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ARTICLE

An excess of chromosome 1 breakpoints in male infertility

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In a search for potential infertility loci, which might be revealed by clustering of chromosomal breakpoints, we compiled 464 infertile males with a balanced rearrangement from Mendelian Cytogenetics Network database (MCN*db*) and compared their karyotypes with those of a Danish nation-wide cohort. We excluded Robertsonian translocations, rearrangements involving sex chromosomes and common variants. We identified 10 autosomal bands, five of which were on chromosome 1, with a large excess of breakpoints in the infertility group. Some of these could potentially harbour a male-specific infertility locus. However, a general excess of breakpoints almost everywhere on chromosome 1 was observed among the infertile males: 26.5 *versus* 14.5% in the cohort. This excess was observed both for translocation and inversion carriers, especially pericentric inversions, both for published and unpublished cases, and was significantly associated with azoospermia. The largest number of breakpoints was reported in 1q21; FISH mapping of four of these breakpoints revealed that they did not involve the same region at the molecular level. We suggest that chromosome 1 harbours a critical domain whose integrity is essential for male fertility.
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Introduction

Infertility affects an estimated 15% of couples and in roughly half of the cases male-related factors are involved, frequently associated with impaired spermatogenesis, e.g. oligospermia or azoospermia.[1,2] The knowledge of specific genetic factors causing human male infertility is still scarce, in part because linkage analyses are of limited value for the mapping of infertility loci due to the frequent lack of familial transmission.

The first male-specific infertility factor identified, the azoospermic factor (AZF), was revealed by the cytogenetic detection of deletions of the distal part of the long-arm of the Y chromosome.[3] In contrast to linkage-based methods, chromosomal-based approaches may offer specific advantages in the search for genetic factors associated with male infertility. Chromosomal defects can be identified even in sporadic cases as *de novo* mutations, and chromosomal aberrations are frequent in infertility. A 10-fold increased incidence of structural chromosome abnormalities has been found in infertile males (on average 5.1%) compared to the normal population (0.5%), where 3.8% involve the sex chromosomes and 1.3% autosomes. The overall incidence of structural chromosome anomalies is 4.6% in oligospermic men compared to 13.7% in azoospermic men: autosome anomalies are the most frequent in the oligospermic group (3%), whereas sex chromosome aberrations predominate among azoospermic men (12.6%).[4]

The causal relation between chromosomal rearrangements and impaired sperm production has been suggested to be a structural effect related to alterations in the process of chromosome synapsis during meiosis.[5] In mice, asynapsed regions may trigger the meiotic checkpoint machinery to eliminate spermatocytes.[6] A similar mechanism might explain why some chromosomal abnormalities in humans are associated with deficient spermatogenesis.

Another mechanism might be related to the presumably multitude of genes involved in spermatogenesis, some of which may be dosage-sensitive and potential mutational targets for chromosomal breakpoints. Disease-associated balanced chromosome rearrangements (DBCRs) that truncate, delete or otherwise inactivate specific genes have been found in many kinds of disorders. In many cases, these breakpoints have been instrumental in the identification of the disease gene by bridging the gap between the phenotype and the alterations at the molecular level.[7,8]

Since dosage-sensitive mutational targets and putative male germ cell-associated chromosomal domains might be revealed by nonrandom clustering of chromosomal breakpoints, we have compiled 464 balanced autosomal rearrangements associated with male infertility. These data were compared with the breakpoint distribution of a nation-wide cohort of carriers with balanced chromosomal rearrangements and analysed in relation to parental inheritance and available sperm parameters.

Systematic molecular mapping of the involved breakpoints will be needed to assess whether clustering of breakpoints within specific chromosomal regions reflects the presence of specific infertility genes or is related to other factors. We have initiated this for 1q21, which is the autosomal band with the highest number of reported breakpoints at the cytogenetic level.

