syndrome (MECP2)	• 1/10 000 female neonates (Hagberg, 1985) • 2.8% of females with autism (Carney *et al.*, 2003)
Neuroligins 3 & 4	• Unable to establish
Creatine transporter disorder (SLC6A8)	• 2.1% in males with X-linked mental retardation (Rosenberg *et al.*, 2004) • 1% of males with unspecified mental retardation (Clark *et al.*, 2006) • No population estimates

of chromosomal material. Numerical abnormalities are associated with either the loss or gain of chromosomal material (as in trisomy 21, or 47,XXY). Structural anomalies are subtler, and usually involve microdeletions of a few thousand nucleotide bases, or more rarely the loss of a substantial part of a chromosome (such as the short arm of an X chromosome). Structural anomalies may be associated with the doubling or tripling of chromosomal segments. Such anomalies may occur during the formation of the gamete, during the formation of the zygote or after cell division begins in early embryonic development. In the former situation, the abnormality is going to be represented in every somatic cell. If the structural problem occurs later, in a segment of developing cells, then the individual will be a mosaic for the abnormality. In these cases, the distribution of the affected cells will be clonal, reflecting the original population of normal or abnormal cells from which they were derived. We do not expect the distribution of those clones to be random. Distribution depends on whether the abnormal cells in question are able to contribute to the development of the particular tissue in which they are expressed. If they are not capable of functioning to a certain minimal degree of efficiency, they could be selected against as the tissue develops and thus would not be represented.

X-monosomy: Turner syndrome

TS, a numeric chromosome anomaly associated with the inheritance of a single X chromosome (45X), was originally described in the 1930s by Henry Turner. It is a common chromosomal aneuploidy with a prevalence of 5/10 000 live female births (Gravholt, 2005). The most striking physical characteristics are short stature, webbed neck and out-turned arms. In addition, individuals have degeneration of the ovaries soon after birth ('streak ovaries') leading to absent secondary sexual characteristics in the absence of estrogen replacement therapy. Verbal intelligence is often normal in X-monosomy, but there are associated deficits in non-verbal skills and in arithmetical abilities. Their deficits are mainly in the domains of social reciprocity and communication, with relative preservation of formal language skills and verbal intelligence. In addition, there is a substantial increase in the risk of autism for children with TS (Creswell and Skuse, 1999).

Genetic anomaly and phenotype correlation. TS is due to the partial or complete loss of one of the sex chromosomes, either the second X chromosome or the Y chromosome. The observed phenotype appears to result from two influences: haploinsufficiency and sex hormone effects. Haploinsufficiency occurs when an individual lacks the genetic contribution from the paired gene, from either the autosomes or sex chromosomes. In this case, females with TS have an unpaired X chromosome. Genes in the pseudoautosomal regions (PAR1 and PAR2) at the tips of the long and short arms of the X chromosome, have complete homology with the equivalent regions on the Y chromosome. There are also many genes that lie outside the PAR which escape X-inactivation thus may need two copies for normal female development (Carrel and Willard, 2005). Hormonal factors are also likely to play a role as non-inactivated genes on the X chromosome contribute to the development and maintenance of ovarian tissues. Early degeneration of the ovaries, in turn, leads to estrogen insufficiency. The effects of haploinsufficiency and sex hormone deficiency will be milder in patients with mosaic anomalies as a portion of the individual's cells will have a normal female karyotype (46, XX). Occasionally, individuals will have a structural anomaly or a partial deletion of the Y chromosome, which also may lead to milder expression of the disease.

Parent of origin is another factor which may affect genotype–phenotype correlation in TS. Males invariably inherit a single X chromosome from their mothers. Consequently, X-linked genes may have sexually dimorphic expression due to a process of imprinting where expression is dependent on the parent of origin. In other words, certain X-linked genes may only be expressed from paternally inherited genes thus only in females. Alternatively, expression could be exclusively from the maternally inherited X chromosome and would be sexually dimorphic if the gene concerned was subject to X-inactivation (Davies *et al.*, 2005a). Skuse *et al.* (1997) investigated this question in a study contrasting X-monosomic females whose single X was either maternal or paternal in origin (Skuse *et al.*, 1997). The paternally expressed allele appeared to confer superior social–cognitive abilities. In addition, recent work with mouse models of X-monosomy has identified a paternally imprinted, maternally expressed gene as having a circumscribed impact on the cognitive–behavioral phenotype. More work is needed to understand the genetic mechanisms involved and their impact on social cognition (Davies *et al.*, 2005b).

