X inactivation as a mechanism of selection against lethal alleles: further investigation of incontinentia pigmenti and X linked lymphoproliferative disease

Ann Harris, Jane Collins, David Vetrie, Charlotte Cole, Martin Bobrow

Abstract
Thirty-one females with incontinentia pigmenti (IP), 42 controls, and 11 females from four families segregating for X linked lymphoproliferative disease (XLP) were studied for evidence of skewed X inactivation by analysis of methylation at sites in the HPRT, PGK, and M278 (DXS755) regions of the X chromosome. Extensive skewing of X inactivation was present in blood from 4/42 (9.5%) control females and 11/31 (35%) of those with IP. This frequency of skewed inactivation was seen in both familial and sporadic cases of IP. Analysis of inactivation in mother/daughter pairs, both affected and control subjects, showed no familial consistency of pattern, arguing against specific mutations being associated with particular patterns of inactivation. In the only informative family where both mother and daughter were affected by IP and showed skewed inactivation, the IP mutation was on the active X chromosome. This argues against cell selection during early embryogenesis being the explanation for the skewed inactivation observed.

These data confirm that skewed inactivation of one X is observed in lymphocytes from a significant minority of normal females, and is seen with raised frequency in IP heterozygotes. It is not, however, a universally observed phenomenon, and the relationship of X inactivity to the IP mutation appears to be complex.

In the case of XLP, though skewed X inactivation patterns are seen in most disease carriers, the frequency with which this phenomenon occurs in normal females renders it an unreliable diagnostic marker for XLP carriers.

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The phenomenon of random inactivation of one X chromosome in all somatic tissues of a female was first reported nearly 30 years ago. Since then, certain exceptions to the random nature of this inactivation have become apparent. Of particular interest are those X linked, male lethal diseases in which carrier females show skewed patterns of X inactivation in certain tissues. It has been suggested that in these cases cells carrying the lethal allele on their active X chromosome might be selected against.1 The skewed patterns of X inactivation, which might result from this type of selection might either be observed in a variety of tissues in the female body, or only within those cell lineages in which the normal counterpart of the lethal allele is functionally important. The precise pattern of skewed X inactivation might also reflect the role of a particular gene earlier in development. Further, the pool size in the stem cell population at the time of selection for X activity is likely to be of significance.

X linked disorders in which skewed inactivation patterns have been observed in specific cell lineages in carrier females include several of the recessive X linked immunodeficiency diseases.

Recently, Migeon et al2 reported selection against mutant alleles in certain cell lineages in the X linked dominant disorder incontinentia pigmenti (IP) (Bloch Schultzberger). Skewed X inactivation was observed in five females from three IP families. This is a particularly interesting disorder which is nearly always lethal in males and can manifest with a range of severities in females. As its name implies, IP is characterised by a swirling pigmentation of the skin that follows a perinatal skin blistering phase. IP females may also show abnormal dentition, frequently having peg-like teeth, other skin related disorders such as partial alopecia, eye abnormalities, and sometimes mental retardation.

IP may either present sporadically or in a familial form following an X linked dominant pattern of inheritance. A total of six sporadic females apparently with IP and specific chromosomal translocations involving the same breakpoint at Xp11.2, but a variety of different autosomal sites, and one case carrying a ring chromosome encompassing the centromere and Xp11.2, have been reported.4-9 There has been some controversy as to whether two of
these translocation patients actually have IP and not Ito's syndrome, a disorder resulting in similar disrupted skin pigmentation. However, it is clear that the majority do have IP, hence it seems highly probable that there is a locus at Xp11.2 whose disruption is responsible for one form of this disorder. However, studies of IP families failed to show linkage of the disease to any of the polymorphic markers mapping to the Xp11.2 region.\textsuperscript{10,11} More recently another family study has shown linkage of IP to the marker St-14 (DXS552) located at Xq26–28 with a lod score of 3.5 at $\theta = 0.05$.\textsuperscript{12} There would therefore appear to be at least two X linked loci which can determine what is currently recognised as the IP phenotype.