Subjects and methods

Subjects

Mendelian Cytogenetics Network database (MCN*db:* http://www.mcndb.org) was searched for balanced chromosomal rearrangements associated with male infertility. Mendelian Cytogenetics Network is a systematic effort for the collection of data on DBCRs, with approximately 300 participating laboratories at present. The participating laboratories fill in appropriate forms with information about the patients: karyotype (including parental origin if known), age when karyotyped, year for karyotyping, reason for clinical referral, known clinical data and availability of cells. Literature data (surveys and case reports) concerning male infertility were also entered into MCN*db*. Familial cases were submitted once. Robertsonian translocations, the recurrent translocation t(11;22)(q23;q11) and 'common variants', as defined by Gardner and Sutherland,[9] were excluded from this study. Association between male infertility and rearrangements involving a sex chromosome is well known and were therefore excluded too. Thus the present study is based on 464 infertile males with a balanced autosomal rearrangement (MCN*db* September 2003, Supplementary Information 2). Of these cases, 228 were published and 236 were unpublished. The sperm parameters (quantity, motility and morphology) were received from the respective centres or extracted from the literature. The compilation of data in MCN*db* is approved by The Danish Ethical committees and reported to The Danish Data Protection Agency.

A nation-wide cohort was established by data drawn from the Danish Cytogenetic Registry, which receives reports from all cytogenetic laboratories in Denmark and is believed to have an almost complete coverage of the constitutional chromosomal abnormalities diagnosed in Denmark since 1961.[10] All carriers of balanced chromosomal rearrangements with the exceptions of Robertsonian translocations, t(11;22)(q23;q11), ''common variants'' and rearrangements involving a sex chromosome were retrieved. Since a proportion of individuals with identical inversions and translocations are likely to be identical by descent,[11] such cases were included only once in the cohort, resulting in 912 unique rearrangements. All carriers were included irrespective of their phenotypes.

Statistical analyses

Statistical analyses were carried out using SAS statistical package.[12] For the cohort, frequency of breakpoints was related to the size of the chromosomes by linear regression. The sizes of the individual chromosomes were drawn from National Center for Biotechnology Information (NCBI, Genome Assembly, build 34: http://ncbi.nlm.nih.gov). χ^2 test was used to test for differences in the distributions of breakpoints in the case and the cohort group and to test for differences in reported sperm parameters. The distribution of parental origin for the infertile males with breakpoint on chromosome 1 was tested with binomial test.

Test for an overall difference in the distribution of band-specific breakpoints in the two groups was performed using χ^2 test and evaluated by permutation test. Since the band resolution of the reported karyotypes varied among cases and laboratories, we used the minimum ~300 band pattern resolution for the analysis (e.g. bands 1p22.1, 1p22.2 and 1p22.3 were compiled under 1p22). Bands inconsistent with the ISCN (1995)[13] and bands on the centromeric regions (p10 and q10) were excluded. In addition, individuals without breakpoints in specified chromosomal bands were excluded from the band-specific analyses, leaving 451 infertile males and 843 in the cohort. To search for potential autosomal loci associated with male infertility, differences in the number of breakpoints were tested for each band with Fisher's exact test and adjusted with Bonferroni correction.

Cytogenetic and molecular analyses of cases with karyotypes involving 1q21

Metaphase chromosomes were prepared from peripheral blood lymphocytes from four individuals who were reported to MCN*db* as oligoasthenozoospermic and with one breakpoint in 1q21. Their karyotypes were: 46,XY,inv(1)(p36q21); 46,XY,inv(1)(q21.2q42); 46,XY,t(1;16)(q21;p11); and 46,XY,t(1;4)(q21;q33)mat. Fluorescence in situ hybridisation (FISH) was carried out using standard procedures[14] and the bacterial artificial chromosome (BAC) clones were obtained from the MCN Reference Centre at the Max Planck-Institute for Molecular Genetics, Berlin (http://www.molgen.mpg.de/~cytogen/). AZFa, AZFb, AZFc and AZFd deletions were screened for by 11 STS markers (SY84, SY95, SY113, SY117, SY121, SY129, SY145, SY242, SY239, SY208 and SY255)[15].

