Global screening and extended nomenclature for 230 aphidicolin-inducible fragile sites, including 61 yet unreported ones

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Abstract. Since the first description of human fragile sites (FS) more than 40 years ago, a variety of substances were reported to induce chromosomal breaks at non-random, breakage-prone regions. According to information available from human genome browsers aphidicolin, an inhibitor of DNA replication induces 77 of 88 known common FS. However, in the literature additional FS are reported, which are also, at least in part, inducible by aphidicolin. To the best of our knowledge, here we present the first and largest ever done systematic, whole genome-directed and comprehensive screening for aphidicolin-inducible breakage-prone regions. The study was performed on stimulated peripheral blood lymphocytes of 3 unrelated healthy individuals. Twenty-five thousand metaphase spreads were analyzed and overall 22,537 FS located in 230 different loci were recorded. Sixty-one of those FS were never observed before and 52 were already previously reported but not included in genome browsers and yet verified. Interestingly, aphidicolin was able to induce all types of rare and common FS, suggesting that these breakage-prone regions are less dependent on the inducing chemicals than originally supposed. Overall, we provide the first comprehensive genome wide map for FS and studied possible correlations of chromosome length and GTG-banding level with FS-frequency. To handle FS better in future, an extension of the already existing alphabetical nomenclature for FS on single chromosomes is suggested.

Introduction

The first description of human fragile sites (FS) dates back more than 40 years (1), and the term fragile site was introduced by Magenis five years later (2). Since that time fragile sites continued as an active area of research in cytogenetics and their definition and classification was subject for several controversies with the increasing knowledge (3,4). Today FS are understood as specific loci that preferentially exhibit gaps and break on metaphase chromosomes following partial inhibition of DNA synthesis. Rare FS are present in a small proportion of individuals and are inherited in a Mendelian manner. In contrast, common FS are present in all individuals and represent the largest class of FS (5). A number of substances are in use to induce common FS e.g. the bromodeoxyuridine (BrdU) or 5-azacytidine (5-azaC) (5,6). Common FS are also induceable by aphidicolin that acts as an inhibitor of DNA polymerase α and σ (7). Common FS are part of the normal mammalian chromosome structure. With a frequency of >5% in normal population they are usually not associated with disease, except for a possible correlation of some FS with cancer-related breakpoints (e.g. 5,7,8,9) or a molecular cytogenetic co-localization with breakpoints in Fanconi anemia patients (10).

According to the present knowledge, only 77 of 88 common FS listed in human genome browsers like NCBI (http://www.ncbi.nlm.nih.gov/) are induceable by aphidicolin. The three most frequently observed such FS are FRA3B in 3p14, FRA16D in 16q23 and FRAXB in Xp22. Therefore, it is not surprising that they are also part of the up to now 22 molecular characterized common FS: FRA1H, FRA1E, FRA2G, FRA3B, FRA4F, FRA6E, FRA6F, FRA7E, FRA7G, FRA7H, FRA7K, FRA7I, FRA8C, FRA9E, FRA9G, FRA11E, FRA11F, FRA11G, FRA13A, FRA16D, FRA18C and FRAXB. Interestingly, not all common FS are necessarily detectable in only one individual. Additionally, differences in the observable FS-frequency within different individuals are well known (11,12). Besides these 88 official, database-annotated common FS others were observed and reported, including six recently reported new sites, particularly FRA4F (13), FRA7K (14), FRA6H (15), FRA9G (16), FRA13E (15) and FRA18C (17).

Thus, with a systematic genome-wide screening for aphidicolin inducible FS in lymphocytes of 3 unrelated healthy individuals here the question should be solved which regions of the human genome are really breakage prone and to what extent they are affected when applying aphidicolin. Twenty-five thousand metaphase spreads presenting 22,537 break events were analyzed by cytogenetics. As a result, 61 new, by now unreported and unclassified FS were detected and the existence of 52 further FS (18,19) was proven.

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Materials and methods

Cytogenetics and induction of FS. Peripheral blood lymphocytes from three unrelated, healthy persons with normal female karyotype were studied (proband I, II and III). Proband I and II were Caucasian and proband III Caucasian-Asian in origin. Using cytogenetic standard protocols lymphocyte cultures were incubated for 72 h at 37˚C in RPMI medium supplemented with 10% fetal calf serum and 2% phytohemoagglutinin. Aphidicolin (0.2 μM) dissolved in dimethylsulfoxide was added 24 h before harvesting (20). Colcemid (0.04 μg/ml) was added 2 h before culture termination. Chromosome preparation was done according to standard protocols (21). Additionally lymphocytes from all three subjects were cultivated without aphidicolin and 100 metaphase spreads were evaluated, each, as negative controls, without finding any spontaneous break events. The chromosomes were banded by diamidinophenylindol (DAPI) and evaluation was done using ISIS software (MetaSystems, Altlussheim, Germany) based inverted DAPI-banding.

The 25,000 analyzed metaphase spreads were categorized according to their band level in 300-350, 400-500, 550-650 and >650 bands per haploid karyotype. Fifty-eight percent of the metaphase spreads were arrested at a 400-500 band level, 29% at 550-650, 11% at 300-350 and 2% at a band level of >650.

A chromosomal lesion was counted when it appeared as a chromatide or chromosome gap or break according to ISCN 2009 (22). A chromosomal site was determined to be a fragile site when it appeared more than once in this study or was reported before elsewhere in the literature.

Results

New aphidicolin-induced FS. A total of 25,000 metaphase spreads from three female subjects were analyzed after aphidicolin-induced lymphocyte culture. Overall, cytogenetic analysis revealed 22,537 break-events, i.e. 0.91 breaks per metaphase spread. Two hundred and thirty different FS including 61 until now not reported break-prone regions could be described (Table I, entries in bold, and Figs. 2 and 3). A FS was included here as new in case it was observed in this study at least twice. If a break-event was observed at least once in this study and also previously reported as FS it was also included into the 230 ones described here.

The mean frequency of all FS detected in this study was 0.279% and only 21 out of 230 detected FS appeared with a frequency over 1%. In contrast 92 specific FS have a frequency between 0.1 and 1% and the main part of 109 FS is expressed between 0.009 and 0.099% (Table I). The most frequently observed FS were FRA3B (14.153%), FRA16D (7.576%), FRAXB (5.494%), FRA2H (3.905%), FRA1M (3.333%) and FRA1I (2.299%) whereas FS that were described before and detected only once in this study had a frequency of 0.004% like FRA8F and FRA17D.

Additionally, single-break events were observed in the current study and not described in literature before. These breaks were not rated as new FS. In detail these were 1p34 (proband II), 2q14.2-14.3 (proband II), 3q23 (proband III), 4q32 (proband II), 15q24 (proband II), 20q13.3 (proband I) and 21q11.2 (proband I). Aphidicolin was able to induce different types of known rare and common FS that were also classified as 5-azacytidine, BrdU, folate sensitive and distamycin type before (Table I).

