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Heritability of X Chromosome–Inactivation Phenotype in a Large Family

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Summary

One of the two X chromosomes in each somatic cell of normal human females becomes inactivated very early in embryonic development. Although the inactivation of an X chromosome in any particular somatic cell of the embryonic lineage is thought to be a stochastic and epigenetic event, a strong genetic influence on this process has been described in the mouse. We have attempted to uncover evidence for genetic control of X-chromosome inactivation in the human by examining X chromosome–inactivation patterns in 255 females from 36 three-generation pedigrees, to determine whether this quantitative character exhibits evidence of heritability. We have found one family in which all seven daughters of one male and the mother of this male have highly skewed patterns of X-chromosome inactivation, suggesting strongly that this quantitative character is controlled by one or more X-linked genes in some families.

Introduction

Any sample of somatic cells from an individual female will consist of two populations of cells; one in which the maternal X chromosome has been inactivated and another in which the paternal X chromosome has been inactivated (Lyon 1988). If the choice of which X chromosome becomes inactivated during embryonic development is truly stochastic, with a probability of .5 that either is inactivated, then a collection of samples from a population of females should approximate a normal distribution, varying about a mean of individuals who have 50% of cells with an inactive maternal X chromosome and 50% of cells with an inactive paternal X chromosome. Individuals whose somatic-cell populations deviate greatly from the mean may occur by chance, by selection against X chromosomes that carry a mutation in an X-linked gene (Lyon 1988; Allen et al. 1992; Gibbons et al. 1992; Brown and Brown 1993), by selection in favor of the activity of an X chromosome involved in a balanced X autosome translocation (Leisti et al. 1975; Zabel et al. 1978; Schmidt and Du Sart 1992), by selection in favor of an X chromosome carrying a gene that results in increased proliferation (Migeon 1993) or survival, or as a result of genetic differences that affect X-chromosome inactivation per se (Willard 1995).

Although the X chromosome–inactivation phenotype of humans is not known to be heritable, it is reasonable to suspect that genetic variation in the propensity of particular X chromosomes to be inactivated may exist among humans, as has been demonstrated in the mouse (Cattanach and Williams 1972). There is, overall, strong similarity between the X-chromosome dosage-compensation system in the mouse and that in the human. The X-inactivation process in both is thought to require a unique region of the X chromosome, the X chromosome–inactivation center (*XIC*). Within the cytogenetic region defined to be the *XIC* in both the human (Brown et al. 1991) and the mouse (Borsani et al. 1991; Brockdorff et al. 1991), there is a gene (*XIST* in human and *Xist* in mouse) that is transcribed specifically from the inactive X chromosome. In the mouse, another locus, called *Xce* (X chromosome–controlling element), has been demonstrated to influence X-chromosome inactivation, in *cis* (Cattanach and Williams 1972). This locus also resides within the cytogenetically defined *XIC* region but is distinct from *Xist* (Simmler et al. 1993). There are at least four variants described at the *Xce* locus: Xce^a, Xce^b, Xce^c, and the less well-described Xce^d allele from *Mus spretus* (Cattanach and Raspberry 1991). This allelic series represents a gradation of increasing tendency for an X chromosome to remain ac-

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tive. Allele Xce^c is stronger than Xce^b, and Xce^b is stronger than Xce^a (Cattanach and Williams 1972; West and Chapman 1978; Johnston and Cattanach 1981). For example, in an Xce^c/Xce^a heterozygote, the chromosome bearing the Xce^c allele is more likely to remain active; and, in an Xce^b/Xce^a heterozygote, the chromosome bearing the Xce^b allele is more likely to remain active.

In addition to these well-documented genetic effects, there is a strong epigenetic effect of parental imprinting on the inactivation of an X chromosome in both the mouse and the human. In the extraembryonic tissues of both organisms, the paternal X chromosome is inactivated preferentially (Takagi and Sasaki 1975; West et al. 1977; Ropers et al. 1978; Harrison and Warburton 1986; Harrison 1989). The epigenetic effect of parental imprinting on X inactivation may be overcome, in at least some cases, by manipulating the parental origin of "strong" *Xce* alleles; that is, a paternally derived, "strong" Xce^c allele is likely to remain active in extraembryonic tissues (Kay et al. 1993).

The possibility that X-chromosome inactivation in humans is also under genetic control has not been investigated extensively. A number of examples of skewed X inactivation in families or in MZ twins (reviewed by Willard 1995) have been observed, but these have generally been ascribed to chance, to selection against an X chromosome bearing a recessive lethal gene, or to selection in favor of an allele resulting in a proliferative advantage for cells with a particular X chromosome active (Migeon 1993), even when the presence of such an allele cannot be demonstrated (Clarke et al. 1993).

