Common fragile genes

A. Matsuyama, C.M. Croce, K. Huebner
Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Penn, USA

Common chromosome fragile sites show susceptibility to DNA damage, leading to alterations that contribute to cancer development. The cloning and characterization of fragile sites have demonstrated that fragile sites are associated with genes that relate to tumorigenesis. Identification of the basis of instability at fragile sites and the related genes provides an entree to understanding of important aspects of chromosomal instability, a prominent feature of neoplastic genomes. FHIT/FRA3B and WWOX/FRA16D, the most sensitive common fragile genes in the human genome, function as tumor suppressor genes. The common features of these two common fragile genes are summarized, and suggest clues to understanding the relation between genomic instability and tumor biology.

Key words: common fragile site, tumor suppressor gene, tumorigenesis, genomic rearrangement

Correspondence: Ayumi Matsuyama, Kimmel Cancer Center, Thomas Jefferson University, 233 South 10th Street, BLSB Room 1006, Philadelphia, Pennsylvania 19107, USA.
Phone: international +1.215.5034655.
Fax: international +1.215.9234498.
E-mail: ayumi.matsuyama@mail.jci.tju.edu

Paper accepted January 8, 2004

European Journal of Histochemistry
2004; vol. 48:29-36

Chromosomal fragile sites are non-randomly distributed regions that show gaps, breaks or rearrangements in metaphase chromosomes under specific culture conditions, generally through inhibited or delayed DNA replication (Sutherland, 1979). Now 119 loci have been identified as chromosomal fragile sites in the Human Genome Database(*http://www.ncbi.nlm.nih.gov/LocusLink/*. Chromosomal fragile sites are classified into two categories on the basis of their frequency in the population and the chemistry of induction (Table 1). Rare fragile sites are observed in less than 5% of individuals and most (23/31) are induced by folic acid deficiency, whereas common fragile sites are found in all individuals and most (76/88) are induced by aphidicolin, an inhibitor of DNA polymerases (Glover et al., 1984). Unstable repeats are expand at the rare fragile sites and lead to chromosome breakage; for example, folate-sensitive FRA11B is associated with CGG triplets repeats (Jones et al., 1995), and BrdU-induced FRA10B and distamycin A-induced FRA16B with AT-rich minisatellite repeats (Hewett et al., 1998; Yu et al., 1997). The CGG repeats lead to unusual secondary DNA structures, such as hairpins (Gacy et al., 1995), slipped strand (S)-DNA (Pearson et al., 1998), or quadruplex DNA (Fry and Loeb, 1994), these structures tend to inhibit or delay DNA replication *in vitro* and *in vivo* (Usdin and Woodford, 1995; Samadashwily et al., 1997). AT-rich minisatellite repeats can also form hairpin structures (Handt et al., 2000).

Common fragile sites

On the other hand, several common fragile sites have been cloned and characterized: FRA3B (Ohta et al., 1996; Inoue et al., 1997; Mimori et al., 1999), FRA6E (Cesari et al., 2003; Denison et al., 2003), FRA6F (Morelli et al., 2002), FRA7G (Huang et al., 1999; Tatarelli et al., 2000), FRA7H (Mishmar et al., 1998), FRA16D (Mangelsdorf et

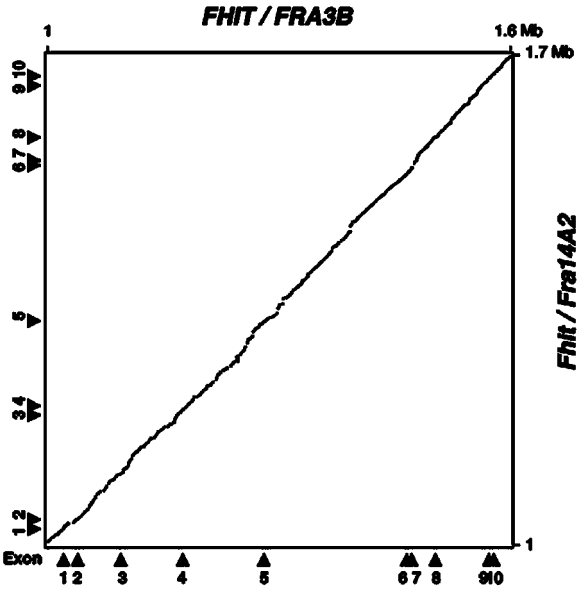

Figure 1. Comparison of human and murine orthologous sequences. The PipMaker program (*http://bio.cse.psu.edu/pipmaker/, Schwartz et al., 2000*) was applied for the large-scale comparison between human FHIT and mouse Fhit sequences, that computes alignments of similar regions in two DNA sequences. The horizontal and vertical axes represent the nucleotide number of human and mouse sequences, respectively, and the locations of exons in FHIT and Fhit are also provided as arrowheads.