Frequency of FS per metaphase spread and per haploid band level. Not surprisingly the highest amount of breaks per metaphase were found in the highest band levels (>650: 3-12; 550-650: 3-8) compared to 300-350 and 400-500 band level with 1-4 and 2-5 breaks per metaphase, respectively.

Frequency of FS in different individuals. The FS-frequencies found in the three different studied individuals are detailed in Table I. Interindividual frequency differences for the expression of FS were found. As typical example for individual-specific FS inducibility results obtained for chromosome 10 are summarized in Fig. 1. Especially proband III expressed the highest rate of chromosome 10-specific FS while proband II showed here an extremely low rate and e.g. no expression of FRA10K. Overall, 41% of all here detected FS were found in proband I, 38% in proband III and only 21% in proband II. Strong FS-expression variation rates were also found e.g. for FRA1I, FRA2J, FRA4C, FRA4H, FRA6B, FRA10F and FRA16C. However, beside these strong variations, there were also sites with practically identical expression in all three studied subjects, such as FRA1A, FRA3C or FRA11C (Table I).

Distribution of FS. As shown in Figs. 2 and 3 the break-prone areas are almost equally distributed along each chromosome. Table II summarizes the number of FS per chromosome and the average interval size in megabases (Mb) between the individual FS. The latter is on average 13.63 Mb. The chromosome with the highest number of FS is human chromosome 2 with twenty-one break-prone regions. The number of FS is not correlated with chromosomal size as can be exemplified on chromosome 16, which is the most unstable chromosome with FS every 9.89 Mb. The smallest human chromosomes 21 and 22 show only two FS each and are the most stable chromosomes with an average breaking distance of 23.50 and 25.00 Mb besides the Y chromosome, which was not studied here and has only one FS reported in the literature. Moreover, half of human chromosomes contain a FS at the centomere including chromosomes 1, 2, 3, 5, 6, 8, 11, 12, 16, 17, 19 and X. Breakage-prone regions were also detected in heterochromatic blocks of human chromosome 1, 9 and the Y-chromosome.

Of the FS, 24.5% are located in GTG dark bands, 62% in light bands, 5% in centromeres and 8.5% at the boarder of light and dark bands. The major part of breaks result from chromatide breaks and only a small portion show chromosome breaks or reunions.

Nomenclature of FS. The nomenclature of FS is chromosome-specific and starts with the abbreviation FRA followed by chromosome number and a capital letter according to the appearance of the FS description from pter to qter, starting from A to Z e.g. FRA1A in 1p36. In accordance with these rules, the here reported new FS and also recently reported FS that were not included in human genome browsers before were

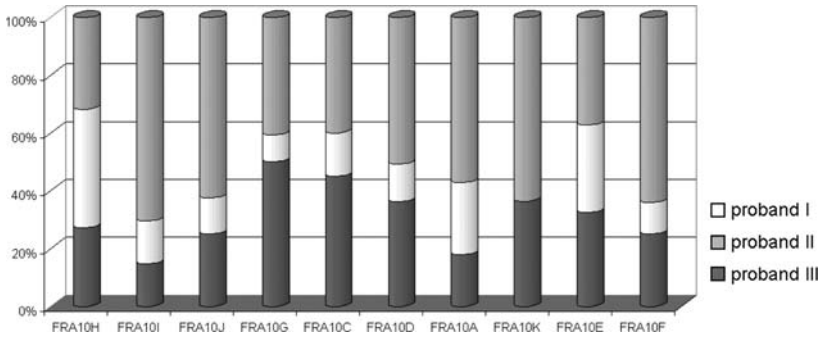

Figure 1. Aphidicolin-induced FS of human chromosome 10 and their frequency in three different individuals (I, II and III). The absolute numbers are given in Table I.

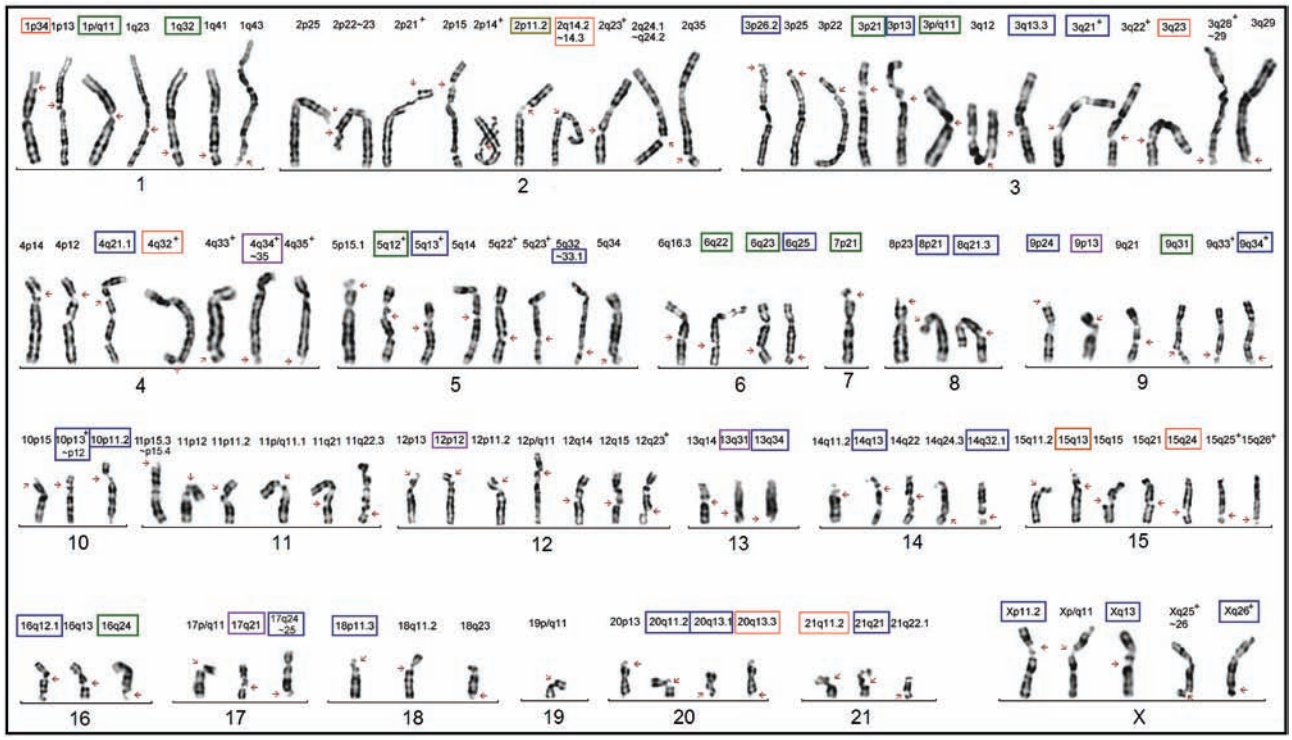

Figure 2. Cytogenetic detection of new aphidicolin-induced FS. Also shown are FS that were not included in the genome browser but described before. For each site the cytoband is given and an arrow indicates the FS. +, FS that might be larger and fused with a neighbor FS; green frame, taken from (37); blue frame, taken from (19); olive frame, taken from (34); brown frame, taken from (38); purple frame, taken from (29); red frame, single break events in the current study.

added to the existing 88 genome browser entries (Table I and Fig. 3).

