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Report

Skewed X-Chromosome Inactivation Is a Common Feature of X-Linked Mental Retardation Disorders

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Some deleterious X-linked mutations may result in a growth disadvantage for those cells in which the mutation, when on the active X chromosome, affects cell proliferation or viability. To explore the relationship between skewed X-chromosome inactivation and X-linked mental retardation (XLMR) disorders, we used the androgen receptor X-inactivation assay to determine X-inactivation patterns in 155 female subjects from 24 families segregating 20 distinct XLMR disorders. Among XLMR carriers, ~50% demonstrate markedly skewed X inactivation (i.e., patterns ⩾80:20), compared with only ~10% of female control subjects ($P < .001$). Thus, skewed X inactivation is a relatively common feature of XLMR disorders. Of the 20 distinct XLMR disorders, 4 demonstrate a strong association with skewed X inactivation, since all carriers of these mutations demonstrate X-inactivation patterns ⩾80:20. The XLMR mutations are present on the preferentially inactive X chromosome in all 20 informative female subjects from these families, indicating that skewing is due to selection against those cells in which the XLMR mutation is on the active X chromosome.

As a result of X-chromosome inactivation (Lyon 1961), heterozygous females are mosaic for X-linked gene expression, with one population of cells expressing genes from the maternal X chromosome and the other population expressing genes from the paternal X chromosome (Nance 1964). The relative ratio of these two cell populations in a given female is frequently referred to as the "X-inactivation pattern." For female carriers of an X-linked mutation or structural abnormality, one cell population may be at a selective growth disadvantage, resulting in clonal outgrowth of cells with one or the other parental X chromosome active (Belmont 1996; Puck and Willard 1998; Willard 2000). Because the choice of one or the other X chromosome early in the process of X inactivation is generally random (Lyon 1961), significant deviation or skewing from an expected mean X-inactivation pattern (i.e., 50:50) in a specific population of female carriers suggests that the X-linked mutation alters in vivo cell viability or proliferation (Lyon 1968; Nyhan et al. 1970).

Mental retardation is a phenotypic component common to several of the disorders associated with skewed X inactivation (Willard 2000). Because of this anecdotal association, we sought to explore the possibility that a general defect in cell viability or proliferation, as measured by skewed X inactivation in peripheral blood cells, is commonly associated with X-linked mental retardation (XLMR). XLMR represents a diverse class of genetic mutations. There are ~150 XLMR disorders, which fall into three classes: X-linked recessive and partly dominant disorders (including syndromes, neuromuscular disorders, and metabolic disorders), X-linked dominant lethal disorders, and nonspecific XLMR disorders (Cabezas et al. 1999; Stevenson et al. 1999; Hamel et al. 2000; Chelly and Mandel 2001). Although >30 XLMR genes have been cloned to date (Chelly and Mandel 2001), the commonality of defects leading to mental retardation is not understood at the cellular or molecular level. Our data dem-

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onstrate that skewed X inactivation is a consistent feature of at least half of all families with XLMR, suggesting that XLMR mutations represent a unique class of X-linked mutations characterized by a general defect in cell viability or proliferation. The data further suggest that, despite the most prominent clinical feature being restricted to the central nervous system, the responsible genes are likely to be expressed in peripheral blood cells, where they will be more accessible to experimental study.

To investigate a possible association between skewed X inactivation and XLMR, we studied 24 families segregating 20 distinct XLMR disorders (table 1). These families were selected solely on the basis of the clinical presentation in affected males and therefore represent an apparently unbiased collection of carrier female subjects. Using the androgen receptor (*AR*) X-inactivation assay (Allen et al. 1992), we determined the X-inactivation patterns of all available female subjects, both carriers and noncarriers, from the families with XLMR ($n = 155$ female subjects).

Illustrative examples of *AR* X-inactivation tracings are shown in figure 1*a*. The distribution of X-inactivation patterns for XLMR carriers ($n = 94$) and noncarriers ($n = 61$) is shown in figure 1*b*. Approximately 9% of female control subjects ($n = 205$) demonstrate skewed X-inactivation patterns ⩾80:20, which is generally consistent with previous estimates (Nance 1964; Gale et al. 1994; Naumova et al. 1996; Plenge et al. 1997, 1999). In contrast, ~50% of the XLMR carriers demonstrate X-inactivation patterns that are ⩾80:20 (tables 1 and 2; $P < .001$). Analysis of the XLMR carrier distribution at other thresholds of skewed X inactivation are also statistically highly significant (table 2). The effect is most dramatic at patterns of X inactivation ⩾90:10; nearly a third of XLMR carriers show such skewing, compared with only a few percent of female control subjects. Thus, these data establish that, in peripheral blood cells, skewed X inactivation is a common feature of XLMR carriers.