Because we were interested in the genetic control of X-chromosome inactivation, as well as in other phenotypes that may be responsive to parental origin, we screened 36 three-generation families with no known genetic disorder for the X chromosome–inactivation phenotype of the female members. As a result of this search, we have identified one family in which a paternal grandmother and all seven of her granddaughters have highly skewed patterns of X-chromosome inactivation in their lymphocytes. We have also demonstrated that, despite the clearly X-linked pattern of transmission of this effect, the skewed X chromosome–inactivation phenotype found in lymphocytes in this family does not cosegregate with a variant allele at the *XIST* locus.

Subjects and Methods

Subjects

DNA samples from lymphocytes from 241 females from 33 three-generation families from the Salt Lake City collection, 14 samples from 3 families, and samples from 64 unrelated females collected by our laboratory and from 169 unaffected mothers of patients who have sporadic cases of either Wilms tumor, retinoblastoma, or Beckwith-Wiedemann syndrome were used to study X inactivation. A total of 365 females who have no known genetic disorders and who were informative for alleles that could be quantitated by densitometry have been screened in this study.

Androgen-Receptor (AR) Gene Methylation Assay

DNA isolated from lymphocytes was used in this assay. A 200-ng portion of DNA from each sample was digested with *Alu*I for 1.5 h, and then one-half of the sample was digested with *Hha*I or *Hpa*II for 2 h. Both digested DNA samples were used as template for amplification of the highly polymorphic *AR* $(CAG)_n$ repeat (Edwards et al. 1992). PCR was run in the presence of α-^{32}[P] dCTP (98°C 1 min, 68°C 1 min, 75°C 2 min) for 26 cycles in an Ericomp Easycycler (primers 5′-GCTGTGAAGGTTGCTGTTCCTC [La Spada et al. 1991] and 5′-AGAGGCCGCGAGCGCAGCACCTC). PCR products were separated in 5% denaturing polyacrylamide gels, as described elsewhere (Naumova et al. 1995).

DNA, RNA, and cDNA Preparation from Lymphoblast Cell Lines

Genomic DNA was extracted from cultured cell lines by a salting-out method, and RNA was extracted as described elsewhere (Chomczynski 1989). All cDNAs were prepared by reverse transcription of 1.0 μg total RNA primed with random hexamers with 5 units of reverse transcriptase as described elsewhere (Brown et al. 1990). Cell lines 11993 and 10860 (National Institute of General Medical Sciences' Human Genetic Mutant Cell Repository) were grown at 37°C in RPMI medium (GIBCO) supplemented with 15% FCS, glutamine, penicillin, and streptomycin (GIBCO).

XIST *PCR Amplification and Sequence Analysis*

A 50-ng portion of genomic DNA or cDNA was amplified in a Perkin Elmer 9600 GeneAmp in 25-μl reaction volumes with C4-3 (5′CTGCCACCCATATATAAGpchCT3′) and C23-3 (5′AGCATGTATCTTCTGGACAG3′) primers (Rupert et al. 1995) in a reaction mixture containing 20 mM Tris (pH 8.4), 50 mM KCl, 200 μM dNTPs, 1.5 mM $MgCl_2$, 0.5 μM each primer, and 2.5 units GIBCO *Taq* DNA polymerase. Amplifications consisted of an initial 2-min denaturation at 95°C, a 15-s annealing at 55°C, and a 45-s elongation at 72°C, with a final 7-min elongation at 72°C after the 30th cycle.

A 15-μl portion of the PCR reaction was purified with Qiagen QIAquick Spin PCR Purification Kit and eluted in 50 μl water. An 8.5-μl portion of the purified fraction was sequenced with 3.8 pmol of the C4-3 primer according to the conditions outlined in the ABI 373 Sequencing System. Sequence data were stored and analyzed by use of ABI SeqEd software.

Quantitation of AR *Methylation Assay*

To quantitate the methylation of *AR* alleles and the skewing of X inactivation in females, we scanned autoradiograms on a laser densitometer (LKB; Ultrascan). Some gels also were exposed to Fuji phosphorimaging screens for 1–16 h, and the intensity of bands (alleles) was quantified by using the Fuji BAS 2000 phosphorimager. Comparable results were obtained when both methods were used on the same gels (data not shown).

As a way of quantifying the degree of skewing—that is, the degree to which the somatic cells of an individual female deviate from a 1:1 ratio of cells having the paternal versus the maternal X chromosome active—we have used two measures: (1) the density of the lower allele, divided by the sum of the densities of both alleles ($m/[m + s]$) in *Hha*I-digested samples, and (2) the absolute value of the difference between the ratios of the nondigested and digested samples. This latter measure, the absolute-difference score (ADS), is defined as ADS $= |[(m/(m + s)]^{HhaI-} - [m'/(m' + s')]^{HhaI+}|$, where m and m' are the densities or phosphorimage counts corresponding to the smaller allele, and s and s' are the values for the larger allele of the *AR* PCR product (Naumova et al. 1995). This latter measure was used in this study only to allow quantitation of skewing in individuals who were heterozygous for alleles that differ in length by only a single CAG repeat. In such cases, a minor band of the upper allele comigrates with the major band of the lower allele (see fig. 2). In the cases reported in table 1, the density of the minor band of the lower allele, rather than that of the major band of the lower allele, was used, and the densitometric fraction observed in the nondigested sample was used to correct the fraction observed in the digested sample.