Cognitive and behavioral phenotype. Females with TS are known to have specific cognitive and behavioral deficits that both overlap and are distinct from children with idiopathic autism. Females with X-monosomy may have preserved verbal intelligence with up to 80% having deficits in non-verbal abilities. These are typified by impaired copying of complex designs, poor visuospatial memory and slow motoric processing (Bishop *et al.*, 2000). Like individuals with idiopathic autism, females with X-monosomy similarly show deficits in socio-perceptual processing as measured by impaired recognition of faces, facial emotion and direction of gaze (Elgar *et al.*, 2002; Lawrence *et al.*, 2003a; Lawrence *et al.*, 2003b). In addition, women with X-monosomy are severely impaired on abilities which require mentalizing or intuiting another's 'theory of mind'. This has been measured by the ability to infer a person's emotional state from looking at photographs of their eye regions and from the ability to attribute mental states to animated shapes (Frith, 2003). Comparatively, the deficit in 'reading the mind from the eyes' is more severe in women with TS than people with autism, while the deficit in processing 'fear' from faces appears to be at least as profound in women with TS as in high functioning people with autism (Lawrence *et al.*, 2003a; Lawrence *et al.*, 2003b). These claims of relative difference must however be taken as indicative rather than authoritative. These tasks are sensitive to verbal and non-verbal intelligence, and show age-related differences, as they do in autism (Lawrence *et al.*, 2006). The etiology of the deficits in 45,X females may not be the same as in idiopathic autistic disorders, despite their superficial similarity as endophenotypes.

Behaviorally, girls with TS frequently have problems forming and maintaining peer relationships and these become all the more complex in adolescence. As adults, many women experience difficulties with family and partner relationships as well as the ability to fit into a work environment with other adults. The social deficits of the syndrome are very striking, but may not be immediately obvious to pediatricians or endocrinologists who are primarily responsible for the care of people with the syndrome.

Neural phenotype. Many studies have attempted to correlate these cognitive and behavioral changes with cerebral abnormalities. Cortical structural and functional imaging in women with X-monosomy has indicated abnormalities in specific neural regions posited to play a role in mentalizing abilities. These include abnormalities of the superior temporal sulcus together with abnormal connectivity, especially between anterior and posterior temporal regions (Molko *et al.*, 2004). Volumetric gray matter differences are found in the amygdala and orbito-frontal cortex (Good *et al.*, 2003; Kesler *et al.*, 2003; Kesler *et al.*, 2004). Functional anomalies in the amygdala have also been reported in relation to facial expression processing (Skuse *et al.*, 2005). Functional imaging has confirmed that (medial) orbitofrontal cortex and superior temporal sulcus regions are involved in mentalizing (Castelli, 2005; Castelli *et al.*, 2000; Sabbagh, 2004). Activation in regions adjacent to the amygdala (anterior medio-temporal) has also been reported for the animations task under 'theory of mind' presentation conditions. All these findings suggest that the anomalous processing of 'theory of mind' measures may reflect atypical cortical structure and function of the 'social brain' in women with TS.

There is also evidence supporting the theory that X-linked imprinted genes may play a role in the X-monosomic social phenotype. X-linked imprinting appears to influence the volumes of the superior temporal gyrus as well as occipital white matter and cerebellar gray matter. Cutter *et al.* (2006) employed magnetic resonance imaging and proton magnetic resonance spectroscopy to investigate brain anatomy and metabolism in X-monosomy. Using both a hand-traced region of interest approach and Voxel-based morphometry, $45,X^{m(maternal)}$ women were shown to possess a significantly larger adjusted right hippocampal volume than $45,X^{p(paternal)}$ subjects. This description may explain a prior finding that $45,X^{p}$ females have poorer visual memory than $45,X^{m}$ females, despite their better social adjustment (Bishop *et al.*, 2000). In addition, $45,X^{m}$ females had significantly smaller caudate nucleus and thalamus than those with a single paternal X-chromosome. Dysfunction of the caudate nucleus could play a role in the observed abnormal executive function with impaired working memory, planning ability, set-shifting and social cooperation. Maternally expressed X-linked genes might, therefore, influence hippocampal development, while paternally expressed genes influence the normal development of the caudate nucleus and thalamus in females. This ongoing research is encouraging and has contributed to investigation of this disorder as well as other disorders of social cognition.