al., 2000; Paige et al., 2000; Ried et al., 2000), and FRAXB (Arlt et al., 2002) (Figure 1). It is now known that these sites are actually huge regions, encompassing megabases, with proneness to forming gaps or breaks, and that sequences of these sites are rich in adenosine and thymidine with many sequence features, such as long interspersed nuclear element 1 (LINE1), high flexibility regions (HFRs), and matrix association regions (MARs), that are sensitive to genomic rearrangements (Inoue et al., 1997; Mimori et al., 1999; Mishmar et al., 1998; Tatarelli et al., 2000; Ried et al., 2000; Shiraishi et al., 2001; Matsuyama et al., 2003). Common fragile sites are induced also by caffeine and alcohol, and environmental carcinogens, such as in cigarette smoke, can enhance their expression (Yunis and Soreng, 1984; Kuwano and Kajii, 1987; Chen et al., 1989; Ban et al., 1995). Fragile sites are recombinogenic and sensitive to agents that inhibit DNA replication. At fragile sites, DNA replication occurs late in the cell cycle, so accumulation of incomplete replication leads to gaps/breaks in metaphase chromosome (Laird et al., 1987). Studies have revealed that the fragile sequences must be responsible for late/delayed DNA replication (Le Beau et al., 1998; Wang et al., 1999; Hellman et al., 2000), however, no specific sequences that could perturb replication have been identified. A recent study reported that the replication checkpoint kinase ATR is critical for genome stability at fragile sites (Casper et al., 2002). Fragility of common fragile regions occurs spontaneously in ATR deficient cells. Thus, stalling of DNA replication forks that escape ATR checkpoint could lead to persistence of single-stranded regions through G2 and mitosis, appearing as gaps in mitotic chromosomes. Zlotorynski et al (2003) reported that aphidicolin-induced common fragile sites contain flexible sequences, composed of interrupted runs of AT-dinucleotides, which can affect DNA replication by forming secondary structures, and these sequences are similar to the AT-rich minisatellite repeats in rare fragile sites, FRA16B and FRA10B. Rare and common fragile sites, thus, may both depend upon flexible sequences for their fragility. We have determined that the human and mouse fragile histidine triad (FHIT/Fhit) genes, encompassing the FRA3B/Fra14A2 common fragile regions, respectively, have more HFRs in their sequences than the flanking human and mouse receptor protein tyrosine phosphatase γ (PTPRG/Ptprg) genes (Matsuyama et al., 2003). The results suggest that AT-rich flexible sequences might play an important role in the fragility mechanism in the mammalian genome. If fragility is based on specific sequences, then the specific hallmark sequences of fragile regions, the HFRs, must be responsible for the late/delayed replication phenomenon, as well as the occurrence of single-strandedness that can escape the ATR DNA damage checkpoint detection.

Fragile sites have been reported also in mouse, rat, hamster, cow, cat, and dog (Djalali et al., 1987; Lin et al., 1984; Smeets and van de Klundert, 1990; Stone et al., 1991; Vitetta et al., 1991; Robinson and Elder, 1987). They appear to be inherent and universal structures of the mammalian genome, and specific fragile regions seem to be conserved across species (Yunis and Soreng, 1984). We already reported that human and mouse orthologous common fragile sites, FRA3B and Fra14A2, are highly conserved (Shiraishi et al., 2001). Sequence comparison revealed that human and mouse orthologous genes, FHIT and Fhit, are more highly conserved through evolution than flanking PTPRG/Ptprg genes (Figure 1, Matsuyama et al.,

