Forty-eight new cases with infertility due to balanced chromosomal rearrangements: Detailed molecular cytogenetic analysis of the 90 involved breakpoints

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Abstract. A molecular cytogenetic study was performed on 48 infertile patients who were identified as carriers of balanced translocations (40 cases), inversions (6 cases) or insertions (2 cases) by means of banding cytogenetics. Cases with a Robertsonian translocation or pericentric inversion 2 or 9 were not included. In summary, 100 break-events occurred in these patients, and 90 different chromosomal regions were involved. Thus, this study confirmed the presence of abnormal karyotypes in a subgroup of patients seeking infertility treatment. Breaks were demonstrated to appear preferentially in GTG-light bands in these patients. Furthermore, the observed breakpoints were associated with genomic regions prone to instability due to the presence of segmental duplications. Nonetheless, further detailed molecular analysis will be necessary in the future to characterize the mechanisms and genetic basis for this phenomenon.

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Introduction

According to Shah *et al* (1) infertility is defined as the inability to conceive after one year of regular unprotected intercourse and accounts for one in six couples wishing to start a family. A range of factors may influence fertility. Besides hormone status, age, lack of exercise, obesity or infectious disease, infertility factors may be immunological, psychological, resulting from surgery or blockage, or associated with defined abnormalities in the gametes (such as azoospermia). In ~20% of couples the reason for infertility remains unexplained (2). As most, if not all, of the above listed factors are likely to have a genetic component, it is difficult to consider accurately the genetic contribution to reduced fertility. Nevertheless, genetic and/or karyotypic analysis revealed association of specific (cyto)genetic conditions with infertility phenotypes, such as mutations in the cystic fibrosis (CFTR) gene, mutations or microdeletions in Y chromosome genes, or the presence of constitutional numerical or structural chromosomal aberrations (1). The latter, such as sex-chromosome aberrations, the presence of small supernumerary marker chromosomes (3), constitutional inversions or translocations can lead both to fertility problems and repeated abortions (1). In translocation and in inversion carriers reduced fertility is mediated by the fact that the rearranged chromosomes need to synapse through

a pairing cross, in order to progress through meiosis. Therefore, in the presence of a rearrangement a multitude of different mechanisms can lead to pairing mistakes during meiosis I or II; only one of these possibilities builds an imbalance to partial trisomy and partial monosomy in regions that are involved in reciprocal translocation or inversion (reviewed in ref. 1). Thus, a balanced chromosomal abnormality in one of the parents is found in up to 5-7% of couples who experience multiple spontaneous abortions; these patients form a special subgroup within the heterogeneous clinical group 'infertile couples' (4).

To the best of our knowledge, here we present the largest molecular cytogenetic study on otherwise healthy persons with 'uncommon' balanced cytogenetic aberrations detected due to infertility problems. Cases with the more common Robertsonian translocations or pericentric inversions of chromosomes 2 or 9 (5-6) were not included here. Twelve males and 36 females with a family history of repeated abortions and/or a child with congenital malformations, or unexplained infertility were studied by banding and molecular high-resolution cytogenetics. The breakpoints were characterized in detail and are discussed concerning the type of detected rearrangements, distribution of breaks within GTG-light and -dark bands, and the possible correlation of characterized breakpoints and known location of segmental duplications within the human genome.

Materials and methods

The 48 patients with fertility problems included in this study are summarized in Table I. In 21 cases a history of repeated abortions was observed in partnership, in 4 cases each an unbalanced rearrangement was detected in an unborn fetus or a newborn child with congenital malformations, and in 19 cases there was unexplained infertility. Metaphase chromosome preparations were obtained from PHA-stimulated lymphocyte cultures for each of the studied patients according to standard procedures. Only carriers of 'uncommon' balanced chromosomal rearrangements were included in this study; i.e. no carriers of Robertsonian translocations or pericentric inversions of chromosomes 2 or 9.