XXY syndrome: Klinefelter syndrome

Klinefelter syndrome, like TS, is a sex chromosome aneuploidy (47,XXY). This disease, first described by Harry Klinefelter in 1942, occurs in as many as one in 500 male live births. The condition is associated with an additional X chromosome, which can either be maternal or paternal in origin (each being equally common.) The physical phenotype is often not apparent until after puberty. In contrast to TS, tall stature and long limbs characterize affected males. There is also gonadal dysfunction with inadequate testis development and prominent infertility.

Genetic anomaly and phenotype correlation. There is wide phenotypic heterogeneity and it is likely that up to two-thirds of Klinefelter syndrome cases are never identified clinically (Lanfranco *et al.*, 2004). In contrast to TS, this variability is not due to mosaicism. In fact, mosaicism with a normal 46,XY cell line is very unusual in Klinefelter syndrome. While, there does not appear to be a relationship

between paternal age and disease prevalence, there is some suggestion that those with an additional paternal X chromosome are more likely to suffer impairments of motor, speech and language skills than 47,XXY males with an additional maternal X chromosome (Stemkens *et al.*, 2006). This finding is consistent with the Skuse *et al.* (1997) hypothesis concerning X-linked imprinting and disease expression as typical males would never inherit a paternal X chromosome. The implication is that by doing so, one would expect a higher dosage effect with two paternally expressing X-chromosomes and hence a more severe phenotype.

In aneuploides of the X chromosome only one of the additional X chromosomes is active, however there may be many. The process of chromosome counting seems to work in an equivalent way to female X-inactivation, although it has to be remembered that many X-linked genes escape inactivation and therefore are being over expressed in the syndrome. There is no clear candidate for the contribution to a behavioral or cognitive phenotype in the syndrome, but Lopes *et al.* (2006) has suggested that PCDH11X could be a contributory factor. In humans, it has a homologue on the Y chromosome in contrast to other mammals (Lopes *et al.*, 2006). This neuronally expressed gene is very likely to escape X-inactivation on the additional X chromosome in 47,XXY males (Ross *et al.*, 2006). This pattern of expression is not influenced by the parental origin of the additional X chromosome. This may be the first hint of a genetic mechanism for the Klinefelter cognitive phenotype.

Cognitive and behavioral phenotype. Individuals with Klinefelter Syndrome, unlike those with TS, have relative deficits in verbal skills, particularly those used for verbal working memory, verbal processing speed, reading and language comprehension (Bender *et al.*, 2001; Fales *et al.*, 2003; Itti *et al.*, 2006). Individuals may present with specific learning disabilities such as dyslexia (Bender *et al.*, 2001; DeLisi *et al.*, 2005). Non-verbal abilities tend to be less impaired. This contradistinction to TS invokes the hypothesis that tight modulation of X-linked gene expression is critical for verbal and non-verbal intelligence development.

There has been a resurgence of interest in the behavioral phenotype of Klinefelter syndrome in recent years. One reason is the possibility that the condition is associated with an increased risk of schizophrenia or affective disorder with psychotic features especially auditory hallucinations (Bojesen *et al.*, 2006; DeLisi *et al.*, 2005). 47,XXY men sometimes find social interactions relatively difficult. They may appear introverted, anxious, impulsive, quiet, unassertive and socially withdrawn (Geschwind *et al.*, 2000). Socially inappropriate and anti-social behaviors were once thought to be more common in those with Klinefelter syndrome, but this is probably due to ascertainment bias. It is important to bear in mind that the majority of cases in the general population are never identified, but imprisonment is associated with checks for chromosomal anomalies (Bojesen 2003).

As with TS social difficulties in Klinefelter syndrome arise from deficits in social cognitive processing (van Rijn *et al.*, 2006). These include disturbances in perception, experience and expression of social cognitive information. Specifically, 47,XXY men may have increased emotional arousal in response to emotion-inducing events; they may appear more influenced by their emotions in strategic decision making; and they may find it difficult to perceive their own emotions. Small studies have found deficits in executive functions such as response inhibition which is essential in these social interactions (Temple and Sanfilippo, 2003).

Neural phenotype. There appears to be central nervous system changes associated with Klinefelter syndrome. In particular, whole brain volumes, frontal lobe and limbic-associated regions (superior temporal gyrus, amygdala, insula and the anterior cingulate gyrus) appear diminutive (Patwardhan *et al.*, 2000; Shen *et al.*, 2004). The temporal lobe changes which show decreased volume of the posterior aspect of the superior temporal gyrus are interesting in light of its association with social deficits and language processing disorders (DeLisi *et al.*, 2005). In addition, abnormalities were found in neural connectivity to the frontal and temporal lobes using Diffusion Tensor Imaging. Finally, there have been suggestions that individuals with Klinefelter syndrome lack the usual asymmetry found in controls during language processing functional imaging tasks, implicating the upper left temporal and parietal regions (Itti *et al.*, 2003). Both TS and Klinefelter syndrome highlight the likely importance of multiple genes and epigenetic factors in the ultimate expression of brain-based social and cognitive disorders.