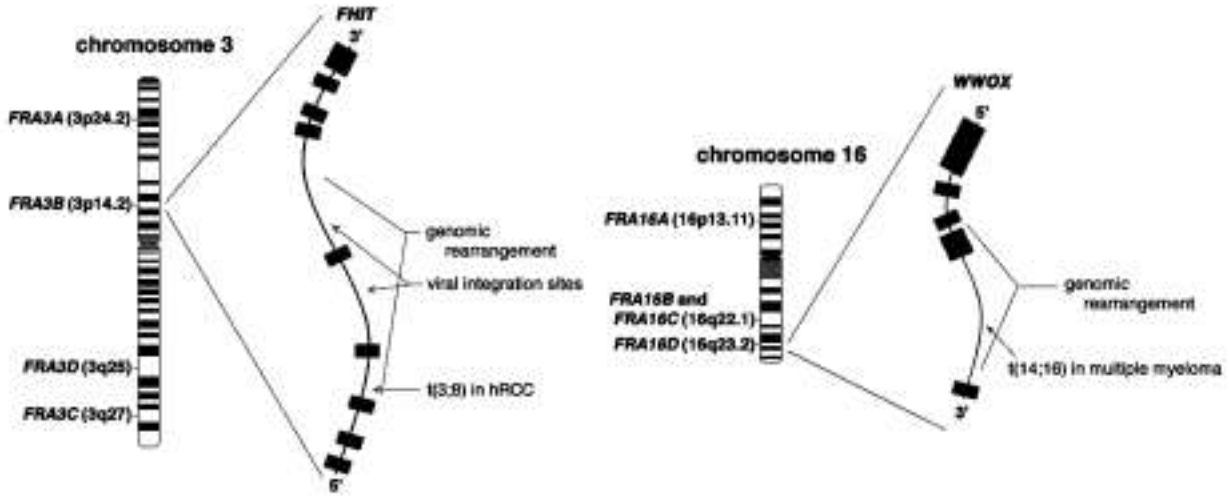

Figure 2. The FHIT and WWOX loci. The FHIT gene encompasses FRA3B, at 3p14.2 on chromosome 3, and the WWOX gene spans 16q23.2 on chromosome 16. FRA3A, FRA3C, FRA3D, and FRA16C are also common fragile sites, and FRA16A and FRA16B are rare ones. Gray boxes represent the location of exons in the sequences.

2003). The human PTPRG gene is located about 1.5 Mbp centromeric to FHIT on chromosome 3; and the mouse ortholog, Ptprg, is located 1.7 Mbp centromeric to Fhit on chromosome 14. The results indicate that not only common fragile sites but also the fragile genes encompassing common fragile sites are recombinogenic.

Chromosome locations of many common fragile sites coincide with locations of cancer breakpoints and/or cancer-associated genes, and the hypothesis that they play an important role in chromosomal instability in cancer development was proposed (Yunis and Soreng, 1984). This hypothesis has produced many studies of recombinigenicity involving common fragile sites, reporting observations of sister chromatid exchanges, translocations, deletions, viral integrations, intrachromosomal gene amplifications, at or near fragile sites in cancer (Glover and Stein, 1988; Popescu et al., 1990; Coquelle et al., 1997; Mishmar et al., 1998). Several genes at an encompassing common fragile sites have been shown to or suggested to function as tumor suppressor genes. Two genes, a tumor suppressor and candidate suppressor associated with the most active common fragile sites, FHIT and WWOX are reviewed below.

FHIT/FRA3B

FRA3B at chromosomal band 3p14.2 is the most active common fragile site in the human genome, and chromosomal abnormalities in this region are observed in a wide variety of human malignant diseases (reviewed in Huebner et al., 1998; Huebner and Croce, 2001, 2003). FRA3B exhibits hallmarks of fragile site recombinigenicity, such as the translocation, t(3;8)(p14.2;q24), breakpoint at 3p14.2 in familial clear cell renal carcinoma (Cohen et al., 1979), plasmid integration sites (Rassool et al., 1991) and a papilloma virus integration site (Wilke et al.,1996) (Figure 2). The fragile histidine triad (FHIT) gene was found to span FRA3B (Ohta et al., 1996). The FHIT locus spans over 1.5 Mb pairs (Mbp) of human genome, including FRA3B, but the mRNA is only 1.1 kb in size and consists of 10 small exons; exons 5 to 9 encode the Fhit protein (Ohta et al., 1996; Zimonjic et al., 1997) (Figure 2). According to the sequence and features of FRA3B; most cancer associated rearrangements thus far defined occur within introns 3, 4, and 5 of the FHIT gene (Inoue et al., 1997; Mimori et al., 1999), and not only the orthologous fragile sites, human FRA3B and mouse Fra14A2, but also the orthologous genes, FHIT and Fhit, are highly conserved over evolution (Shiraishi et al., 2001; Matsuyama et al., 2003). The region from exon 3 through 6 has been considered the epicenter of fragility for FRA3B and thus this region is referred to as FRA3B and Fra14A2.

FHIT is frequently deleted in cancers and cancer cell lines, such as lung, digestive tract, kidney, breast, liver, and pancreatic cancers, and Fhit protein is absent or reduced in most cancers (reviewed

Table 1. Classification of human chromosomal fragile sites and features.

Classification (n)	*Common fragile sites (88)*	*Rare fragile sites (31)*
Frequency in the population	observed in all individuals	observed in less than 5% of individuals
Inducing chemistry (n)	aphidicolin (76) 5- bromodeoxyuridine (BrdU) (7) 5-azacytidine (4) unclassified (1)	folic acid deficiency (23) distamycin A (5) BrdU (2) unclassified (1)
Feature of sequence	common feature is still unknown (AT-rich flexible sequence ?)	unstable repeat expansion (AT-rich minisatellite repeat, CGG triplets repeat,.....) n, number of identified human loci.