We have examined further the patterns of X inactivation in a group of females with IP, both sporadic and familial. If selection against expression of the IP mutation was a common phenomenon in heterozygous females, then we would expect to observe skewed X inactivation in the majority of cases. If the IP gene has a functional role in early embryonic development then skewed X inactivation might be seen in a large number of cell lineages, possibly including peripheral blood cells. Alternatively, non-random inactivation might only be seen in tissues expressing the IP gene. Unfortunately, it is not clear which of the many cell types making up the skin might actually be involved in the initial IP blistering phase and subsequent damage related abnormal pigmentation. Owing to inaccessibility of pure populations of most skin cells, we have been limited to the study of skin derived fibroblasts and epithelial cells. In the five females from three families investigated by Migeon et al.,\textsuperscript{4} skewed X inactivation was observed in skin fibroblasts and in some cases also in peripheral blood cells.

We have also analysed a large cohort of control females who were not known to be carrying any X linked disease, and 11 females from four families segregating for X linked lymphoproliferative disease (XLP, LYP, IMD9). XLP is a rare, recessive disorder that is characterised by the inability of affected subjects to mount a normal immune response to Epstein–Barr virus (EBV).\textsuperscript{13} Affected subjects and presumed obligate carrier females usually show abnormal serum antibody responses to EB viral antigens. EBV infection in XLP patients generally causes an initial infectious mononucleosis that progresses rapidly into malignant, polyclonal lymphoproliferative disease.

The method we have used to establish patterns of X inactivation is the analysis of methylation patterns at specific chromosomal sequences.\textsuperscript{14} It is known that at the DNA level the phenomenon of X inactivation can be observed through differential methylation at particular Cpg sites. Polymorphisms in the housekeeping genes hypoxanthine phosphoribosyl transferase (HPRT)\textsuperscript{15} and phosphoglycerate kinase (PGK),\textsuperscript{16} or the hypervariable locus DXS255 detected by the probe M27B,\textsuperscript{17–19} have been used to distinguish active and inactive X chromosomes. The methylation state of the two polymorphic DNA fragments is then investigated using methylation sensitive enzymes that will not cleave methylated DNA.

**Materials and methods**

Twenty-three familial cases and eight sporadic cases of IP were examined. The family structures are shown in fig 1. All sporadic cases were IP females born to apparently unaffected mothers and with no family history of the disease. Family 1 is resident in the South-East Thames region, families 2 and 3 in South Australia, families 4 to 9 in Hungary, family 10 in Oxford, and family 11 (not shown) in the East Midland region (see acknowledgements). All sporadic cases are Hungarian.

The control females included 28 cystic fibrosis (CF) carriers, nine carriers of known other (non-X linked) diseases including Huntington's chorea (5), Tay-Sachs disease (2), DIDMOAD (diabetes insipidus, diabetes mellitus, and optic atrophy) (1), and ataxia cerebellum (1), and five females not known to be carriers of any genetic disease.

Eleven females from four families segregating for XLP were examined (fig 2) using peripheral blood cells (families 1 and 2) or EBV transformed lymphoblastoid cell lines (families 3 and 4 from whom peripheral blood samples were not available). EBV transformed lymphoblastoid cell lines are polyclonal and have been shown not to bias X inactivation patterns,\textsuperscript{20,21} though Migeon et al.\textsuperscript{4} have suggested this polyclonality may be distorted after prolonged periods in culture. Three of these families (fig 2, Nos 2, 3, and 4) have been reported previously.\textsuperscript{22–26} In family 4, serological responses to EBV are abnormal in the female marked C (carrier) and normal in those marked NC (non-carrier). In the other families no immunological data are available to establish carrier status, so the mother and maternal grandmother of one or more affected boys are presumed to be carriers. It is of course possible that a new mutation has arisen either in the XLP patient himself or in his mother, rather than having been inherited from his maternal grandmother.