Results

We have examined X chromosome–inactivation patterns in lymphocytes (*not* lymphoblastoid cell lines) from 365 "normal" females (>400 females were, in fact, analyzed, but only 365 were informative for quantitative analysis; see Subjects and Methods). As a quantitative measure of the proportion of each female's cells that had one or the other of their X chromosomes active, we used an assay that is responsive to methylation of cytosine residues at *Hpa*II or *Hha*I restriction-endonuclease cleavage sites adjacent to the highly polymorphic trinucleotide repeat within the *AR* (Allen et al. 1992). These CpG sites have been demonstrated previously to be methylated on the inactive X chromosome, and a sensitive PCR-based X chromosome–inactivation assay has been described and used by a number of laboratories (Allen et al. 1992, 1994; Pegoraro et al. 1994; Naumova et al. 1995; Orstavik et al. 1995; Wengler et al. 1995). The distribution of these females, as a function of the percent of each female's lymphocytes that have the same X chromosome active, is given in figure 1.

We note that a substantial minority of these females appear in the two most extreme phenotypic categories (80%–90% and 90%–100% of cells with the same X chromosome active). Twenty-two percent, or 80 of the 365 women, were found to have ≥80% of their lymphocytes with the same X chromosome active. Initially we found this observation surprising, but this estimate is similar to that reported by other investigators using either the *AR* methylation assay on total lymphocytes from normal females (3 [15%] of 20 of normal women were found to have ≥80% of lymphocytes having the same X chromosome active [Pegoraro et al. 1994]) or independent measures of X inactivation (Nance [1964] observed 8 [27%] of 30 normal women expressing the same G6PD allele in ≥80% of their lymphocytes). Some investigators have found a smaller proportion of normal females to be in the two most extreme categories when the *AR* methylation assay was used (2 [11%] of 19 T cell samples from normal females [Puck et al. 1992], and 3 [12.5%] of 24 $CD19^-$ samples and 1 [4.2%] of 24 $CD19^+$ samples of normal females), but these studies examined only subpopulations of lymphocytes and small numbers of women. In our population sample, if we consider only those 64 unrelated women who were sampled specifically as a control group and are not members of the 36 three-generation families that we have analyzed for heritability of X-inactivation phenotype or unaffected mothers of pediatric disease patients, then 9 (14%) of these 64 women have ≥80% of their lymphocytes with the same X chromosome active. We conclude that, overall, the results of our analysis of X chromosome–inactivation patterns in "normal" females do not differ substantially from those reported by other investigators.

Two hundred fifty-five of the females analyzed are members of 36 three-generation families. We examined these families for evidence of heritability of a skewed pattern of X-chromosome inactivation. Because the X chromosome–inactivation phenotype of an individual female is a quantitative character but the simple type of segregation analysis that we wished to do is most easily performed with a qualitative character (i.e., skewed X inactivation vs. nonskewed X inactivation), we chose an operational and historical definition for a "skewed" X-inactivation phenotype (Takagi and Sasaki 1975; see also Naumova et al. 1995). If ≥80% of an individual's lymphocytes had the same X chromosome active, that individual was designated as "skewed." This value was chosen on the basis of the original description, by Takagi and Sasaki (1975), of nonrandom X-chromosome inactivation in extraembryonic tissues of the mouse. The minimum value for the fraction of cells observed by these investigators to have preferential inactivation of

Table 1

Analysis of Skewing of X Inactivation in Lymphocytes of Females from Family K1362

		X Inactivation[a]					
		*Hha*I			*Hpa*II		
			Cells with Active X (%)			Cells with Active X (%)	
Individual	*AR* Alleles	ADS	Maternal	Paternal	ADS	Maternal	Paternal
I-1	B, C[b]	.43[c]	7[c]	93[c]	.45[c]	5[c]	95[c]
I-2	A, B[b]	.02[c]	52[c]	48[c]			
II-2	A, D		47	53		No data	
III-1	A, C		6	94		11	89
III-2	C, D	.39	11	89		No data	
III-3	A, C		6	94		12	88
III-4	A, C		4	96		15	85
III-5	C, D	.25	25	75	.44	6	94
III-6	A, C		13	87		No data	
III-7	A, C		15	85		15	85

[a] Estimation of the proportion of cells with the same X chromosome active, based on quantitation of *AR* assay. The *Hha*I[+] assay represents the methylation status of two *Hha*I sites within the first exon of the *AR* gene, and the *Hpa*II assay represents the methylation status of one *Hpa*II site within the same region; these sites have been shown to correlate with the inactivation status of the X chromosome (*AR* alleles being methylated on the inactive X) (Allen et al. 1992).

[b] Parental origin of the active and inactive alleles could not be determined (see footnote c).