Fluorescence *in situ* hybridization (FISH) was performed using well-established protocol. High-resolution multicolor banding (MCB) and subcentromere-specific multicolor FISH (subcenM-FISH) were performed as described previously (7-8). Commercially available probes were used according to the manufacturer's instructions (Abbott/Vysis) and are listed in Table I. Ten to twenty metaphase spreads were analyzed, each using a fluorescence microscope (Axioplan 2 mot, Zeiss) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes and the counterstain DAPI (diaminophenylindol). Image capturing and processing were carried out using an isis mFISH imaging system (MetaSystems, Altlussheim, Germany) for the MCB evaluation.

Results

The obtained results are listed in Table I. In the 48 reported cases all 24 human chromosomes were involved in the observed rearrangements, each single chromosome at least one time (Fig. 1). In summary, 100 break-events were observed in 90 different chromosomal regions. Five breakpoints were molecularly cytogenetically identical in two cases each, and one breakpoint even in three cases (Table I; Fig. 1). All rearrangements were molecular cytogenetically balanced.

The chromosomal breakpoints were detected in an initial step by GTG-banding. In a second step they were characterized in detail by MCB and, if convenient, additional locus-specific probes were applied (Table I). In summary, only 17/100 breakpoints could be confirmed as determined by GTG-banding alone; 47/100 breakpoints had to be corrected and 36/100 were determined exclusively by MCB; namely, 47 of the 64 breakpoints (73%) were established incorrectly after GTG-banding alone.

In 38 cases a 'simple' balanced translocation between two chromosomes was present. Two cases each showed either a complex balanced translocation involving three different chromosomes or an insertion of chromosomal material into another chromosome, and in 6 cases an inversion took place.

Evident from Fig. 1 and Table II, 57 of the break-events occurred in GTG-light bands, 3 in hemiheterochromatic regions, 25 in GTG-dark bands and 5 were localized in the transient area of a GTG-dark to a GTG-light band.

Discussion

One hundred break-events were studied in 48 infertility patients by multicolor banding. One hundred break-events in 48 patients referred for molecular-cytogenetic characterization due to unexplained infertility, repeated abortions and/or born or unborn children with an 'uncommon' chromosomal imbalance are reported here. All patients, except 'unexplained infertility' were clinically normal and not previously reported in the literature; only case 26 was previously described (9). To the best of our knowledge this is the largest such study ever reported.

As previously shown, molecular cytogenetics, especially high-resolution multicolor banding (MCB) (7) enabled a much better characterization of chromosomal breakpoints than GTG-banding. It is striking that 73% of the GTG-banding-characterized breakpoints had to be reassessed after application of MCB. However, similar data was obtained in previous comparable MCB studies of leukemia and clinical genetics cases (10-12). The high reliability of MCB results has been proven previously by checking all (12) or a subset of the results by other locus-specific probes [(11) and present study, Table I]. Thus, in summary the high rate of GTG-banding results needing revision is alarming and underlines the need for molecular cytogenetic validation. This is preferable in all cases but especially in those which are published and are intended to be used for later genotype-phenotype correlations.

As all rearrangements were balanced according to cytogenetics and molecular cytogenetics and all patients were clinically normal (apart from infertility), no microarray-based comparative genomic hybridization (array CGH) analysis (13) of the breakpoints was deemed necessary (14). However, this type of analysis must be applied in cases of

