

## EVIDENCE FOR CORRELATION OF FRAGILE SITES AND CHROMOSOMAL BREAKPOINTS IN CARRIERS OF CONSTITUTIONAL BALANCED CHROMOSOMAL REARRANGEMENTS

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### ABSTRACT

A molecular cytogenetic study of 251 cases with balanced chromosomal rearrangements detected due to infertility of unclear origin or in prenatal diagnostics with a later normal outcome was done. Balanced translocations (127 cases), inversions (105 cases), insertions (three cases), balanced complex rearrangements (four cases), or derivative chromosomes leading to no imbalance (12 cases), were studied by multicolor banding (MCB) and/or subcentromeric multicolor fluorescence *in situ* hybridization (subcenM-FISH). Five-hundred and twenty-nine break-events were characterized by molecular cytogenetics. Only 150 of these were unique breakpoints, the remainder were observed between two and 10 times. According to the results obtained, there was cytogenetic co-localization of fragile site (FS) in ~71% of the studied 529 break-events. Nine selected cases with evidence for breakpoints within FS were further analyzed by FS-specific bacterial artificial chromosome (BAC) probes; only one did not show a co-localization. Further detailed molecular analysis will be necessary to characterize the mechanisms and genetic basis for this phenomenon.

**Key words:** Fragile sites (FS), Constitutional balanced chromosomal rearrangements, Fluorescent *in situ* hybridization (FISH), Co-localization.

### INTRODUCTION

The observation of balanced chromosomal aberration is common in patients with fertility problems and/or repeated abortions. There is evidence that the chromosomal breakpoints involved in constitutional balanced chromosomal rearrangements such as balanced translocations, inversions, insertions, balanced complex rearrangements, or derivative chromosomes leading to no imbalance, appear in a non random distribution along the human karyotype [1]. In a previous study, we showed that ~88% of such break-events arose in GTG-light bands, 21% co-localized with intrachromosomal telomeric-like sequences (ITS), 35.8% were at or near the Mariner transposon-like elements (MTLE), and at least 45% could have had a correlation with fragile sites (FS) [1]. Surprisingly, the idea that such “breakpoint prone” regions could be involved in chromosomal rearrangements in general, and thus also in constitutional balanced chromosomal rearrangements, is relatively new [2,3]. Also, the possible link of low-copy repeat clusters and recurrent human translocations is discussed [4].

Recently, 230 FS were reported, including 61 as yet unreported ones [5]. Thus, these FS were aligned with breakpoints involved in constitutional chromosomal rearrangements.

Supplementary material available online at:  
<http://www.bjmg.edu.mk/record.asp?recordid=589>

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**Table 1.** Test for co-localization of fragile sites and breakpoints in selected cases.

Case	Karyotype	Fragile Site	Co-localization (BAC probe)
D-9	46,XX,der(1)t(1;acro)(p36.33;p10)	FRA1A	[+] (RP11-19M4)
T10	46,XX,t(1;4)p36.3;q31.3)	FRA1A	[+] (RP11-19M4)
T-10	46,XX,t(1;4)p36.3;q31.3)	FRA4C	[+] (RP11-1289C17)
T-92	46,XX,t(9;14)(q21.1;q11.1)	FRA9K	[+] (RP11-280P22)
T-61	46,XX,t(5;10)(q33.3;q26.1)	FRA10F	[+] (RP11-310M21)
T-68	46,XY,t(6;11)(q21.3;q22.3~23.1)	FRA11G	[+] (RP11-172C16)
T-100	46,XY,t(11;13)(q23.3;q34)	FRA11G	[-] (RP11-172C16)
T-107	46,XY,t(11;18)(q23.1~23.2;p11.32)	FRA11G	[++] (RP11-172C16)
C-1	46,XX,der(1)t(10;11;1)(10pter→10p11.2::11q25→11q23::1p34.3→1qter),der(10)t(10;11)(p11.2;q25),der(11)t(1;11)(p34.3;q23),t(13;18)(q14;11.32)	FRA11G	[+] (RP11-172C16)

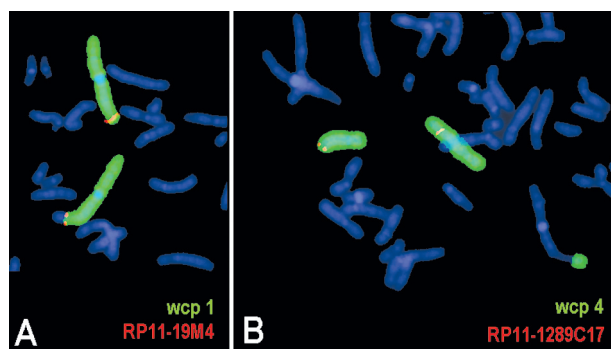
[+]: signal less than 1 diameter of BAC signal away from breakpoint; [-]: signal more than 1 diameter of BAC signal away from breakpoint; [++]: split signal.