DNA was extracted from peripheral blood lymphocytes by standard methods. A fibroblast culture was set up from a skin biopsy from one IP patient by standard methods. Epithelial cells were grown from the same skin biopsies by a modification of the technique of Harris and Coleman.\textsuperscript{27} The HPRT and M27B probes detect polymorphisms including a CCGG restriction enzyme site which is methylated on the active X chromosome and therefore resistant to cleavage by HpaII (but not MspI). The PGK probe detects a polymorphism involving a HpaII/MspI site which is methylated on the inactive X chromosome.

Details of the HPRT and PGK polymorphisms are described elsewhere.\textsuperscript{14–16,28,29} Briefly, the HPRT probe detects a polymorphism after simultaneous cleavage with BamH1 and PstI11 giving band sizes of 18 and 12 kb. The PGK probe detects a polymor-
Figure 1. Family pedigrees (1-10) of IP subjects included in the study and three control families (C1-3). NR = extremely skewed, NRp = moderately skewed, R = symmetrical.

Figure 2. X inactivation patterns in females from four families segregating for X linked lymphoproliferative disease. C = XLP carrier, NC = non-carrier, R = symmetrical, NRp = moderately skewed inactivation, NRc = extremely skewed. h denotes HPRT data, m denotes M27β data, p denotes PKG data.
was cleaved first with *PvuII* in 50 mmol/l NaCl and then with *BamHI* in 150 mmol/l NaCl. For PGK, 20 μg of genomic DNA was cleaved first with *EcoRI* and *BglII* simultaneously in 50 mmol/l NaCl and then with *BglII* in 100 mmol/l NaCl. For both HPRT and PGK DNA samples were then ethanol precipitated, resuspended in NaCl free buffer, and samples divided into two 10 μg aliquots. One of these was further restricted with 60 U *HpaII* for 18 hours.

After digestion of genomic DNA with *PstI*, the M27β probe shows one fragment from each X chromosome, the precise band size depending on the number of tandemly repeated 26 bp units at the DXS255 locus on each chromosome; 30 μg of DNA were digested with *PstI* and then the sample was divided into three aliquots of 10 μg each. One aliquot was further digested with *MspI* to show the fragment sizes expected after digestion of both X chromosome DNAs and another aliquot was digested with 60 U of *HpaII* to distinguish between active and inactive X chromosomes. With the M27β polymorphism, the active X chromosome appears in some cases to show partial methylation (resistance to *HpaII* digestion) so totally skewed inactivation was defined as one allele being completely resistant to cleavage by *HpaII* (fig. 3).

Digested DNA was resolved by electrophoresis through 0.8% (PGK) or 0.7% (HPRT) agarose (BRL) gels in Tris/acetate/EDTA buffer and transferred to Hybond N (Amersham) membranes by standard procedures. Membranes were hybridised at 65°C in 0·5 mol/l sodium phosphate, pH 7·5, 5 × Denhardt’s, 1% SDS, 100 μg/ml sonicated calf thymus DNA, and 10% dextran sulphate. Genomic DNA inserts from the HPRT (1·7 kb *PstI-BamHI* insert of pBl1.71), PGK (0·8 kb *BamHI* insert of psPT19.1114), and M27β (2·3 kb *EcoRI* insert)28 probes were excised and gel purified on low gelling temperature agarose (BRL) gels and then 32P labelled by standard methods to a specific activity of 1 × 106 cpm/μg. The HPRT probe (100 ng) was pre-reassociated with human placental DNA (300 μg) in 5 × SSC for 30 minutes at 65°C. Filters were washed to a final stringency of 0·2% SSC, 0·1% SDS at 65°C, and then exposed to Kodak XAR5 film.

A total of 31 females with familial (23) or sporadic (eight) IP and 42 non-IP females have been analysed with respect to their patterns of X inactivation. Among the IP patients, 14 were informative at the PGK locus, four at the HPRT locus, and 13 who were uninformative at both these loci were tested at the M27β locus. The 42 non-IP females were all chosen to be informative at the PGK locus. The data are summarised in tables 1 to 3.