[c] Because parental origin of the active and inactive alleles could not be determined (see footnote b), this entry represents the proportion of cells with lower ("maternal") or upper ("paternal") allele active.

the paternal X chromosome was 79% (Takagi and Sasaki 1975, table 1). Although 22% of the 365 normal females whom we examined have skewing scores greater than or equal to this value, if this degree of skewing is assumed to occur randomly, then the probability that individuals within the same family exhibit this level of skewing is given by the binomial distribution with $P = .22$. In the case of all n individuals within a family being skewed to this level, this probability simplifies to $(.22)^n$.

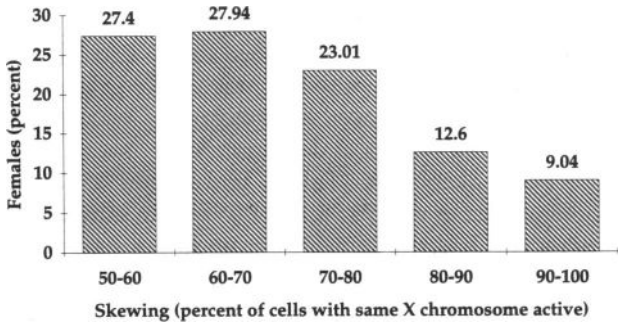

Figure 1 Frequency distribution of 365 normal females with respect to X chromosome–inactivation phenotype found in lymphocytes. The percentage of cells with the same X chromosome active (*abscissa*) is as measured by the *AR* methylation assay.

We found several families in which X chromosome–inactivation profiles were unlikely, on a statistical basis, to be random. Segregation analysis of all but one of these families is complicated by additional factors (analytical criteria, choice of segregation model, potential for nonpenetrance of trait or for occurrence of "sporadics" within families, etc.), and these pedigrees, as well as a heritability analysis of X inactivation for all of the families, will be reported elsewhere (K. Morgan, unpublished data); but one family provides strong evidence of an X-linked pattern of transmission for the phenotype of highly skewed X-chromosome inactivation (fig. 2).

The *AR*-locus PCR-based X-inactivation assay for each female in K1362 is shown beneath the pedigree in figure 2. (Note that only the assays for females are shown in the figure but that all of the males in this pedigree also have been typed at the *AR* locus. All seven of the daughters have the same *AR* allele as their father, as well as their father's alleles at *DXYS17* [data not shown], as expected.) Neither the mother (II-2; fig. 2, lanes 5 and 6) nor the maternal grandmother (I-2; fig. 2, lanes 3 and 4) in this family exhibits a skewed pattern of X-chromosome inactivation, as can be seen either by inspection of the autoradiograph (fig. 2) or by quantitative densitometry (table 1). The paternal grandmother, on the other hand, shows a highly skewed pattern of X

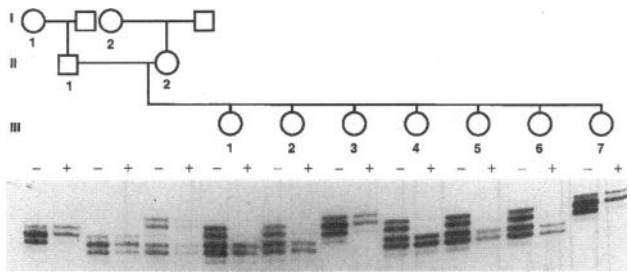

Figure 2 *AR*–methylation PCR assay in females from family K1362. The *HhaI*$^+$ assay represents the methylation status of two *HhaI* sites within the first exon of the *AR* gene, which has been shown to correlate with the inactivation status of the X chromosome, (*AR* alleles being methylated on the inactive X) (Allen et al. 1992). The inactive *AR* allele (i.e., the better-amplified allele) in I-1 is inherited by II-1 but is active (i.e., poorly amplified) in all seven females in generation III.

inactivation, as do all seven of her granddaughters (fig. 2 and table 1). In addition, the paternally derived X chromosome is active preferentially in all seven of the females in generation III.

Although the phenotypic trait of skewed X-chromosome inactivation in the granddaughters (III-1–III-7) appears to have been inherited from the paternal grandmother (I-1), through the father (II-1), the autoradiogram in figure 2 indicates that the *AR* allele that is located on the preferentially active X chromosome in the seven females in generation III was located on the preferentially inactive X chromosome in their paternal grandmother. This observation indicates that, if the phenotype of skewed X-chromosome inactivation is linked, in *cis*, to the inactive X chromosome, then a recombination event must have occurred between the *AR* locus and the gene that is responsible for skewed X inactivation in the paternal grandmother (I-1). This recombinant chromosome would then be transmitted to the son (II-1), who would transmit this X chromosome to his daughters (generation-III females).

