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Evidence That Mutations in the X-linked *DDP* Gene Cause Incompletely Penetrant and Variable Skewed X Inactivation

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Summary

X chromosome inactivation results in the random transcriptional silencing of one of the two X chromosomes early in female development. After random inactivation, certain deleterious X-linked mutations can create a selective disadvantage for cells in which the mutation is on the active X chromosome, leading to X inactivation patterns with the mutation on the inactive X chromosome in nearly 100% of the individual's cells. In contrast to the homogeneous patterns of complete skewed inactivation noted for many X-linked disorders, here we describe a family segregating a mutation in the dystonia-deafness peptide (*DDP*) gene, in which female carriers show incompletely penetrant and variable X inactivation patterns in peripheral blood leukocytes, ranging between 50:50 and >95:5. To address the genetic basis for the unusual pattern of skewing in this family, we first mapped the locus responsible for the variable skewing to the proximal long arm (Xq12-q22) of the X chromosome ($Z = 5.7$, $P = .002$, LOD score 3.57), a region that includes both the *DDP* and the *XIST* genes. Examination of multiple cell types from women carrying a *DDP* mutation and of peripheral blood leukocytes from women from two unrelated families who carry different mutations in the *DDP* gene suggests that the skewed X inactivation is the result of selection against cells containing the mutant *DDP* gene on the active X chromosome, although skewing is apparently not as severe as that seen for many other deleterious X-linked mutations. Thus, *DDP* is an example of an X-linked gene for which mutations cause partial cell selection and thus incompletely skewed X inactivation in peripheral blood leukocytes.

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Introduction

X chromosome inactivation is the mammalian dosage compensation system that results in transcriptional equality from the two X chromosomes in females as compared to males (Lyon 1961). Whereas X inactivation is thought to be a random process, with ~50% of cells containing the maternal X inactive and 50% of the cells containing the paternal X inactive, significant deviation from a 50:50 X inactivation pattern is occasionally observed among normal females in the population, a phenomenon referred to as skewed X inactivation (Nance 1964; Naumova et al. 1996; Plenge et al. 1997). It is generally thought that the broad distribution of X inactivation patterns reflects random fluctuation on a relatively small number of progenitor cells at the time an X is designated to be the inactive X (Nance 1964). However, genetic control of the inactivation process may result in nonrandom X inactivation—that is, preferential inactivation of one X chromosome over the other X chromosome (Willard 1995; Belmont 1996). Characterization of the genetic control of X inactivation is important for understanding the molecular events of the X inactivation pathway, the genetic control of cell proliferation, and the health status of female carriers of X-linked mutations (Willard 1995; Puck and Willard 1998).

Nonrandom X inactivation can occur by either primary or secondary mechanisms (Belmont 1996). Primary mechanisms disrupt the probability that an X will be the active X in a given cell. The best-characterized example of primary skewed X inactivation occurs in mice heterozygous for an X-linked locus, the X-controlling element (*Xce*) (Cattanach and Isaacson 1967). In these mice, preferential choice of one X chromosome occurs, resulting in skewed X inactivation in multiple tissue types (Johnston and Cattanach 1981; Krietsch et al. 1986). Whereas it is unclear whether common genetic factors in humans significantly influence choice in an *Xce*-like fashion, familial skewed X inactivation has been observed (Naumova et al. 1996; Pegoraro et al. 1997; Plenge et al. 1997; Parolini et al. 1998). In two

unrelated families, the genetic basis for skewed X inactivation in multiple tissues was determined to be a rare point mutation in the promoter of the *XIST* gene (Plenge et al. 1997). The *XIST* gene (*Xist,* in the mouse) maps to the critical region of the X chromosome that is essential for X inactivation, the X inactivation center in Xq13.2. In the correct developmental context, *XIST* is both necessary and sufficient for X inactivation (Lee et al. 1996; Penny et al. 1996; Herzing et al. 1997), and targeted mutations in the mouse *Xist* gene can result in primary nonrandom X inactivation (Marahrens et al. 1997; Carrel and Willard 1998; Clerc and Avner 1998; Marahrens et al. 1998). Autosomal factors are also thought to be involved in the choice process, although no such factors have yet been identified.