Results

In this study, 464 infertile men with balanced chromosome rearrangements involving autosomes were included. Among the karyotypes, there were 357 two-way reciprocal translocations, 81 inversions and 26 complex rearrangements (Table 1). All reported autosomal rearrangements were unique at the cytogenetic level except from seven inversion 1 rearrangements and two simple translocations (Supplementary Information 2).

Table 1 Subjects with breakpoint involving a particular chromosome reported for each type of rearrangement

	Inversions		*Two-way reciprocal translocations*		*Complex karyotypes*[a]		*Total*	
Chromosome	*Infertile No.*	*Cohort No.*	*Infertile No.*	*Cohort No.*	*Infertile No.*	*Cohort No.*	*Infertile No. (%)*[b]	*Cohort No (%)*[b]
1	31	18	84	102	8	12	123 (26.5)	132 (14.5)
2	3	23	46	76	3	8	52 (11.2)	107 (11.7)
3	7	10	40	80	4	14	51 (11.0)	104 (11.4)
4	0	17	39	66	6	4	45 (9.7)	87 (9.5)
5	4	16	31	82	4	9	39 (8.4)	107 (11.7)
6	6	16	40	88	3	6	49 (10.6)	110 (12.1)
7	7	11	22	69	4	17	33 (7.1)	97 (10.6)
8	1	10	24	63	2	5	27 (5.8)	78 (8.6)
9	4	8	36	71	7	15	47 (10.1)	94 (10.3)
10	7	9	32	53	1	4	40 (8.6)	66 (7.2)
11	2	14	31	70	5	5	38 (8.2)	89 (9.8)
12	4	24	36	50	7	9	47 (10.1)	83 (9.1)
13	1	4	33	62	6	10	40 (8.6)	76 (8.3)
14	0	1	36	38	1	6	37 (8.0)	45 (4.9)
15	0	0	28	46	1	4	29 (6.3)	50 (5.5)
16	1	4	16	43	2	1	19 (4.1)	48 (5.3)
17	0	2	26	47	0	4	26 (5.6)	53 (5.8)
18	2	7	20	57	1	4	23 (5.0)	68 (7.5)
19	0	4	18	31	0	2	18 (3.9)	37 (4.1)
20	0	3	26	36	3	4	29 (6.3)	43 (4.7)
21	1	0	18	33	2	1	21 (4.5)	34 (3.7)
22	0	1	29	38	0	1	29 (6.3)	40 (4.4)
Subjects	81	202	357	651	26	59	464	912

[a]Defined as karyotypes involving more than two breakpoints.
[b]The percentages of individuals with a breakpoint on a specific chromosome were calculated by dividing the observed number with the total number of individuals.

The nation-wide cohort of 912 individuals was used as a comparison group when analysing the rearrangements of the infertile men. This cohort involved 651 two-way reciprocal translocations, 202 inversions and 59 complex rearrangements (Table 1).

Chromosomes involved

The number of individuals with breakpoint(s) involving a particular chromosome were plotted against the length of the chromosomes involved for the infertile males and for the cohort (Figure 1). Regression analysis of the cohort showed a significant linear relation between number of breakpoints and chromosome size ($r^2 = 84.01\%$, $P < 0.001$, test for nonlinearity: $P = 0.26$). The calculated equation of the line is $y = 16.58 + 4.48 \times 10^{-7}x$ but is shown as percentage in Figure 1.

The distribution of breakpoints among the infertile males was significantly different from the cohort, with a χ^2 sum = 49.03 (21 degrees of freedom (df), $P < 0.001$). The significance was based on a great excess of breakpoints on chromosome 1, thus the χ^2 sum decreased to 25.31 (20 df, $P = 0.19$) when chromosome 1 was excluded. The subgroup of translocation carriers showed the same pattern with only chromosome 1 breakpoints at a significant excess (χ^2 sum = 36.46, 21 df, $P = 0.02$; without chromosome 1: $P = 0.11$). Due to the small number of inversions not involving chromosome 1 (Table 1), these were pooled and compared with the number of inversion 1 carriers, and a significant excess of chromosome 1 inversions was found among the infertile males ($\chi^2 = 34.81$, 1 df, $P < 0.0001$).

