Mini Review

The clinical significance of fragile sites on human chromosomes


Fragile X syndrome is now a well established common clinical entity and most of those who are aware of the condition probably know that it takes its name from a rare fragile site (FRAXA) on the X chromosome. This is the best known fragile site and its clinical significance is clear. Similar, but a little less known is FRAXE, a fragile site close to that associated with fragile X syndrome, but in this case associated with a mild form of non-specific X-linked mental retardation. These are the only two fragile sites that are unequivocally of clinical significance. A fragile site within the CBL2 oncogene on chromosome 11 has been mapped very close to the deletion breakpoint in a handful of patients with Jacobsen syndrome. It is doubtful that parents with FRA11B are at increased risk of having children with Jacobsen syndrome, but this cannot be ruled out.

The common fragile sites have been implicated in oncogenesis since shortly after their discovery in the early 1980s. While a couple of these are within genes that have been implicated in cancer it is unclear whether either the fragile sites, or the genes in which they are located are important in cancer. It may be that the common fragile sites are regions of genomic instability and that this instability is increased in malignant cells, analogous to the enhanced instability seen at microsatellite loci in a number of tumours. Since we all have the common fragile sites there is no suggestion that they give anyone an increased risk of developing malignant disease.

In dealing with patients who are found to have fragile sites, other than FRAXA, FRAXE and possibly FRA11B, considerable reassurance can be given that they are not at increased risk of having children with congenital disease or developing disease themselves because of their fragile sites.

The discovery of a fragile site in the chromosomes of a patient often causes anxiety for the patient and uncertainty on the part of professionals dealing with the patient. We are also aware of situations at prenatal diagnosis where clones of cells have been found with a deletion at or close to the position of a known fragile site. Again this creates uncertainties for all involved. We have also been consulted from time to time about apparently novel fragile sites and their potential significance in a variety of clinical situations. Common fragile sites are now being implicated as regions of genomic instability associated with somatic chromosomal changes in cancer. We will briefly examine these situations and provide guidelines for their resolution.

Fragile sites on the X chromosome

There are three rare fragile sites on the X chromosome, indistinguishable from each other on routine cytogenetics but readily separated by molecular genetics. Two of these are well known causes of mental retardation, FRAXA with fragile X syndrome and FRAXE with non-specific mild X-linked mental retardation. The third fragile site, FRAXF, has not had any clinical problem associated with it and, unlike the previous two fragile sites, is not known to be associated with a gene on the X chromosome. There is a common fragile site FRAXD which is in band Xq27.2 that can be confused with the rare fragile sites. Being a common fragile site it is probably present on all X chromosomes.
The finding of a fragile site in the Xq27-28 region of the X chromosome is potentially highly clinically significant and easy to resolve. Molecular analysis for the FRAXA and FRAXE CCG repeat expansions will determine whether either of these fragile sites is present. If either is present, appropriate genetic counselling and treatment can be provided (1). If neither of these fragile sites is present, whatever fragile site has been seen cytogenetically is not of clinical significance and can be ignored. While there are a number of common fragile sites on the X chromosome, like the other common fragile sites they are of no pathological significance.

**Premutation carriers of FRAXA**

The situation regarding premutations at FRAXA (and possibly FRAXE) is unclear. Until recently it was assumed that premutation carriers of FRAXA, either male or female, were unaffected by their premutation status. Their expanded repeat is not methylated and is transcribed and produces protein. It has gradually emerged that premutation carrier women (but not full mutation carriers) are susceptible to premature ovarian failure (POF) and there is no obvious reason for this (2).

Tassone et al. (3) have shown that males with FRAXA premutations have considerable over-expression of FMR1 mRNA. This is the first molecular pathology associated with premutations, although it had been shown that FMR1 transcripts with large premutations were not efficiently translated (4).

Hundscheid et al. (5) have shown that it is primarily those women who inherit their premutation from their fathers who are at risk of POF. They provide, from a small data set, risks of POF for such women of 4% by age 30 and 25% by age 40. Women with maternally inherited premutations do not appear to have a risk of POF discernibly greater than non-carrier women.

**Rare autosomal fragile sites**

The rare fragile sites can be classified according to the conditions of tissue culture under which they are induced (Table 1). While there are a number of groups, the first division is between those that are folate-sensitive (i.e. among other conditions, are expressed in folate-deficient culture media) and those that require other conditions to induce their expression. The rare X-linked fragile sites come into the folate-sensitive group.

