Chapter 6

Role of Skewed X-Chromosome Inactivation in Recurrent Early Pregnancy loss
Role of skewed X- chromosome inactivation in recurrent early pregnancy loss

6.1 Introduction

In mammals, dosage differences in X linked genes between the male and female are compensated by inactivating one female X chromosome during the early stages of embryonic development, a process widely known as X-chromosome inactivation (XCI) or lyonization, after the name of its discoverer. Normally, XCI is a random process, making females mosaic for X linked gene expression. However, mutations in \textit{XIST} locus, imprinted inactivation patterns (as is the case with paternally derived X in extra embryonic lineages in mouse), can lead to primary non-random XCI. On the other hand, selection against a chromosomal abnormality in X can result in secondary XCI. Non random XCI has also been implicated in the increased prevalence of autoimmune disorders seen in females (Brix 2005). Apart from the cytogenetically detectable X chromosome abnormalities, nonrandom patterns of X inactivation may also result from single gene mutations as seen in X linked immunodeficiencies (Schmucker 1995). In any case, a nonrandom X inactivation event indicates an underlying abnormality in the X chromosome. Apart from these, it has also been reported that aging can lead to non random X inactivation through clonal deletion. The role of nonrandom XCI was shown in a wide variety of complex reproductive abnormalities such as polycystic ovary syndrome, ovarian failure and recurrent pregnancy loss (Bagislar 2006, Hickey 2002, Bione 2006).
Recurrent early pregnancy loss refers to the occurrence of three or more consecutive miscarriages before 12 weeks of gestation. It can be attributed to anatomical, chromosomal, immunological and environmental etiological factors. Chromosomal abnormalities contribute to approximately half of the spontaneous early pregnancy loss. Of these, X chromosome abnormalities alone contribute to about 10% (greater than any other individual chromosomal abnormalities). A number of association studies encompassing a multitude of candidate genes were carried out to explain the idiopathic REPL cases. A study of X inactivation status in idiopathic REPL subjects will be useful in estimating the contribution of deleterious mutations in X linked genes. A number of genes present on X chromosome are involved in establishment and maintenance of pregnancy. In addition, X mutations can also result in poor or inefficient ovarian reserves which results in REPL. Hence, in the present study we tried to assess the relationship between the random X inactivation and the risk of idiopathic REPL.

6.2 Results

Individuals heterozygous for the CAG repeat of androgen receptor were chosen for the X-inactivation screening. Heterozygous individuals with a difference of at least two repeats were included in the present study. The study was carried out using 114 cases and 68 controls. The degree of non randomness of X-inactivation was measured using the differences in ratios of gel band intensities obtained with and without HpaII digestion. A typical denaturing PAGE image with varying degrees of skewing is given in Fig 6.1. 47% (n= 32) normal women and 21% (n=24) of cases showed >80% skewing and the differences are significant at the 99% level (OR 0.30 CI: 0.15-0.58).
Fig 6.1 A poly acryl amide gel stained with ethidium bromide showing the various ranges of skewing in X-chromosome inactivation

A: PCR products from genomic DNA
B: PCR products from Hpa II digest of genomic DNA

Another interesting thing to note is that in 70% (n= 78) of the individuals (both cases and controls summed up), the lower CAG repeat containing allele is inactivated (skewing was considered when ≥60%). In the absence of a relationship between repeat length and the choice of X-chromosome to be inactivated, a 50% of higher and 50% of lower repeat containing alleles should get inactivated. However, the results indicated a significant association between the repeat number and the choice of X to be inactivated (OR: 2.33, CI: 1.26-4.35, P: 0.006) as mentioned in Table 6.1.

<table>
<thead>
<tr>
<th>Degree of skewing</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;60%</td>
<td>50 (43.8)</td>
<td>20 (29.4)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>60-80%</td>
<td>40 (35.1)</td>
<td>16 (23.5)</td>
<td>1.00 (0.46-2.14)</td>
<td>0.001†</td>
</tr>
<tr>
<td>80%&lt;</td>
<td>24 (21.1)</td>
<td>32 (47.1)</td>
<td>0.29 (0.14-0.62)</td>
<td></td>
</tr>
</tbody>
</table>

† indicates significance at 99% level.

Values in the brackets indicate the frequencies.
6.3 Discussion

The extent of skewing varies with age and when the age difference was considered. Case and control groups in the present study do not differ significantly in terms of age. Hence, the association observed in the present study is a true association with the risk of REPL and is not confounded by age. The result observed in Indian population is not in line with the reports from other populations. Association as well as lack of association reports from various populations exists with the risk of REPL. The distinguishing feature of the present result is that skewing is more seen in controls than in cases. This observation could not be explained from a point of chromosomal abnormalities resulting in skewing. Hence, we tried to correlate the degree of skewing with the repeat number in androgen receptor gene. A strong association suggests that the lower repeat containing allele is selectively inactivated among the individuals who exhibited skewed X-inactivation. A recent report regarding the role of androgen receptor length, skewed X- inactivation in Klinefelter syndrome also stated a similar observation (Suzuki 2001). Many reports exist in the literature regarding the association between non random X-inactivation and hyperandrogenic conditions such as polycystic ovaries, hirsutism suggesting a possible link between androgen levels and or androgen receptor activity with the choice of X. DNA methyl transferase 3a (Dnmt3a) is responsible for the methylation of embryonic stem cells and its known that this enzyme can methylate non CpG sequences with a preference for CpA dinucleotide (Ramsahoye 2000). As the X-inactivation is an embryonic event, the role played by Dnmt3a in X-inactivation must be significant.
It is known that exon 1 of androgen receptor contains CAG repeats and they can get methylated by Dnmt3a. The bias can be due to simple competition between the uneven CAG containing X chromosomes to get methylated by the limited number of Dnmt3a. Within any given time, more number of shorter repeats can be methylated resulting in a selective inactivation of those X chromosomes. As a cause or consequence, the biased inactivation of low repeat allele offers a protection against the possible damage caused by excess androgens. As the larger repeat makes the androgen receptor less sensitive to androgens, by inactivating the more active receptor gene (with less repeats), the female gets the advantage of becoming tolerant to elevated androgen levels. These results are further supported by our results regarding the association between CAG repeat length and the risk of REPL. Hence, this study found a significant association between the non-random X inactivation and the risk of recurrent abortions.