Because the X chromosome–inactivation center (*XIC*) is believed (by definition) to be required, in *cis*, for the inactivation of the X chromosome (Russel 1963; Mattei et al. 1981; Brown et al. 1991), we addressed the possibility that the preferentially inactive X chromosomes in female I-1 and in generation III share the same copy of *XIC*. This hypothesis requires a recombination event to have occurred between *AR* and the *XIC* in Xq13.2, which maps ~8 cM distal to *AR* (Fain et al. 1995). To test this hypothesis, we used an expressed, single-base-substitution polymorphism within the *XIST* gene (Brown et al. 1992; Rupert et al. 1995). This gene is expressed only from the inactive X chromosome and is located within the cytogenetic region currently defined as the *XIC* (Brown et al. 1991; Heard and Avner 1994). Both DNA and RNA were obtained from lymphoblast cell lines of the paternal grandmother (I-1) and her son (II-1). The paternal grandmother was determined to be heterozygous for *XIST* alleles containing either A or G at position 15944 (fig. 3). The phase of the *AR* and *XIST* alleles on the paternal grandmother's X chromosomes was determined by exploiting the facts (1) that both the lymphocytes and the lymphoblast cell line from this female had strongly skewed patterns of X inactivation and that the *XIST* gene is expressed from only the inactive X chromosome and (2) that the *AR* allele on the inactive X chromosome is methylated. *XIST* cDNA derived from the lymphoblast cell line of the paternal grandmother consisted of only the G-containing allele, indicating that this allele ($XIST^G$) was present on the inactive X chromosome. Analysis of the alleles at the *AR* locus by the differential methylation assay revealed that the same allele (defined as allele "C" [AR^C in table 1]) that was present on the preferentially inactive X chromosome in the lymphocytes of individual I-1 is also present on the inactive X chromosome in the lymphoblast cell line from this female (fig. 1 and data not shown), indicating that the phase of these markers in female I-1 is centromere–AR^C–$XIST^G$. The father (II-1) inherited allele C at the *AR* locus and inherited the G-containing allele at *XIST*, indicating that the phase of these markers in the son and his mother are the same and that no recombination event has occurred between *AR* and *XIST* on the X chromosome inherited by the son. In addition, these results imply that the *XIST* allele that was located on the preferentially inactive X chromosome in the paternal grandmother is located on the X chromosome that is preferentially active in all seven of her granddaughters. Because the paternal grandmother and her granddaughters are discordant for which X chromosome is preferentially active in their lymphocytes, this phenotype does not map to the X chromosome interval between *AR* and the *XIST* polymorphic site in this family.

Discussion

We have entertained three categories of models to explain these observations: nongenetic, autosomal dominant, and X-linked inheritance of skewed X inactivation. We do not believe that the skewed X chromosome–inactivation pattern observed in the seven daughters occurred by chance, because our observations (fig. 1; also see Results) indicate that the probability that this would occur in all seven daughters of one family is $<2.5 \times 10^{-5}$ ($.22^7$).

The second possibility is that the trait is genetic but not X-linked. We are unable to eliminate this possibility at this time, but, if there were an autosomal gene cosegregating with skewed X-inactivation phenotype in this family, the probability that the gene would be trans-

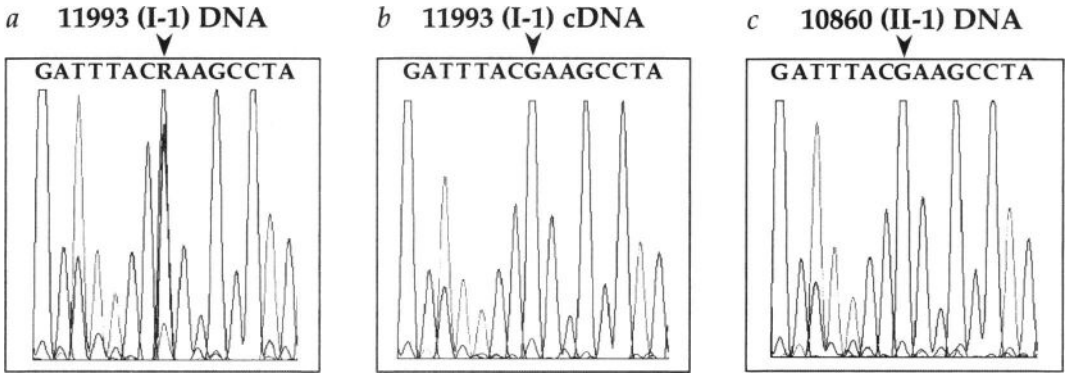

Figure 3 Analysis of the *XIST* polymorphism (Rupert et al., in press) in DNA and cDNA. DNA and RNA were obtained from lymphoblast cell lines (National Institute of General Medical Sciences' Human Genetic Mutant Cell Repository) 10860 (II-1) and 11993 (I-1), and ABI sequence analysis was performed. *a*, Analysis of genomic DNA, which demonstrates that I-1 has both the "A" and "G" *XIST* alleles (*arrow*; R-purines). *b*, cDNA sequence from I-1, which demonstrates that the "G" allele is preferentially expressed (*arrow*) and therefore is present on the inactive X chromosome. *c*, DNA sequence analysis from II-1, which shows the inheritance of the "G" allele (*arrow*) from I-1. Therefore, the *XIST* allele located on the preferentially inactivated X chromosome in I-1 is inherited by II-1 and, on the basis of its being in *cis* with the *AR* allele, is on the preferentially active X chromosome in all females in generation III.

mitted from the grandmother (I-1) to the father and then to all seven granddaughters is .004 ($[^1/_2]^8$). In addition, our observation that the paternal chromosome is preferentially active in all seven daughters would impose the requirement that any autosomal gene involved in this phenomenon must act, in *trans*, to preferentially inactivate the maternal X chromosome (except in the father) or to keep the paternal X chromosome active.