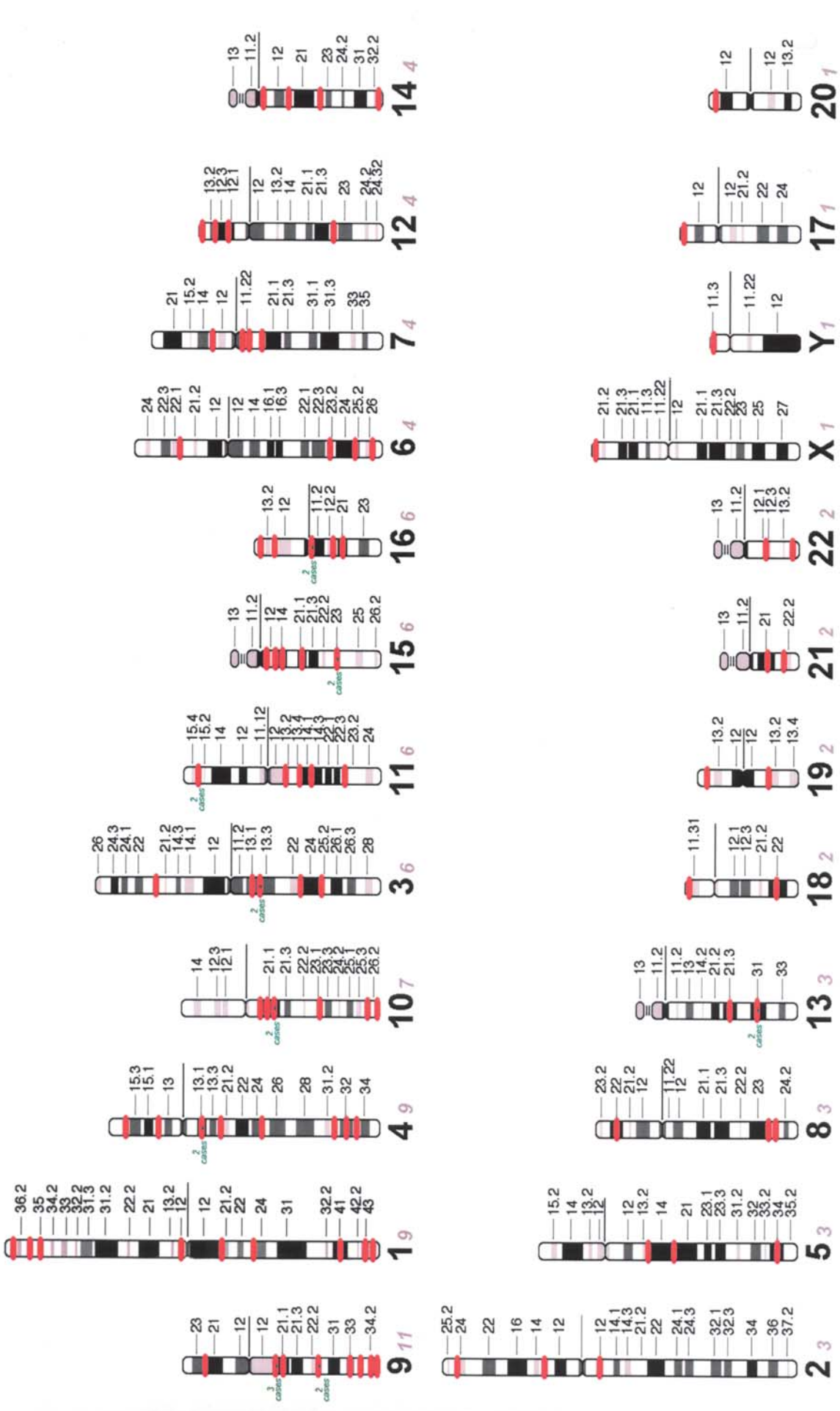

Figure 1. Depiction of breakpoint distribution based on MCB data collected in Table II. The chromosomes are ordered according to the number of breaks observed in this study, starting with chromosome 9 (11 breaks) and finishing with chromosome 20 (1 break). Below each chromosome the black numeral indicates the chromosome number and the smaller grey one, the number of breaks present in the specific chromosome. Green lettering indicates if a breakpoint was observed in more than one case.

Table I. Listed are the 48 studied infertility cases.[a]