Discussion

Frequency of FS. The common classification of FS is based on their induction with specific chemicals or frequencies in the population. The latter is divided into common and rare FS. Common FS are thought to be part of the normal chromosome structure and can be expressed in every individual (11). Nevertheless, they were also differentially expressed between different individuals, ethnic groups, sex and inducibility (12,19,20,23,24,25). According to the literature, the most frequently-detected FS are FRA3B (3p14.2) and FRA16D (16q23.2), which was confirmed in the current study. Even within the three investigated individuals the frequencies for those two FS were almost the same. Contrary, it is known that not all common FS were inducible in every individual (26). This could also be shown in this study; although a huge number of metaphases were analyzed certain FS were exclusively observed only in one of the investigated test persons e.g. FRA17D (Table I). Additionally, most FS vary in their inter-individual frequency; examples are given in Fig. 1 and Table I. Only a few authors discuss the ethnic influence on FS expression (27). In this study proband III was Caucasian-Asian descent whereas probands I and II were of Caucasian origin. Inter-individual frequency variations in FS-expression were also observed in other mammals like the mouse (28) and also between different tissues (29,30). Although aphidicolin-induced FS were previously studied, for a closer

Table I. Summary of all known and newly described aphidicolin-induced FS analyzed in 25,000 metaphase spreads with the extended nomenclature, cytogenetic localization, absolute numbers in different band levels (300-350, 400-450, 550-650, >650 bands per haploid karyotype) for three different subjects (I, II and III) and the frequency of every FS after aphidicolin induction in the current study.

		Number of FS per band level													
		300-350			400-500			550-650			>650				
All FS	Cytogenetic localization	III	I	II	III	I	II	III	I	II	III	I	II	∑ (all subjects and band levels)	Frequency in % (all subjects and band levels)
FRA1A	1p36	10	2	2	25	17	7	21	15	6	2	2	1	110	0.488
FRA1[h]	**1p34**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**0.004**
FRA1B	1p32	25	5	1	81	55	38	51	37	26	7	5	5	336	1.491
FRA1L	1p31	9	3	1	18	28	8	8	20	9	2	1	1	108	0.479
FRA1D	1p22	-	-	-	-	-	-	-	1	-	1	-	-	2	0.009
FRA1M[d]	1p21.3	55	7	13	178	118	75	73	116	83	10	12	11	751	3.333
FRA1E	1p21.2	-	-	-	5	2	1	2	3	1	1	-	-	15	0.067
FRA1N	**1p13**	**2**	**-**	**-**	**8**	**1**	**-**	**1**	**-**	**1**	**-**	**-**	**-**	**13**	**0.058**
FRA1O[e]	1p11/q11	4	3	4	20	23	9	8	15	9	-	1	3	99	0.439
FRA1J[a]	1q12	2	-	-	8	5	1	3	6	-	-	1	1	27	0.120
FRA1F	1q21	-	1	-	4	7	5	1	3	1	-	-	-	22	0.098
FRA1P	**1q23**	**1**	**-**	**1**	**3**	**-**	**-**	**1**	**-**	**1**	**-**	**1**	**-**	**8**	**0.036**
FRA1G	1q25.1	17	2	2	39	25	26	14	26	19	2	4	4	180	0.799
FRA1K	1q31	5	-	-	7	2	4	4	7	1	-	1	-	31	0.138
FRA1Q[e]	1q32	1	-	-	9	5	4	1	2	-	-	-	-	22	0.098
FRA1R	**1q41**	**2**	**2**	**-**	**3**	**4**	**-**	**4**	**1**	**1**	**1**	**-**	**1**	**19**	**0.084**
FRA1H[a]	1q42	1	1	-	6	3	1	9	5	1	1	1	-	29	0.129
FRA1S	**1q43**	**-**	**-**	**-**	**1**	**1**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**3**	**0.013**
FRA1I	1q44	16	10	2	84	97	52	39	115	74	2	9	18	518	2.299
FRA2M	**2p25**	**-**	**-**	**2**	**7**	**4**	**1**	**2**	**6**	**3**	**-**	**-**	**1**	**26**	**0.115**
FRA2C	2p24.2	19	7	4	65	99	25	24	50	27	8	7	6	341	1.513
FRA2N	**2p22-23**	**1**	**-**	**-**	**7**	**5**	**2**	**3**	**4**	**2**	**-**	**-**	**-**	**24**	**0.107**
FRA2O	**2p21**	**-**	**1**	**-**	**1**	**1**	**-**	**1**	**3**	**1**	**1**	**-**	**-**	**9**	**0.040**
FRA2D	2p16.2	38	11	6	158	93	27	53	80	39	4	6	9	524	2.325
FRA2P	**2p15**	**-**	**-**	**-**	**4**	**-**	**-**	**2**	**-**	**2**	**3**	**-**	**-**	**11**	**0.049**
FRA2Q	**2p14**	**-**	**-**	**-**	**4**	**-**	**-**	**2**	**-**	**-**	**-**	**-**	**-**	**6**	**0.027**
FRA2E	2p13	3	2	-	14	11	5	5	6	5	-	1	1	53	0.235
FRA2L[i]	2p11.2	-	-	-	3	-	4	1	-	4	-	-	1	13	0.058
FRA2R	2p11/2q11	3	2	2	11	6	2	6	5	2	-	-	1	40	0.178
FRA2A[d]	2q11.2	-	-	-	11	-	4	1	4	-	-	-	-	20	0.089
FRA2B[d]	2q13	-	-	-	4	3	3	1	1	3	-	-	-	15	0.067
FRA2[h]	**2q14.2-14.3**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**1**	**0.004**
FRA2F	2q21.3	12	4	1	49	60	24	19	33	16	-	3	3	224	0.994
FRA2K[d]	2q22.3	15	5	2	33	22	11	13	17	20	2	-	1	141	0.626
FRA2S	**2q23**	**4**	**-**	**1**	**8**	**4**	**3**	**2**	**1**	**-**	**-**	**-**	**-**	**23**	**0.102**
FRA2T	**2q24**	**1**	**-**	**-**	**1**	**4**	**-**	**-**	**4**	**1**	**-**	**-**	**1**	**12**	**0.053**
FRA2G	2q31	8	1	2	16	8	4	11	11	2	1	1	4	69	0.306
FRA2H	2q32.1	69	29	17	223	182	74	73	139	49	10	9	6	880	3.905
FRA2I	2q33	27	11	6	75	104	27	28	73	25	5	1	6	388	1.722
FRA2U	**2q35**	**-**	**1**	**1**	**5**	**7**	**-**	**3**	**3**	**2**	**-**	**-**	**1**	**23**	**0.102**
FRA2J	2q37.3	9	2	5	39	22	9	24	18	9	3	4	4	148	0.657
FRA3E[g]	3p26	3	-	1	13	13	8	5	14	12	1	1	-	71	0.315

Table I. Continued.

