LETTER TO JMG

FRA18C: a new aphidicolin-inducible fragile site on chromosome 18q22, possibly associated with in vivo chromosome breakage

Kim Debacker, Birgitta Winnepeninckx, Neta Ben-Porat, David FitzPatrick, Rob Van Lwijk, Stefaan Scheers, Batsheva Kerem, R Frank Kooy

J Med Genet 2007;44:347–352. doi: 10.1136/jmg.2006.044628

Fragile sites are specific genomic loci that form gaps, constrictions and breaks on chromosomes exposed to replication stress conditions. In the father of a patient with Beckwith-Wiedemann syndrome and a pure truncation of 18q22-qter, a new aphidicolin-sensitive fragile site on chromosome 18q22.2 (FRA18C) is described. The region in 18q22 appears highly enriched in flexibility islands previously found to be the characteristic of common fragile site regions. The breakpoint was cloned in this patient. The break disrupts the *DOK6* gene and was immediately followed by a repetitive telomere motif, (TTAGGG)_n. Using fluorescent in situ hybridisation, the breakpoint in the daughter was found to coincide with the fragile site in the father. The breakpoint region was highly enriched in AT-rich sequences. It is the first report of an aphidicolin-sensitive fragile site that coincides with an in vivo chromosome truncation in the progeny.

Fragile sites are specific genomic regions that appear as gaps, constrictions or breaks on chromosomes in cells grown under conditions that perturb DNA replication.¹ According to their frequency within the population, fragile sites are divided into two groups: rare and common. Rare fragile sites have a maximum frequency of 1/20 in the human population, whereas common fragile sites are considered as an intrinsic component of the chromosome structure, present in all individuals. To date, 30 rare and 88 common fragile sites have been described.^{2–4} The majority of rare fragile sites are expressed when cells are grown in folic acid deficient medium, whereas some are induced by bromodeoxyuridine or distamycin A. Most common fragile sites are induced by aphidicolin, which inhibits DNA polymerases α and δ .

Repeat expansions form the molecular basis of rare fragile sites. Six of the folate-sensitive type have been cloned to date, including FRAXA, FRAXE, FRAXF, FRA11B, FRA16A and FRA10A, and are all due to a CGG-repeat expansion. The distamycin-sensitive site FRA16B and the bromodeoxyuridine-inducible site FRA10B are caused by expansion of similar AT-rich minisatellites.^{5–6} All cloned common fragile sites to date (n=15) are characterised by enrichment of highly flexible AT-dinucleotide rich sequences^{7–8} and reviewed by Schwartz *et al.*⁴

Rare fragile sites are often associated with human diseases. An expression of the fragile site FRAXA causes fragile X syndrome, the most frequent cause of inherited mental retardation.⁹ Expansion of the FRAXE repeat is associated with a mild form of mental retardation.¹⁰ In vivo chromosome breakage at or near the FRA11B locus has been implicated in Jacobsen syndrome, characterised by mental retardation and specific associated abnormalities.¹¹

Common fragile sites have often been associated with chromosome breakpoints in tumour cells. First evidence for this was provided by statistical analyses,^{12–13} later confirmed by the cloning of common fragile sites, containing genes often deleted in tumour cells, reviewed in Arlt *et al.*¹⁴ For example, the fragile site FRA3B maps within the large *FHIT* gene, and is often rearranged in tumour cells.¹⁵ Next to deletions, amplification events and/or translocations are also associated with chromosomal breakage in common fragile sites.¹⁶ Jamieson *et al.*¹⁷ described a family with a translocation 16q23.2 breakpoint, which transects the common fragile site FRA16D.

We investigated a patient with an 18q22-qter truncation and Beckwith-Wiedemann syndrome, a fetal overgrowth syndrome.¹⁸ The father of the patient had a normal karyotype, but expressed a novel fragile site at 18q22. To investigate whether the paternal fragile site might be associated with the chromosome break in the progeny, we cloned the chromosomal breakpoint and analysed this fragile site. A possible link between the 18q22 chromosome breakage and fragile site expression is discussed.