Table 2. Cloned human common fragile sites related with malignancies.

Locus	*Location*	*Related genes*	*Alteration in malignancies*
FRA3B	3p14.2	*FHIT*	deletion in a wide variety of cancers FHIT product is reduced/absent in most cancers translocation in renal cancer
FRA6E	6q26	Parkin	LOH in ovarian and breast cancer duplication or deletion of exons
FRA6F	6q21	19 known genes are related	deletion in several types of leukemias and solid tumors
FRA7G	7q31.2	CAV1, CAV2, TES	LOH in ovarian, breast and prostate cancer
FRA16D	16q23.3~24.1	WWOX/WOX1/FOR	LOH in liver, ovarian, breast, esophageal, prostate, and lung cancer deletion in ovarian and liver cancer translocations in multiple myeloma
FRAXB	Xp22.31	STS, GS1, TLR5a	deletion in esophageal cancer cells LOH, loss of heterozygosity

in Huebner et al., 1998; Huebner and Croce, 2001, 2003; Richards, 2001). The mouse Fhit ortholog also encompasses a common fragile site, Fra14A2 on murine chromosome 14 (Glover et al., 1998), and sustains homozygous deletions in murine cancer cell lines (Pekarsky et al., 1998). FHIT expression is lost/reduced at an early stage, both in esophageal cancer, adenocarcinoma and squamous cell carcinoma, cancers strongly influenced by environmental carcinogens, such as in tobacco smoke and alcohol consumption (Michael et al., 1997; Mori et al., 2000). Thus, loss of FHIT function is involved in carcinogenesis at an early stage of cancer progression. Exogeneous FHIT can suppress tumor growth (Siprashvili et al., 1997), and exogenous Fhit protein expression induces apoptosis, directly or indirectly, in cancer cells (Ji et al., 1999; Sard et al., 1999). Furthermore, Fhit knockout mice are more susceptible to tumor formation (Fong et al., 2000; Zanesi et al., 2001) and delivery of FHIT in viral vectors prevents and reverses cancer development (Dumon et al., 2001; Ishii et al., 2001; Ishii et al., 2003). Thus, Fhit functions as a tumor suppressor, consistently with the idea that fragile sites may harbor genes that, when altered, contribute to cancer development.

WWOX/FRA16D

Human WW domain containing oxidoreductase (WWOX, also known as WOX1 or FOR) gene was identified from chromosome region 16q23.3-24.1 at the aphidicolin-sensitive common fragile site FRA16D (Bednarek et al., 2000; Ried et al., 2000). At that time FRA16D had been partially characterized, and, within the region, homozygous deletion in gastric, ovarian, colon cancer, and loss of heterozygosity (LOH) in prostate and breast cancer had been reported (Mangelsdorf et al., 2000; Paige et al., 2000). The murine orthologous Wwox gene also encompasses a common fragile site, Fra8E1, and FRA16D/Fra8E1 are highly conserved, like FRA3B/Fra14A2, across species (Krummel et al., 2002). The WWOX gene encompasses a genomic locus > 1 Mb, consists of 9 small exons with 2.3 kb mRNA (Bednarek et al., 2000). The translocation, t(14;16)(q32;q23), breakpoint in multiple myeloma (Chesi et al., 1998; Krummel et al., 2000) is observed in intron 8 (Figure 2). Homozygous deletions have been defined between exons 4-9 of WWOX in some cancers, liver (Yakicier et al., 2001), ovarian (Paige et al., 2001), breast (Driouch et al., 2002), esophageal (Kuroki et al., 2002), and lung cancer (Yendamuri et al., 2003). WWOX mRNA expression has been examined in tumors and cancer cell lines and aberrant products observed in many tumors, including breast (Bednarek et al., 2001), ovary (Paige et al., 2001), esophageal (Kuroki et al., 2002), and lung cancers (Yendamuri et al., 2003). Wwox protein may engage a proapoptotic pathway that inhibits tumor cell growth *in vitro* and *in vivo* (Bednarek et al., 2001), perhaps through c-Jun NH2-terminal kinase interaction (Chang et al., 2003). WWOX is involved in stress and apoptotic responses, and the presence of WW domains in the structure of WWOX indicates that it physically interacts with other proteins (Bednarek et al., 200). WWOX protein has been reported to interact with both p53 and JNK1. The

two fragile genes, FHIT and WWOX, have common features; both encompass a genomic locus of more than 1 Mb with an associated fragile region, show frequent altered expression in cancers, and are suspected tumor suppressor genes.