All FS	Cytogenetic localization	Number of FS per band level												∑ (all subjects and band levels)	Frequency in % (all subjects and band levels)
		300-350			400-500			550-650			>650				
		III	I	II	III	I	II	III	I	II	III	I	II		
FRA3F	**3p25**	**4**	**2**	**3**	**7**	**9**	**10**	**6**	**3**	**4**	**2**	**2**	**1**	**53**	**0.235**
FRA3A	3p24.2	21	10	2	137	35	19	24	29	11	8	2	1	299	1.327
FRA3G	**3p22**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**2**	**1**	**-**	**-**	**4**	**0.018**
FRA3H[e]	3p21	2	1	-	-	10	5	2	9	3	-	-	2	34	0.151
FRA3B	3p14.2	202	71	53	682	751	256	289	544	233	36	24	48	3189	14.153
FRA3I[g]	3p13	2	1	-	11	7	4	4	5	3	1	-	1	39	0.173
FRA3J[e]	3p/q11	-	-	-	1	5	3	4	3	-	-	-	-	16	0.071
FRA3K	**3q12**	**-**	**-**	**-**	**-**	**1**	**2**	**-**	**1**	**1**	**-**	**-**	**-**	**5**	**0.022**
FRA3L[g]	3q13.3	6	1	4	13	28	12	14	24	6	1	2	2	113	0.501
FRA3M[g]	3q21	-	-	-	5	-	-	2	3	-	-	-	-	10	0.044
FRA3N	**3q22**	**-**	**-**	**-**	**3**	**2**	**1**	**2**	**3**	**-**	**1**	**-**	**-**	**12**	**0.053**
FRA3[h]	**3q23**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**1**	**0.004**
FRA3D	3q25	3	3	2	43	15	10	12	22	9	2	1	1	123	0.546
FRA3O[f]	3q26	-	-	-	-	-	-	-	-	-	-	-	-	0	0.000
FRA3C	3q27	12	8	4	50	45	14	24	45	24	5	4	4	239	1.061
FRA3P	**3q28-29**	**-**	**1**	**1**	**3**	**1**	**1**	**2**	**-**	**-**	**-**	**-**	**1**	**10**	**0.044**
FRA3Q	**3q29**	**-**	**-**	**-**	**2**	**-**	**1**	**-**	**1**	**-**	**-**	**-**	**-**	**4**	**0.018**
FRA4A	4p16.1	8	1	4	35	32	8	8	20	12	3	3	4	138	0.612
FRA4D	4p15	1	2	-	8	5	-	3	2	2	-	1	1	25	0.111
FRA4G	**4p14**	**-**	**-**	**-**	**-**	**-**	**2**	**1**	**-**	**1**	**-**	**-**	**-**	**4**	**0.018**
FRA4H	**4p12**	**1**	**-**	**-**	**1**	**2**	**-**	**-**	**2**	**-**	**-**	**-**	**-**	**6**	**0.027**
FRA4B[b]	4q12	1	1	-	9	5	4	3	5	2	1	-	1	32	0.142
FRA4I[g]	4q21	4	1	2	23	4	17	10	4	13	-	1	2	81	0.359
FRA4F[i]	4q22	27	5	6	35	34	11	11	17	6	1	1	-	154	0.683
FRA4J[e]	4q23	-	-	-	-	-	-	-	-	-	-	-	-	0	0.000
FRA4E	4q27	14	4	3	25	14	8	6	6	4	-	2	-	86	0.382
FRA4C	4q31.1	34	25	6	95	135	41	19	92	28	2	8	5	490	2.175
FRA4[h]	**4q32**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**1**	**0.004**
FRA4K	**4q33**	**-**	**-**	**1**	**3**	**1**	**-**	**3**	**-**	**-**	**-**	**-**	**-**	**8**	**0.036**
FRA4L[f]	4q34-35	-	-	-	1	1	-	-	3	-	-	-	-	5	0.022
FRA4M[g]	4q35	1	-	1	2	1	-	1	1	1	-	-	1	9	0.040
FRA5H	**5p15**	**-**	**-**	**2**	**8**	**7**	**2**	**3**	**6**	**1**	**-**	**-**	**-**	**29**	**0.129**
FRA5E	5p14	7	3	1	6	22	6	4	7	4	1	3	2	66	0.293
FRA5A	5p13	2	2	1	13	8	6	1	7	3	-	-	-	43	0.191
FRA5I	5p11/5q11	7	2	1	19	11	7	9	12	6	1	1	1	77	0.342
FRA5J[e]	5q12	-	-	-	3	1	1	2	-	1	-	-	1	9	0.040
FRA5K[g]	5q13	-	1	-	-	6	3	-	6	3	-	-	-	19	0.084
FRA5L	**5q14**	**-**	**-**	**-**	**4**	**3**	**2**	**1**	**6**	**1**	**-**	**1**	**-**	**18**	**0.080**
FRA5D	5q15	13	11	10	33	59	24	13	20	5	-	-	-	188	0.834
FRA5F	5q21	5	2	3	24	15	23	8	12	18	1	1	4	116	0.515
FRA5M	**5q22**	**-**	**-**	**-**	**5**	**1**	**1**	**1**	**-**	**1**	**-**	**-**	**-**	**9**	**0.040**
FRA5N	**5q23**	**-**	**-**	**1**	**1**	**1**	**-**	**2**	**1**	**-**	**1**	**-**	**-**	**7**	**0.031**
FRA5C	5q31.1	2	2	1	19	19	8	4	19	6	1	2	1	84	0.373
FRA5O[g]	5q33	-	-	-	-	-	1	-	1	1	-	-	1	4	0.018
FRA5P	**5q34**	**-**	**-**	**-**	**4**	**1**	**1**	**4**	**1**	**-**	**-**	**-**	**-**	**11**	**0.049**

Table I. Continued.