To address whether the increased incidence of skewed inactivation was due to an association with skewing in only a subset of families, we examined separately each family with XLMR in which there were at least three female carriers (fig. 2 and table 1). Of the 20 distinct XLMR disorders examined in this way, 4 show a strong association with skewed X inactivation, in that all female carriers within each family demonstrate an X-inactivation pattern ⩾80:20. This is particularly striking for families K8435, K8300, and K8135, in which either all seven or all five carriers show such extreme skewing ($P \ll .0001$ for each family) (table 1). An additional seven families show an incomplete association, with at least two—but not all—carriers demonstrating highly skewed patterns of inactivation. Given the rarity of highly skewed patterns in the general female population, however, each of these patterns is statistically significant ($P < .01$). Of the remaining families, only two (K8450 and K8295) had a large number of carriers with no apparent association between carrier status and skewing.

For certain X-linked conditions, secondary cell selection is believed to occur after an initially random X-inactivation pattern has been established (Belmont 1996; Puck and Willard 1998; Willard 2000). For X-linked disorders associated with skewing, cell selection most likely occurs against those cells in which the mutation is on the active X chromosome; the mutation is therefore predicted to be associated with the preferentially inactive X chromosome. To determine whether the XLMR mutations in our families reside on the preferentially active or inactive X chromosome, we followed the cosegregation of the XLMR mutation and the differentially methylated *AR* allele (i.e., the allele associated with the inactive X chromosome). Of the XLMR carriers with X-inactivation patterns ⩾80:20, the XLMR mutation was on the preferentially inactive X chromosome in all 20 informative carriers (table 1). These data provide strong evidence that the differential growth advantage does, in fact, occur in favor of cells in which the XLMR mutation is on the active X chromosome.

The possibility of cell selection against certain X-linked mutations has been appreciated by geneticists for some time (Lyon 1968; Nyhan et al. 1970). However, previous studies have focused on either a specific X-linked disorder or a specific family, with emphasis on the clinical presentation of carrier females, and thus demonstrate a potentially significant ascertainment bias. In the present study, we have ascertained families with XLMR through a male index patient, without regard to the phenotype of female carriers.

The most important question raised by our study is whether skewed X inactivation is specific to particular classes of X-linked mutation (such as XLMR and the immune-deficiency syndromes [Belmont 1995]) or whether this phenomenon applies more generally to mutations in all X-linked genes. Although limited, available studies favor the hypothesis that skewing is restricted to certain classes of X-linked disorders (Belmont 1996; Willard 2000). If skewing were common to X-linked mutations generally, one would predict a diversity of phenotypes in disorders associated with skewed inactivation. However, of approximately a dozen X-linked disorders demonstrating either a complete or partial association with skewing (Willard 2000), only one—focal dermal hypoplasia (Gorski 1991)—does not have a mental-retardation or immune-deficiency phenotype.

If X-linked mutations were commonly associated with variable or incompletely penetrant skewed inactivation, as was observed for ~50% of the families with XLMR in the present study, one would predict the detection of skewing for carriers of many (and perhaps all) X-linked