Case no.	Cytogenetic results	Molecular cytogenetic results	Applied FISH method
Females with repeated abortions			
1	46,XX,t(1;2)(p35;p14)	t(1;2)(p36.1;p13)	MCB 1, 2
2	46,XX,t(1;10)(q23.1;q11.2)	t(1;10)(q23.3;q11.23)	MCB 1, 10; subcenM 10
3	46,XX,t(2;6)(p24.2;q25.1)	t(2;6)(p24.2;q25.1)	MCB 2, 6
4	46,XX,t(3;6)	t(3;6)(q13.2;q23)	MCB 3, 6
5	46,XX,t(3;9)	t(3;9)(q25.1;p22)	MCB 3, 9; subtel 9p
6	46,XX,t(3;19)(p21.3;q13.1)	t(3;19)(p21.3;q13.13)	MCB 3, 19
7	46,XX,t(4;9)(q35;q22)	t(4;9)(q33;q13)	MCB 4, 9
8	46,XX,t(4;10)(q12;q22.3)	t(4;10)(q13.1;q23.1)	MCB 4, 10; subcenM 4
9	46,XX,t(4;22)(q21.2;q11.2)	t(4;22)(q32;q12)	MCB 4; subcenM 22
10	46,XX,t(5;7;12)	t(5;7;12)(q34;q11.21;p12.2)	MCB 5, 7, 12
11	46,XX,t(5;8)	t(5;8)(q15;q23~24.1)	MCB 5, 8
12	46,XX,t(5;16)(q13;p13.1~13.3)	t(5;16)(q13.3;p13.3)	MCB 5, 16; subtel 16p
13	46,XX,t(7;9)(p13;q22)	t(7;9)(p13;q22.3)	MCB 7, 9
14	46,XX,t(7;14)(q11.23;q22)	t(7;14)(q11.23;q22)	MCB 7, 14
15	46,XX,t(8;10)(p23;q22.1)	t(8;10)(p22;q21.2)	MCB 8, 10
16	46,XX,t(9;15)(q21;q21)	t(9;15)(q13;q21.1)	MCB 9, 15; subcenM 9
17	46,XX,t(9;16)(q34;p13.1)	t(9;16)(q34.3;p13.1)	MCB 9, 16
18	46,XX,t(11;15)(p15;q25)	t(11;15)(p15.3;q23)	MCB 11, 15
19	46,XX,t(11;15)(q13.5;q13)	t(11;15)(q13.1;q14)	MCB 11, 15
20	46,XX,t(11;18)(q22;q22)	t(11;18)(q14.2;q22)	MCB 11, 18
Partner of female with repeated abortions			
21	46,XY,t(2;14)	t(2;14)(q12;q32.33)	MCB 2, 14; subcenM 2; subtel 14q
Aberration detected in pregnancy with fetus carrying an imbalance			
22	46,XX,ins(1;3)(q44;q21q25)	ins(1;3)(q43;q13.2q23)	MCB 1, 3
23	46,XX,t(1;4)(p36.3;q31.3)	t(1;4)(p36.3;q31.3)	MCB 1, 4; subtel 1p
24	46,XY,t(12;20)(q22;p11.2)	t(12;20)(q22;p13)	MCB 12; subcenM 20; subtel 20p
25	46,XY,ins(15;13)(q13;q32q34)	ins(15;13)(q13;q31.2q21.3)	MCB 13, 15; LSI UBE3A; LSI PML; Subtel 13q
Aberration detected in parent of abnormal child carrying an imbalance			
26	46,XY,t(1;17)	t(1;17)(q44;p13.2)	MCB 1, 17; subtel 1q, 17p
27	46,XX,t(6;22)	t(6;22)(q26~q27;q13.32)	MCB 6; subtel 6q, 22q
28	46,XX,t(9;14)	t(9;14)(q21.1;q11.1)	MCB 9, 14; subcenM 9, 14
29	46,XX,t(10;13)	t(10;13)(q26.3;q31.2)	MCB 10, 13; subtel 10q
Unexplained infertility in partnership			
30	46,X,t(X;11)[28]/45,t(X;11)	46,X,der(X)t(X;11)(Xqter->Xp22.32~22.33::11q13.5->11qter),der(11)t(Y;?X;11)(Ypter->Yp11.3::?Xp22.32~22.33->?Xp22.33::11q13.5->11pter)[65]/45,der(X)t(X;11)(Xqter->Xp22.32~22.33::11q13.5->11qter),der(11)t(Y;?X;11)(Ypter->Yp11.3::?Xp22.32~22.33->?Xp22.33::11q13.5->11pter)[5]	MCB X, 11; subcenM 11; LSI KAL; LSI SRY; subtel Xp~Yp
31	46,XX,inv(1)	inv(1)(p12q21.1)	MCB 1

Table I. Continued.