In contrast to primary nonrandom X inactivation, secondary mechanisms of skewed X inactivation occur after random inactivation because a particular X inactivation pattern confers a selective advantage or disadvantage to a cell. Several X-linked mutations have been described that result in skewed X inactivation due to postinactivation cell selection (Belmont 1996). Clear examples of secondary cell selection occur in heterozygous carriers of X-linked immunodeficiency syndromes (reviewed in Belmont 1995), incontinentia pigmenti type II (*IP2*) (Parrish et al. 1996), dyskeratosis congenita (*DK*) (Devriendt et al. 1997), and α-thalassemia with mental retardation (*ATRX*) (Gibbons et al. 1992). For these X-linked disorders, selection is against cells in which the mutant gene is on the active X chromosome, leading to complete skewing in peripheral blood cells with essentially 100% penetrance (i.e., X inactivation patterns are >95:5 in all adult female carriers). For some X-linked disorders, complete skewing may be restricted to specific blood lineages (Belmont 1995).

In a family described elsewhere (Orstavik et al. 1996), many females showed skewed X inactivation. This family also segregates a mutation in the X-linked dystonia-deafness peptide (*DDP*) gene. In addition to dystonia and deafness, affected males have visual impairment, fractures, and mental retardation (Tranebjaerg et al. 1995). The obligate female carriers did not have any significant clinical manifestations. In contrast to X-linked conditions discussed above, the X inactivation patterns of the female carriers in this family were incomplete, ranging between 50:50 and >95:5, thus calling into question the relationship between the *DDP* mutation and skewed X inactivation.

We considered four possibilities to explain why the familial skewed X inactivation is not complete and not 100% penetrant in women who are carriers. First, it seemed possible, although unlikely, that the familial skewing occurred simply by chance or as a result of shared environmental factors. Second, cell selection may occur against cells in which the mutant *DDP* allele is on the active X chromosome, but the selection is partial, thus leading to variable skewing in female carriers. Third, there may be a primary alteration in the X inactivation pathway that disrupts the choice of which X is to be the active X (e.g., mutations in *XIST* or a novel gene in the X inactivation pathway). Under this model, the *DDP* mutation would be unrelated to X inactivation but simply accounts for the family's ascertainment. And fourth, there may be a more complex scenario that includes stochastic fluctuation, cell selection, and primary nonrandom X inactivation.

To address these possibilities, we have used linkage analysis to establish that skewed X inactivation in this family is under genetic control and to map the skewing locus to the proximal long arm of the X chromosome. Further, we provide evidence that mutations in the *DDP* gene represent the molecular defect that results in partial skewed X inactivation in peripheral blood leukocytes. In contrast to previous findings for X-linked mutations, our data suggest that, within a given family and for a specific X-linked mutation, secondary cell selection may lead to incompletely penetrant and variable skewed X inactivation. This altered cell viability may be responsible in part for the dystonia-deafness phenotype observed in affected men and may explain the lack of clinical manifestations in heterozygous females.

Subjects, Material, and Methods

Families

Family K8160 segregates an X-linked recessive deafness syndrome with dystonia, fractures, progressive mental deficiency, and visual deterioration due to optic atrophy (DFN-1 [MIM 304700]) (Tranebjaerg et al. 1995; Orstavik et al. 1996). The mutant *DDP* gene in family K8160 carries a 1-bp deletion in exon 1 (151delT) (Jin et al. 1996). Females who are carriers show no overt clinical manifestation, including one woman with a 50:50 X inactivation pattern (individual II-4; fig. 1); four women (aged 42–72 years) were investigated more thoroughly, and two of four carriers were found to manifest mild hearing loss, decreased Achilles tendon reflexes, and mild reduction of pain and temperature sensation. The Jensen syndrome family and family K8190 have been described elsewhere (Jensen 1981; Jin et al. 1996).

DNA Extraction

DNA was extracted from peripheral blood cells of all available carrier and noncarrier females as described elsewhere (Orstavik et al. 1996). A fibroblast cell line was created from a forearm skin biopsy sample for all available women.