MATERIAL AND METHODS

Family description

The patient is an 8-year-old girl, diagnosed with Beckwith-Wiedemann syndrome, characterised by overgrowth and loss of insulin-like growth factor 2 imprinting. A detailed clinical description is given by Brewer *et al.*¹⁸ The girl has an 18q22.1 truncation. The child does not show any clinical features of the 18q syndrome (microcephaly, short stature and hypotonia) often caused by 18qter deletions.¹⁹ Both parents are healthy, but the father carries a fragile site at 18q22.1.

Epstein-Barr-transformed lymphoblastoid cell lines (CM0035 and CM0036) from the patient and her father were obtained from the European Collection of Cell Cultures (Cambridge, UK). A blood sample of the mother was used with informed consent.

Markers to ascertain the origin of the truncation in the proband were taken from the Genetic Location Database (http://cedar.genetics.soton.ac.uk/public_html). Marker analysis was performed on an ABI 3100 automated DNA sequencer (Perkin-Elmer, Foster City, California, USA).

Fragile site induction and fluorescent in situ hybridisation experiments

For fragile site induction, cells of the father (CM0035) were cultured in the presence of 0.4 μ M aphidicolin for 24 h before harvesting the cell. Metaphase chromosomes were prepared following standard cytogenetic procedures. Probes were

Abbreviations: DAPI, 4',6-diamino-2-phenylindole; DIG, digoxigenin; FITC, fluorescein isothiocyanate

labelled by nick translation and hybridised according to standard protocols. Three probes were used: the biotin-labelled probe (RP11-64C15) detected by an ALEXA-594-conjugated streptavidin detection system, the digoxigenin (DIG)-labelled probe (18q telomeric: GS-964-M9) detected by a FITC (fluorescein isothiocyanate)-conjugated antibody α -DIG-detection system and the aqua-labelled probe (18q centromeric: CEP 18; Vysis). Slides were counterstained with VECTASHIELD (Vector Laboratories, Inc., Burlington, California, USA) containing 4',6-diamino-2-phenylindole (DAPI). Image capture and analyses were performed using a Leica fluorescence microscope (Leica DFC 350FX, Leica Microsystems, Wetzlar, Germany) equipped with the appropriate filter combination for detecting ALEXA-594, FITC and DAPI. The images were captured by a cooled CCD camera equipped with a Leica image analysis system.

Computational analysis of DNA flexibility

To evaluate DNA flexibility, we used the TwistFlex program. The program measures the potential averaged potential variation in the twist angle of each dinucleotide step in the sequence. The analysis was performed in overlapping windows of 100 bp. Windows with values of $>13.7^\circ$ were considered as flexibility peaks. Since the length of the window is 100 bp, flexibility peaks that were <100 bp apart were considered as one unified flexibility peak.

Fluorescent in situ hybridisation experiments and chromosome painting

A TelVision 18q subtelomeric probe (Vysis; orange labelled) was hybridised to metaphase spreads of the father and the patient. For identification, the chromosome 18 was painted green (paint 18 Euro-Diagnostica (Malmö, Sweden) for the patient; paint 18 Oncor (Intergen Company, Purchase, New York) for the father). For the patient, the subtelomeric probe was DIG-labelled and visualised by indirect labelling with α -DIG-FITC; for the father a direct fluo labelling was used. All hybridisations were performed according to standard protocols.

Cloning the 18q22 breakpoint

Repeat markers to refine the candidate region of the chromosome breakpoint were taken from the Genetic Location Database (http://cedar.genetics.soton.ac.uk/public_html) or developed on the basis of the human genome sequence (<http://www.ncbi.nlm.nih.gov>). For polymerase chain reaction (PCR)-based fluodosage, 20 primersets were obtained from the genome database (<http://www.ncbi.nlm.nih.gov>).²⁰ Marker and PCR-based fluodosage analysis were performed on an ABI 3100 automated DNA sequencer (Perkin-Elmer). All sequences of primers that we developed for the refinement of the breakpoint region can be obtained from the authors on request.