X inactivation patterns were scored as symmetrical with band intensity ratios (defined by a Biorad 620 video densitometer) after *HpaII* digestion of 50:50–65:35%, moderately skewed (NRc) 65:35–80:20, and extremely skewed (NRs) > 80:20 (fig 3). These values

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**Table 1 Patterns of X inactivation in incontinentia pigmenti females.**

<table>
<thead>
<tr>
<th>Probe</th>
<th>Symmetrical</th>
<th>Skewed</th>
<th>Extreme</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>HPRT</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>M27β</td>
<td>3</td>
<td>2</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>8 (42%)</td>
<td>11 (58%)</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>12 (39%)</td>
<td>19 (61%)</td>
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</table>
Table 2A  Patterns of X inactivation in familial or sporadic incontinentia pigmenti.

<table>
<thead>
<tr>
<th>Probe</th>
<th>Familial</th>
<th>Sporadic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGK</td>
<td>4 symmetrical</td>
<td>2 symmetrical</td>
<td>6</td>
</tr>
<tr>
<td>HPRT</td>
<td>7 skewed</td>
<td>1 skewed</td>
<td>8</td>
</tr>
<tr>
<td>M27β</td>
<td>3 symmetrical</td>
<td>0 informative</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1 skewed</td>
<td>0 informative</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6 skewed</td>
<td>4 skewed</td>
<td>10</td>
</tr>
<tr>
<td>Symmetrical</td>
<td>9 (39%)</td>
<td>3 (38%)</td>
<td>12</td>
</tr>
<tr>
<td>Skewed</td>
<td>14 (61%)</td>
<td>5 (62%)</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>8</td>
<td>31</td>
</tr>
</tbody>
</table>

Table 2B  Degree of skewed inactivation in familial or sporadic incontinentia pigmenti.

<table>
<thead>
<tr>
<th>Probe</th>
<th>Familial</th>
<th>Sporadic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGK</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>M27β</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>HPRT</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Moderato (NR₄)</td>
<td>PGK</td>
<td>4</td>
<td>1</td>
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<tr>
<td></td>
<td>M27β</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>HPRT</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

NR₄ = extremely skewed, NR₃ = moderately skewed.

Table 3  Patterns of X inactivation at PGK locus in control females.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Symmetrical</th>
<th>Skewed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate</td>
<td>Extreme</td>
<td></td>
</tr>
<tr>
<td>CF carriers</td>
<td>15</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Other autosomal diseases</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Normal controls</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>15</td>
<td>4</td>
</tr>
</tbody>
</table>

| | (55%) | (45%) | |
| Total | 23 | 19 | 42 |

were consistent with visually obvious distinctions. The extremely skewed group did not warrant further subdivision.

Results

Among the IP females (table 1), 39% (12/31) show symmetrical patterns of X inactivation in their peripheral blood lymphocytes and 61% (19/31) show skewed patterns. These 61% have been further subdivided into two groups as illustrated in fig 3. Forty-two percent (8/19) of IP females exhibiting skewed patterns of X inactivation show moderate skewing and 58% (11/19) show inactivation of the same X chromosome in nearly all their peripheral blood lymphocytes.

In table 2A the IP females have been grouped according to whether they have familial or sporadic types of the disease. Thirty-nine percent (9/23) of familial IP cases show symmetrical X inactivation and 61% (14/23) show skewed patterns. Among sporadic cases, 38% (3/8) show symmetrical X inactivation and 62% (5/8) showed skewed patterns. However, within the skewed group the percentage of females showing moderate skewing or total inactivation of one X chromosome differs somewhat in familial and sporadic IP. Sixty-four percent (9/14) of familial IP females with skewed pattern and 40% (2/5) of sporadic IP show extremely skewed patterns (table 2B). However, when measured in terms of the total familial or sporadic population, 39% (9/23) of familial IP females and 25% (2/8) of sporadic IPs are extremely skewed.

