High frequency of skewed X inactivation in young breast cancer patients


ORIGINAL ARTICLE

Introduction: Patients with invasive ovarian cancer were recently shown to have a higher frequency of skewed X chromosome inactivation in peripheral blood cells compared to patients with borderline cancer patients.

Methods: X inactivation analysis was performed using HpaII predigestion of DNA followed by PCR of the highly polymorphic CAG repeat of the androgen receptor gene (AR), which amplifies the undigested inactive X chromosome only. The X inactivation pattern was classified as skewed when 90% or more of the cells preferentially used one X chromosome.

Results: Young breast cancer patients (27-45 years) had a higher frequency of skewed X inactivation than young controls (13 and 1%, respectively) (p=0.009), whereas no difference was found for middle aged and older patients compared to controls of a similar age.

Conclusion: A germline mutation in an X linked tumour suppressor gene may give a proliferative advantage to cells with this mutation on the active X chromosome, thus causing skewed X inactivation and an increased risk for developing cancer. Another possible explanation could be that females with a constitutionally skewed X inactivation pattern are more susceptible to develop breast cancer because of an X linked low penetrance susceptibility allele that is affected by the inactivation pattern.

SUBJECTS

Breast cancer patients

The breast cancer cases were part of a consecutive series of blood and tumour samples that had been collected after informed consent at the Norwegian Radium Hospital and Ullevål Hospital, respectively, from 1984 to 1994. Median age at diagnosis was 60 years, range 27-90 years.

Controls

Since there is a tendency for X inactivation to be more skewed with advancing age, it was necessary to have controls of different age groups. The control populations were 144 Norwegian blood donors aged 20-40 years, 138 blood donors aged 19-65 years (median 40 years), and 202 females aged 73-93 years (median 77 years). In addition, we had a control population of 91 females aged 55-72 (median 65 years), who were part of a routine mammography screening programme where blood samples were collected after two negative screenings (table 1).

METHODS

DNA isolation

DNA was extracted from peripheral blood cells and from tumour tissue according to standard procedures, using the automated phenol extraction method (Nucleic Acid Extractor 340A, Applied Biosystems).

X chromosome inactivation analysis

The X inactivation pattern was determined by PCR analysis of a polymorphic CAG repeat in the first exon of the AR gene.14 Methylation of HpaII sites in close proximity to this repeat correlates with X chromosome inactivation. After digestion with the methylation sensitive enzyme HpaII, a PCR product is obtained from the inactive X chromosome only. PCR products

Abbreviations: AR, androgen receptor
from undigested and digested DNA were separated on an ABI 373 automated sequencer and analysed by GeneScan software (Applied Biosystems) (fig 1).

X inactivation pattern was recorded as the relative amount of the PCR product of the smallest allele, where 0 indicates a pattern where the smallest allele is the predominating active X chromosome and 100 indicates a pattern where the largest allele is the predominating active X chromosome. The X inactivation pattern was classified as skewed when 90% or more of the cells preferentially used one X chromosome.

Statistical methods
The Pearson chi-square test was used for testing categorical variables. The Fisher two tailed exact test was used where appropriate; p values less than 0.05 were taken as statistical significance.

RESULTS
X inactivation in breast cancer patients
Two hundred and sixteen patients were heterozygous for the CAG repeat in the AR gene and therefore informative in the X inactivation assay. Since females aged 60 years or older have a much higher frequency of skewed X inactivation than younger females, the frequency of patients with a skewed X inactivation was determined separately for the various age groups (fig 1). A skewed pattern was found in the younger patients (<48 years) and in the elderly patients (≥64 years) only. The frequency of skewed X inactivation in the patients was significantly higher than in the controls, both when the youngest patients (27-40 years) were compared to the young control group (20-40 years) (22% and 2%, respectively) (p=0.003), and when the patients aged 27-65 years were compared to the blood donor control group aged 19-65 years (7% and 0.7%, respectively) (p=0.005) (table 1). When presumably premenopausal patients only (<45 years) were compared to blood donors of the same age group, the frequencies were also different (13% and 1%, respectively) (p=0.009). Information on the absence of breast cancer after mammography screening was available in the controls aged 55-72 only. When this group was compared with patients of the same age group, a difference in the frequency of skewed X inactivation was also found (4% and 8%, respectively), but the difference was not significant (p=0.35). In the elderly patients (73-90 years), the frequency of skewed X inactivation was lower than in a population of elderly controls (73-93 years) (14% and 21%, respectively), but the difference was not significant (p=0.27) (table 1).

Tumour tissue from four young patients (31-44 years) only was available and informative for X inactivation analysis. The X inactivation in tumour tissue was compared to the X inactivation in peripheral blood cells. Two patients with random X inactivation in peripheral blood cells had a similar pattern in tumour tissue. Two patients had a skewed X inactivation in blood cells (>90%) and a skewing of 80% in tumour cells with a preference for the same cell line as observed in blood.