Homozygotes for three of the non-folate sensitive fragile sites (FRA10B, FRA16B, FRA17A) have been identified and are normal individuals (6). This indicates that these three fragile sites do not interfere with the function of genes that are important in development.

Homozgyotes for the autosomal folate-sensitive fragile sites have not been documented. However by analogy with the rare fragile sites on the X, where hemizygosity is deleterious for two of the three, it is likely that homozgyosity for some of the autosomal sites of this type could be significant. It will not be until each of these has been characterised and the gene in which it is located, if indeed it is in a gene, has been identified that more definitive hypotheses could be offered on likely effects in homozygotes. The only practical implication of this is the potential situation in which both members of a couple were heterozygotes for the same fragile site. Here they might be at risk of having offspring with some genetic disorder, or possibly spontaneous abortion of lethal homozygotes.

FRA11B is located within the 5'-end of the CBL2 oncogene and has been implicated as the break point in a small number of patients with Jacobsen syndrome (11q-) (8). The evidence that this has occurred is strong, the mothers were carriers of FRA11B pre- or full mutations, the haplotype of the deleted #11 chromosome indicated that it originated from the FRA11B chromosome and the breakpoint mapped very close to if not at the fragile site. However it has also been shown that the majority of Jacobsen syndrome deletion breakpoints map away from the fragile site (9, 10).

We have estimated that about 1 in 5 000 individuals will carry FRA11B. Yet Jacobsen syndrome is very rare (estimated incidence less than 1 in 100 000) and most cases are not associated with FRA11B. Consequently the risk to a FRA11B carrier of having a child with Jacobsen syndrome must be very small, although it might not be zero.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare fragile sites</td>
<td>n = 22, includes FRAXA, FRAXE, FRA11B</td>
</tr>
<tr>
<td>Distamycin A-inducible</td>
<td>n = 2, FRA16B, FRA17A</td>
</tr>
<tr>
<td>a) also induced by BrdU</td>
<td>n = 3, recorded only in Japanese subjects</td>
</tr>
<tr>
<td>b) not induced by BrdU</td>
<td></td>
</tr>
<tr>
<td>BrdU-requiring</td>
<td>n = 2, FRA10B, FRA12C</td>
</tr>
<tr>
<td>Common fragile sites</td>
<td></td>
</tr>
<tr>
<td>Aphidicolin inducible</td>
<td>n = 76, includes FRA3B</td>
</tr>
<tr>
<td>5-azacytidine-inducible</td>
<td>n = 4</td>
</tr>
<tr>
<td>BrdU-inducible</td>
<td>n = 7</td>
</tr>
</tbody>
</table>

Table 1. Classification of fragile sites (7)
In practice FRA11B carriers will rarely be encountered, however if they do request genetic counselling prenatal cytogenetic diagnosis could be offered because of a very small but unquantifiable risk of having a child with Jacobsen syndrome.

No other rare fragile site has ever been implicated in the generation of offspring with constitutional chromosome abnormalities. There is a single case report of a woman with FRA16B who had a son and a grandson with an extra small marker chromosome interpreted as coming from distal 16q (11). FRA16B is carried by about 5% of caucasians and if it carried any significant risk of producing derivative chromosomes this would be well established. FRA16B must therefore be regarded as of no pathological significance.

Common fragile sites

There are over 80 common fragile sites recognised in the human genome (Table 1). These have been implicated as breakpoints in chromosomal rearrangements in cancer cells since the early 1980s (12). While a number of these common fragile sites have been sequenced there is no recognised sequence element that is sufficient to produce a fragile site. These sites are clearly regions of potential genome instability as evidenced by them being targets for increased rates of SCE, viral integration and possibly being amplicon boundaries (13). FRA3B has been shown to be within a large intron of the FHIT gene at 3p14.2 (14). In a number of tumours deletions within the fragile site region have been demonstrated; abnormal transcripts of FHIT have been found in tumours and normal tissues (15). There is ongoing controversy about whether FHIT is a tumour suppressor gene and whether the deletions and abnormal transcripts demonstrated have any role in oncogenic processes.