In familial IP the X inactivation patterns of mother/daughter pairs have been investigated to see whether the IP lesion is causing consistent patterns from one generation to the next (table 4). Thirteen mother and daughter pairs have been analysed (fig 1). Of these, seven show identical patterns of X inactivation (four symmetrical and three skewed) while six do not. In the latter cases the mother shows symmetrical inactivation and the daughter skewed inactivation in four pairs (two mothers each with two daughters, though in one case these are by two different fathers) while two mothers show skewed patterns but have daughters with symmetrical patterns. In one of the three pairs where both mother and daughter show skewed X inactivation, it has been possible to establish whether the paternal or maternal X has been preferentially inactivated in the daughter. In this case the IP lesion is on the active X chromosome.

In the only IP female from whom a skin sample was obtained (fig 1, family 1, subject marked *) skin fibroblasts and skin derived epithelial cells showed the same symmetrical pattern of inactivation at the HPRT locus as was seen in peripheral blood cells (data not shown).

Among 42 control females (table 3), 55% (23/42) show symmetrical patterns of X inactivation and 45% (19/42) skewed patterns. Of the 45% (19/42) showing skewed patterns of X inactivation, 79% (15/19) show moderate skewing and 21% (4/19) inactivation of the same X chromosome in all their peripheral blood cells. Slightly less than 10% (9/5%, 4/42) of the whole control population show extremely skewed patterns. In three control mother and daughter pairs (all CF carriers) where X inactivation patterns have been analysed, two show symmetrical patterns in mother and daughter while one symmetrical pattern mother has a moderately skewed pattern daughter (table 4).

Data for XLP patients are summarised in fig 2. In kindred 1 the maternal grandmother of the XLP patient shows an extremely skewed pattern, while the mother shows a moderately skewed pattern and the sister (who may or may not be a carrier) shows a symmetrical pattern. In kindred 2 the maternal grandmother has a symmetrical inactivation pattern but the mother shows a moderately skewed pattern. In kindred 4 the mother of the XLP boys, who as well as being an obligate carrier has the immunological characteristics of a carrier, shows a moderately skewed inactivation pattern as does the sister of the affected boys, whose carrier
status is unknown. In the same kindred, the maternal aunt of the affected boys has a normal immunological response to EBV antigens, hence is thought not to be an XLP carrier, and has a symmetrical X inactivation pattern. However, her daughter, who also appears to be a non-carrier, shows by immunological criteria, shows a moderately skewed inactivation pattern. In kindred 3, the identical twin sisters of the affected boy, whose carrier status is unknown, show different X inactivation patterns. One has a symmetrical pattern, while the other has a moderately skewed pattern, a not uncommon phenomenon.

Discussion

We have assessed X inactivation patterns by the relative changes in density of polymorphic bands on Southern blots, with and without digestion with a methylation sensitive enzyme, HpaII. We have classified each informative female as showing either symmetrical X inactivation (both bands remain of approximately equal intensity after HpaII digestion, 50:50–65:35), extremely skewed inactivation (one band disappears virtually completely causing band density ratios of >80:20), or moderate skewing (where the ratio of band densities varies between about 65:35 and 80:20). Previous reports have suggested that about 5% of normal females show extremely skewed X inactivation, while a significantly larger number shows some degree of skewing. Skewed X inactivation has been reported with older techniques to occur regularly in females with X chromosome abnormalities, and with this technique has been reported in heterozygotes for some X linked recessive disorders, including the X linked immune deficiencies, Lesch–Nyhan syndrome, and incontinentia pigmenti.

Our data show that though 61% of females with incontinentia pigmeni show some degree of skewing of X inactivation patterns in their peripheral blood lymphocytes, so do 45% of control females who are not known to carry X linked disorders.

When data are analysed with respect to degree of skewing, a marked difference is observed between IP females and controls. Among IP females, 35% (11/31) of subjects show extreme skewing. In the control females, only 9-5% (4/42) show extreme skewing, somewhat higher than but not incompatible with previous estimates of about 5%. This might suggest that there is a group of IP patients who do indeed show extreme skewing of X inactivation, possibly on the basis of early selection against cells carrying the mutant allele. These data are not incompatible with the observations of Migeon et al. that five females in three families with IP showed skewed X inactivation, predominantly on evidence from fibroblast cultures. However, the larger present series emphasises that this is not a consistent phenomenon, and is not necessarily related to selection against cells expressing the mutant allele. The reason for the heterogeneity among IP females in this regard clearly warrants further investigation.