Case no.	Cytogenetic results	Molecular cytogenetic results	Applied FISH method
32	46,XX,t(1;7)(q24.1;q11.23)	t(1;7)(q41;q11.22)	MCB 1, 7; LSI ELN; subcenM 7
33	46,XX,t(1;9)	t(1;9)(p35;q22.3)	MCB 1, 9
34	46,XX,t(3;4)(q12;q12)	t(3;4)(q13.1;q21.1)	MCB 3, 4; subcenM 3
35	46,XX,der(4)(q)	inv(4)(q13.1q25)	MCB 4
36	46,XY,t(4;16)(p16.1;p11.2)	t(4;16)(p16.1;q11.1)	MCB 4; subcenM 16
37	46,XY,t(4;21)(p14;q22.1)	t(4;21)(p14;q22.1)	MCB 4, 21
38	46,XX,t(6;19)(p12.1;p12)	t(6;19)(p21.3;p13.3)	MCB 6, 19; subcenM 19
39	46,XY,t(8;12)	t(8;12)(q24.1;p13.1)	MCB 8, 12
40	46,XX,der(9)(q)	inv(9)(q33q34.2)	MCB 9; LSI bcr/abl; subtel 9q
41	46,XX,der(9)	inv(9)(q13q34.1)	MCB 9
42	46,XX,inv(10)	inv(10)(q21.1q26.1)	MCB 10
43	46,XX,t(10;16)(q11.2;q12.1)	t(10;16)(q21.2;q12.2~13)	MCB 10, 16; subcenM 10, 16
44	46,XY,t(11;15)(p15;p13)	t(11;15)(p15.3;p11.2)	MCB 11, 15
45	46,XY,t(11;18)(q23.3;p11.32)	t(11;18)(q23.1;p11.32)	MCB 11, 18; subtel 11q, 18p
46	46,XY,t(12;15)(p13.3;q24)	t(12;15)(p13.3;q23)	MCB 12, 15/3
47	46,XY,t(14;21)(q22;q22.1)	t(14;21)(q13;q21)	MCB 14, 21; subcenM 14, 21
48	46,XX,inv(16)(q11.2q13)	inv(16)(q11.1q21)	MCB 16; subcenM 16

[a]The patients were grouped according to the reason for cytogenetic study. The results of GTG-banding (cytogenetic results) and the subsequent application of molecular cytogenetics are listed together with the applied FISH methods. abl, abl-oncogene in 9q34; bcr, breakpoint cluster region in 22q11.2; ELN, elastin gene in 7q11.2; FISH, fluorescence *in situ* hybridization; KAL, Kallmann syndrome 1 gene in Xp22.3; LSI, commercially available locus-specific probe (Abbott/Vysis); MCB, multicolor banding; PML, inducer of acute promyelocytic leukemia gene in 15q22; SRY, sex-determining region Y in Yp11.3; subtel, commercially available subtelomeric probe (Abbott/Vysis); subcenM, subcentromere-specific multicolor FISH; and UBE3A, ubiquitin-protein ligase E3A in 15q13.

developmental delay and dysmorphic features and normal or apparently balanced karyotypes (15).

Types of rearrangements observed

Translocations. As expected the largest proportion of detected rearrangements in the studied 48 patients constituted cytogenetically balanced translocations; 83% of the cases had such a kind of rearrangement. In the majority of them only two chromosomes were involved, while in 2/40 balanced translocation cases three different chromosomes were contributing breakpoints. In the literature there are many reported cases on infertile couples in which one partner had such a rearrangement. However, similar to the aforementioned and discussed discordance of GTG-banding and molecular cytogenetic results confronted within this study there are few comparable cases available in the literature. What can be deduced from the literature is, that complex chromosomal rearrangements as in cases 10 and 30 are relatively rare events in infertility cases and can involve up to 5 or more different chromosomes (16-17). The most complex rearrangement observed in this study was case 30 involving chromosome X, Y and 11. The SRY-region was translocated here to the der(11); no similar cytogenetic case of an 'XX-male' has been previously reported.

Insertions. Similar to complex chromosomal rearrangements, insertions of chromosomal material into another chromosome are extremely rare events. Here two such cases were found among the reported 48. To the best of our knowledge only three cytogenetically comparable cases with balanced insertion and infertility problems have been previously reported (18-20).

Inversions. In concordance with the literature, inversions were seen more frequently in connection with infertility; 6/48 cases (12.5%) had an inversion. Apart from the frequently reported pericentric inversions of chromosomes 2 and 9 in connection with infertility (8) other unique inversions were reported in ~22% of these cases (20).