## MATERIALS AND METHODS

Two-hundred and fifty-one patients were studied cytogenetically due to different reasons such as infertility of unclear origin, previous pregnancies with unbalanced outcome, or detection of a balanced rearrangement in prenatal diagnostics with later birth of normal children. In all of them different cytogenetic aberrations were detected such as balanced translocations (127 cases), inversions (105 cases), insertions (three cases), balanced complex rearrangements (four cases), or derivative chromosomes leading to no imbalance (12 cases) (Supplementary Table 1). Cytogenetic preparations were done according to standard proce-

dures and the results are listed in the Supplementary Table 1.

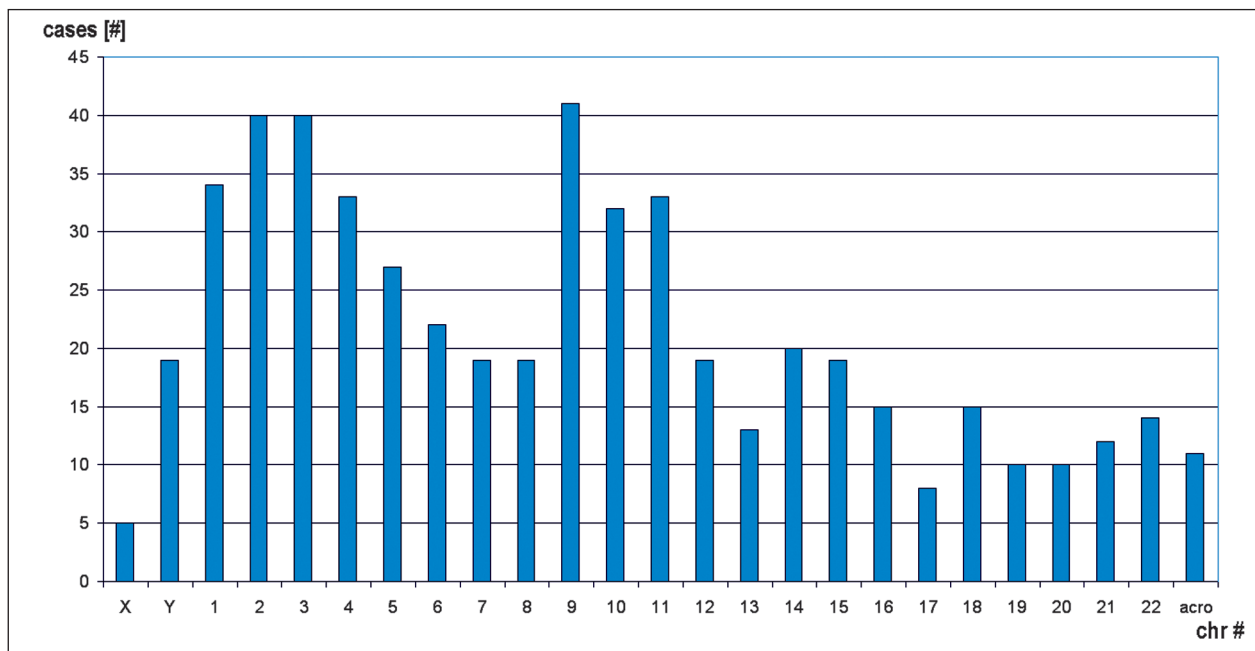
To further characterize the chromosomal breakpoints involved, multicolor banding (MCB) [6,7] and/or subcentromeric multicolor fluorescence *in situ* hybridization (subcenM-FISH) [8] were applied. To study a possible correlation of FS and constitutional chromosomal breakpoints (Supplementary Table 2), bacterial artificial chromosome (BAC) probes specific for FRA1A (RP11-19M4), FRA4C (RP11-1289C17), FRA9K (RP11-280P22), FRA10F (RP11-310M21) and FRA11G (RP11-172C16) were applied together with corresponding whole chromosome painting (wcp) probes in two-color-FISH experiments in nine selected cases, in which still cell suspension was available (Figure 1; Table 1). As FS usually span several megabases of DNA [5], a co-localization was not only suggested if a signal splitting appeared (as in case T-107), but also if the specific signal was less than 1 diameter of BAC signal away from the breakpoint itself, as highlighted by the corresponding wcp probe. This cut-off was chosen, as it is known that wcp probes have a flaring effect, and apparently label a larger chromosomal part by fluorescence rather than “biologically true.”



**Figure 1.** Partial metaphases of case T-10 having a t(1;4)(p36.3;q31.3). Two-color FISH using FS-spanning BAC probes with wcp showed evidence for a co-localization of FS FRA1A (RP11-19M4) and FRA4C (RP11-1289C17).

## RESULTS AND DISCUSSION

For 251 patients with different constitutional chromosomal aberrations, MCB and/or subcenM-FISH



**Figure 2.** Chromosomal distribution of breaks along all 24 human chromosomes, including the 12 break-events in short arms derived from any of the acrocentric chromosomes (acro).