TRINUCLEOTIDE REPEAT EXPANSIONS

Some autosomal-dominant and X-linked conditions become more severe in successive generations. Often these diseases result from genetic 'anticipation' or the expansion of a repeat sequence from parent to child. Trinucleotide repeats are triplets of nucleotides (i.e. CTG, CAG, etc.) that can occur within a gene or its regulatory region. They can interrupt either the actual coding sequence (the exons) or the areas between the coding regions (the introns.) In addition, such as in the case of FXS, they can reside in front of a coding region which directly influences the methylation of the gene and can lead to transcriptional silence. A large proportion of triplet repeat diseases such as Fragile X Syndrome, Huntington's disease and myotonic dystrophy have severe neurological impairment. The considerable phenotypic heterogeneity is believed to result in part from the variable length of the repeat sequence.

FMR1: Fragile X syndrome

Fragile X syndrome (FXS) is the leading cause of inherited mental retardation. It occurs in approximately one in 4000 males. Up to 30% of those children are also diagnosed with

autism spectrum disorders, however, only 2% of autistic children overall will have the fragile X mental retardation protein 1 (FMR1) full mutation (Pembrey *et al.*, 2001; Reddy, 2005). Females are also affected at a rate of one in 8000 and often with a milder phenotype. As the disease anticipates through the maternal germ line, male offspring are often far more severely affected than their mothers. Expansion, or anticipation, typically occurs with maternal transmission (carriers with greater than 90 repeats) rather than through the paternal germ line. Affected individuals may have dysmorphic features such as a long narrow face, large protruding ears and macro-orchidism. However, this is not universally true. Individuals with FXS are often brought to clinical attention due to developmental delay and autistic features.

Genetic anomaly and phenotype correlations. Identified by Herbert Lubs in 1969, FXS is caused by the deficiency or absence of the FMR1 gene (Verkerk *et al.*, 1991). In well over 99% of cases, the molecular defect is an expansion of cytosine–guanine–guanine (CGG) repeats in the 5′ untranslated region of the FMR1 gene. This gene lies on the long arm of the X chromosome at Xq27.3. Typical individuals have 5–40 CGG repeats. Men and women with 55–200 repeats are said to have the FMR1 premutation which is now known to cause the Fragile X, Tremor and Ataxia syndrome (FXTAS) (Hagerman and Hagerman, 2004). Expansions greater than 200 repeats are known as full mutations. These mutations leads to hypermethylation of the FRM1 gene which inhibits transcription and leads to decreased production of its protein product (Snow *et al.*, 1993).

The FMR1 protein (FMRP) is strongly expressed in neurons. It is believed to function as an RNA binding protein that may suppress messenger RNA, which is important for dendrite formation and signal transduction proteins (Hagerman *et al.*, 2005). It is thus an important element of translational regulation of messages that are involved with synaptic maturation, synaptic plasticity, axonal guidance and experience-dependent learning and synaptic pruning (Hagerman *et al.*, 2005). In addition, FMRP binds to glucocorticoid receptor mRNA and subunits of the γ-aminobutyric acid A ($GABA_A$) receptor, which may help to explain the enhanced stress response and seizure predisposition in individuals with FXS.

Multiple determinants of phenotypic severity, including genetic and environmental factors, are being investigated through human and animal research. Clearly, expansion length correlates directly with disease severity and inversely with age of onset. In addition, methylation mosaics and repeat-size mosaics can exist which are associated with a more benign expression of the disease. Methylation mosaicism exists when the gene is not fully methylated in all cells. Repeat-size mosaicism occurs in cases of post-conception expansion of this unstable region, leading to variation of triplet length in different cell lines. Finally, a female's expression of the disease is likely to be affected by the skewing of her X-inactivation (Heine-Suner *et al.*, 2003; Wolff *et al.*, 2000). If a higher proportion of the wild type X chromosome is inactivated, the female will show signs of disease.