Other common fragile sites, genes, cancers, and future directions

Cancer-related common fragile sites are summarized in Table 2. FRA7G at 7q31.2 is involved in LOH in ovarian, breast and prostate cancers; three genes near this fragile region, the caveolin-1 and -2 (CAV1 and 2), and the TESTIN (TES) genes, have been reported to show altered expression in cancers (Engelman et al., 1998; Huang et al., 1999; Tatarelli et al., 2000). In addition, CAV1 and 2 have been reported as tumor suppressor genes (Hurlstone et al., 1999).

FRAXB, at Xp22.31, spans a large genomic region of approximately 500 kb, three known genes, including GS1 (a gene of unknown function), the microsomal steroid sulfatase locus (STS), and Toll-like receptor 5a (TLR5a), map near or within this fragile site region (Arlt et al., 2002). In this study, multiple deletions in cancer cell lines within four fragile sites, FRAXB, FRA7G, FRA7H and FRA16D, were reported, supporting the hypothesis that common fragile sites and their associated genes are unstable in some cancer cells.

The common fragile site FRA6F, located at 6q21, is an extended region of about 1.2 Mb; deletion in this region has been recognized in several types of leukemia and solid tumors. Transcription mapping of the FRA6F region identified 19 known genes (Morelli et al., 2002).

Recently, in the third most active common fragile site, FRA6E (6q26), Parkin, the gene involved in the pathogenesis of juvenile Parkinson's disease has been identified (Cesari et al., 2003; Denison et al., 2003). Alterations in FRA6E and Parkin are observed in ovarian, breast, lung and other cancers. Thus, Parkin is a large gene located in a large common fragile site, and is also a candidate tumor suppressor gene, like FHIT/FRA3B and WWOX/FRA16D.

To date, many findings have revealed the relationship between common fragile sites, related genes, and cancer (Table 2). Genomic rearrangements in several genes, located in or encompassing common fragile sites, lead to tumorigenesis and tumor progression; thus, these genes can have roles as tumor suppressors. Further detailed analyses of genes at common chromosome fragile sites will determine whether genes at these sites commonly have roles as fragile tumor suppressor genes. Since fragile genes are likely to be inactivated in parallel in cancer, the characteristics of biological functions of these fragile genes will contribute to a further understanding of the natural history of cancer progression.

References

Arlt MF, Miller DE, Beer DG, Glover TW. Molecular characterization of FRAXB and comparative common fragile site instability in cancer cells. Genes Chromosomes Cancer 2002;33:82-92.

Ban S, Cologne JB, Neriishi K. Effect of radiation and cigarette smoking on expression of FUdR-inducible common fragile sites in human peripheral lymphocytes. Mutat Res 1995;334:197-203.

Bednarek AK, Laflin KJ, Daniel RL, Liao Q, Hawkins KA, Aldaz CM. WWOX, a novel WW domain-containing protein mapping to human chromosome 16q23.3-24.1, a region frequently affected in breast cancer. Cancer Res 2000;60:2140-5.

Bednarek AK, Keck-Waggoner CL, Daniel RL, Laflin KJ, Bergsagel PL, Kiguchi K, et al. WWOX, the FRA16D gene, behaves as a suppressor of tumor growth. Cancer Res 2001;61:8068-73.

Casper AM, Nghiem P, Arlt MF, Glover TW. ATR regulates fragile site stability. Cell 2002;111:779-89.

Cesari R, Martin ES, Calin GA, Pentimalli F, Bichi R, McAdams H, et al. Parkin, a gene implicated in autosomal recessive juvenile parkinsonism, is a candidate tumor suppressor gene on chromosome 6q25-q27. Proc Natl Acad Sci USA 2003;100:5956-61.

Chang NS, Doherty J, Ensign A. JNK1 physically interacts with WW domain-containing oxidoreductase (WOX1) and inhibits WOX1-mediated apoptosis. J Biol Chem 2003;278:9195-202.

Chen AT, Reidy JA, Annest JL, Welty TK, Zhou HG. Increased chromosome fragility as a consequence of blood folate levels, smoking status, and coffee consumption.Environ Mol Mutagen 1989; 13:319-24.

Chesi M, Bergsagel PL, Shonukan OO, Martelli ML, Brents LA, Chen T, et al. Frequent dysregulation of the c-maf proto-oncogene at 16q23 by translocation to an Ig locus in multiple myeloma. Blood 1998;91:4457-63.

Cohen AJ, Li FP, Berg S, Marchetto DJ, Tsai S, Jacobs S., et al. Hereditary renal-cell carcinoma associated with a chromosomal translocation. N Engl J Med 1979;301:592-5.

Coquelle A, Pipiras E, Toledo F, Buttin G, Debatisse M. Expression of fragile sites triggers intrachromosomal mammalian gene amplification and sets boundaries to early amplicons. Cell 1997;89:215-25.

Denison SR, Wang F, Becker NA, Schule B, Kock N, Phillips LA, et al. Alterations in the common fragile site gene Parkin in ovarian and other cancers. Oncogene 2003;22:8370-8.