AR CAG repeat length
There was no difference in CAG repeat size between patients and controls. The median repeat length for the shorter allele was 20 (range 6-26) for cases and 20 (range 12-27) for controls. The median repeat length for the longer allele was 24 (range 19-31) for cases and 23 (range 18-31) for controls. Two cut off points, six and 30 repeats, were determined to evaluate if CAG repeat extremes were associated with breast cancer. A repeat size of 30 or more was more frequent in the young Table 1  X chromosome inactivation in breast cancer patients and controls

<table>
<thead>
<tr>
<th>Age range (median)</th>
<th>Subjects with skewed X inactivation No (%)</th>
<th>Total number</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients 27–40 (38)</td>
<td>4 (22)</td>
<td>18</td>
<td>0.003</td>
</tr>
<tr>
<td>Controls 20–40</td>
<td>3 (2)</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td>Patients 27–45 (41)</td>
<td>5 (13)</td>
<td>40</td>
<td>0.009</td>
</tr>
<tr>
<td>Controls 19–45 (33)</td>
<td>1 (1)</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Patients 27–65 (51)</td>
<td>10 (7)</td>
<td>136</td>
<td>0.005</td>
</tr>
<tr>
<td>Controls 19–65 (40)</td>
<td>1 (0.7)</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>Patients 55–72 (63)</td>
<td>7 (8)</td>
<td>91</td>
<td>0.35</td>
</tr>
<tr>
<td>Controls 55–72 (65)</td>
<td>4 (4)</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Patients 73–90 (78)</td>
<td>6 (14)</td>
<td>43</td>
<td>0.27</td>
</tr>
<tr>
<td>Controls 73–93 (77)</td>
<td>43 (21)</td>
<td>202</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1  X inactivation in breast cancer patients. Hpall− indicates no predigestion and Hpall+ indicates predigestion with Hpall. (A) Random X inactivation pattern. (B) Skewed X inactivation pattern. (C) Male control. A PCR product is obtained for the inactive X chromosomes only. Note lack of PCR product after Hpall digestion of male control.
patient group (7.5%) compared to the control group (2%), but
the difference was not significant (p=0.12).

The possibility existed that there was a preferential
inactivation of the shorter and most functional allele, or a
preferential inactivation of the longer and less functional
allele in the breast cancer patients. No such preferential in-
avtivation was found. In the patients, the shorter allele was the
preferentially active allele in 49%, whereas in the total control
colation, the shorter allele was the preferentially active
allele in 52%.

DISCUSSION

In this study, we found a higher frequency of skewed X inac-
vitation in young breast cancer patients than in control
females. Middle aged and old patients did not have a higher
frequency than their respective controls.

Buller et al.11 found that patients with invasive ovarian cancer
had an increased frequency of skewed X inactivation compared
to patients with borderline tumours or controls. Furthermore,
they found a skewed X inactivation in nine of the 11 patients
where a BRCA1 mutation was identified. Our results are in
agreement with their findings. However, in our study, an
increase in skewing was found for the younger patients only
(table 1). In the report by Buller et al11 age was not considered.

The authors suggested that an X linked gene is a risk factor
for the development of ovarian cancer and discussed two
models, both involving a mutation in an X linked tumour sup-
pressor gene. In the first model, skewed X inactivation is a
chance occurrence, inactivating the X chromosome with the
normal copy of the gene, and thus leading to inactivation of
both copies. In the second model, cells with the mutated copy
of the gene on the active X chromosome have a proliferative
advantage, thus leading to a skewed X inactivation.

The lack of an increased frequency of skewed X inactivation
in older breast cancer patients could imply that a proportion of
those females who are born with skewed X inactivation
develop cancer at younger ages, and are therefore not included
in the older patient group. This supports the hypothesis that
skewed X inactivation, or a factor associated with it, is a risk
factor for the development of early onset breast cancer.

The possibility existed that the skewed X inactivation in the
patients is related to chemotherapy, since chemotherapy may
cause neutropenia and lymphopenia. Information on the tim-
ing of blood sampling in relation to therapy was not available
for the patients in our study. However, no difference in X inac-
vitation pattern was found between females who had received
chemotherapy and controls in a study by Gale et al.13 Further-
more, in the report by Buller et al.,11 several breast cancer
patients who had been given chemotherapy were examined
for X inactivation pattern more than a year after chemo-
therapy, with no change in X inactivation pattern. A
significant effect of chemotherapy on X inactivation pattern
would also be expected to affect the X inactivation in middle
aged and elderly patients, where no increase in X inactivation
pattern was found.