Cognitive and behavioral phenotype. Evidence is emerging to suggest FXS is associated with a complex pattern of cognitive disabilities, with areas of preserved function and areas of greater deficit. In contrast to TS and Klinefelter syndrome, global (verbal and nonverbal) mental retardation exists in most cases. There is relative sparing of specific skills, such as face recognition, emotion processing and 'theory of mind' (Cornish *et al.*, 1999, 2001, 2004). Studies have shown deficits in sequential online processing, short-term visual spatial memory, motor planning and impulse control. The repetitive and impulsive behavior also translates into speech production with frequent stuttering. Executive function skills, such as planning, attention and set-shifting are also impaired in females with the full or premutation, and these seem to worsen with chronological age.

The association between FXS and autism has intrigued researchers and clinicians for many years. Similar to children with idiopathic autism, children with FXS find it difficult to use verbal and non-verbal communications effectively. They may also have other signs and symptoms reminiscent of autistic children, including hyperarousal, social anxiety, withdrawal, stereotypic behaviors, gaze aversion and impaired social reciprocity (Hessl *et al.*, 2006). There is preliminary evidence to suggest that both girls and boys with the full mutation find direct gaze aversive, and that it is associated with increased stress reflected by cortisol reactivity. For other children, the avoidance of direct eye contact may reflect a lack of social interest. Investigators are continuing to pursue the etiology of social difficulty in this population.

Neural phenotype. A series of studies using structural neuroimaging of individuals with FXS have found anomalies associated with the full mutation (Hessl *et al.*, 2004). These include enlargement of the hippocampus, amygdala, caudate nucleus and thalamus. The cerebellar vermis and superior temporal gyrus are reduced in size. Many of these regions are recruited during social engagement and the processing of social information in typical individuals, including direction of gaze and 'theory of mind' tasks. In a series of functional neuroimaging studies, females with a premutation show an inadequacy to recruit brain regions that are associated with certain specific cognitive tasks, such as arithmetic processing. This is particularly interesting as arithmetic skills may be relatively more impaired in females with the full and premutation when compared with reading and spelling skills. However, while suggestive, this has not been replicated in all studies (Fisch, 2006; Lachiewicz *et al.*, 2006). The in-depth study of FXS is ongoing with the hope of targeting behavioral and pharmacologic treatments for affected individuals.

SINGLE NUCLEOTIDE DISORDERS

Many disorders arise from inherited and spontaneous mutation of a single or group of nucleotides affecting a particular gene. Disease causing mutations can result from basepair substitutions, insertions, deletions or rearrangements. Furthermore, these alterations can arise in multiple locations including regulatory elements (i.e. the promoter region), coding regions and the intronic regions that affect splicing control of transcribed mRNA. The location and extent of a change will determine the pathogenicity and severity of the resulting clinical phenotype. For example, promoter mutations that completely block transcription of a gene are generally more significant than a basepair change that does not affect a coding sequence or does not change a specified amino acid. Once disease-causing mutations have been identified, the candidate gene is often screened in at-risk populations. This process helps to determine disease prevalence attributed to particular genes. Several genes have been investigated in this way for both mental retardation and autistic features.

MECP2: Rett syndrome

Rett syndrome, originally described by Andreas Rett in 1966, is a progressive neurodevelopmental X-linked dominant disorder that predominantly affects females. It occurs in one/10 000–15 000 female neonates (Hagberg, 1985). Although originally believed to be lethal to male carriers, mutations in the causative gene have now been identified in surviving males. The disease presentation and progression can be variable but ultimately the clinical phenotype is quite distinctive. Development in the first year is generally on target. After this initial stage, development is marked by acquired microcephaly, stereotypic hand wringing and the regression of motor, language and social skills. Over 80% of affected females have epilepsy (Steffenburg *et al.*, 2001). Following early neurodegeneration, there is a period of stabilization.

Rett syndrome which has long been recognized to have phenotypic overlap with autism, has been clinically grouped with classic autism and Asperger syndrome in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) (American Psychiatric Association, 2000). Verbal and non-verbal communication regression are a core feature of the disorder, early signs of delay in social and play skills such as not recognizing familiar adults and appearing socially disinterested have also been reported (Charman *et al.*, 2002). Despite these clinical similarities, MECP2 mutations are relatively rare causes of classic autism (Carney *et al.*, 2003; Lobo-Menendez *et al.*, 2003). Certainly the presence of autistic regression and seizures warrants genetic evaluation for MECP2 mutations which is now clinically available.