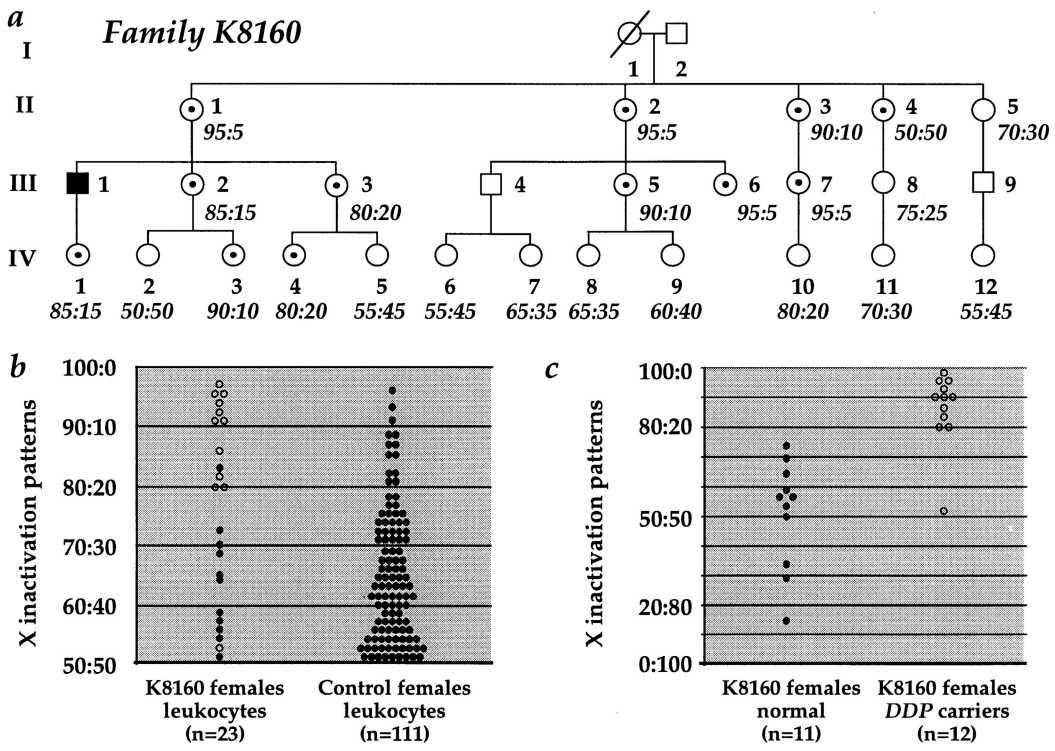

Figure 1 Familial skewed X chromosome inactivation. *a,* The X inactivation patterns in all available females from family K8160 were determined from peripheral blood leukocyte DNA by using the AR assay. The X inactivation patterns are indicated. Females who are carriers of the mutant *DDP* allele, and an affected male, are indicated. *b,* Scatter plot of the X inactivation patterns of the females from family K8160 (n = 23) and unrelated women (control group, n = 111). Blackened circles indicate women who carry the normal *DDP* gene, and unblackened circles indicate women who carry the mutant *DDP* allele. *c,* Scatter plot of X inactivation patterns from peripheral blood leukocyte DNA expressed relative to alleles inherited from individual I-1. As in *b,* blackened circles indicate mutant *DDP* carriers, and unblackened circles indicate noncarriers.

Androgen Receptor X-Inactivation Assay

The androgen receptor (*AR*) X inactivation assay has been described elsewhere (Allen et al. 1992; Plenge et al. 1997). In brief, digestion of genomic DNA with the methylation-sensitive restriction enzyme *Hpa*II results in digestion of alleles residing on the active X chromosome. Subsequent PCR with primers flanking the *Hpa*II restriction site and a polymorphic CAG repeat results in amplification of alleles on the inactive X only. In females heterozygous for the *AR* polymorphism, the two amplification products can be compared directly and an individual's X inactivation pattern determined quantitatively.

Genotyping

A panel of 14 fluorescently labeled microsatellite markers spanning the X chromosome at ~10-cM resolution was used for linkage analysis (Research Genetics; see fig. 2 for genetic map). DNA (100 ng) was amplified in a reaction mixture containing 20 mM Tris (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.5 μM primers, and 2.5 U *Taq* DNA polymerase (Gibco BRL) in a Perkin Elmer 9600 for 30 cycles of 95°C for 20 s, 55°C for 15 s, and 72°C for 30 s. An initial denaturation was performed at 95°C for 2 min and a final extension at 72°C for 7 min. PCR products were individually diluted and analyzed on an ABI 373A Sequencer by use of GENESCAN 672 software according to the manufacturer's specifications. The approximate positions of the *AR, XIST,* and *DDP* genes were determined from information supplied by flanking markers.