Distribution of breakpoints in GTG-light and -dark bands. As summarized in Fig. 1 the 48 reported cases exhibited 100 breaks in 90 different chromosomal regions. In Table II the detected breakpoints were aligned with their localization within GTG-dark and GTG-light bands. Breakpoints detected in infertile patients with chromosomal rearrangements (Tables I and II) were prone to be in genetically more or less irrelevant regions; otherwise more severe clinical problems would have had to be expected in the rearrangement carriers. In discordance with this idea, 57/90 described breakpoints were located in GTG-light bands, which are known to be relatively gene-rich compared to the GTG-dark bands (21). Three out of ninety breaks appeared in the gene-poor hemi-heterochromatic centromere-near regions of chromosomes 1, 14 or 16. Of the remaining observed breakpoints four were localized in the transient area of a GTG-dark to a GTG-light

Table II. The molecular cytogenetic breakpoints from Table I are listed according to their chromosomal origin.[a]

Breakpoint	GTG-light band	Fragile sites	Mariner transposon-like elements	Intrachromosomal telomeric-like sequences	Involvement	
					Cancer	Evolution
Xp22.32~22.33	mix	+Xp22.3[b] (30)	+Xp22[b] (22)		+Xp22.3[b] (35)	
Yp11.3	-mix					
1p36.3	+	+1p36[b] (23,28,30)	+1p36[b] (22)	+1p36[b] (23)	+ (33)	+ (33)
1p36.1	+	+1p36[b] (23,28,30)		+1p36[b] (23)		+ (33)
1p35	-		+ (22)	+ (23)		
1p12	-(hemihet)					
1q21.1	+	+1q21[b] (23,28,30)		+1q21[b] (23)	+ (42)	+ (41)
1q23.3	+			+1q23[b] (23)	+ (33)	+ (33)
1q41	-					
1q43	-					
1q44	+	+(23,28,30)			+ (43)	+ (33)
2p24.2	+	+2p24[b] (23,28,30)				+ (41)
2p13	+	+(23,28,30)		+ (23)	+ (33)	+ (33)
2q12	-mix		+ (22)			
3p21.3	+	+3p21[b] (28)	+3p21[b] (22)		+ (44)	
3q13.1	-mix	+3q13[b] (28)	+3q13[b] (22)			
3q13.2 {2x}	+	+3q13[b] (28)	+3q13[b] (22)			
3q23	+					
3q25.1	+	+3q25[b] (28,30)				
4p16.1	+	+4p16[b] (23,28,30)	+4p16[b] (22)			
4p14	+			+ (23)		
4q13.1 {2x}	-mix			+4q13[b] (23)		
4q21.1	+	+4q21[b] (28)		+4q21[b] (23)	+ (33)	+ (33)
4q25	+			+ (23)		
4q31.3	+	+4q31[b] (28)				
4q32	-mix			+ (23)		
4q33	+					
5q13.3	+					
5q15	+	+(23,28,30)				+ (41)
5q34	-		+ (22)			
6p21.3	+		+6q21[b] (22)			
6q23	+					
6q25.1	+					
6q26~q27	mix	+6q26[b] (28,30)	+ (22)		+ (26)	+ (41)
7p13	+	+ (28,30)				
7q11.21	+	+7q11[b] (28,30)	+7q11[b] (22)		+7q11.2[b] (45)	+7q11.2[b] (41)
7q11.22	-	+7q11[b] (28,30)	+7q11[b] (22)		+7q11.2[b] (45)	+7q11.2[b] (41)
7q11.23	+	+7q11[b] (28,30)	+7q11[b] (22)		+7q11.2[b] (45)	+7q11.2[b] (41)
8p22	-					
8q23~24.1	mix		+8q23[b] (22)			
8q24.1	+	+ (28,30)	+8q24.1[b] (22,46)		+ (46)	+ (41)
9p22	+					
9q13 {3x}	+	+ (23)				
9q21.1	-mix					
9q22.3 {2x}	+	+9q22[b] (23,28,30)	+9q22[b] (22)	+9q22[b] (23)		
9q33	-mix					
9q34.1	+					
9q34.2	-					
9q34.3	+				+ (33)	+ (33)
10q11.23	+					+10q11.2[b] (41)
10q21.1	-	+10q21[b] (23,28,30)	+ (22)	+10q21[b] (23)	+10q21[b] (47)	

Table II. Continued.