The third and, in our opinion, most likely possibility is that the phenotype may be controlled, in *cis*, by a locus on the X chromosome. We believe that this is the most likely possibility because, if the paternal grandmother's X chromosome carried a "skewing" allele at this locus, then the probability that her son would receive this allele is .5, which is the same as the probability that *all* of her granddaughters would receive the allele. The fact that the X chromosome that the granddaughters inherited from their grandmother is active in the granddaughters but inactive in the grandmother may be explained most easily by a recombination event between the *cis*-acting "skewing" control locus and the X-chromosome region that we have examined. Although our current data do not rule out localization of the putative control locus to a place within the *XIC*, such a locus must be distal to the *XIST* polymorphic site. Although sequences in the 5′ region of *XIST* or upstream of *XIST* may (if, e.g., an *Xce*-homologous locus exists in the human) still be considered candidates for elements controlling the skewed X-inactivation phenotype, we cannot eliminate the possibility that a gene at some other location on the X chromosome is involved.

With regard to other possible locations for such a gene, it is interesting to note that Schmidt et al. (1990), Clarke et al. (1991, 1992), and Dahl et al. (1995) have proposed that a gene affecting X-chromosome inactivation is present in the vicinity of Xq27. Additional mapping information that would address the location of a putative X-linked gene might be derived by determining more extensive X-chromosome haplotypes for individual I-1. However, this would require the isolation of this female's individual X chromosomes in somatic-cell hybrids. Such an effort is not, at present, justified, because only a single informative meiosis (individual II-1) is available for analysis.

It is very unlikely that our observation is due to selection against a maternal X chromosome carrying a mutation in an X-linked gene. Neither the X chromosome–inactivation pattern of the mother nor the X-inactivation pattern of the maternal grandmother indicates the presence of such an allele (fig. 1 and table 1). It is also unlikely that the paternally derived X chromosome has been selected for on the basis that the father carries a balanced X autosome translocation, such that cells that inactivate the translocation chromosome fail to survive. Each female in generation III has a small but easily observed (fig. 1) subpopulation of cells that must have an inactive paternal X chromosome. In addition, this male fathered 11 children, including 7 daughters to whom he transmitted his single X chromosome, and there are no indications of infertility or other abnormalities in this family.

It is also possible that the X-inactivation skewing that we observe in this family may be due to the presence of a gene that results in a proliferative advantage (Migeon 1993), or increased relative survival, of lymphoid precursor cells that have the X chromosome bearing this gene active. If this "proliferative advantage" or "increased survival" gene were hypothesized to be specific to lymphoid cells and were able to alter the cell-division rate of lymphoid precursors without giving rise to any

disease phenotype, then one might expect that nonlymphoid cells from these females would not exhibit a skewed pattern of X inactivation. However, if the effect of the "proliferative advantage" or "increased survival" gene was not restricted to lymphoid cells, then, in the absence of detailed biochemical knowledge of the mechanism by which such a gene exerted its effects, it would not be possible to distinguish between a gene that acted at the level of X inactivation per se and one that affected either the rate of cell division or the probability of survival. Unfortunately, at this time we are unable to obtain samples of other tissues from this family in order to test the first possibility.

An intriguing model of an effect of a mutation in the *XIST* gene on patterns of X inactivation—a model that would explain the discordance between the *XIST* polymorphism and the skewed phenotype in this family—would posit a "reverse parental origin effect" on the expression of *XIST*, such that the mutant *XIST* allele would be much more likely to be expressed when maternally derived than when paternally derived. The mutant *XIST* gene on the X chromosome carrying this allele would be expressed in I-1 (if it is assumed both that the relevant *XIST* mutation was distal to the *XIST* polymorphic site that we have examined and that the paternal grandmother received her preferentially inactive X chromosome from her mother), but not in generation III, because the hypothesized mutant *XIST* allele is paternally derived in these instances. The daughters in K1362, therefore, would show nonrandom inactivation of the other (maternal) X chromosome. Although at present there are no data to support or refute this model, it is worth pointing out that *Xist* shows clear evidence of imprinting effects in normal gametogenesis and in early development in the mouse (Ariel et al. 1995; Zuccotti and Monk 1995). Thus it does not seem unreasonable to consider possible effects of *XIST* mutations that interfere with the imprinting process.