The frequency of infertile males with a breakpoint on chromosome 1 was not significantly lower among the unpublished infertile males compared with the published (23.3 *versus* 29.8%, $P = 0.10$). When excluding the published cases, the difference between the cases and the cohort was still significant and based on the excess of breakpoints on chromosome 1 only (χ^2 sum = 34.89 with 21 df, $P = 0.03$; without chromosome 1: χ^2 sum = 28.06, 20 df, $P = 0.11$).

The most frequent rearrangement among the infertile males was inv(1), representing 6.7% of the total cases and among these, pericentric inversions were significantly more frequent than in the cohort ($P = 0.01$). Among all other autosomal inversions, no significant difference between peri- and paracentric inversions was found ($P = 0.45$).

Parental inheritance

Data on carrier status of the parents were available in 54 infertile males, where 31 cases were of maternal and 23 of paternal inheritance. Among the 14 rearrangements involving chromosome 1, 11 were of maternal and three of paternal inheritance. This may suggest an over-representation of maternal inheritance although the difference was not significant ($P = 0.057$).

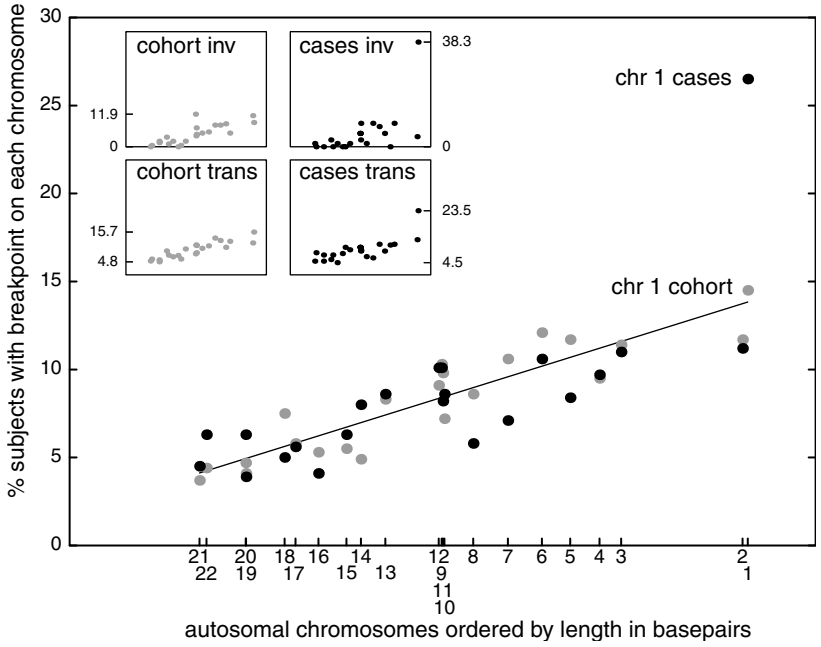

Figure 1 The relationship between chromosome size and the percentage of number of chromosomal breakpoints for infertile males (black) and for the nation-based cohort (grey). The distributions are shown for the subgroups, inversion (inv) and translocation (trans) carriers too.