Linkage Analysis

We performed parametric linkage analysis with LINKAGE (Lathrop et al. 1984) by using the mutant *DDP* allele as a genetic marker (the mutant *DDP* allele frequency was estimated to be .0001). Inheritance was assumed to be X-linked dominant, and a variety of penetrance values were considered. Affection status was defined as preferential inactivation of one X chromosome (i.e., skewed X inactivation) at one of three different thresholds, X inactivation patterns ≥90:10, ≥80:

20, or ⩾70:30. X inactivation patterns were expressed both without regard to the parent-of-origin of the preferentially inactive X (X inactivation patterns ranged from 50:50 to 100:0) and relative to alleles inherited from individual I-1 (X inactivation patterns ranged from 0:100 to 100:0). Nonparametric linkage analysis was performed by GENEHUNTER (Kruglyak et al. 1996). Affection status was assigned as described above. All microsatellite marker alleles were considered to be of equal frequency.

Results

Familial Skewed X Inactivation Is under X-Linked Genetic Control

Familial skewed X inactivation was previously reported in a family segregating mutations in the X-linked *DDP* gene (family K8160, fig. 1*a*; Orstavik et al. 1996), although it was not clear from the initial report that the skewing was under genetic control. To expand these data, we determined X inactivation patterns on all available females in family K8160 (fig. 1*a*), confirming and quantitating the previously described patterns and increasing the number of females assayed. The distribution of X inactivation patterns shown in figure 1*b* is reported without regard to parent-of-origin or another anchoring trait, and therefore ranges from 50:50 to 100:0.

To classify skewed X inactivation as a phenotype, we chose several thresholds to assign affection status: X inactivation patterns ⩾70:30, ⩾80:20, and ⩾90:10. The percentages of females from the general population who show such patterns are ~33%, 12%, and 3%, respectively (fig. 1*b;* Plenge et al. 1997). In contrast, the percentages of females in this family who show such patterns are 65% (15/23), 52% (12/23), and 30% (7/23), respectively (fig. 1*b*).

A priori, the observation that ~50% of females in this family show X inactivation patterns ⩾80:20 is consistent with a single dominant "skewing" locus, either autosomal or X-linked. To determine whether the skewing locus was autosomal or X-linked, we performed linkage analysis with the mutant *DDP* allele (inherited from individual I-1 in family K8160) as a genetic marker. The LOD scores for various parameters are shown in table 1. The most significant LOD scores were obtained with X inactivation patterns for affection status of ⩾80:20 (table 1, LOD score 2.50, recombination fraction [θ] = 0.1 at 100% penetrance; LOD score 2.83, θ = 0.0 at 80% penetrance). This analysis strongly suggests linkage of skewed inactivation to the mutant *DDP* allele and thus shows genetic control of skewed X inactivation in this family.