We have provided evidence that the choice of which X chromosome is inactivated in somatic cells of normal human females may be influenced by genetic factors. We believe that the family reported here is the first clear example of this phenomenon in the human. Although this, in itself, is not unexpected, our observations differ from those made in the mouse, in that the present report does not show that there is any clear-cut genetic linkage to the *XIC*. Whether this may be taken either as evidence for the existence of other loci that affect the X chromosome–inactivation process per se or as an indication of X-linked genes that are involved in controlling either cell proliferation (Migeon 1993) or cell survival is unclear. With respect to the possible existence of other loci that affect X inactivation per se, it is noteworthy that *Xist* has been shown to have a clear, *cis*-acting role in the process of X inactivation in the mouse but has not been demonstrated to be involved in the counting of X chromosomes or in the choice of which X chromosome becomes inactivated in an individual cell (Penny et al. 1996). Our studies indicate (Naumova et al. 1995; present report; A. K. Naumova, L. M. Bird, and C. Sapienza, unpublished data) that female individuals in which the X-inactivation phenotype is strongly biased may occur with sufficient frequency that family studies designed to locate genes that affect this process may be possible.

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References

Allen RC, Nachtman RG, Rosenblatt HM, Belmont JW (1994) Application of carrier testing to genetic counseling for X-linked agammaglobulinemia. Am J Human Genet 54:25–35

Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW (1992) Methylation of *Hpa*II and *Hha*I sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet 51:1229–1239

Ariel M, Robinson E, McCarrey JR, Cedar H (1995) Gamete-specific methylation correlates with imprinting of the murine *Xist* gene. Nat Genet 9:312–315

Borsani B, Tonlorenzi R, Simmler M-C, Dandolo L, Arnaud C, Willard HF, Avner P, et al (1991) Characterization of a murine gene expressed from the inactive X chromosome. Nature 351:325–329

Brockdorff N, Ashworth A, Kay GF, Cooper P, Smith S (1991) Conservation of position and exclusive expression of mouse *Xist* from the inactive X chromosome. Nature 351:329–331

Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R, Willard HF (1991) A gene from the region of the human X-inactivation center is expressed exclusively from the inactive X chromosome. Nature 349:38–44

Brown, CJ, Flenniken AM, Williams BRG, Willard HF (1990) X chromosome inactivation of the human *TIMP* gene. Nucleic Acids Res 18:4191–4195

Brown CJ, Hendrich BD, Rupert JL, Lafreniere RG, Xing Y, Lawrence J, Willard HF (1992) The human *XIST* gene: analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. Cell 71:527–542

Brown RM, Brown GK (1993) X chromosome inactivation and the diagnosis of X-linked disease in females. J Med Genet 30:177–184

Cattanach BM, Raspberry C (1991) Identification of the *Mus spretus Xce* allele. Mouse Genome 89:565–566

Cattanach BM, Williams CE (1972) Evidence of non-random X-chromosome activity in the mouse. Genet Res 19:229–240

Chomczynski P(1989) The RNAzol method. Cinna/Biotecx bulletin 3. Biotecx Laboratories, Houston

Clarke JTR, Greer WL, Strasberg PM, Pearce RD, Skomorowski MA, Ray PN (1991) Hunter disease (mucopolysaccharidosis type II) associated with unbalanced inactivation of the X chromosomes in a karyotypically normal girl. Am J Hum Genet 49:289–297

Clarke JTR, Wilson PJ, Morris CP, Hopwood JJ, Richards RI, Sutherland GR, Ray PN (1992) Characterization of a deletion at Xq27-q28 associated with unbalanced inactivation of the nonmutant X chromosome. Am J Hum Genet 51:316–322

——— (1993) Reply to Migeon. Am J Hum Genet 52:433–434

Dahl N, Hu LJ, Chery M, Fardeau M, Gilgenkrantz S, Nivelon-Chevallier A, Sidaner-Noisette I, et al (1995) Myotubular myopathy in a girl with a deletion at Xq27-q28 and unbalanced X inactivation assigns the MTM1 gene to a 600-kb region. Am J Hum Genet 56:1108–1115

Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12:241–253

Fain PR, Kort EN, Chance PF, Nguyen K, Redd DF, Econs MJ, Barker DF (1995) A 2D crossover-based map of the human X chromosome as a model for map integration. Nat Genet 9:261–266

Gibbons RJ, Suthers GK, Wilkie AOM, Buckle VJ, Higgs DR (1992) X-linked α-thalassemia/mental retardation (ATR-X) syndrome: localization to Xq12-q21.31 by X inactivation and linkage analysis. Am J Hum Genet 51:1136–1149

Harrison KB (1989) X-chromosome inactivation in the human cytotrophoblast. Cytogenet Cell Genet 52:37–41

Harrison KB, Warburton D (1986) Preferential X chromosome activity in human female placental tissues. Cytogenet Cell Genet 41:163–168

Heard E, Avner P (1994) Role play in X-inactivation. Hum Mol Genet 3:1481–1485

Johnston PG, Cattanach BM (1981) Controlling elements in the mouse. IV. Evidence of non-random X-inactivation. Genet Res 37:151–160