All FS	Cytogenetic localization	Number of FS per band level												∑ (all subjects and band levels)	Frequency in % (all subjects and band levels)
		300-350			400-500			550-650			>650				
		III	I	II	III	I	II	III	I	II	III	I	II		
FRA5G	5q35	1	1	-	6	6	3	1	4	3	-	2		27	0.120
FRA6B	6p25.1	7	5	5	30	41	32	12	36	42	3	2	7	222	0.985
FRA6A[d]	6p23	-	-	-	-	-	-	-	2	-	-	-	-	2	0.009
FRA6C	6p22.2	-	-	-	2	1	1	1	4	5	-	-	-	14	0.062
FRA6H[i]	6p21.1	1	-	-	1	4	3	1	7	5	-	-	-	22	0.098
FRA6I	6p11/q11	1	2	1	7	4	4	6	7	2	1	1	1	37	0.164
FRA6D[b]	6q13	2	1	-	-	1	-	1	2	-	-	-	-	7	0.031
FRA6G	6q15	3	-	2	5	3	2	2	3	4	-	-	2	26	0.115
FRA6J	**6q16.3**	**3**	**-**	**-**	**9**	**2**	**-**	**3**	**-**	**2**	**-**	**-**	**-**	**19**	**0.084**
FRA6F	6q21	15	7	2	30	27	11	7	22	7	8	-	1	137	0.608
FRA6K[e]	6q22	1	2	-	8	8	7	4	8	10	2	-	2	52	0.231
FRA6L[e]	6q23	1	1	2	10	8	2	4	1	1	-	-	1	31	0.138
FRA6M[g]	6q25	1	1	2	6	4	4	2	4	1	-	2	1	28	0.124
FRA6E	6q26	50	21	5	155	153	19	56	128	25	10	4	10	636	2.823
FRA7B	7p22	3	2	1	41	21	8	16	30	37	3	3	15	180	0.799
FRA7L[e]	7p21	2	-	-	3	1	1	-	-	-	-			7	0.031
FRA7C	7p14.2	7	3	2	26	33	13	10	21	4	1	1	1	122	0.541
FRA7D	7p13	2	2	-	35	23	10	15	27	6	-	3	1	124	0.550
FRA7A[d]	7p11.2	1	-	-	3	1	-	4	-	2	-	2	-	13	0.058
FRA7J	7q11.23	19	8	2	41	49	22	10	26	6	1	-	3	187	0.830
FRA7E	7q21.2	6	1	2	45	11	8	23	7	7	1	2	-	113	0.501
FRA7F	7q22	1	-	1	7	5	4	4	1	1	-	-	-	24	0.107
FRA7K[i]	7q22-31.1	36	21	13	111	160	81	42	100	49	7	9	6	635	2.818
FRA7G	7q31.2	2	-	1	4	4	-	4	3	5	-	1	1	25	0.111
FRA7H	7q32.3	20	11	5	104	91	73	33	104	67	7	8	12	535	2.374
FRA7M	7q34	7	2	2	26	7	17	21	11	14	2	-	1	110	0.488
FRA7I	7q36	1	-	-	6	5	2	1	3	1	-	-	-	19	0.084
FRA8G	**8p23**	**1**	**-**	**-**	**4**	**3**	**-**	**2**	**4**	**1**	**-**	**-**	**-**	**15**	**0.067**
FRA8H[g]	8p21	-	-	1	3	1	2	1	1	-	-	-	-	9	0.040
FRA8I	8p11/q11	-	1	-	8	5	9	4	7	3	-	-	4	41	0.182
FRA8F	8q13	-	-	-	-	-	-	-	1	-	-	-	-	1	0.004
FRA8J[g]	8q21.3	-	-	-	3	-	-	2	2	-	-	-	-	7	0.031
FRA8B	8q22.1	20	1	1	52	27	30	23	34	14	-	-	6	208	0.923
FRA8A[d]	8q22.3	-	-	-	2	-	1	-	1	-	-	-	-	4	0.018
FRA8C	8q24.1	9	3	3	28	30	20	23	27	23	4	2	8	180	0.799
FRA8D	8q24.3	3	-	-	10	2	4	3	8	8	2	1	-	41	0.182
FRA9H[g]	9p24	-	-	-	-	2	1	2	2	-	-	-	-	7	0.031
FRA9G[i]	9p22	1	-	-	4	-	1	2	-	1	-	-	-	9	0.040
FRA9A[d]	9p21	-	1	-	4	7	3	2	5	-	1	-	-	23	0.102
FRA9I[f]	9p13	-	-	-	-	-	1	1	-	1	-	-	-	3	0.013
FRA9F[a]	9q12	6	-	2	13	22	11	14	18	9	4	2	1	102	0.453
FRA9J[e]	9q13	-	-	-	-	-	-	-	-	-	-	-	-	0	0,000
FRA9K	**9q21**	**1**	**1**	**-**	**2**	**4**	**3**	**1**	**2**	**3**	**-**	**-**	**-**	**17**	**0.075**
FRA9D	9q22.1	4	1	1	11	15	4	3	12	1	-	-	-	52	0.231
FRA9L[e]	9q31	2	-	-	1	-	1	2	1	1	-	-	-	8	0.036

Table I. Continued.