Djalali M, Adolph S, Steinbach P, Winking H, Hameister H. A comparative mapping study of fragile sites in the human and murine genomes. Hum Genet 1987;77:157-62.

Driouch K, Prydz H, Monese R, Johansen H, Lidereau R, Frengen E. Alternative transcripts of the candidate tumor suppressor gene, WWOX, are expressed at high levels in human breast tumors. Oncogene 2002;21:1832-40.

Dumon KR, Ishii H, Fong LY, Zanesi N, Fidanza V, Mancini R, et al. FHIT gene therapy prevents tumor development in Fhit-deficient mice. Proc Natl Acad Sci USA 2001;98:3346-51.

Engelman JA, Zhang XL, Lisanti MP. Genes encoding human caveolin-1 and -2 are co-localized to the D7S522 locus (7q31.1), a known fragile site (FRA7G) that is frequently deleted in human cancers. FEBS Lett 1998;436:403-10.

Fong LY, Fidanza V, Zanesi N, Lock LF, Siracusa LD, Mancini R, et al. Muir-Torre-like syndrome in Fhit-deficient mice. Proc Natl Acad Sci USA. 2000;97:4742-7.

Fry M, Loeb LA. The fragile X syndrome d(CGG)n nucleotide repeats form a stable tetrahelical structure. Proc Natl Acad Sci USA 1994; 91:4950-4.

Gacy AM, Goellner G, Juranic N, Macura S, McMurray CT. Trinucleotide repeats that expand in human disease form hairpin structures in vitro. Cell 1995;81:533-40.

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.

Glover TW, Stein CK. Chromosome breakage and recombination at fragile sites. Am J Hum Genet 1988;43:265-73.

Glover TW, Hoge AW, Miller DE, Ascara-Wilke JE, Adam AN, Dagenais SL, et al. The murine Fhit gene is highly similar to its human orthologue and maps to a common fragile site region. Cancer Res 1998;58:3409-14.

Handt O, Sutherland GR, Richards RI. Fragile sites and minisatellite repeat instability. Mol Genet Metab 2000;70:99-105.

Hellman A, Rahat A, Scherer SW, Darvasi A, Tsui LC, Kerem B. Replication delay along FRA7H, a common fragile site on human chromosome 7, leads to chromosomal instability. Mol Cell Biol 2000;20:4420-7.

Hewett DR, Handt O, Hobson L, Mangelsdorf M, Eyre HJ, Baker E, et al. FRA10B structure reveals common elements in repeat expansion and chromosomal fragile site genesis. Mol Cell 1998;1:773-81.

Huang H, Reed CP, Mordi A, Lomberk G, Wang L, Shridhar V, et al. Frequent deletions within FRA7G at 7q31.2 in invasive epithelial ovarian cancer. Genes Chromosomes Cancer 1999;24:48-55.

Huebner K, Garrison PN, Barnes LD, Croce CM. The role of the FHIT/FRA3B locus in cancer. Annu Rev Genet 1998;32:7-31.

Huebner K, Croce CM. FRA3B and other common fragile sites: the weakest links. Nat Rev Cancer 2001;1:214-21.

Huebner K, Croce CM. Cancer and the FRA3B/FHIT fragile locus: it's a HIT. Br J Cancer 2003;88:1501-6.

Hurlstone AF, Reid G, Reeves JR, Fraser J, Strathdee G, Rahilly M, et al. Analysis of the CAVEOLIN-1 gene at human chromosome 7q31.1 in primary tumours and tumour-derived cell lines. Oncogene 1999;18:1881-90.

Inoue H, Ishii H, Alder H, Snyder E, Druck T, Huebner K, et al. Sequence of the FRA3B common fragile region: implications for the mechanism of FHIT deletion. Proc Natl Acad Sci USA 1997; 94:14584-9.

Ishii H, Dumon KR, Vecchione A, Trapasso F, Mimori K, Alder H, et al. Effect of adenoviral transduction of the fragile histidine triad gene into esophageal cancer cells. Cancer Res 2001;61:1578-84.

Ishii H, Zanesi N, Vecchione A, Trapasso F, Yendamuri S, Sarti M, et al. Regression of upper gastric cancer in mice by FHIT gene delivery. FASEB J 2003;17:1768-70.

Ji L, Fang B, Yen N, Fong K, Minna JD, Roth JA. Induction of apoptosis and inhibition of tumorigenicity and tumor growth by adenovirus vector-mediated fragile histidine triad (FHIT) gene overexpression. Cancer Res 1999;59:3333-9.

Jones C, Penny L, Mattina T, Yu S, Baker E, Voullaire L, et al. Association of a chromosome deletion syndrome with a fragile site within the proto-oncogene CBL2. Nature 1995;376:145-9.