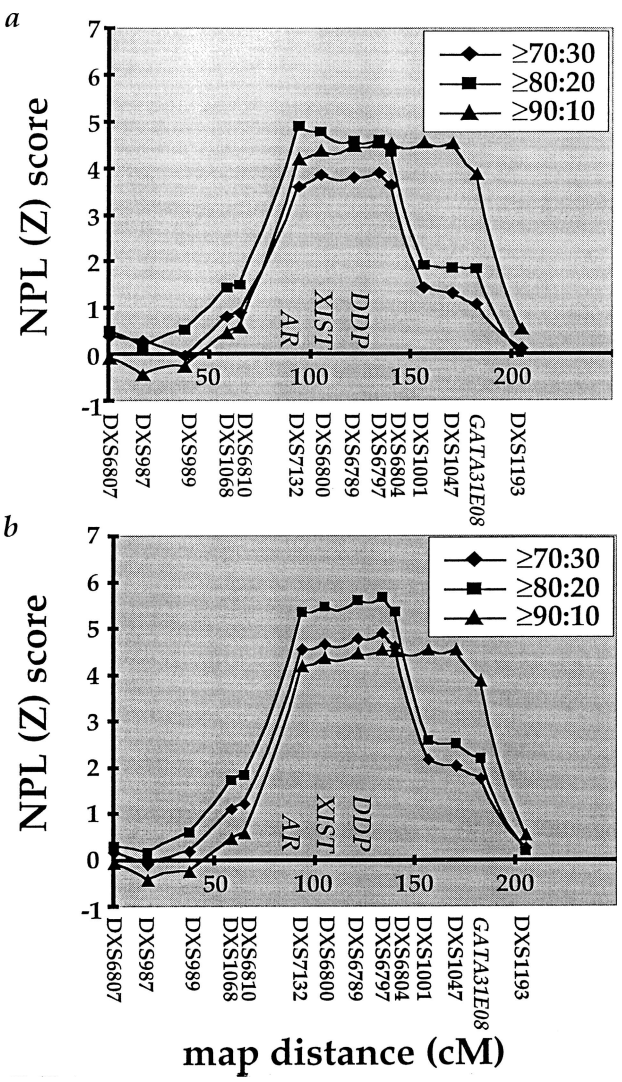

Figure 2 Nonparametric linkage analysis from family K8160. *a,* X inactivation patterns are expressed without regard to a genetic marker. *b,* X inactivation patterns are expressed relative to alleles inherited from individual I-1. Affection status was determined by use of X inactivation patterns of ⩾70:30, ⩾80:20, and ⩾90:10. The approximate positions of the *AR, XIST,* and *DDP* genes are shown.

Skewed X Inactivation Defined Relative to Alleles from Individual I-1

To more precisely define the skewed X inactivation phenotype in this family, we hypothesized, on the basis of the model predicted above, that the skewing locus must also be inherited from individual I-1. By determining the region of the X chromosome that cosegregates with skewed X inactivation, we could express X inactivation patterns in a biologically significant manner (i.e., relative to parental origin) while simultaneously mapping the skewing locus (see below). Therefore, we genotyped all available family members with markers along the entire length of the X chromosome and, in

Table 1

LOD Score Analysis with Mutant *DDP* Allele

X Inactivation Pattern	Female Penetrance	LOD Score at θ = .00	.10	.20	.40
⩾90:10	1.00	−1.14	.19	.50	.29
	.90	.09	1.08	1.04	.40
	.80	1.10	1.34	1.15	.40
⩾80:20	1.00	−2.12	2.50	2.14	.80
	.90	2.82	2.55	2.07	.73
	.80	2.83	2.44	1.92	.62
⩾70:30	1.00	−.01	.20	.13	.01
	.90	.27	.19	.09	.01
	.80	.16	.09	.04	.00

NOTE.—LOD score results from family K8160, obtained by use of the mutant *DDP* allele as a genetic marker. X inactivation patterns are expressed without regard to any genetic marker. Affection status was assigned by use of three X inactivation patterns: ⩾90:10, ⩾80:20, and ⩾70:30.

particular, with markers around the *XIST* and *DDP* genes. In total, 14 polymorphic markers were used, spaced ~10 cM apart.

The X inactivation patterns shown in figure 1*c* are presented with respect to X-linked alleles inherited from individual I-1. Because, in a given female, the inheritance of the differentially methylated *AR* alleles can be determined, it is possible to determine whether the inactive X is of maternal or paternal origin. By following the cosegregation of the differentially methylated *AR* alleles, together with the alleles inherited from individual I-1, it is possible to determine whether the region of the X chromosome inherited from individual I-1 is on the preferentially active or inactive X chromosome in each individual. As shown in figure 1*c*, it is clear that the skewing locus results in preferential inactivation of the associated chromosome: 11 of 12 women with X inactivation patterns ⩾80:20 inherited alleles from I-1 on their preferentially inactive X chromosome; only individual IV-10 inherited alleles from I-1 on her preferentially active X chromosome, which suggests that her X inactivation pattern either occurred by chance or is under the control of another genetic locus.

Skewed X Inactivation Maps to Xq12-q22

Defining skewed X inactivation relative to alleles inherited from individual I-1, we recalculated LOD scores by using the same parameters described in table 1. Only females with X inactivation patterns ⩾70:30, ⩾80:20, or ⩾90:10 were considered affected. A peak LOD score of 3.57 was obtained by use of the mutant *DDP* allele as a genetic marker (table 2, $\theta = 0.0$ at 90% penetrance; LOD score 3.07, $\theta = 0.1$ at 100% penetrance). The only carrier of a mutant *DDP* allele to show a pattern less extreme than 80:20 was individual II-4.