Breakpoint	GTG-light band	Fragile sites	Mariner transposon-like elements	Intrachromosomal telomeric-like sequences	Involvement	
					Cancer	Evolution
10q21.2 {2x}	+	+10q21[b] (23,28,30)		+10q21[b] (23)		
10q23.1	-	+ 10q23[b] (28)		+ 10q23[b] (23)	+10q23[b] (48)	
10q26.1	+	+ (30)	+10q26[b] (22)		+ 10q26[b] (49)	+ (41)
10q26.3	+	+10q26[b] (23,28,30)				
11p15.3 {2x}	+	+11p15[b] (23,28)	+11p15~pter[b] (22)			
11q13.1	+	+11q13[b] (23,28,30)	+11q13[b] (22)	+11q13[b] (23)		+11q13[b] (41)
11q13.5	+	+11q13[b] (23,28,30)	+11q13[b] (22)	+11q13[b] (23)		+11q13[b] (41)
11q14.2	+	+ (30)				+ (41)
11q23.1	+	+11q23[b] (23,28)		+11q23[b] (23)	+11q23[b] (50)	
12p13.3	+		+12p13[b] (22)		+ (33)	+ (33)
12p13.1	+		+12p13[b] (22)		+ 12p13[b] (51)	
12p12.2	+				+ (33)	+ (33)
12q22	+					
13q21.3	-mix	+13q21[b] (23,28)				+ (33)
13q31.2 {2x}	+					
14q11.1	-(hemihet)					
14q13	+					
14q22	+					
14q32.33	+		+14q32[b] (22)		+14q32[b] (52)	
15p11.2	+					
15q13	+					
15q14	-					
15q21.1	-					
15q23 {2x}	-					
16p13.3	+	+16p13[b] (28)	+16p13.3[b] (22)			
16p13.1	+	+16p13[b] (28)	+ 16p13.3[b] (22)	+ (23)	+ (33)	+ (33)
16q11.1 {2x}	-(hemihet)	+ (30)				
16q12.2~13	mix					
16q21	-					
17p13.2	-		+17p13[b] (29)			
18p11.32	+					
18q22	-mix		+ (22)			
19p13.3	+		+ (22)			
19q13.13	+	+19q13.1[b] (28)	+ (22)			
20p13	+					
21q21	-mix		+21q22[b] (22)			
21q22.1	+					
22q12	-mix	+ (23,28)			+ (42)	+ (41)
22q13.32	-	+22q13[b] (23,28)				

[a]The molecular cytogenetic breakpoints are aligned with their location within a GTG-light chromosomal band if they are located at or near a fragile site, a Mariner transposon-like element or an intrachromosomal telomeric-like sequence and according to involvement of the corresponding region in cancer- or evolution-related breakpoints. The references are given in parentheses. hemihet, hemiheterochromatic band in centromeric or centromere-near region; mix, breakpoint localized in the transient area of a GTG-dark to a GTG-light band. [b]Breakpoint of patient not exactly in concordance with fragile site, site of Mariner transposon-like element or intrachromosomal telomeric-like sequence; {2x} or {3x}, same breakpoint detected 2 or 3 times in 48 patients.

band and twenty-six were located in GTG-dark bands. However, eleven of these last mentioned twenty-six breakpoints were located in GTG-dark bands known to split in GTG-light and -dark subbands. Because of this no clear conclusion could be drawn for these cases when the breakpoint was in a GTG-dark or -light subband. Thus, in summary only 11/90 breakpoints (~12%) were undoubtfully located in GTG-dark bands, while the great majority were

detected in the relatively gene-richer GTG-light bands of the karyotype. Thus, other reasons must be responsible for the observed breakpoint distribution.