FRA7G is a region of instability and LOH in several tumours and this region contains the caveolin genes, one of which (caveolin 1) has been proposed as a tumour suppressor gene (16). FRA16D is one of the regions of the long arm of chromosome 16 reported to show LOH in breast cancer (17). Arlt et al. (18) have hypothesised that deletions at fragile sites in malignant cells may be a non-specific manifestation of genomic instability rather than a specific etiological factor.

What does all this mean in dealing with individual patients? We are probably all homozygous for all the common fragile sites. These have not yet been shown to be polymorphic, indeed the minimal DNA sequence requirement (if there is one) has not been determined for common fragile sites. Hence there is no way of demonstrating individual differences between common fragile sites at the same locus.

Most patient queries about common fragile sites arise when a particular site has been shown to be present in a conspicuous proportion of cells. At this time such patients should be strongly reassured that this is of no clinical significance and in particular, that there is no evidence such a patient is at increased risk of malignant disease.

Prenatal diagnostic problems

We are aware of a number of instances where prenatal diagnostic laboratories have found a clone of cells in an amniotic fluid culture that has a chromosome with a deletion at or about the location of FRA10A (Table 2). We can offer no obvious explanation for these findings. We do however make the point that probably around 1 in 500 fetuses will have FRA10A yet we are aware of only a single report of a mosaic deletion (and no non-mosaic ones) in a woman who also had mosaic Turner syndrome. She was reported to have FRA10A, but no clinical anomalies that could not be attributed to her sex chromosome abnormality (20). Furthermore, about 1% of the population have a rare autosomal folate-sensitive fragile site, and another 6–8% will have one of the other rare non-folate sensitive fragile sites. With the possible
exception of Jacobsen syndrome and FRA11B (mentioned above), children who have deletions at the loci of rare autosomal fragile sites or who are mosaics for such deletions are almost unknown, and ascertainment bias would certainly have brought even rare cases to attention.

When individuals with rare fragile sites are studied cytogenetically, following induction of the fragile site, a proportion of cells are found with various duplications or deletion of material distal to the fragile site. This is the result of breakage at the fragile site followed by non-disjunction of the distal chromosomal material (21). There is no evidence that this is likely to occur in somatic cells in vico or in non-induced tissue cultures, although the Jacobsen syndrome instances and the data in Table 2 suggest that this might occur very rarely.

How should apparent deletions at fragile sites encountered in prenatal diagnostic cultures be interpreted? A non-mosaic deletion is likely to indicate serious maldevelopment in the fetus. A mosaic deletion may reflect something that has occurred in very few cells, in tissues (eg the kidney) that may not imply fetal abnormality. In the absence of data other than that in Table 2 we would urge caution. While a normal fetus is a likely outcome without further study, fetal blood chromosome studies may be reassuring if normal or if FRA10A is present. While the absence of a fragile site in either parent would suggest that a deletion is more likely to be significant, this cannot be certain. One instance of a mother, with a premutation expansion at FRA11B and a child with Jacobsen syndrome with the breakpoint putatively at FRA11B is on record (8). The mother would have had molecular but not cytogenetic evidence of CCG expansion at the fragile site locus, and molecular evidence of such expansion cannot be sought for most of the rare fragile sites at this time.

**Novel fragile sites**

All accepted fragile sites (with the exception of some cases of FRA16B and FRA17A) require some induction process during tissue culture before they will be apparent. Fragile sites are never present in 100% of metaphases examined (again FRA16B can approach this under some conditions of induction). Hence, putative novel fragile sites that are present in all cells and do not require induction, are unlikely to be fragile sites. Rare reports of such phenomena occur and their origins and nature are obscure (22–24). Some could be satellite stalks that have been inserted into a chromosome (25). A number of chromosome arms have been reported with a satellited appearance (26, 27), and it is not clear whether these are fragile sites close to telomeres or some other phenomenon.

It is difficult to offer any information on the significance of these types of observation. Ascertainment bias can lead to their identification in developmentally abnormal children and they can be found at prenatal diagnosis. Studies of other family members if available may help resolve such situations.

**Conclusion**

With the definite exceptions of FRAXA and FRAXE, and possibly FRA11B, patients with any other fragile site either rare or common can be strongly reassured the fragile site will not affect their personal health or increase their risk of having chromosomally abnormal children.

**Acknowledgements**

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**References**

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