All FS	Cytogenetic localization	Number of FS per band level												∑ (all subjects and band levels)	Frequency in % (all subjects and band levels)
		300-350			400-500			550-650			>650				
		III	I	II	III	I	II	III	I	II	III	I	II		
FRA9B	9q32	22	5	3	93	65	24	44	49	28	5	-	3	341	1.513
FRA9M	**9q33**	**1**	**-**	**-**	**2**	**-**	**2**	**-**	**2**	**2**	**-**	**-**	**-**	**9**	**0.040**
FRA9N[g]	9q34	-	1	1	2	5	4	3	2	4	-	1	-	23	0.102
FRA10H	**10p15**	**2**	**1**	**1**	**4**	**4**	**4**	**1**	**1**	**3**	**-**	**-**	**1**	**22**	**0.098**
FRA10I[g]	10p12-13	2	1	1	14	1	3	2	2	-	1	-	-	27	0.120
FRA10J[g]	10p11.2	2	-	-	10	2	1	3	4	2	-	-	-	24	0.107
FRA10G	10q11.2	-	1	-	9	9	1	4	6	2	-	-	-	32	0.142
FRA10C[b]	10q21	-	-	-	7	4	2	1	5	-	-	-	1	20	0.089
FRA10D	10q22.1	7	3	1	23	16	5	5	6	3	-	-	-	69	0.306
FRA10A[a]	10q23.3	1	-	2	7	3	2	8	2	2	-	-	1	28	0.124
FRA10K[d]	10q24.2	2	-	-	7	5	-	5	3	-	-	-	-	22	0.098
FRA10E	10q25.2	-	-	1	10	7	8	6	7	2	-	-	2	43	0.191
FRA10F	10q26.1	5	1	1	32	11	4	20	10	3	2	1	2	92	0.408
FRA11J	**11p15.3-p15.4**	**1**	**-**	**-**	**1**	**3**	**-**	**1**	**1**	**1**	**-**	**-**	**-**	**8**	**0.036**
FRA11C	11p15.1	6	5	-	23	25	13	10	22	3	2	1	5	115	0.510
FRA11D	11p14.2	17	5	1	53	40	13	22	34	14	4	1	5	209	0.928
FRA11E	11p13	6	3	4	28	32	7	17	29	8	-	3	3	140	0.621
FRA11K	**11p12**	**-**	**-**	**-**	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**0.009**
FRA11L	**11p11.2**	**-**	**-**	**-**	**2**	**1**	**1**	**-**	**1**	**1**	**-**	**-**	**-**	**6**	**0.027**
FRA11M	**11p11/q11**	**1**	**-**	**1**	**8**	**7**	**2**	**7**	**3**	**2**	**2**	**-**	**2**	**35**	**0.155**
FRA11H	11q13.3	-	1	1	2	7	-	2	2	2	-	-	-	17	0.075
FRA11F	11q14.2	24	12	9	122	64	30	38	55	31	7	6	1	399	1.771
FRA11N	**11q21**	**-**	**-**	**-**	**1**	**5**	**1**	**1**	**8**	**-**	**-**	**1**	**-**	**17**	**0.075**
FRA11O	**11q22**	**1**	**-**	**-**	**3**	**-**	**1**	**2**	**2**	**-**	**-**	**-**	**-**	**9**	**0.040**
FRA11G	11q23.3	1	1	1	4	5	-	4	2	2	1	1	-	22	0.098
FRA12F	**12p13**	**1**	**-**	**-**	**1**	**2**	**-**	**1**	**-**	**1**	**-**	**-**	**-**	**6**	**0.027**
FRA12G[f]	12p12	-	-	-	2	4	-	2	-	1	1	-	-	10	0.044
FRA12H	**12p11.2**	**1**	**1**	**-**	**-**	**1**	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**5**	**0.022**
FRA12I	**12p11/q11**	**1**	**-**	**-**	**2**	**6**	**-**	**1**	**4**	**-**	**-**	**-**	**2**	**16**	**0.071**
FRA12A	12q13.1	1	-	-	1	4	3	1	2	4	1	-	-	17	0.075
FRA12J	**12q14**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**2**	**-**	**-**	**1**	**4**	**0.018**
FRA12K	**12q15**	**-**	**1**	**-**	**2**	**-**	**-**	**-**	**3**	**-**	**2**	**1**	**-**	**9**	**0.040**
FRA12B	12q21.3	7	-	1	26	8	6	13	10	6	2	1	2	82	0.364
FRA12L	**12q23.1**	**3**	**-**	**-**	**7**	**-**	**2**	**3**	**6**	**5**	**-**	**-**	**-**	**26**	**0.115**
FRA12E	12q24	4	1	3	12	14	11	6	6	6	1	-	1	65	0.288
FRA13A	13q13.2	15	7	2	63	45	23	28	32	19	2	1	2	239	1.061
FRA13G	**13q14**	**1**	**-**	**1**	**3**	**1**	**-**	**1**	**1**	**-**	**-**	**-**	**-**	**8**	**0.036**
FRA13C	13q21.2	1	-	-	6	2	6	4	5	2	-	-	2	28	0.124
FRA13E[i]	13q22	-	1	-	4	4	2	2	6	2	-	-	-	21	0.093
FRA13H[f]	13q31	-	-	-	-	-	2	-	-	1	-	-	-	3	0.013
FRA13D	13q32	1	-	2	6	10	3	4	7	3	-	1	3	40	0.178
FRA13I[g]	13q34	3	-	1	10	4	3	9	6	7	1	1	1	46	0.204
FRA14D	**14q11.2**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**1**	**1**	**-**	**-**	**-**	**3**	**0.013**
FRA14E[g]	14q13	1	-	-	9	6	4	5	8	4	1	-	1	39	0.173
FRA14A	14q21.2	3	-	1	9	4	3	3	4	3	-	-	1	31	0.138

Table I. Continued.

All FS	Cytogenetic localization	Number of FS per band level												∑ (all subjects and band levels)	Frequency in % (all subjects and band levels)
		300-350			400-500			550-650			>650				
		III	I	II	III	I	II	III	I	II	III	I	II		
FRA14F	**14q22**	**-**	**-**	**-**	**7**	**3**	**3**	**-**	**1**	**3**	**1**	**-**	**1**	**19**	**0.084**
FRA14B	14q23	6	5	4	39	54	45	23	87	35	4	7	13	322	1.429
FRA14C	14q24.1	4	3	3	12	20	18	8	9	8	1	-	-	86	0.382
FRA14G	**14q24.3**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**2**	**0.009**
FRA14H[g]	14q32	-	1	-	4	-	3	-	1	-	-	-	-	9	0.040
FRA15C	**15q11.2**	**-**	**-**	**-**	**1**	**-**	**1**	**-**	**-**	**1**	**-**	**-**	**-**	**3**	**0.013**
FRA15B[i]	15q13	1	1	-	6	2	-	-	1	-	-	-	1	12	0.053
FRA15D	**15q15**	**2**	**-**	**-**	**4**	**9**	**2**	**-**	**2**	**1**	**-**	**-**	**-**	**20**	**0.089**
FRA15E	**15q21**	**-**	**-**	**-**	**-**	**1**	**2**	**-**	**1**	**1**	**-**	**-**	**-**	**5**	**0.022**
FRA15A	15q22	-	1	-	5	2	5	1	-	1	1	-	2	18	0.080
FRA15[h]	**15q24**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**0.004**
FRA15F	**15q25**	**-**	**-**	**-**	**2**	**-**	**-**	**1**	**1**	**1**	**-**	**-**	**-**	**5**	**0.022**
FRA15G	**15q26**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**1**	**1**	**-**	**-**	**-**	**3**	**0.013**
FRA16A[d]	16p13.11	-	-	-	-	2	1	1	4	1	-	1	2	12	0.053
FRA16E[c]	16p12.1	1	-	-	-	1	1	-	1	-	-	1	1	6	0.027
FRA16F	16p11/16q11	-	-	-	3	4	3	2	1	1	-	-	1	15	0.067
FRA16G[g]	16q12	-	-	-	-	1	1	-	2	-	-	1	-	5	0.022
FRA16H	**16q13**	**-**	**-**	**-**	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**0.099**
FRA16I[e]	16q21	-	-	-	1	-	-	-	1	-	-	-	-	2	0.099
FRA16C	16q22.1	4	5	-	14	18	17	9	23	15	1	3	1	110	0.488
FRA16D	16q23.2	78	36	18	283	443	110	133	381	146	17	26	36	1707	7.576
FRA16J[e]	16q24	-	-	-	3	3	-	-	1	2	-	2	1	12	0.053
FRA17A[c]	17p12	-	-	-	2	-	4	2	2	1	-	-	-	11	0.049
FRA17C	**17p11/17q11**	**-**	**-**	**-**	**1**	**2**	**-**	**1**	**4**	**-**	**-**	**-**	**-**	**8**	**0.036**
FRA17D[f]	17q21	-	-	-	1	-	-	-	-	-	-	-	-	1	0.004
FRA17B	17q23.1	-	-	-	4	4	1	2	2	1	1	1	-	16	0.071
FRA17E[g]	17q24-25	3	-	-	10	12	4	5	9	8	1	2	-	54	0.240
FRA18D[g]	18p11.3	-	-	-	3	3	1	-	5	1	-	-	1	14	0.062
FRA18E	**18q11.2**	**-**	**-**	**-**	**1**	**1**	**1**	**1**	**2**	**-**	**-**	**1**	**1**	**8**	**0.036**
FRA18A	18q12.2	2	1	5	29	18	3	18	23	8	-	-	-	107	0.475
FRA18B	18q21.3	3	2	1	7	7	6	3	8	4	-	-	-	41	0.182
FRA18C[i]	18q22	1	-	1	2	2	1	3	1	4	-	-	-	15	0.067
FRA18F	**18q23**	**2**	**-**	**-**	**1**	**2**	**2**	**1**	**2**	**-**	**-**	**-**	**1**	**11**	**0.049**
FRA19B[d]	19p13.1	-	-	-	1	3	-	2	-	2	-	-	-	8	0.036
FRA19C	**19p11/q11**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**1**	**-**	**-**	**-**	**1**	**3**	**0.013**
FRA19A[a]	19q13	-	-	-	3	3	1	2	2	-	-	-	1	12	0.053
FRA20C	**20p13**	**-**	**-**	**-**	**2**	**7**	**4**	**-**	**9**	**1**	**-**	**-**	**1**	**24**	**0.107**
FRA20B	20p12.2	1	-	-	7	10	8	4	13	10	-	-	2	55	0.244
FRA20A[d]	20p11.23	-	-	-	-	4	1	-	-	1	-	-	-	6	0.027
FRA20D[g]	20q11.2	-	-	-	1	2	-	1	1	-	-	-	-	5	0.022
FRA20E[g]	20q13.1	1	-	-	3	4	2	3	5	1	3	-	1	23	0.102
FRA20[h]	**20q13.3**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**1**	**0.004**
FRA21[h]	**21q11.2**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**0.004**
FRA21A[g]	21q21	-	-	-	1	1	-	1	2	-	-	-	-	5	0.022
FRA21B	**21q22.1**	**1**	**-**	**-**	**3**	**1**	**1**	**-**	**1**	**3**	**-**	**1**	**-**	**11**	**0.049**