Table 2 Sperm data for 285 infertile males with a chromosomal rearrangement, reported according to the chromosomes involved[a]

Chromosome	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
Abnormal sperm quantity																						
Azoospermia	31	6	6	5	1	8	6	2	5	7	9	3	7	3	3	3	3	2	2	5	6	9
Oligozoospermia[b]	45	17	26	15	14	19	9	8	19	16	11	17	11	18	15	10	9	11	9	13	8	11
Abnormal motility	29	11	16	12	9	11	8	4	12	7	10	13	8	10	10	5	7	4	5	7	7	9
Abnormal morphology	28	10	17	12	11	10	4	4	12	9	8	14	8	12	9	5	8	5	4	11	5	9
Total cases with reported sperm data	79	29	34	24	19	28	17	12	27	27	22	25	19	24	19	15	15	14	19	21	14	21

[a]Inversion carriers are recorded once and translocation carriers are recorded for each involved chromosome.
[b]Oligozoospermia is defined as $<20 \times 10^6$/ml.

Sperm parameters

Sperm parameters were available for 285 patients with an autosomal rearrangement (130 unpublished cases and 155 published). Among the 71 known azoospermic males, we found a significant excess of individuals with karyotypes involving chromosome 1 (χ^2 sum = 34.86, 21 df, $P = 0.03$) (Table 2). No significant differences were found among subjects with oligozoospermia, abnormal sperm motility or abnormal morphology according to the chromosomes involved.

Band-specific clustering

When testing the difference in the overall distribution of band-specific breakpoints in the two groups, a χ^2-value of 411.62. was found. Permutation test with 10 000 simulations revealed a median χ^2-value of 300.8 and a maximum value of 390.70, thus confirming a significant difference in the distribution of the band-specific breakpoints. When adjusted by Bonferroni correction, none of the individual bands were found to be significant, indicating that the overall significant difference is based on many bands contributing with small differences between the two groups. In order to report the bands contributing most to the observed difference, we arbitrarily tabulated the bands with a χ^2-value above 6.635, resulting in 10 bands with an excess and two bands with fewer breakpoints among the infertile males (Table 3). The relative frequency of azoospermic males with a breakpoint in the five chromosome 1 bands listed in Table 3 does not differ from those with a breakpoint elsewhere on chromosome 1 (χ^2 sum = 1.86, 5 df, $P = 0.87$).

When summarising the number of individuals with a breakpoint on an acrocentric p-arm, an excess was observed among the infertile males (37 of 443 infertile males compared with 38 of 843 in the cohort, $\chi^2 = 7.35$, 1df, $P = 0.007$).

Cytogenetic and molecular analyses of cases with karyotypes involving 1q21

The highest number of reported breakpoints among the infertile males was found on 1q21, thus this chromosomal band was selected for FISH studies. AZF deletions were not found in any of the four patients studied and FISH mapping showed that the assumed 1q21 breakpoints were on three different chromosome bands: 1q21.3 (spanning BAC: RP11-662B1), 1q22 (spanning BAC: AL592078), 1q23.1 (spanning BAC: AL365181) and 1q23.3 (spanning BAC: AL590714). The genes within the four breakpoint regions are listed in Figure 2.

Table 3 Chromosomal regions contributing to a significant difference in the distribution of breakpoints between infertile males and cohort

Band	*Infertile males* n = *451*[a]	*Cohort* n = *843*[a]	*χ^2-value for the individual bands* df = *1*
1p32	14	6	11.05
1q21	20	12	11.05
17q11	7	1	9.83
1q24	5	0	9.38
3q29	5	0	9.38
11q23[b]	2	22	7.57
14q11	8	3	7.01
1p22	14	9	6.98
1q12	9	4	6.84
10q22	12	7	6.80
9p24	10	5	6.76
5p15[b]	4	27	6.74

[a]Number of individuals with breakpoints in specified chromosomal bands.
[b]Bands with fewer breakpoints among the infertile males compared to the cohort.