[6-8] exactly characterized the involved breakpoints; the corresponding results are summarized in the Supplementary Table 1. Overall, 529 break-events were characterized by molecular cytogenetics. It turned out that only 150 of these were unique break-events, the remainder have been observed between two and 10 times within the same chromosomal sub-bands (see Supplementary Table 2). Based on the FS published in [5] there was (molecular) cytogenetic co-localization in ~71% of the studied break-events, *i.e.*, in 318 of 529 (Supplementary Table 2).

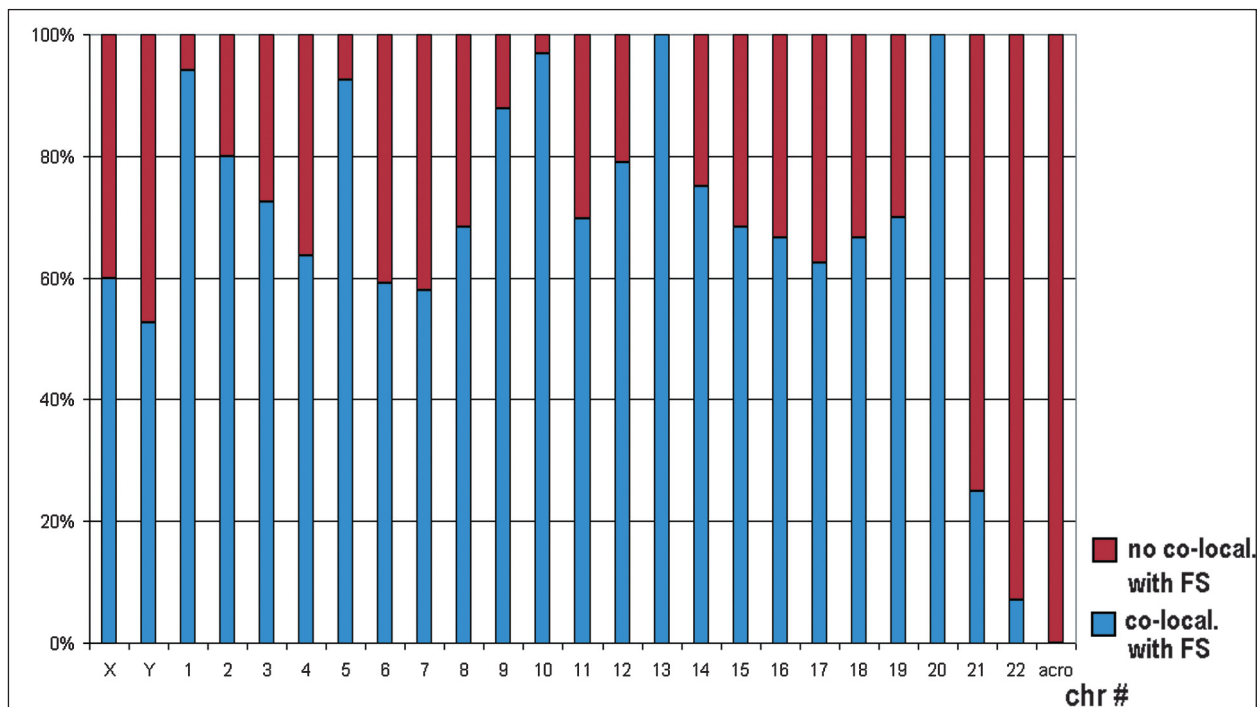
As summarized in Figure 2, the breakpoints detected in the 251 studied cases were not distributed according to the size of the chromosomes, as one might expect. On the contrary, the chromosomes most frequently hit by chromosomal breaks were #9, #2 and #3, followed by #1, #4, #11, #10 and #5. The rarest involved chromosomes were the X-chromosome and chromosomes #17, #19-22 and #13. This supports the hypothesis that there are mechanisms preferably producing chromosomal breaks at special regions, such as those recently shown for low-copy repeats [4], and for FS in this study and also a previous one [1].

Thus, in Figure 3 (molecular) cytogenetic co-localization of FS and the 529 observed breakpoints are visualized per chromosome. For chromosomes #1, #9

and #10, which are in the group with high involvement in constitutional chromosomal rearrangements, there are also high percentages of cases with a correlation of breakpoint- and FS-co-localization. The same holds true, in reverse, for chromosomes #21 and #22, which are not often involved in the studied chromosomal break-events, and having below 25% of association with FS in the break-prone regions (Figure 3).

The finding that FS play a role in formation of constitutional chromosomal rearrangements was further supported by the following experimental setup: nine selected cases with evidence for breakpoints within or near FS were additionally analyzed by FS-specific BAC probes (example in Figure 1), and strikingly, only one (case T-100), did not show a co-localization with the corresponding FS in chromosome 11. All other eight cases showed either a complete overlap (breakpoint spanning the BAC probe) or a tight co-localization of FS-BAC and studied breakpoint (Table 1).

Further detailed molecular analysis is necessary to characterize the mechanisms and genetic basis for the phenomenon described here. Pathways such as those discussed by Mani and Chinnaiyan [9] must be involved, however, these models still lack proteins/enzymes involved FS-formation.



**Figure 3.** Co-localization of observed breakpoints with FS per chromosome.

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