It is possible that the use of the probe M27β in the IP population may be biasing observations of X inactivation patterns. Eight out of 11 IP females with extremely skewed X inactivation patterns were assessed with M27β. When the IP and control populations are compared on the basis of PGK data alone, the percentage of females showing extremely skewed X inactivation is 4/42 (9.5%) in normal and 3/14 (21%) in IP females. X inactivation patterns in four control females have been analysed with both PGK and M27β (A Coffey, unpublished observations). Three of these gave a concordant pattern at the two loci but one female who gave a symmetrical pattern with PGK had a moderately skewed profile with M27β. Other data would support this observation (H Hinds, personal communication).

Both IP affected and normal mother/ daughter pairs (table 4) show concordance or discordance of X inactivation patterns. In the only informative case where an IP mother and her IP daughter showed extremely skewed inactivation (family 8), the chromosome carrying the IP mutation was active in both subjects. This argues against cell selection being a means of masking the IP mutation in carrier females.

We have analysed our data with respect to the family history of IP manifested by the females studied. Twenty-three cases had classical familial IP where the disease being inherited over two or more generations (fig 1). Eight females had sporadic IP. Among the 23 cases of familial IP, nine (39%) showed symmetrical X inactivation, five (22%) were moderately skewed, and nine (39%) extremely skewed. Among the eight sporadic IP females, three (38%) showed symmetrical inactivation, three (38%) showed moderate skewing, and two (25%) showed extreme skewing. A raised frequency of extremely skewed X inactivation therefore appears to be present among both sporadic and familial IP. (It remains possible that some cases of sporadic IP may be carrying an as yet unlocalised autosomal form of the disease.)

Since we have clearly shown that selection against IP alleles does not occur invariably in lymphocytes, we were interested to find out whether this might be a more general phenomenon in tissues that directly express the IP phenotype. It is not clear which cell lineage in the skin might be involved in the blistering lesions seen early in life in IP that lead on to abnormal skin pigmentation. Skin fibroblasts and epithelial cells were examined from one subject (marked * in pedigree 1, fig 1). A comparison was made of the X inactivation states at the HPRT locus in the lymphocyte, fibroblast, and epithelial cell DNA of this girl, and lymphocyte DNA from her mother, father, and maternal grandmother. Both mother and maternal grandmother also have IP. Blood samples from the grandmother, mother, and child all showed symmetrical patterns of X inactivation, as did skin fibroblasts and epithelial cells from the child. There appears to be no evidence in this case for a
selection process resulting in biased inactivation profiles.

Though there is clear evidence for an unusually high frequency of skewed X inactivation in incontinentia pigmenti, it is not so consistent in phenotypes in readily available tissues as to be clinically useful in genetic counselling.

In the case of XLP, the three mothers of XLP boys (two obligate carriers) analysed in this study all show moderately skewed X inactivation patterns. It is not certain whether the genetic defect in XLP is expressed in B cells and T cells. Since T cells make up about 80% of the peripheral blood lymphocytes it is possible that if selection against XLP-bearing chromosomes were only occurring in B cells, and causing an extremely skewed pattern in these cells, this would appear as a moderately skewed pattern in DNA extracted from whole blood (which will be derived from many cell types). However, in families 3 and 4, EBV-transformed lymphoblastoid cell lines (B cells) were analysed. Moderate rather than extreme skewing is seen in the XLP carrier mother in family 4. This would argue against selection in XLP B cells being masked by a symmetrical pattern in other whole blood cells.

With as many as 5 to 10% of normal females showing highly skewed X inactivation, clinical application for carrier detection could have a significant error rate, even in those disorders of the immune system where all heterozygotes appear to show this phenomenon.

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