For Southern blot analysis, a probe specific for the breakage region, probe 2 (forward: 5'-TAATCAACACCTTCCACTGC-3' and reverse: 5'-CTGATTTTAAGTACTTGAGCT-3') was developed. Genomic DNA of the patient and her father were digested with HindII. The digested DNA was separated by electrophoresis on a 0.7% agarose gel and, after denaturation and neutralisation, transferred to Hybond N⁺ membranes. Hybridisation was performed at 65°C.

To determine the breakpoint, PCR amplification and sequencing were performed using the following primers: a telomere-specific reverse primer (5'-TATGGATCCCTAACCC TGACCCTAACCC-3') and a forward primer (5'-GTGCAGTGGC ACCATCA-3') developed on the basis of the sequence flanking the chromosomal breakpoint. The sequencing product was analysed using an ABI 3100 automated DNA sequencer (Perkin-Elmer).

Selection, Southern blotting and hybridisation of the candidate repeats

Several repeats in the chromosome breakage region were selected by using the computer program Tandem Repeat Finder (<http://c3.biomath.mssm.edu/trf.html>). The selection criterion was based on the amount of repetitions of the uninterrupted repeat.

Southern blots were created by digesting 8 μ g DNA, extracted from Epstein-Barr virus transformed cells, with an appropriate restriction enzyme. The digested DNA was then separated by electrophoresis on a 0.7% agarose gel, and after denaturation and neutralisation transferred to Hybond N⁺ membranes. Hybridisations were performed at 65°C using a single PCR probe specific for each candidate repeat. Sequences of the primers, used to generate probes, and the appropriate restriction enzymes can be obtained from the authors on request.

RESULTS

The fragile site observed in the father is aphidicolin inducible

Previously, expression of the paternal fragile site at 18q22 in the father I.1 (fig 1) was found in 3/200 cells, analysed in two different experiments, grown in a folate-deficient medium.¹⁸ However, we could not observe FRA18C expression under low folate conditions and moreover the 18q22 sequence appeared to be AT-rich—a feature of common fragile sites. Therefore we cultivated lymphoblastoid cells in the presence of 0.4 μ M aphidicolin to activate common fragile site expression, and observed 18q22 fragile sites in 5/100 metaphases scored (fig 2). No fragile sites on chromosome 18 were found when cells were cultured in the absence of aphidicolin. This indicates that the fragile site can be induced by aphidicolin and is probably previously undescribed aphidicolin-induced fragile site, which was assigned the name FRA18C.

The 18q22 region is highly enriched in flexibility islands

Since aphidicolin-induced fragile sites are enriched with highly flexible AT-dinucleotide rich sequences, a flexibility analysis of

Figure 1 Pedigree of the family studied in this paper. The father (I.1) showed fragile site expression at 18q22, the daughter (II.2) has an 18q22.2 truncation. By analysing three additional markers (marked green), the candidate region was refined to a 181 kb interval between D18S1092 and D18S61. The numbers represent the genotypes of the markers present on the chromosomes of the father, the mother and the daughter. Using the marker D18S61, we also showed that the truncated chromosome in the daughter (II.2) originated from one of the two chromosomes 18 of the father. Physical position of the markers: D18S477 (63.25M), D18S466 (64.57M), D18S1131 (64.96M), D18S1092 (65.40M) and D18S61 (65.58M)

Figure 2 The FRA18C fragile site. (A) The 18q22.2 fragile site after reverse 4',6-diamino-2-phenylindole (DAPI) staining. The arrow indicates the fragile site. (B) The same chromosome after DAPI staining. Localisation of the bacterial artificial chromosome clone RP11-64C15, spanning the breakpoint (labelled red) on the 18q22.2 paternal fragile site. Two reference probes CEP18 (labelled blue) and GS-964-M9 (labelled green) were also used.