Discussion

In a compilation of more than 10 000 translocations, Bickmore and Teague[16] found that the sizes of the chromosomes involved in the translocation is the most important determinant of translocation frequency. In line with this, the nation-wide cohort used as a comparison group in this study shows a linear relation between the number of breakpoints and the size of the individual

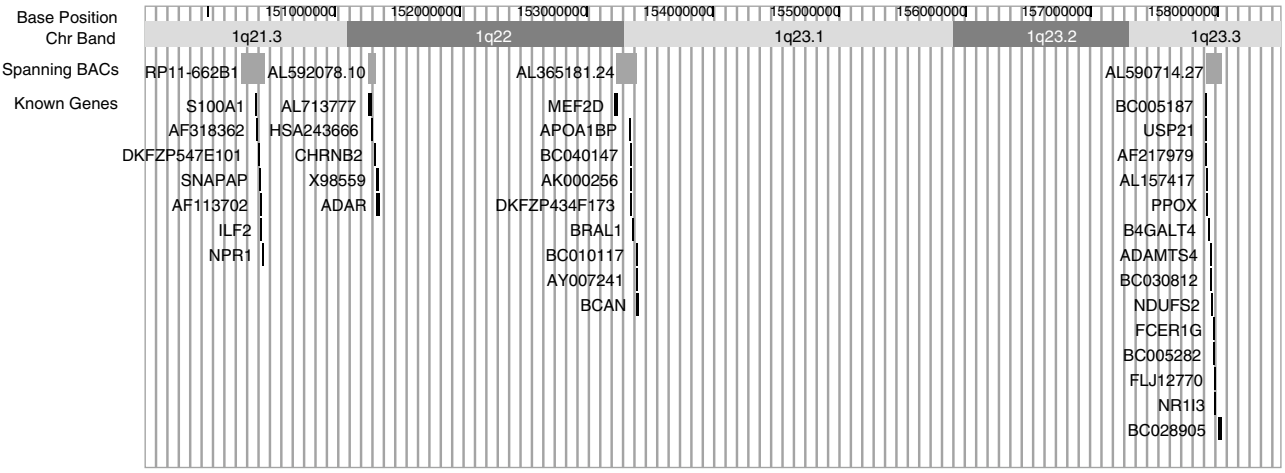

Figure 2 FISH mapping of four infertile males with reported oligoasthenospermia and 1q21 breakpoints. The breakpoints are mapped to the size of a BAC and the annotated genes within the breakpoint regions are listed (Human Genome Browser, UCSC, build 34: http://genome.ucsc.edu/).

chromosome involved. In general, the number of cases with breakpoints per chromosome in infertile males corresponded well with the cohort, but with one main exception: the highly significant over-representation of breakpoints on chromosome 1. Previously, there have been several sporadic reports of infertile males carrying an inversion or a translocation specifically involving chromosome 1,[17] with a potential risk of publication bias for the involvement of chromosome 1. Although we detected a slightly lower frequency of chromosome 1 breakpoints in the unpublished cases compared to the published ones, the frequency was still significantly elevated, indicating that any publication bias is insignificant, and that this over-representation must reflect a real phenomenon. Chromosome 1 is the largest chromosome, but chromosome size *per se* was not found to be the major determinant of this surplus, for example, the number of breakpoints on chromosome 2, which is nearly as large as chromosome 1, was almost half of those observed on chromosome 1.

About 5% of the human genome is composed of evolutionarily recent segmental duplications,[18] which conceivably might predispose to inter- and intrachromosomal rearrangements.[19] Since chromosomal breakage involves recombination, it would be relevant to compare the number of band-specific breakpoints with segmental duplications and recombination frequencies. However, this would require an exact knowledge of the molecular position of the breakpoints. As shown by our FISH mapping of four breakpoints within 1q21, where three out of four were misplaced with regard to the cytogenetic map, the localisation of the breakpoints based on the karyotypes is not accurate enough.

The effect of chromosome 1 appears to be related to male germ cell development since we found the expected number of cases with breakpoints on chromosome 1 (12.9%) among 240 karyotypes associated with any kind of female gonadal dysgenesis compiled from MCN*db* (data not shown). A male-specific nature of breakpoints on chromosome 1 might also be supported by the few available data related to the carrier status of the parents: although it was not statistically significant, among those with a known parental inheritance of chromosome 1 rearrangement, the majority was found to be inherited from the mother.