Genetic anomaly and phenotype correlation. It is now known that Rett syndrome results from mutations in the methyl-CpG-Binding Protein2 (MECP2) in 96% of classic Rett syndrome cases (Amir *et al.*, 1999). MECP2 lies on the long arm of the X chromosome (Xq28) and has widespread expression within the central nervous system. Sequencing of clinically affected patients has revealed over 200 types of mutations including: missense mutations, non-sense mutations, frameshift mutations, large deletions and splice site mutations. Mutations that are either frameshift or nonsense in nature may be associated with more severe phenotypes than missense mutations, however, the protein domain affected has also been shown to have clinical ramifications (Chae *et al.*, 2004; Charman *et al.*, 2005; Dotti *et al.*, 2002; Gomot *et al.*, 2003; Schanen *et al.*, 2004; Weaving *et al.*, 2003).

MECP2 protein appears to play a role in targeted transcriptional gene silencing as well as RNA splicing. The methylation-binding domain (MBD) of the normal MECP2 protein binds to methylated CpG islands at the 5-prime end (promoter region) of other genes. This potentially leads to the formation of a co-repression complex that prevents transcription of adjacent chromatin (Martinowich *et al.*, 2003; Segawa & Nomura, 2005). Specifically, mouse and rat models have suggested that this repressor complex may be targeted by both brain derived neurotropic factor (BDNF), which is implicated in synaptic plasticity, and Hairy2a, which is involved in neurogenesis (Chen *et al.*, 2003). MECP2 mutant mice have also been shown to exhibit aberrant RNA splicing patterns. The mouse model in this disease is proving especially useful given its close phenotypic similarity to the human phenotype including delayed onset of symptoms, forepaw stereotypies, and progressive impairment of social behavior and cognition.

Mutations in MECP2 have now been found in both males and females with a wide range of severity and traits. This heterogeneity appears to be a consequence of the type of mutation and the X-inactivation pattern. Until recently, MECP2 mutations were thought to be an X-linked dominant mutation with lethality in hemizygous males. There is now evidence that the female predominance is in large part caused by sporadic paternal germ line mutations (Trappe *et al.*, 2001). The implication is that only female offspring inherit the mutated paternal X chromosome while the males inherit a normal paternal Y chromosome from their father and a normal X chromosome from their mother. As affected females rarely reproduce, this is most commonly a sporadic condition, predominantly affecting girls. That being said, we are now recognizing that males can inherit an affected X chromosome from carrier mothers if these females have a significant skew towards their normal X chromosome and thus are mild or asymptomatic carriers of the disease due to skewed X-inactivation (Orrico *et al.*, 2000).

Cognitive and behavioral phenotype. There are several stages in the development of Rett syndrome and clinical symptomatology is affected unevenly through

the progression of this disease. During the period of developmental arrest and regression in classic Rett syndrome, children will have deterioration of visual processing, memory, and expressive language function (von Tetzchner *et al.*, 1996). Girls will often lose all ability to communicate verbally and with expressive gestures, other than eye movement or facilitated communication boards. In comparative studies of girls with Rett syndrome relative to IQ matched females, girls with Rett syndrome were noted to have less communicative behavior and function (Woodyatt and Ozanne, 1997). Finding alternate means of communication becomes crucial, as many of the challenging behaviors prevalent in this syndrome are a result of frustration engendered by inability to get needs and wants communicated to care providers. This issue is magnified when there are multiple changing providers.

Behavioral features reminiscent of autism are often seen during the period of early arrest. These include stereotypic repetitive behaviors, including but not limited to hand wringing. Furthermore, diminished social and communicative intention and interaction are the hallmarks of both disorders and have lead to the lumping of these diseases under the autism spectrum disorder heading. There has been considerable effort to correlate these severe disabilities with their neural underpinnings.

Neural phenotype. Rett syndrome, like autism, has been reported to show decreased neuronal size and increased neuronal packing (Armstrong, 2005). In addition, there are reports of reduced arborization of dendritic spines (Segawa and Nomura, 2005; Shibayama *et al.*, 2004). These abnormalities are thought to affect the cortex as well as the basal ganglia, the basal forebrain and the brainstem given the manifestations of abnormal breathing, cardiac pacing, swallowing and autonomic dysregulation. Both groups may have seizures, but the EEG findings in Rett syndrome are more reliable and robust, including an occipital dominant rhythm, slowed background and multifocal spike and wave activity. Rett syndrome has highlighted the importance of seeking out a neurogenetic diagnosis for an atypical autism presentation. In addition, the study of this disease has brought into sharp focus the range of presentation and severity of autistic features caused by a single gene.