Table I. Continued.

All FS	Cytogenetic localization	Number of FS per band level												∑ (all subjects and band levels)	Frequency in % (all subjects and band levels)
		300-350			400-500			550-650			>650				
		III	I	II	III	I	II	III	I	II	III	I	II		
FRA22B	22q12.2	2	-	2	12	10	4	5	13	11	-	-	3	62	0.275
FRA22A[d]	22q13.2	3	-	-	17	10	4	5	28	5	1	-	1	74	0.328
FRAXB	Xp22.31	70	24	30	261	267	130	93	201	116	13	13	20	1238	5.494
FRAXG[g]	Xp11.2	-	-	-	1	-	-	-	1	-	-	-	-	2	0.009
FRAXH	**Xp11/q11**	**-**	**1**	**-**	**7**	**5**	**-**	**4**	**9**	**2**	**1**	**2**	**-**	**31**	**0.138**
FRAXI[g]	Xq13	-	-	2	11	6	2	2	8	4	1	-	1	37	0.164
FRAXC	Xq22.1	31	13	8	109	90	40	40	83	44	6	3	11	478	2.121
FRAXJ	**Xq25**	**1**	**-**	**-**	**3**	**1**	**-**	**1**	**2**	**1**	**1**	**-**	**-**	**10**	**0.044**
FRAXK[g]	Xq26	-	-	-	-	1	1	-	-	-	-	-	-	2	0.009
FRAXD	Xq27.2	2	1	1	7	17	2	2	14	-	1	-	-	47	0.209
FRAXE	Xq28	-	-	-	-	1	-	-	1	-	-	1	1	4	0.018
∑ (all FS)		1344	516	358	4978	4625	2032	2059	3806	1871	282	260	406	22537	0.276

Entries in bold are all new identified FS of the current study with new nomenclature. [a]5-azacytidin type, [b]BrdU type, [c]distamycin A type, [d]folic acid type, [e](37) with new nomenclature, [f](23) with new nomenclature, [g](19) with new nomenclature, [h]new identified FS but only one time recorded and not included in the nomenclature, [i]FS that were already assigned to the nomenclature by other authors but not included in genome browsers.

Table II. Number of FS and average distance in Mb between FS for single chromosomes.

Chromosome	1	2	3	4	5	6	7	8	9	10	11	12
Number of FS	17	21	17	13	15	13	13	9	12	10	12	10
Interval in Mb	14.53	11.52	11.76	14.69	12.07	13.15	12.23	16.22	11.67	13.35	11.17	13.20
Chromosome	13	14	15	16	17	18	19	20	21	22	X	Y
Number of FS	8	8	7	9	5	6	3	5	2	2	9	1
Interval in Mb	14.25	13.25	14.29	9.89	15.80	12.67	21.33	12.40	23.50	25.00	17.22	29.00

look on inter-individual and/or ethnic influences on FS frequency variation, larger population studies on comparable conditions would be needed.

New FS. The current study identified 61 until now not reported FS and 7 unique break events that were not reported before. The frequency of these new sites varies between 0.009% (FRA14G) and 0.231% (FRA3E). Other, well-established aphidicolin-induced sites range from 0.067% (FRA1E) to 14.153% (FRA3B), indicating that the newly described sites have a low expression rate and therefore were not detected or reported previously. Although some authors stress the necessity for fragility to be defined as a statistical phenomenon (e.g. 12,31), the main part of fragile sites is expressed in low frequencies, sometimes limited to single individuals and depends on several other factors such as aphidicolin concentration, gender or ethnic origin. Furthermore, complicating is the definition of a fragile site since several authors use different definitions or count also single breaks as fragile sites (11,30,32). Therefore, we decided to use a more practical definition where all sites are included that were observed more than once in this study or were already published elsewhere to minimize false positive random background breaks.

Only little is known about the influence of local chromatin structure for FS expression. Obviously FS are overrepresented in GTG light bands. This holds also true for the new identified FS, 59% of which appears in GTG-light bands. Recently, an unusual chromatin structure or failure of chromatin condensation was postulated as inductors of FS (33).