Data from both mouse and man suggest that male meiosis may be more susceptible to the effect of chromosomal rearrangements compared to female meiosis.[20,21] Homozygosity for mutations in a number of genes coding for proteins involved in recombination and in the synaptonemal complex has been observed to lead to meiotic arrest in male mice, while a continued fertility was observed for female mice.[22] Whether any of these male-specific checkpoints are more likely to be triggered by a chromosomal rearrangement involving chromosome 1 is unknown.

One possible explanation could be that the breakpoints disrupt male-specific fertility genes on chromosome 1. Five regions on chromosome 1 (1p32, 1p22, 1q12, 1q21, 1q24) were among the autosomal regions that contributed most to the significant difference in the distribution of breakpoints between infertile males and the cohort (Table 3). However, even when these five regions are excluded, an excess of individuals with chromosome 1 breakpoints remain among the infertile males (15.8 *versus* 10.9%). In addition, FISH mapping in four patients where the karyotypes indicated involvement of 1q21, which is one of the most significant clusters on chromosome 1, revealed that the breakpoints within this region were scattered at

the molecular level too. Within the four breakpoints spanning regions, only one known candidate gene, *ILF2*, was observed,[23] and defect of this single gene cannot explain the causal relation between breakpoints on chromosome 1 and male infertility in general. Furthermore, among the genes with male-specific effect on meiosis listed by Hunt and Hassold, only one of the human orthologues, *Msh4*, is localised on chromosome 1[22] (Human Genome Browser, UCSC, build 34: http://genome.ucsc.edu).

A relatively high number of t(Y;1) was observed in MCN*db* (data not shown), which suggest a close physical relation between chromosome 1 and Y during meiosis. In addition, chromosomes 1 and Y have been reported to be in close proximity in mature human sperm cells.[24,25] Thus, another hypothesis explaining our findings could be related to a possible association between the chromosome 1 rearrangements and the XY-body. Previous studies have shown an interaction of autosomal aberrations and the XY-bivalent during male meiosis in mice[26] and in humans carrying a Robertsonian or reciprocal translocation.[27,28] It has been suggested that this unusual association causes spermatogenic arrest. However, none of these studies have studied reciprocal translocations involving chromosome 1, and the studies of three different inversions involving chromosome 1 have failed to confirm any association to the XY-body.[17,29,30] An association with the XY-body is especially frequent when acrocentric chromosomes are involved in the rearrangements.[28] In the present study, we observed a significant excess of infertile males with a breakpoint on an acrocentric p-arm. This might support a relation between the XY-body and male infertility, but might not explain the effect of chromosome 1.

A third hypothesis is that the synaptonemal disturbances observed in a number of infertile males carrying a pericentric inversion 1[17,30,31] might be related to the large heterochromatic block, which normally is delayed in pairing and therefore might disturb the alignment of the adjacent euchromatic areas.[30] However, no such effect is demonstrated for chromosomes 9 and 16, which also have very large heterochromatic blocks. Whatever the hypothesis is, it must explain that the significant excess of breakpoints on chromosome 1 are seen both in translocation and inversion carriers, and more frequently in carriers of pericentric than paracentric inversions.

In conclusion, we have demonstrated a large excess of male-specific breakpoints on chromosome 1 associated with infertility, and especially with azoospermia. To some extent, this resembles the situation with X-autosome translocations in females, where breakpoints within the so-called critical region at Xq13–Xq26 are associated with gonadal dysgenesis.[32] Molecular mapping of several of these breakpoints have shown that they are scattered over the entire critical region, supporting the idea that integrity of a specific chromosomal domain may be important for the development of female germ cells.[33] Since the chromosome 1 breakpoints in male infertility do not seem to be associated with any specific chromosomal region(s), either at the cytogenetic or at the molecular level, we suggest that chromosome 1 harbours a large chromosomal domain, the integrity of which is important for normal spermatogenesis.

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