the entire 18q22 region (chr18: 59 800 000–71 300 000) (<http://genome.ucsc.edu/cgi-bin/hgGateway>), to which the fragile site was mapped, was performed.^{7,8} For this, we used the Twistflex program—a computer program that identifies DNA sequences with a potential high flexibility at the twist angle.⁸ This region is composed of three sub-bands: 18q22.1 (G band), 18q22.2 (R band) and 18q22.3 (G band). We previously showed that R and G bands differ in the mean number of flexibility islands.⁸ Genomic regions mapped to G bands have significantly higher number of flexibility islands relative to R bands, hence each of the 18q22 sub-bands were analysed separately. As can be seen in figure 3, the G band 18q22.1 and most of G band 18q22.3 are highly enriched in flexibility islands (an average of 6.4 and 6.1 islands per 100 kb, respectively), relative to non-fragile regions mapped to G bands (an average of 3.3 islands per 100 kb), as well as relative to other aphidicolin-induced fragile sites mapped to this band type (an average of 5.7 islands per 100 kb).⁸ The R band 18q22.2 is also highly enriched in flexibility islands (an average of 4.2 islands per 100 kb), both relative to non-fragile (an average of 1.8 islands per 100 kb) and fragile regions (an average of 3.4 islands per 100 kb) mapped to this band type. These results suggest that the flexibility of a substantial part of 18q22 is characteristic for common fragile sites.

The 18q22-qter deletion is a pure truncation

The chromosome breakpoint in the proband II.1 has been mapped previously to the interval between markers D18S477 and D18S61, a region of approximately 2.3 Mb.¹⁸ By analysing additional markers in this family, we refined the candidate region to a 181 kb interval between markers D18S1092 and D18S61 and confirmed that the breakpoint occurred on the paternal chromosome (fig 1). To investigate whether the 18q22-qter deletion in the patient II.1 is a pure truncation or rather part of a more complex chromosomal rearrangement, we performed fluorescent in situ hybridisation experiments on metaphase spreads in combination with whole chromosome 18 painting using an 18q subtelomeric probe. The paint stained both copies of chromosome 18 in the father I.1 and the intact chromosome 18 as well as the derivative chromosome 18 in the patient II.1. The subtelomeric probe was detectable on the intact chromosome 18, but not on the derivative chromosome 18 in the patient II.1 (fig 4A).

Cloning of the 18q22 breakpoint in the patient

By analysing additional newly developed polymorphic CA-repeat markers in the family, the candidate region for chromosome breakage in patient II.1 was narrowed to the 157 kb interval between markers D18S61 and the newly

developed marker 18q22_di 34, located proximal to D18S61. The breakage region was further refined to a 2.2 kb interval by performing a quantitative PCR-based fluodisage assay using 20 primersets developed in the D18S61–18q22_di34 interval. Using Southern blots of genomic DNA of the father I.1 and the patient II.1 digested with restriction endonucleases and hybridised with a probe specific for the region, we further refined the location of the breakpoint. Using probe 2, we detected an additional Hind II fragment of 4.8 kb in patient II.1 in addition to the fragment of 5.5 kb present in controls and the father I.1, suggesting that the breakpoint is situated approximately 700 bp from the Hind II site. The sequence of the breakpoint was determined after PCR amplification with a primer designed $\pm$ 500 bp from the expected breakpoint and a telomere-specific primer.²¹ The sequence showed the expected, pure 18q sequence, up until base position 15 185 171 in contig NT_025028.13 (<http://www.ncbi.nlm.nih.gov>), immediately followed by the repetitive telomeric sequence (TTAGGG)_n (fig 5).

The breakpoint disrupts the *DOK6* gene involved in the activation of the receptor tyrosine kinases.²² The breakpoint is situated in intron 4 of the *DOK6* gene. According to the NCBI database (<http://www.ncbi.nlm.nih.gov>), the entire *DOK6* gene (441 kb) maps to 18q22.2 indicating that the breakpoint is positioned in 18q22.2 and not in 18q22.1 as suggested previously.¹⁸

The breakpoint in the patient coincides with the fragile site in the father

To investigate a possible association between the novel fragile site FRA18C in the father I.1 and the chromosomal breakpoint in the daughter II.1 of our family, fluorescent in situ hybridisation analysis on FRA18C-expressing cells of the father I.1 was performed with bacterial artificial chromosome clone RP11-64C15, spanning the breakpoint in combination with the reference probes CEP18 and GS-964-M9, located in the centromeric and telomeric region of chromosome 18, respectively. The results showed the colocalisation of the paternal fragile site with the chromosomal breakpoint in the progeny (II.1) in five of five analysed fragile sites (fig 2B). As shown in figure 3, the 18q22.2 region is highly enriched in flexibility islands. Interestingly, the breakpoint occurred in a genomic region of 300 kb within the R band 18q22.2, which shows even higher DNA flexibility (21 islands/300 kb) than the rest of this sub-band. Together, the results suggest that the breakpoint occurred within a fragile site region, which has high DNA flexibility.