Krummel KA, Roberts LR, Kawakami M, Glover TW, Smith DI. The characterization of the common fragile site FRA16D and its involvement in multiple myeloma translocations. Genomics 2000;69:37-46.

Krummel KA, Denison SR, Calhoun E, Phillips LA, Smith DI. The common fragile site FRA16D and its associated gene WWOX are highly conserved in the mouse at Fra8E1. Genes Chromosomes Cancer 2002;34:154-67.

Kuroki T, Trapasso F, Shiraishi T, Alder H, Mimori K, Mori M, et al. Genetic alterations of the tumor suppressor gene WWOX in esophageal squamous cell carcinoma. Cancer Res 2002; 62:2258-60.

Kuwano A, Kajii T. Synergistic effect of aphidicolin and ethanol on the induction of common fragile sites. Hum Genet 1987;75:75-8.

Laird C, Jaffe E, Karpen G, Lamb M, Nelson R. Fragile sites in human chromosomes as regions of late-replicating DNA. Trends Genet 1987;3:274-81.

Le Beau MM, Rassool FV, Neilly ME, Espinosa R 3rd, Glover TW, Smith DI, et al. Replication of a common fragile site, FRA3B, occurs late in S phase and is delayed further upon induction: implications for the mechanism of fragile site induction. Hum Mol Genet 1998; 7:755-61.

Lin MS, Takabayashi T, Wilson MG, Marchese CA. An in vitro and in vivo study of a BrdU-sensitive fragile site in the Chinese hamster. Cytogenet Cell Genet 1984;38:211-5.

Mangelsdorf M, Ried K, Woollatt E, Dayan S, Eyre H, Finnis M, et al. Chromosomal fragile site FRA16D and DNA instability in cancer. Cancer Res 2000; 60:1683-9.

Matsuyama A, Shiraishi T, Trapasso F, Kuroki T, Alder H, Mori M, et al. Fragile sitorthologs FHIT/FRA3B and Fhit/Fra14A2: evolutionarily conserved but highly recombinogenic. Proc Natl Acad Sci USA 2003 100:14988-93.

Mimori K, Druck T, Inoue H, Alder H, Berk L, Mori M, et al. Cancer-specific chromosome alterations in the constitutive fragile region FRA3B. Proc Natl Acad Sci USA 1999;96:7456-61.

Mishmar D, Rahat A, Scherer SW, Nyakatura G, Hinzmann B, Kohwi Y, et al. 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 USA 1998;95:8141-6.

Morelli C, Karayianni E, Magnanini C, Mungall AJ, Thorland E, Negrini M, et al. Cloning and characterization of the common fragile site FRA6F harboring a replicative senescence gene and frequently deleted in human tumors. Oncogene 2002;21:7266-76.

Mori M, Mimori K, Shiraishi T, Alder H, Inoue H, Tanaka Y, et al. Altered expression of Fhit in carcinoma and precarcinomatous lesions of the esophagus. Cancer Res 2000;60:1177-82.

Michael D, Beer DG, Wilke CW, Miller DE, Glover TW. Frequent deletions of FHIT and FRA3B in Barrett's metaplasia and esophageal adenocarcinomas. Oncogene 1997;15:1653-9.

Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, et al. 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.

Paige AJ, Taylor KJ, Stewart A, Sgouros JG, Gabra H, Sellar GC, et al. A 700-kb physical map of a region of 16q23.2 homozygously deleted in multiple cancers and spanning the common fragile site FRA16D. Cancer Res 2000;60:1690-7.

Paige AJ, Taylor KJ, Taylor C, Hillier SG, Farrington S, Scott D, et al. WWOX: a candidate tumor suppressor gene involved in multiple tumor types. Proc Natl Acad Sci USA 2001;98:11417-22.

Pearson CE, Wang YH, Griffith JD, Sinden RR. Structural analysis of slipped-strand DNA (S-DNA) formed in (CTG)n. (CAG)n repeats from the myotonic dystrophy locus. Nucleic Acids Res 1998; 26:816-23.

Pekarsky Y, Druck T, Cotticelli MG, Ohta M, Shou J, Mendrola J, et al. The murine Fhit locus: isolation, characterization, and expression in normal and tumor cells. Cancer Res 1998;58:3401-8.

Popescu NC, Zimonjic D, DiPaolo JA. Viral integration, fragile sites, and proto-oncogenes in human neoplasia. Hum Genet 1990; 84:383-6.

Rassool FV, McKeithan TW, Neilly ME, van Melle E, Espinosa R 3rd, Le Beau MM. Preferential integration of marker DNA into the chromosomal fragile site at 3p14: an approach to cloning fragile sites. Proc Natl Acad Sci USA 1991;88:6657-61.