It would be of interest to study the relationship between the
X inactivation pattern in blood and tumour tissue. In this
study, we found skewing in the same direction in blood and
tumour cells in the only two young skewed cancer patients
where tumour tissue was available, but no conclusions may be
drawn from this limited material.

The role of the AR gene in breast cancer development is
poorly understood.14-16 Both short and long AR CAG repeats
have been associated with breast cancer development.14-16 Yu et
al.17 found that shorter CAG repeat length was associated with
more aggressive forms of breast cancer. Spurdle et al.18 did not
find any difference in mean AR CAG repeat length between
females who developed breast cancer before the age of 40 and
controls. In this study, we found no evidence for a different
distribution of alleles between young breast cancer patients
and controls. However, we found a tendency for extreme CAG
repeats of 30 or more to be more frequent in the young breast
cancer patients. This finding is in agreement with Rebbeck et
al.,19 who found that longer AR CAG repeats were associated
with an increased risk of developing breast cancer at an early
age in BRCA1 mutation carriers.

We found a higher incidence of skewed X inactivation in
young patients with breast cancer. These results need to be
verified in a larger sample. Since patients with familial breast
cancer have an earlier onset of breast cancer than the sporadic
cases,20 it will be of interest to see whether the increased fre-
cquency of skewed X inactivation is limited to patients with
BRCA1 or BRCA2 mutations. It will also be of interest to exam-
ine the relationship between various histological characteris-
tics of the tumour and X inactivation pattern.

Skewed X inactivation can lead to the expression of
recessive traits in females who are heterozygous for X linked
disorders, either as the result of the chance occurrence of
skewed X inactivation or as the result of a selection process. An
increase in skewing of X inactivation in females with breast
cancer may therefore be an indication of an effect of X linked
genes. It would also be of interest to study the X inactivation
pattern in females with other cancers.

ACKNOWLEDGEMENTS

This work was supported by The Research Council of Norway, The
Norwegian Cancer Association. Anders Jahre's Foundation for the
Promotion of Science, and EXTRA funds from the Norwegian
Foundation for Health and Rehabilitation.

Authors’ affiliations
M Kristiansen, Institute of Medical Genetics, University of Oslo, Oslo,
Norway
A Langerad, A L Barresen-Dale, Department of Genetics, Institute for
Cancer Research, The Norwegian Radium Hospital, Oslo, Norway
G P Knudsen, K H Ørstavik, Department of Medical Genetics,
Rikshospitalet University Hospital, Oslo, Norway
B L Weber, Cancer Centre, University of Pennsylvania, Philadelphia,
USA
A L Barresen-Dale, Institute for Laboratory Medicine, University of Oslo,
Norway

REFERENCES

1 Puck J, Willard H. X inactivation in females with Xlinked disease. N

2 Belmont JW. Genetic control of X inactivation and processes leading to

3 Fearon ER, Kohn DB, Winkelstein JA, Vogelstein B, Blaese RM. Carrier

4 Nyhan WL, Bakay B, Connor JD, Marks JF, Kleele DK. Hemispheric
expression of glucose-6-phosphate dehydrogenase in erythrocytes of
heterozygotes for the Lesch-Nyhan syndrome. Proc Natl Acad Sci USA

5 Ørstavik KH, Ørstavik RE, Naumova AD, Gadean A, Bolhuis BA, Barth PG, Tonialo D. X chromosome inactivation in carriers of

6 Allen RC. Naitchman RG, Rosenblatt HM, Belmont JW. Application of
carrier testing to genetic counseling for Xlinked agamaglobulinemia.

7 Busque L, Mio R, Mattoli E, Bias E, Blais N, Lalonde Y, Maragh M, Gilliland DG. Non-random X-inactivation patterns in normal females:

factors regulate hematopoietic stem cell kinetics in females. Blood

9 Brown CJ, Robinson WP. The causes and consequences of random and
nonrandom X chromosome inactivation in humans. Clin Genet

10 Buller RE, Saad AK, Lallas T, Buenker T, Skilling JS. Association between
nonrandom Xchromosome inactivation and BRCA1 mutation in germline

11 Chamberlain NL, Driver ED, Miesfeld RL. The length and location of
CAG trinucleotide repeats in the androgen receptor Nterminal domain

12 Yu H, Bharaj B, Vassilikos EJ, Gai M, Diamandis EP. Shorter CAG
repeat length in the androgen receptor gene is associated with more

www.jmedgenet.com


High frequency of skewed X inactivation in young breast cancer patients


J Med Genet 2002 39: 30-33
doi: 10.1136/jmg.39.1.30

Updated information and services can be found at:
http://jmg.bmj.com/content/39/1/30

These include:

References
This article cites 18 articles, 7 of which you can access for free at:
http://jmg.bmj.com/content/39/1/30#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

Breast cancer (239)
Molecular genetics (1254)
Reproductive medicine (518)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/