Repeats in the 18q22 breakpoint region

Since in vivo chromosome truncation at fragile sites has so far only been reported in case of a single, rare, folate-sensitive fragile site as a result of repeat expansion,^{11,23} we wanted to investigate whether repeat expansions may occur within the FRA18C region. We searched a 400 kb interval surrounding the chromosomal breakpoint for repeats using the computer program Tandem Repeat Finder.²⁴ The breakpoint region appeared enriched in AT-rich repeats and no CGG-repeats were identified. To test whether any of the repeats were expanded in the father I.1, the 29 longest repeats were selected (see supplementary table 1, available at <http://jmg.bmj.com/supplemental>). They were first tested for heterozygosity using PCR with primers flanking the repeats. For six of these repeats (1, 2, 4, 6, 14 and 25) the father I.1 showed two alleles in the normal size range, excluding the possibility of an expansion in one of the alleles. The remaining 23 repeats were analysed using Southern blotting and hybridisation with probes specific for the region. Although the majority of the repeats were polymorphic, no repeat expansions were detected.

Figure 3 Flexibility analysis of the 18q21–23 regions. The y axis represents the number of AT-dinucleotide rich islands per 100 kb. The x axis represents the nucleotide position according to UCSC, hg 17. Yellow lines: average number of AT-dinucleotide islands per 100 kb in non-fragile regions in the human genome. Red lines: average number of AT-dinucleotide islands per 100 kb in fragile regions. Note the difference in the average number of flexibility islands between R and G bands in both fragile and non-fragile regions. So the R band 18q22.2, containing the chromosomal breakpoint, is highly enriched in flexibility islands both relative to non-fragile and fragile regions mapped to this band type. FS, fragile regions.

DISCUSSION

This paper provides evidence that FRA18C is a previously undescribed aphidicolin-inducible fragile site at chromosome 18q22.2. When cells of the father I.1 were cultured in the presence of aphidicolin to activate the expression of common fragile sites, we observed fragile site expression in 5% of metaphases, but none were observed when cells were cultured without aphidicolin. We have no explanation why FRA18C expression is so low. However this does not seem to be unique for this site; Leversha *et al*²⁵ observed the common fragile site FRA6E in up to 3.5% of metaphases. We analysed the DNA flexibility of 18q22 and found that the region is relatively enriched in flexibility islands—characteristics of molecularly cloned common fragile sites.^{8–26} Although this is the only case of FRA18C so far, it shows features of a common fragile site.

Although common fragile sites are considered to be present in all individuals, there is little information concerning their distribution among the individuals. Several reports claim that the frequency of common fragile sites varies among different individuals.^{27–29} Some common fragile sites, like FRA3B and FRA16D, are expressed in almost 100% of individuals, whereas others like FRA13A are less frequent and are only observed in a minority of individuals.²⁸ The latter group is sometimes referred to as the low-frequency common fragile sites. It seems plausible that FRA18C belongs to this group. We do not know why FRA18C was not detected previously. Assuming it has not been overlooked in the past, there are several possibilities why FRA18C is expressed in the father I.1, but not in others. First, the regional DNA composition of the father I.1 may be different as hypothesised by Savelyeva *et al*,³⁰ who suggested that the presence of sequence polymorphisms only in individuals who

Figure 4 Localisation of a chromosome 18q subtelomeric probe (labelled red) on a metaphase spread of (A) the patient (II.1) and (B) the father (I.1). Chromosomes 18 are coloured green.