NLGN3 and NLGN4: neuroligins 3 and 4

Many researchers have searched for single autism associated genes in multiplex autism families and the X-linked mental retardation cohorts. Neuroligin 3 (NLGN3) and neuroligin 4 (NLGN4) were first reported to be associated with autism in 2003 (Jamain *et al.*, 2003). Subsequently, they have been extensively investigated for prevalence in large samples. Despite considerable effort to establish the prevalence of these excellent candidate genes, large sequencing efforts in the United States, Canada and Europe have yielded no additional disease associated mutations (Blasi *et al.*, 2006; Gauthier *et al.*, 2005; Vincent *et al.*, 2004; Ylisaukko-oja *et al.*, 2005). Thus far, the neuroligins appear to represent a rare but informative cause of inherited mental retardation and autism.

Genetic anomalies and phenotype correlations. There remains interest in this gene family given the role of its protein products. The neuroligins are cell adhesion molecules that lead to the formation of functional neural synapses. In particular, NLGN1 is abundant in the post-synaptic membrane of glutamatergic synapses, suggesting specific targeting of excitatory synapses. Furthermore, NLGN 3 (Xq13) and NLGN4 (Xp22.32) appear to promote the formation of presynaptic elements in hippocampal neurons (Chih *et al.*, 2004; Jamain *et al.*, 2003; Laumonnier *et al.*, 2004; Talebizadeh *et al.*, 2006; Yan *et al.*, 2005).

The initial genetic changes in NLGN3 and NLGN4 were found in two Swedish families. The first, in NLGN3, resulted from a missense mutation and the second, in NLGN4, constituted a basepair insertion. Both were in exon 5 and were found to segregate with disease and not in healthy controls (Jamain *et al.*, 2003). An additional disease-associated mutation was found in a French family with multiple family members affected with either X-linked mental retardation or autism spectrum diseases or both (Laumonnier *et al.*, 2004). Given the small numbers of affected individuals and the wide interfamilial heterogeneity even with the same mutation, genotype–phenotype correlations are as yet impossible.

Cognitive, behavioral and neural phenotype. Individuals identified with mutations in the neuroligins have phenotypes that range broadly from normal general intelligence to severe mental retardation. Seizures appear to be associated with NLGN3 mutations but not NLGN4. To date, there are no published neuroimaging or pathologic findings. More work on these disorders is likely to lead to an increased understanding of their relevance to autism and mental retardation as clinical testing for mutations in these genes is now clinically available.

SLC6A8: creatine transport deficiency

In contrast the neuroligins, creatine deficiency syndrome was first detected in 2001 and appears to contribute significantly to males with mental retardation and autistic features (Salomons *et al.*, 2001). Prevalences of 2.1% and 1% have been reported in males with known X-linked mental retardation and unspecified mental retardation, respectively (Clark *et al.*, 2006; Rosenberg *et al.*, 2004). Creatine and phosphocreatine are essential components of neural functions underlying cognitive processes. They assist in the storage and transmission of phosphate bound energy (Salomons *et al.*, 2003). Individuals maintain their creatine pool via biosynthesis and nutritional uptake. The creatine transporter gene allows cells to take up creatine from their blood supply and thus is thought to be crucial to neuronal energy supplies (Nash *et al.*, 1994). There are two other

disorders of creatine metabolism that are also known to lead to neurological dysfunction.

A clear physical phenotype is beginning to emerge for this disease. Individuals affected with creatine transport deficiency tend to have short stature, dysmorphic features, dysphagia, hypotonia and poor motor development. The characteristic facial features for this syndrome are reported to include progressive myotonic facies with ptosis, midface hypoplasia, long narrow face, prominent chin and occasional microcephaly (Clark *et al.*, 2006; Kleefstra *et al.*, 2005). These facial features and a thin body habitus appear to segregate with disease and affect the carrier females (Mancini *et al.*, 2005). In addition, multiple-affected individuals have gastrointestinal immobility, generalized myopathy and motoric (pyramidal and extrapyramidal) abnormalities. These physical features can help clinically support the suspicion of these diseases and prompt a timely evaluation.