Kay GF, Penny GD, Patel D, Ashworth A, Brockdorff N, Rastan S (1993) Expression of Xist during mouse development suggests a role in the initiation of X chromosome inactivation. Cell 72:171–182

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH (1991) Androgen receptor gene mutations in X-linked and bulbar muscular atrophy. Nature 352:77–79

Leisti JT, Kaback MM, Rimoin DL (1975) Human X-autosome translocations: differential inactivation of the X-chromosome in a kindred with an X-9 translocation. Am J Hum Genet 27:441–453

Lyon MF (1988) The William Allan Memorial Award Address: X-chromosome inactivation and the location and expression of X-linked genes. Am J Hum Genet 42:8–16

Mattei MG, Mattei JF, Vidal I, Girauld F (1981) Structural anomalies of the X chromosome and inactivation center. Hum Genet 56:401–408

Migeon BR (1993) The postulated X-inactivation center at Xq27 is most reasonably explained by ascertainment bias: heterozygous expression of recessive mutations is a powerful means of detecting unbalanced X inactivation. Am J Hum Genet 52:431–432

Nance WE (1964) Genetic tests with a sex-linked marker: glucose-6-phosphate dehydrogenase. Cold Spring Harb Symp Quant Biol 29:415–424

Naumova AK, Olien L, Bird LM, Slamka C, Fonseca M, Verner A, Wang M, et al (1995) Transmission-ratio distortion of X chromosomes among the male offspring of females with skewed X-inactivation. Dev Genet 17:198–205

Orstavik RE, Tommerup N, Eiklid K, Orstavik KH (1995) Non-random X chromosome inactivation in an affected twin in a monozygotic twin pair discordant for Wiedemann-Beckwith syndrome. Am J Med Genet 56:210–214

Pegoraro E, Schimke RN, Arahata K, Hayashi Y, Stern H, Marks H, Glasberg MR, et al (1994) Detection of new paternal dystrophin gene mutations in isolated cases of dystrophinopathy in females. Am J Hum Genet 54:989–1003

Penny GD, Kay GF, Sheardown SA, Rastan S, Brockdorff N (1996) Requirement for *Xist* in X chromosome inactivation. Nature 379:131–137

Puck JM, Stewart CC, Nussbaum RL (1992) Maximum-likelihood analysis of human T-cell X chromosome inactivation patterns: normal women versus carriers of X-linked severe combined immunodeficiency. Am J Hum Genet 50:742–748

Ropers H-H, Wolff G, Hitzeroth HW (1978) Preferential X inactivation in human placenta membranes: is the paternal X inactive in early embryonic development of female mammals. Hum Genet 43:265–273

Rupert JL, Brown CJ, Willard HF (1995) Direct detection of non-random X-chromosome inactivation by use of a transcribed polymorphism in the *XIST* gene. Eur J Hum Genet 3:333–343

Russel LB (1963) Mammalian X-chromosome action: inactivation limited in spread and in region of origin. Science 140: 976–978

Schmidt M, Certoma A, Du Sart D, Kalitsis P, Leversha M, Foster K, Sheffield L, et al (1990) Unusual X chromosome inactivation in a mentally retarded girl with an interstitial deletion Xq27: implications for the fragile X syndrome. Hum Genet 84:347–352

Schmidt M, Du Sart D (1992) Functional disomies of the X chromosome influence the cell selection and hence the X-inactivation pattern in females with balanced X-autosome translocations: a review of 122 cases. Am J Med Genet 42: 161–169

Simmler M-C, Cattanach BM, Raspberry C, Rouguelle C, Avner P (1993) Mapping the murine *Xce* locus with $(CA)_n$ repeats. Mamm Genome 4:523–530

Takagi N, Sasaki M (1975) Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes in the mouse. Nature 256:640–641

Wengler G, Gorlin JB, Williamson JM, Rosen FS, Bing DH (1995) Nonrandom inactivation of the X chromosome in

early lineage hematopoietic cells in carriers of Wiskott-Aldrich syndrome. Blood 85:2471–2477

West JD, Chapman VM (1978) Variation for X chromosome expression in mice detected by electrophoresis of phosphoglycerate kinase. Genet Res 32:91–102

West JD, Frels WI, Chapman VM, Papaionnou VE (1977) Preferential expression of the maternally derived X chromosome in the mouse yolk sac. Cell 12:873–882

Willard HF (1995) The sex chromosomes and X-chromosome inactivation. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 7th ed. McGraw-Hill, New York, pp 719–737

Zabel BU, Baumann WA, Pirntke W, Gerhardt-Ratschow K (1978) X-inactivation pattern in three cases of X/autosome translocation. Am J Med Genet 1:309–317

Zuccotti M, Monk M (1995) Methylation of the mouse *Xist* gene in sperm and eggs correlates with imprinted *Xist* expression and paternal X inactivation. Nat Genet 9:316–320