www.jmedgenet.com

- 5 **Yu S**, Mangelsdorf M, Hewett D, Hobson L, Baker E, Eyre HJ, Lapsys N, Le Paslier D, Doggett NA, Sutherland GR, Richards RI. Human chromosomal fragile site FRA16B is an amplified AT-rich minisatellite repeat. *Cell* 1997;**88**:367-74.
- 6 **Hewett DR**, Handt O, Hobson L, Mangelsdorf M, Eyre HJ, Baker E, Sutherland GR, Schuffenhauer S, Mao JJ, Richards RI. FRA10B structure reveals common elements in repeat expansion and chromosomal fragile site genesis. *Mol Cell* 1998;**1**:773-81.
- 7 **Mishmar D**, Rahat A, Scherer SW, Nyakatura G, Hinzmann B, Kohwi Y, Mandel-Gutfroind Y, Lee JR, Drescher B, Sas DE, Margalit H, Platzer M, Weiss A, Tsui LC, Rosenthal A, Kerem B. Molecular characterization of a common fragile site (FRA7H) on human chromosome 7 by the cloning of a simian virus 40 integration site. *Proc Natl Acad Sci U S A* 1998;**95**:8141-6.
- 8 **Zlotorynski E**, Rahat A, Skaug J, Ben-Porat N, Ozeri E, Hershberg R, Levi A, Scherer SW, Margalit H, Kerem B. Molecular basis for expression of common and rare fragile sites. *Mol Cell Biol* 2003;**23**:7143-51.
- 9 **Kremer EJ**, Pritchard M, Lynch M, Yu S, Holman K, Baker E, Warren ST, Schlessinger D, Sutherland GR, Richards RI. Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)_n. *Science* 1991;**252**:1711-14.
- 10 **Knight SJL**, Flannery AV, Hirst MC, Campbell L, Christodoulou Z, Phelps SR, Pointon J, Middleton-Price HR, Barnicoat A, Pembrey ME, Holland J, Oostra BA, Bobrow M, Davies KE. Trinucleotide repeat amplification and hypermethylation of a CpG island in FRA1E mental retardation. *Cell* 1993;**74**:127-34.
- 11 **Jones C**, Penny L, Mattina T, Yu S, Baker E, Voullaire L, Langdon WY, Sutherland GR, Richards RI, Tunnacliffe A. Association of a chromosome deletion syndrome with a fragile site within the proto-oncogene CBL2. *Nature* 1995;**376**:145-9.
- 12 **Hecht F**, Sutherland GR. Fragile sites and cancer breakpoints. *Cancer Genet Cytogenet* 1984;**12**:179-81.
- 13 **Yunis JJ**, Soreng AL. Constitutive fragile sites and cancer. *Science* 1984;**226**:1199-204.
- 14 **Arlt MF**, Casper AM, Glover TW. Common fragile sites. *Cytogenet Genome Res* 2003;**100**:92-100.
- 15 **Ohta M**, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM, Huebner K. The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancers. *Cell* 1996;**84**:587-97.
- 16 **Hellman A**, Zlotorynski E, Scherer SW, Cheung J, Vincent JB, Smith DI, Trakhtenbrot L, Kerem B. A role for common fragile site induction in amplification of human oncogenes. *Cancer Cell* 2002;**1**:89-97.
- 17 **Jamieson RV**, Perveen R, Kerr B, Carette M, Yardley J, Heon E, Wirth MG, van Heyningen V, Donnai D, Munier F, Black GCM. Domain disruption and mutation of the bZIP transcription factor, MAF, associated with cataract, ocular anterior segment dysgenesis and coloboma. *Hum Mol Genet* 2002;**11**:33-42.
- 18 **Brewer CM**, Lam WW, Hayward C, Grace E, Maher ER, FitzPatrick DR. Beckwith-Wiedemann syndrome in a child with chromosome 18q deletion. *J Med Genet* 1998;**35**:162-4.
- 19 **Strathdee G**, Zackai EH, Shapiro R, Kamholz J, Overhauser J. Analysis of clinical variation seen in patients with 18q terminal deletions. *Am J Med Genet* 1995;**59**:476-83.
- 20 **Yau SC**, Bobrow M, Mathew CG, Abbs SJ. Accurate diagnosis of carriers of deletions and duplications in Duchenne/Becker muscular dystrophy by fluorescent dosage analysis. *J Med Genet* 1996;**33**:550-8.