Genetic anomaly and phenotype correlation. Creatine transport deficiency is caused by mutations in the solute carrier family 6 member 8 gene (SLC6A8) at Xq28. In total, 16 families have been found to carry a mutation in the SLC6A8 gene. There does not appear to be any single hot spot and the sites of these mutations are variable, being scattered throughout the exons. They include non-sense mutations, small and large deletions and one missense mutation leading to the use of alternative splice site (Clark *et al.*, 2006). There appears to be less within-family than between-family variation in the phenotype. It is unclear whether this results from a directly genotype–phenotype relationship or whether there are as yet unidentified gene-environment interactions. Female heterozygotes have a milder phenotypic expression than their male relatives. If the mutated gene is subject to X-inactivation then disease severity could be modulated by skewing of gene expression, due to biased inactivation.

Cognitive and behavioral phenotype. The pattern of cognitive and behavioral deficits in creatine transporter deficiency has recently been described. Males with creatine transporter deficiency generally have mild to moderate mental retardation with severe expressive speech and language disorders (Bizzi *et al.*, 2002; Cecil *et al.*, 2003; deGrauw *et al.*, 2003; Salomons *et al.*, 2003) Neuropsychological testing has revealed specific deficits in executive control including impaired attention, poor impulse modulation and aberrant semantic-pragmatic language skills. Female heterozygotes are also reported to have similar but milder learning disabilities. This profile appears distinct from other known mental retardation and autism syndromes (Mancini *et al.*, 2005).

Neural phenotype. Epilepsy is common in creatine-deficient disorders and can be challenging to control. Furthermore, structural abnormalities have been reported. Magnetic resonance imaging has shown primarily white matter abnormalities ranging from a small focus of hyperintense signal in the right posterior periventricular white matter to global ventricular enlargement and reduced area of the corpus callosum (Mancini *et al.*, 2005). There are currently no functional imaging or pathologic studies in this population. This disease can be easily identified if suspected through magnetic resonance spectroscopy of the brain and/or quantification of creatine in the cerebral spinal fluid, blood and urine.

CONCLUSIONS

There has been a long-standing hope that we would be able to discover the neurogenetic basis of major brain behavior disorders. While this is proving challenging, inroads are being made. In our review of syndromes associated with anomalous expression of X-linked genes, we find evidence that such genetic influences do influence the integrated functioning of the 'social brain'. The hope that we can arrive at a more rational system for classifying neuropsychiatric disorders such as autism, by means of identifying genes that increase susceptibility to them, is more elusive.

The difficulty with categorization is exemplified by examining behavioral and cognitive phenotypes that are known to be associated with specific genetic disorders such as those reviewed here. First, even single gene disorders, such as FXS or Rett syndrome, have pleiotropic effects. Some affected individuals have autistic features, but others do not. There is no invariable set of behavioral characteristics, and it is arguable that only non-autistic features (e.g. the cluttered speech of FXS, the hand-wringing of Rett syndrome) are specific to the disorder. Second, these conditions are associated with moderate to severe mental retardation, in people with the full mutation. The prevalence of autistic features increases among people with low IQ, whatever the reason for their condition. Whether there is greater specificity in the phenotype amongst a representative sample of individuals with FXS (for example) than with idiopathic mental retardation is not known. Third, it has proved extraordinarily difficult to identify a clear relationship between the influence of any single gene (out of several potential candidates) and any aspect of the autistic clinical phenotype. There are probably important modifying influences that could be environmental in origin.

So, what insights into the developmental neurobiology of idiopathic autism result from identifying syndromes resulting from anomalies of X-linked genes? The lessons we have learned from these diseases include an understanding of autistic features as a broad phenotype rather than as a single clinical entity, the role of multiple genes either alone or in concert with the manifestation of autistic features and the role of epigenetic factors such as imprinting and X-inactivation in the expression of disease severity. It is likely that the majority of the behavioral and cognitive features associated with idiopathic autism result from the aggregate effect of allelic variation in the normal range, influenced by a substantial number of genes, some of which

may be X-linked. So far, the specific genes we have identified on the X chromosome that lead to autistic features, if mutated, do not seem to possess those qualities of population-wide normal variation. Their non-functionality is an all-or-nothing quality, and the null variants are rare. Furthermore, they are almost invariably associated with a substantial detrimental impact on general intelligence, with the implication that any autistic features could be an epiphenomenon. Thus while the story is a complicated one, the search goes on and progress is being made. Mouse models have recently been described in which dosage-sensitive X-linked genes have remarkably specific effects upon behavioral and cognitive phenotypes independent of an impact on general learning capacity or 'intelligence' (Davies *et al.*, 2005b; Raefski and O'Neill, 2005). Perhaps the first elusive candidate gene for susceptibility to autism is not so far away after all?

Conflict of Interest

None declared.

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