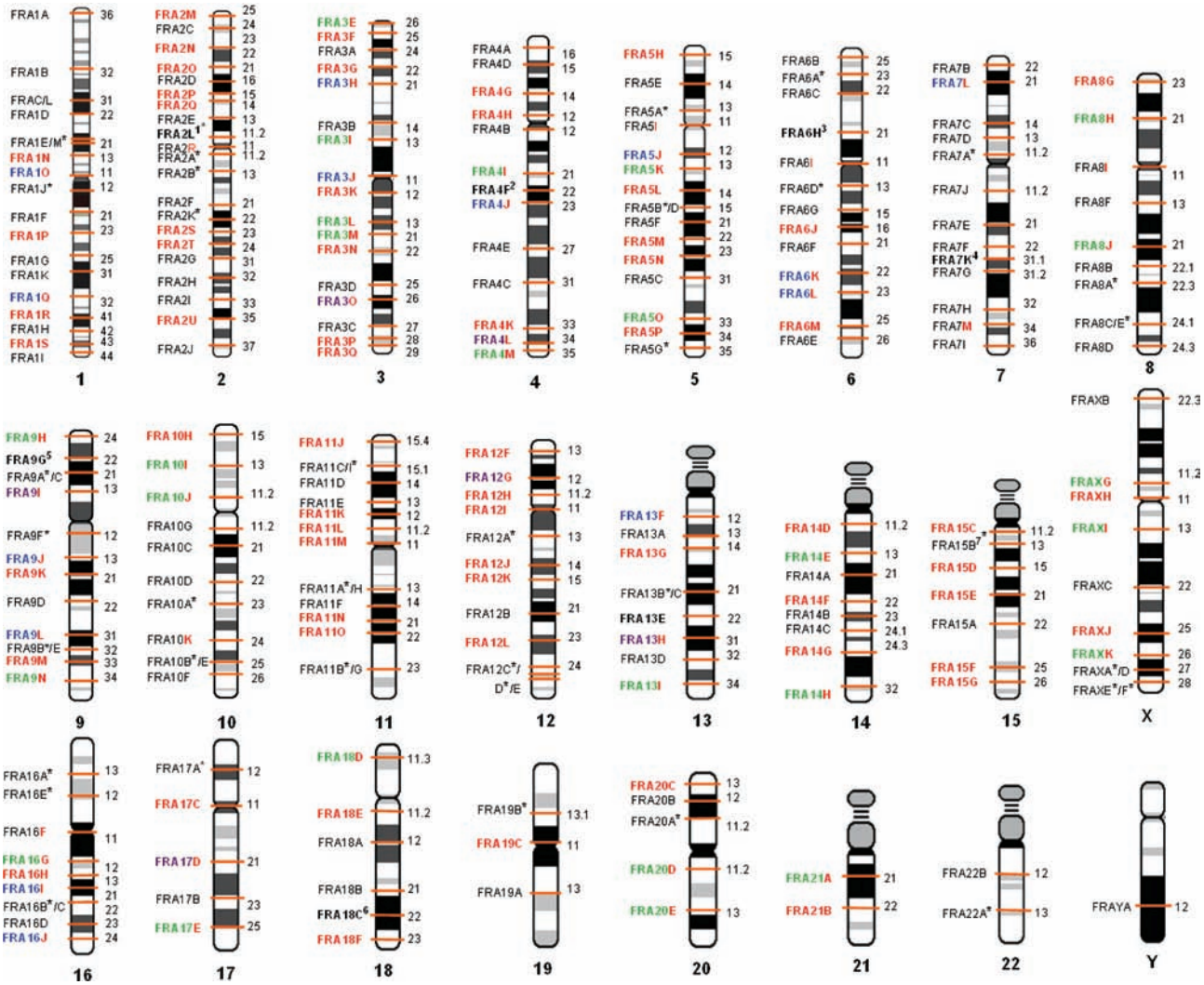

Figure 3. Overview of all known and newly described FS (orange line in ideograms). Left to the ideograms is shown the FS nomenclature, to the right the cytoband. Bold text FS are not listed in NCBI 36.3 genome browser; black, are NCBI 36.3 listed FS; red, are newly described FS in the current study; blue, taken from (37); green, taken from (19); light blue, taken from (19); purple, taken from (23); *FS not reported as aphidicolin type before; 1: (34), 2: (13), 3: (15), 4: (14), 5: (16), 6: (17), 7: (38).

Table III. Comparison of FS, not reported as aphidicolin inducible before, with literature data.[a]

FS	Cytogenetic localization	Frequency in the current study (%) n=25,000	Frequency in (%)			
			Manjunatha *et al*, 2002 (39) n=100	Takahashi *et al*, 1988 (40) n=?	Rao *et al*, 1988 (32) n=800	Tastemir *et al*, 2006 (41) n=1,443
FRA1Q	1q32	0.098	-	-	-	1.68
FRA2A	2q11.2	0.089	1.0	-	-	-
FRA5J	5q12	0.040	-	-	0.4	-
FRA10C	10q21	0.089	-	-	-	1.01
FRA17A	17p12	0.049	-	0.1	-	-
FRAXK	Xq26	0.009	-	-	-	0.33

[a](39): 5-fluorodeoxyuridin (FUdR); (40): distamycin A; (32): FUdR; (41): folate absence; n = number of studied metaphases.

Nomenclature. The common nomenclature system for FS includes the abbreviation FRA followed by chromosome number and a letter starting with A arranged in a linear order from pter to qter. Chromosome and chromatide breaks in this study were counted as new FS and included in the nomenclature when they were observed more than one time. Seven unique breaks were also recorded (Fig. 2 and Table I), but not included in the nomenclature (Fig. 3 and Table I).

The current study identified 230 compared to only 129 rare and common FS, which are listed in the NCBI genome browser. Even more complicating is the fact that 15 of these sites map at the same cytogenetic region but have different names because of different ways of induction (NCBI genome browser, Fig. 3). Additionally, 52 identified and in parts mapped FS were already published, but not included in the current genome browser versions (13,14,15,16,17,19,23,34). Most of these sites were confirmed in this study which underlines their eligibility to be appreciated as FS.

FS classification. FS are commonly classified in rare FS that can be induced by folate absence, distamycin A, 5-fluorodeoxyuridin or BrdU, and in common FS inducible by aphidicolin, BrdU or 5-azacytidine. In the current study exclusively aphidicolin induction was used, and surprisingly nearly every type of rare and common FS could be recorded. Overall, this makes the classification in rare and common FS at least questionable. The main reason to explain this surprising finding might be a relative small amount of FS studied in near-comparable studies. Differences to literature data were also observed in the frequency of single sites when the induction was done in a different way (Table III).

Based on frequency differences within expression rates of so-called common FS from 14.153% (FRA3B, this study) to 0.004% (e.g. FRA17D, this study) some authors (12,24,28) suggested a classification in high (HFFS) and low frequency FS (LFFS). This classification seems to be more reliable than the historical division in common and rare FS. Also a classification based on the chemical induction of FS seems questionable; particularly as in this study every type of FS could be induced by aphidicolin.

Conclusion. Location of FS seems to be independent of the chemical agents used. Thus, a classification in different induction types is no longer warranted. According to Hecht *et al* (4) and others we prefer a classification of high and low frequency FS. In order to prevent double naming of FS in the same cytogenetic location, caused historically by different modes of induction, an expanded definition of FS into fragile regions is helpful as it becomes more and more clear from molecular mapping data that especially aphidicolin inducible FS do not break at defined sequences but in breakage-prone regions up to 10 Mb (35,36) where the break is most likely to appear.

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