Table 1

Families with XLMR: Association with Skewed X Inactivation

Family[a]	XLMR Disorder	Region of Linkage	No. of Carriers[b]			Description[c]
			Total	With Inactivation Pattern ≥80:20	X_i[d]	
Strong association ($n = 4$):						
K8135	XLMR, short stature, tremor	q22-q24	5	5	1/1	Short stature, tremor, behavioral abnormalities
K8210	Williams	q28	4	4	3/3	Muscle hypoplasia, hypotonia, frontal bossing, death at early age
K8300	Pai	q28	7	7	3/3	Profound mental retardation, death at early age, nonambulatory
K8435	Mulvenna-Trotter-Fisher	q27	7	7	4/4	Seizures, IgE deficiency, large head, moderate mental retardation
Incomplete association ($n = 14$):						
K8005	Allan-Herndon Dudley	q13-q21	3	1		Severe mental retardation, severe hypotonia, ataxia, abnormal facies
K8090	Allan-Herndon Dudley	q13-q21	2	0		Severe mental retardation, severe hypotonia, ataxia, abnormal facies
K8225	Allan-Herndon Dudley	q13-q21	2	1		Severe mental retardation, severe hypotonia, ataxia, abnormal facies
K8020	Aarskog-Scott	q11.21	3	0		Short stature, facial, skeletal, and urogenital anomalies
K8250	Aarskog-Scott	q11.21	2	2	2/2	Short stature, facial, skeletal, and urogenital anomalies
K8285	Aarskog-Scott	q11.21	3	0		Short stature, facial, skeletal, and urogenital anomalies
K8765	Agenesis corpus collosum	q28	7	2	1/1	Neuromuscular spasticity, unsteady gait
K8065	MRX7	p11-p14	6	3	2/2	Nonspecific XLMR
K8355	XLMR, seizures, ataxia	p21-p11.2	6	2		Seizures, ataxia, aphasia, autism
K8070	Miles-Carpenter MRSX4	q13-q22	5	3	3/3	Arched fingertips, microcephaly
K8240	XLMR with cleft lip/palate	q12-q21	5	3	1/1	Sloped forehead, short stature, small testicular volume
K8615	XLMR, spastic paraplegia	p12-q12	3	2		Spastic paraplegia, club feet, dystonia
K8075	Wieacker-Wolff	Proximal X q arm	2	1		Neuromuscular, muscle atrophy
K8610	FG syndrome	q13-q21	2	1		Macrocephaly, imperforate anus, and congenital hypotonia
No apparent association ($n = 6$):						
K8035	XLMR, arched fingerprints	q13-q21	3	0		Arched fingerprints, hypotonia, areflexia
K8045	Arena	q22-q25	2	0		Severe spastic paraplegia, ataxia
K8100	Armfield	q28	3	0		Short stature, cleft palate, seizures, glaucoma, severe mental retardation
K8295	Lujan	None	4	0		Marfanoid, triangular facies, narrow palate, hypernasal voice
K8395	XLMR, spastic paraplegia	Proximal X q arm	2	0		Spastic paraplegia, nystagmus; carriers have gait abnormalities
K8450	MRX32	p21-p22.2	6	0		Nonspecific and variable mental retardation
Total	20 distinct disorders		94	44	20/20	

[a] Most of the families have been described elsewhere (Lubs et al. 1996).

[b] Carrier or noncarrier status was determined by linkage analysis, by pedigree analysis (in the case of obligate carriers) and, where possible, by direct mutation screening.

[c] Specific clinical features are provided in the Miami XLMR database (Cabezas et al. 1999).

[d] X_i = inactive X chromosome; data are number of informative carriers in whom the mutation was present on the preferentially inactive X chromosome/total number of informative carriers.

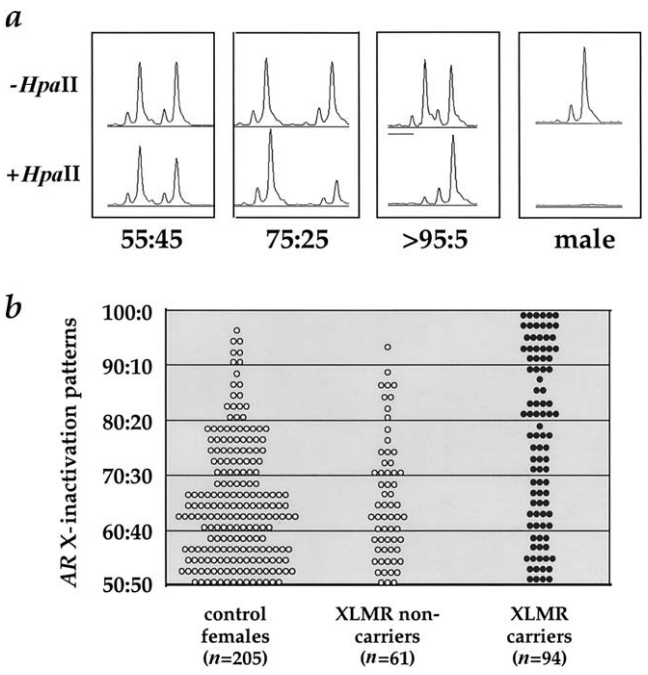

Figure 1 X-inactivation patterns in XLMR disorders. *a*, *AR* X-inactivation–pattern tracings. The top tracings represent the undigested DNA (−*Hpa*II) from three female control subjects and from a male control subject; the bottom tracings represent DNA digested with *Hpa*II prior to PCR (+*Hpa*II). The relative intensity of the two alleles after digestion represents the *AR* X-inactivation pattern for each individual (expressed as a ratio and normalized to the undigested samples). The tracing in males disappears, representing complete digestion of the unmethylated allele on the active X chromosome. Details of the *AR* X-inactivation assay have been described elsewhere, including methods for correcting for unequal peak heights owing to preferential allele amplification (Allen et al. 1992; Naumova et al. 1996; Plenge et al. 1997, 1999). *b*, Distribution of *AR* X-inactivation patterns in families with XLMR and control subjects. The *AR* X-inactivation patterns are shown for two control populations (*unblackened circles*) and for the XLMR carrier population (*blackened circles*).

disorders. In contrast to this prediction, however, several studies have demonstrated apparently *random* X inactivation in many female carriers, through use of a variety of assays (Willard 2000). As part of the present study, we examined X-inactivation patterns in our collection of carriers of Duchenne muscular dystrophy; these patterns did not differ significantly from those of female control individuals (data not shown). Notwithstanding the detection of occasional (usually symptomatic) carriers with demonstrated skewed X inactivation (Puck and Willard 1998), it is clear that skewing is not frequently observed in a high proportion of carriers of most X-linked conditions. Thus, these results also favor the hypothesis that skewing is specific to certain classes of X-linked mutations.