Individual II-4 represents a crucial individual in this analysis, since she carries a mutant *DDP* allele but shows a 50:50 X inactivation pattern. There are two possible explanations for this finding. First, the skewing locus could be fully penetrant and linked to, but distinct from, the mutant *DDP* allele. Second, the skewing locus could show reduced penetrance and map to the vicinity of the mutant *DDP* allele. To map the skewing locus to a region on the X chromosome, we performed nonparametric linkage (NPL) analysis, using GENEHUNTER with the set of polymorphic markers described above. Affection status was assigned by use of the three X inactivation patterns described above, both with and without respect to alleles inherited from individual I-1 (fig. 2). In comparing figures 2*a* and 2*b*, it is clear that expressing the X inactivation patterns relative to alleles inherited from individual I-1 consistently provides more significant evidence of linkage. A region of ~50 cM (DXS7132 to DXS6804) cosegregates with the skewed inactivation phenotype; the most significant NPL scores at each of the three X inactivation values were $Z = 4.5$ (⩾90:10; $P = .008$); $Z = 5.7$ (⩾80:20; $P = .002$); and $Z = 4.9$ (⩾70:30; $P = .0005$). As shown in figure 2, and as predicted from the linkage analysis, this region includes the *XIST* and *DDP* genes. Individual II-4 shared this ~50-cM region with the other women who are *DDP* carriers, and therefore no crossover was detected to explain her 50:50 X inactivation pattern (see the Discussion section).

Additional Families Segregating DDP *Mutations*

Because of the strong association of the mutant *DDP* allele with the preferentially inactive X chromosome, we considered the possibility that the skewed inactivation is due to selection against cells with the mutant *DDP* allele on the active X, despite the fact that such selection, if present, must be, in contrast to other X-linked conditions, incomplete. If this hypothesis is correct, then unrelated women who carry *DDP* mutations may also show skewed X inactivation. We therefore determined X inactivation patterns of four *DDP* carrier females from two additional families (fig. 3). Each family seg-

Table 2

LOD Score Analysis with Mutant *DDP* Allele and X Inactivation Patterns Relative to Alleles Inherited from I-1

X Inactivation Pattern	Female Penetrance	LOD Score at θ = .00	.10	.20	.40
⩾80:20	1.00	−1.27	3.07	2.51	.90
	.90	3.57	3.05	2.39	.80
	.80	3.48	2.87	2.19	.67

NOTE.—LOD score results from family K8160 obtained by use of the mutant *DDP* allele as a genetic marker. X inactivation patterns are expressed relative to alleles inherited from individual I-1 and thus range from 0:100 to 100:0. Affection status was determined by use of the three X inactivation patterns shown in table 1, but only those results for X inactivation patterns ⩾80:20 are shown.

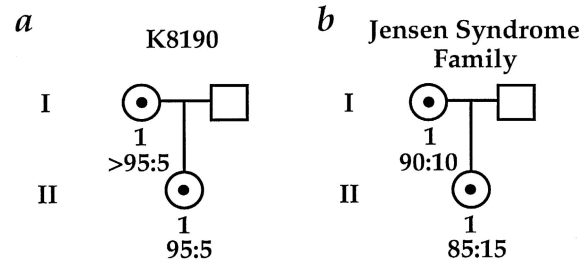

Figure 3 Two unrelated families who carry different mutations in the *DDP* gene. Dots indicate females who are carriers of the mutant *DDP* allele. X inactivation patterns are from peripheral blood leukocyte DNA. *a,* Family K8190 carries a 10-bp deletion in exon 2 (183del10), which creates a frameshift mutation and polypeptide termination (Jin et al. 1996). The mutant *DDP* allele is on the preferentially inactive X in individual II-1. *b,* The Jensen syndrome family carries a 1-bp substitution resulting in a premature stop codon and a predicted truncated protein (L. Tranebjaerg, unpublished data). The mutant *DDP* allele is on the preferentially inactive X in individual II-1.

regates a different mutation in the *DDP* gene. As shown in figure 3, all four individuals show X inactivation patterns ⩾80:20 in peripheral blood leukocytes. In addition, the mutant *DDP* gene was shown to reside on the preferentially inactive X in the two females in whom the analysis could be performed (II-1, fig. 3*a;* and II-1, fig. 3*b*).