Alignment of breakpoints with segmental duplications. Mariner transposon-like elements (MTLE) (22) and intra-chromosomal telomeric-like sequences (ITS) (23) are special subgroups of the recently reported segmental duplications (24), which were shown to be involved in primate evolution and also in cancer development (25-27). Moreover, ITS and MTLE co-localize in parts with fragile sites (22-23,26,28-30). The latter are also reported to hold di-or tri-nucleotide repeats (31). In general, all segmental duplications such as ITS, MTLE and also fragile sites contribute to genomic instability (32). Here the characterized 90 chromosomal breakpoints detected in infertile but otherwise clinically normal patients were aligned with these aforementioned three elements (Table II). In 6/90 breakpoints (6.7%) a cytogenetic co-localization with ITS was observed, and in the additional 13, ITS were located near the observed breakpoints. In 9/90 (8.9%) and 24/90 (26.9%) of the cases the breaks were at or near the localization of MTLE, respectively. Finally, 10/90 (11.1%) and 30/90 (33.3%) of the break-events were at or near known fragile sites. In summary, 21% of the breakpoints in the studied 100 in infertility patients were near ITS; 35.8% co-localized with MTLE and 44.4% with fragile sites.

The relation of these sequences (fragile sites and segmental duplications) to cancer and evolution has been discussed among cytogeneticists (25-26,33-34). In Table II we summarized exemplary literature highlighting the fact that there appears to be a co-localization of fragile sites or other potentially unstable regions of the genome and breakpoints known from cancer or evolution cytogenetics.

The idea that these 'breakpoint-prone' regions could also be involved in chromosomal rearrangements in general and thus also in clinical genetics cases surprisingly is quite new (35-36). Raghavan and Lieber (36) suggest that some of the so-called non-B DNA structures, which are in part single stranded, are vulnerable to structure-specific nucleases and thus are the biological basis for constitutional chromosomal translocations or rearrangements in general.

Repeatedly observed breakpoints and segmental duplications/ fragile sites. Among the 90 breakpoints listed in Table II, 8 breakpoints were molecularly cytogenetically identical in two cases each and one breakpoint even in three cases. These breakpoints were co-localized with fragile sites (3q13.2, 9q13, 9q22.3, 10q21.2, 11p15.3, 16q11.1), ITS (4q13.1, 9q22.3, 10q21.2) and/or MTLE (3q13.2, 9q22.3, 11p15.3) in 77% of the cases (Table II). Only the breakpoints 13q31.2 and 15q23 were not in correlation with any of these segmental duplication/fragile sites. Among the observed 81 breakpoints of the present study, 31 were not correlated with any segmental duplication/fragile sites (Table II). Thus, the correlation rate was 62% in 'single break-events' versus 77% in 'repeated break-events'.

Notably, the most frequently observed 'repeated break-event' was present in 9q13. This band is known to be involved in constitutional pericentric inversions of chromosome 9, which occurs with a frequency of about 2% in the general population (37). Additionally, this region is known to be breakpoint prone and carries segmental duplications (38). Our own studies showed that the constitutional pericentric inversions of chromosome 9 are not due to one or two founder mutations but that they differ on a molecular level (8,39; our own unpublished data). Thus, an involvement of 9p13 and also 9p11 should be expected in cases as reported here. With high probability due to small sample size no 9p11 breaks were described.

The presented data showed a strong positive correlation of breakpoints present in infertile patients with chromosome rearrangements and the location of fragile sites and of segmental duplications such as ITS and MTLE. These specific regions seem to be somewhat breakage prone and further studies are necessary to characterize the specific DNA-sequence base for this kind of feature.

In conclusion, evident from this molecular cytogenetic study of 48 infertility cases, out of a total of 90 involved, different chromosomal breakpoints confirmed a relatively high frequency of abnormal karyotypes found in couples with normovulatory women. This subgroup of patients seeking infertility treatment clearly benefits from a routine karyotype analysis as previously stated by Papanikolaou *et al* (40). Translocations, inversions, but also insertions are likely to be observed both in males and females of these couples. However, in our study these rearrangements were found at a gender-specific ratio of 1:3. Breaks are more likely to appear in GTG-light bands and in general in genomic regions prone to instability due to the presence of segmental duplications. However, further detailed molecular analysis will be necessary to study the mechanisms and genetic basis for this phenomenon.

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