It may appear surprising that a group of disorders affecting the central nervous system would have a negative effect on cell proliferation in an apparently unrelated tissue (peripheral blood cells). One possible explanation is that peripheral blood cells serve as a phenotypic surrogate for cells in the central nervous system. Accordingly, XLMR genes, as a class, might affect in vivo cell viability or proliferation in many tissue types, and a number of examples are consistent with this suggestion. For example, genes responsible for syndromic XLMR are widely expressed and have demonstrated general roles in transcriptional regulation, cell proliferation, and/or development (Chelly and Mandel 2001). Some nonspecific XLMR genes also appear to be involved in cell proliferation and/or global transcriptional regulation (Allen et al. 1998; D'Adamo et al. 1998; Kutsche et al. 2000; Couvert et al. 2001). Thus, the apparent functions of at least some XLMR genes provide support for the hypothesis that these genes affect cell viability or proliferation generally. This finding also has practical significance, since it suggests that, for a substantial subset of XLMR disorders, the relevant loci are likely expressed in peripheral blood and thus are potentially accessible for experimentation (i.e., expression arrays).

In addition to providing potential insight into XLMR pathogenesis, the finding of skewed X inactivation may assist in the mapping of XLMR genes. Other studies have used the phenotype of skewed X inactivation to both establish X linkage (Zoghbi et al. 1990; Krepischi et al. 1998; Amir et al. 1999) and narrow the critical region for mutant genes (Gibbons et al. 1992; Sirianni et al. 1998). In XLMR, this approach would be especially useful in small families in which there are few affected males and in which carrier status is critical to achieving a meaningful LOD score (Lubs et al. 1999).

Assignment of carrier status may also be important for establishing a diagnosis and for genetic counseling in XLMR conditions. In pedigrees with nonspecific mental

Table 2

Skewed X-Inactivation Patterns in XLMR Carriers

X-Inactivation Pattern	Frequency of Skewed X Inactivation[a] (%)		
	Female Control Subjects	XLMR Noncarriers[b]	XLMR Carriers[c]
⩾90:10	3	2	30
⩾80:20	9	15	48
⩾70:30	30	41	63

[a] To assess statistically the distribution of X-inactivation patterns, a χ^2 test was used to compare the number of female carriers above and below a particular threshold value (⩾90:10, ⩾80:20, and ⩾70:30) to that of the control population, as described by Plenge et al. (1999). To control for multiple hypothesis testing, a Bonferroni correction was applied, and the significance value was set at $P < .01$.

[b] Results were not statistically significant.

[c] All results were significant ($P < .001$).

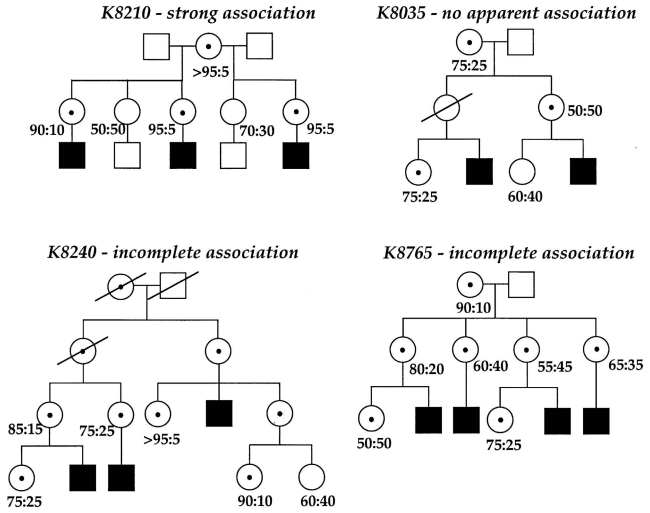

Figure 2 Pedigrees of illustrative families with XLMR. *AR* X-inactivation patterns are shown near each informative female subject. Blackened symbols denote affected individuals, unblackened symbols denote unaffected individuals, and symbols with a black dot denote carriers.

retardation that are so small that it is not possible to distinguish between X-linked and autosomal patterns of inheritance, the detection of multiple females with highly skewed X inactivation (i.e., patterns ⩾90:10)—a decidedly unlikely occurrence for autosomal or non-XLMR mutations—would greatly raise the suspicion that the disorder in question is XLMR.

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