- 21 **Lamb J**, Harris PC, Wilkie AOM, Wood WG, Dauwerse JG, Higgs DR, De Novo Truncation of Chromosome 16p and Healing with (TTAGGG)_n in the α -Thalassemia/Mental Retardation Syndrome (ATR-16). *Am J Hum Genet* 1993;**52**:668-76.
- 22 **Crowder RJ**, Enomoto H, Yang M, Johnson EM Jr, Milbrandt J. Dok-6, a Novel p62 Dok family member, promotes Ret-mediated neurite outgrowth. *J Biol Chem* 2004;**279**:42072-81.
- 23 **Jones C**, Slijepcevic P, Marsh S, Baker E, Langdon WY, Richards RI, Tunnacliffe A. Physical linkage of the fragile site FRA11B and a Jacobsen syndrome chromosome deletion breakpoint in 11q23.3. *Hum Mol Genet* 1994;**3**:2123-30.
- 24 **Benson G**. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res* 1999;**27**:573-80.
- 25 **Leversha MA**, Webb GC, Pavey SM. Chromosome banding required for studies on X-linked mental retardation. *Lancet* 1981;**1**:49.
- 26 **Mishmar D**, Mandel-Gutfroind Y, Margalit H, Rahat A, Kerem B. Common fragile sites: G-band characteristics within an R-band. *Am J Hum Genet* 1994;**55**:908-10.
- 27 **Denison SR**, Simper RK, Greenbaum IF. How common are common fragile sites in humans: interindividual variation in the distribution of aphidicolin-induced fragile sites. *Cytogenet Genome Res* 2003;**101**:8-16.
- 28 **Savelyeva L**, Sagulenko E, Schmitt JG, Schwab M. The neurobeachin gene spans the common fragile site FRA13A. *Hum Genet* 2005;**115**:1-8.
- 29 **Glover TW**, Berger C, Coyle J, Echo B. DNA polymerase alpha inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. *Hum Genet* 1984;**67**:136-42.
- 30 **Savelyeva L**, Sagulenko E, Schmitt JG, Schwab M. Low-frequency common fragile sites: Link to neuropsychiatric disorders? *Cancer Lett* 2006;**232**:58-69.
- 31 **Howlett NG**, Taniguchi T, Durkin SG, D'Andrea AD, Glover TW. The Fanconi anemia pathway is required for the DNA replication stress response and for the regulation of common fragile site stability. *Hum Mol Genet* 2005;**14**:693-701.
- 32 **Schwartz M**, Zlotorynski E, Goldberg M, Ozeri E, Rahat A, le Sage C, Chen BPC, Chen DJ, Agami R, Kerem B. Homologous recombination and nonhomologous end-joining repair pathways regulate fragile site stability. *Genes Dev* 2005;**19**:2715-26.
- 33 **Casper AM**, Nghiem P, Arlt MF, Glover TW. ATR regulates fragile site stability. *Cell* 2002;**111**:779-89.
- 34 **Musio A**, Montagna C, Mariani T, Tilenni M, Focarelli ML, Brait L, Indino E, Benedetti PA, Chessa L, Albertini A, Ried T, Vezzoni P. Smc1 involvement in fragile site expression. *Hum Mol Genet* 2005;**14**:525-33.
- 35 **Arlt MF**, Xu B, Durkin SG, Casper AM, Kastan MB, Glover TW. BRCA1 is required for common-fragile-site stability via its G2/M checkpoint function. *Mol Cell Biol* 2004;**24**:6701-9.
- 36 **Arlt MF**, Durkin SG, Ragland RL, Glover TW. Common fragile sites as targets for chromosome rearrangements. *DNA Repair* 2006;**5**:1126-35.
- 37 **Shaffer LG**, Lupski JR. Molecular mechanisms for constitutional chromosomal rearrangements in humans. *Annu Rev Genet* 2000;**34**:297-329.

Let us assist you in teaching the next generation

Figures from all articles on our website can be downloaded as a PowerPoint slide. This feature is ideal for teaching and saves you valuable time. Just click on the image you need and choose the "PowerPoint Slide for Teaching" option. Save the slide to your hard drive and it is ready to go. This innovative function is an important aid to any clinician, and is completely free to subscribers. (Usual copyright conditions apply.)