Discussion

Genetic control of skewed X inactivation can occur at the time an X chromosome undergoes inactivation ("primary nonrandom X inactivation") or after random X inactivation because of a selective growth advantage ("secondary nonrandom X inactivation"). Secondary cell selection has been well described for several X-linked mutations, with extreme skewing (i.e., X inactivation patterns >95:5) showing essentially 100% penetrance in carrier females; the rare exceptions have been hypothesized to result from a primary defect in the X inactivation pathway (Parolini et al. 1998; Puck and Willard 1998). It was not clear from previous studies whether the skewed X inactivation observed in family K8160 was under genetic control (Orstavik et al. 1996), in part because of the observation that X inactivation patterns in the women who are carriers of the mutant *DDP* allele ranged between 50:50 and >95:5, with only 4 of 12 X inactivation patterns being >95:5. As such variable skewed X inactivation may be due (in theory) to nongenetic factors, primary genetic control, secondary genetic control, or a more complex interaction of factors, we have taken a nonbiased approach to our analysis by first determining that skewed X inactivation is under genetic control and then mapping the responsible skewing locus. In the present study, we provide evidence for secondary cell selection due to mutations in the X-linked gene *DDP* (as opposed to a primary defect in the X inactivation pathway) and discuss the implications of the finding of variable skewing due to partial cell selection in peripheral blood leukocytes.

By using NPL and parametric linkage analysis and several classifications of affection status, we consistently mapped the skewing locus in family K8160 to the proximal long arm of the X chromosome. Importantly, this mapping result was, by design, obtained independent of any assumptions about the potential role of the *DDP* gene in the observed skewing. Parametric linkage analysis mapped the skewing locus to the proximity of the *DDP* gene (LOD score 3.57, $\theta = 0.0$). NPL analysis determined that the skewing locus maps to the proximal long arm (Xq12–q22; fig. 2), a region that includes both the *XIST* and *DDP* genes. Whereas statistically significant NPL scores were obtained ($Z = 5.7$, $P = .002$), no single region of the X chromosome was inherited exclusively by females classified as affected by any of the skewed inactivation classifications described above. For example, individual II-4 shared this region, despite showing a 50:50 X inactivation pattern (data not shown).

As implied above, two likely candidate genes within the region are *XIST* and *DDP. XIST* maps ~10 cM from *DDP* and, in the correct developmental context, is necessary and sufficient for X inactivation (Lee et al. 1996; Penny et al. 1996; Herzing et al. 1997). A mutation in the minimal promoter of the *XIST* gene is associated with skewed X inactivation (Plenge et al. 1997), and targeted mutations of the murine *Xist* gene result in primary nonrandom X inactivation (Marahrens et al. 1997; Carrel and Willard 1998; Clerc and Avner 1998; Marahrens et al. 1998). Whereas complete analysis of the *XIST* gene in K8160 has not been performed, sequencing of the minimal promoter and a highly conserved repeat region in the first exon (Hendrich et al. 1993) showed no nucleotide differences in this family (data not shown).

Whereas we cannot exclude *XIST* or another gene as the responsible skewing locus in family K8160, four lines of evidence strongly implicate the *DDP* gene. First, mutations in several X-linked genes have been shown to cause skewed X inactivation by secondary cell selection (Belmont 1996), although most appear to show 100% penetrance of the skewed inactivation phenotype. Some of these genes are expressed in peripheral blood leukocytes and are subject to X inactivation, characteristics that are also attributable to *DDP* (Brown et al. 1997). Second, four females who are carriers from two unrelated families with different *DDP* mutations also showed skewed X inactivation in peripheral blood leukocytes (fig. 3). When the three families are considered together, 15 of 16 females who are carriers of a *DDP* mutation

show X inactivation patterns ≥80:20. Third, the nature of skewed X inactivation in these three families is consistent with a secondary cell-selection mechanism. In general, cell selection occurs against those cells with the X-linked mutation on the active X chromosome; the mutant gene thus resides on the preferentially inactive X chromosome (Belmont 1996). Here, in all informative females with X inactivation patterns ≥80:20, the mutant *DDP* allele resides on the preferentially inactive X chromosome ($n = 13$). And fourth, secondary cell selection is often restricted to specific tissue types (Belmont 1996; Puck and Willard 1998). In the present study, skewed inactivation is observed in peripheral blood leukocytes but not in fibroblasts (data not shown). Taken together, these data strongly suggest that cell selection occurs against those peripheral blood cells in which the mutant *DDP* allele is on the active X chromosome.