Richards RI. Fragile and unstable chromosomes in cancer: causes and consequences. Trends Genet 2001;17:339-45.

Ried K, Finnis M, Hobson L, Mangelsdorf M, Dayan S, Nancarrow JK, et al. Common chromosomal fragile site FRA16D sequence: identification of the FOR gene spanning FRA16D and homozygous deletions and translocation breakpoints in cancer cells. Hum Mol Genet 2000;9:1651-63.

Robinson TJ, Elder FF. Multiple common fragile sites are expressed in the genome of the laboratory rat. Chromosoma 1987;96:45-9.

Samadashwily GM, Raca G, Mirkin SM. Trinucleotide repeats affect DNA replication in vivo. Nat Genet 1997;17:298-304.

Sard L, Accornero P, Tornielli S, Delia D, Bunone G, Campiglio M, et al. The tumor-suppressor gene FHIT is involved in the regulation of apoptosis and in cell cycle control. Proc Natl Acad Sci USA 1999; 96:8489-92.

Schwartz S, Zhang Z, Frazer KA, Smit A, Riemer C, Bouck J, et al. PipMaker: a web server for aligning two genomic DNA sequences. Genome Res 2000;10:577-86.

Shiraishi T, Druck T, Mimori K, Flomenberg J, Berk L, Alder Het al. Sequence conservation at human and mouse orthologous common fragile regions, FRA3B/FHIT and Fra14A2/Fhit. Proc Natl Acad

Sci USA 2001;98:5722-7.

Siprashvili Z, Sozzi G, Barnes LD, McCue P, Robinson AK, Eryomin V, et al. Replacement of Fhit in cancer cells suppresses tumorigenicity. Proc Natl Acad Sci USA 1997;94:13771-6.

Smeets DF, van de Klundert FA. Common fragile sites in man and three closely related primate species. Cytogenet Cell Genet 1990;53:8-14.

Stone DM, Jacky PB, Hancock DD, Prieur DJ. Chromosomal fragile site expression in dogs: I. Breed specific differences. Am J Med Genet 1991;40:214-22.

Sutherland GR. Heritable fragile sites on human chromosomes. I. Factors affecting expression in lymphocyte culture. Am J Hum Genet 1979;31:125-35.

Tatarelli C, Linnenbach A, Mimori K, Croce CM. Characterization of the human TESTIN gene localized in the FRA7G region at 7q31.2. Genomics 2000;68:1-12.

Usdin K, Woodford KJ. CGG repeats associated with DNA instability and chromosome fragility form structures that block DNA synthesis in vitro. Nucleic Acids Res 1995;23:4202-9.

Vitetta L, Sali A, Little P, Nayman J, Elzarka A. Primary "brown pigment" bile duct stones. HPB Surg 1991;4:209-22.

Wang L, Darling J, Zhang JS, Huang H, Liu W, Smith DI. Allele-specific late replication and fragility of the most active common fragile site, FRA3B. Hum Mol Genet 1999;8:431-7.

Wilke CM, Hall BK, Hoge A, Paradee W, Smith DI, Glover TW. FRA3B extends over a broad region and contains a spontaneous HPV16 integration site: direct evidence for the coincidence of viral integration sites and fragile sites. Hum Mol Genet 1996;5:187-95.

Yakicier MC, Legoix P, Vaury C, Gressin L, Tubacher E, Capron F, et al. Identification of homozygous deletions at chromosome 16q23 in aflatoxin B1 exposed hepatocellular carcinoma. Oncogene 2001; 20:5232-8.

Yendamuri S, Kuroki T, Trapasso F, Henry AC, Dumon KR, Huebner K, et al. WW domain containing oxidoreductase gene expression is altered in non-small cell lung cancer. Cancer Res 2003;63:878-81.

Yunis JJ, Soreng AL. Constitutive fragile sites and cancer. Science 1984;226:1199-204.

Yu S, Mangelsdorf M, Hewett D, Hobson L, Baker E, Eyre HJ, et al. Human chromosomal fragile site FRA16B is an amplified AT-rich minisatellite repeat. Cell 1997;88:367-74.

Zanesi N, Fidanza V, Fong LY, Mancini R, Druck T, Valtieri M, et al. The tumor spectrum in FHIT-deficient mice. Proc Natl Acad Sci USA 2001;98:10250-5.

Zimonjic DB, Druck T, Ohta M, Kastury K, Croce C., Popescu NC, et al. Positions of chromosome 3p14.2 fragile sites (FRA3B) within the FHIT gene. Cancer Res 1997;57:1166-70.

Zlotorynski E, Rahat A, Skaug J, Ben-Porat N, Ozeri E, Hershberg R, et al. Molecular basis for expression of common and rare fragile sites. Mol Cell Biol 2003;23:7143-51.