It is intriguing that, in contrast to previous studies of X inactivation patterns in many other X-linked disorders, the extent of skewing in peripheral blood leukocytes varies among *DDP* mutation carriers, with one woman showing a 50:50 X inactivation pattern. Indeed, it was this original observation that led us to adopt an unbiased strategy in considering the possibility of an additional skewing locus. It has been suggested that women who carry full mutations at *FMR1* (Reiss et al. 1995; de Vries et al. 1996), severe alleles at glucose 6-phosphate dehydrogenase (*G6PD*) (Filosa et al. 1996), and mutations in the Barth syndrome gene (Orstavik et al. 1998) also show variability in the skewing phenotype, although genetic mapping studies in large pedigrees have not been performed for those disorders. In addition, for some X-linked immunodeficiencies, skewed X inactivation is observed only in specific peripheral blood cell types, although the skewing is 100% penetrant (reviewed in Belmont 1995). Here, however, we show that, whereas skewing in peripheral blood leukocytes may be complete (>95:5) in some carriers, significant variability is observed in the cell-selection phenotype of other carriers.

That complete skewing is not observed in all carriers of a mutant *DDP* allele suggests that additional factors, either stochastic or genetic, contribute to the skewed inactivation phenotype. A mutation in the promoter of the *XIST* has been shown to modify X inactivation patterns in carriers of an X-linked mental retardation mutation by increasing the likelihood that the associated X will be the inactive X chromosome in a given cell (Plenge et al. 1997). However, no association was observed between inheritance of a particular *XIST* allele and variation in the skewed X inactivation phenotype in family K8160. Additional families have been described in which inheritance of skewed inactivation appears to be Mendelian, although no specific genes have yet been implicated (Naumova et al. 1996; Pegoraro et al. 1997; Parolini et al. 1998). In individual II-4, the carrier of a *DDP* mutation who showed a 50:50 X inactivation pattern, there was no region unique to her X chromosome that might explain the reduced penetrance (data not shown). Another possible explanation for the variability is age-related skewing (Fey et al. 1994; Busque et al. 1996). In family K8160, however, no consistent trend was detected (e.g., individual II-4 is in the oldest surviving generation). And finally, an X-linked gene may be associated with lymphocyte proliferation (Luzzatto et al. 1979; Abkowitz et al. 1998). However, no region on the X chromosome was inherited exclusively by individual II-4, although it is possible that she inherited a unique autosomal region(s) to explain her 50:50 X inactivation pattern.

It has been suggested that skewed X inactivation may serve a protective function in heterozygous females. For example, it is rare to find a manifesting carrier of the X-linked immunodeficiency Wiscott-Aldrich syndrome, a disorder associated with completely skewed X inactivation in peripheral blood leukocytes (Belmont 1995; Parolini et al. 1998; Puck and Willard 1998). Support for this protective function also comes from the mouse, in which the X-linked mutations "lined" (*Li*) and "stripey" (*Stpy*) show embryonic lethality in males but only coat color variegation and reduced birth weight in heterozygous females (Blair et al. 1998). Consistent with these observations, females who are carriers of a mutant *DDP* allele are essentially asymptomatic.

In conclusion, we have mapped an X inactivation skewing locus to the proximal long arm of the X chromosome and have provided evidence that mutations in the *DDP* gene represent the molecular defect underlying the variable skewing phenotype in peripheral blood leukocytes. In contrast to previous studies of X-linked mutations and skewed inactivation, our study provides an approach to mapping the responsible skewing locus that is independent of prior assumptions about the nature of the responsible locus and that shows the range of variability that may be associated with the skewed X inactivation phenotype in related females who carry the identical mutation.

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Electronic-Database Information

URL and accession number for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for DFN1 [MIM 304700])

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