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# Heterogeneity of Pericentric Inversions of the Human Y Chromosome

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**Key Words**
Ampliconic fertility genes · FISH · Human inv(Y) · Inversion breakpoints

**Abstract**
Pericentric inversions of the human Y chromosome (inv(Y)) are the result of breakpoints in Yp and Yq. Whether these breakpoints occur recurrently on specific hotspots or appear at different locations along the repeat structure of the human Y chromosome is an open question. Employing FISH for a better definition and refinement of the inversion breakpoints in 9 cases of inv(Y) chromosomes, with seemingly unvarying metacentric appearance after banding analysis, unequivocally resulted in heterogeneity of the pericentric inversions of the human Y chromosome. While in all 9 inv(Y) cases the inversion breakpoints in the short arm fall in a gene-poor region of X-transposed sequences proximal to PAR1 and *SRY* in Yp11.2, there are clearly 3 different inversion breakpoints in the long arm. Inv(Y)-types I and II are familial cases showing inversion breakpoints that map in Yq11.23 or in Yq11.223, outside the ampliconic fertility gene cluster of *DAZ* and *CDY* in AZFc. Inv(Y)-type III shows an inversion breakpoint in Yq11.223 that splits the *DAZ* and *CDY* fertility gene-cluster in AZFc. This inversion type is representative of both familial cases and cases with spermatogenetic impairment. In a further familial case of inv(Y), with almost acrocentric morphology, the breakpoints are within the *TSPY* and *RBMY* repeat in Yp and within the heterochromatin in Yq. Therefore, the presence of specific inversion breakpoints leading to impaired fertility in certain inv(Y) cases remains an open question.


Pericentric inversions of the human Y chromosome, inv(Y) [Jacobs et al., 1964; Solomon et al., 1964], are rather common and show an estimated incidence of 0.6–1:1,000 males in the general population [Friedrich and Nielsen, 1973; Zeuthen and Nielsen, 1973; Bochkov et al., 1974; Verma et al., 1982; Shapiro et al., 1984; Bernstein et al., 1986]. Most of the reported cases with inv(Y) are familial [reviewed in Verma et al., 1982; Shapiro et al., 1984; Tóth et al., 1984] and, although they include progeny with aneuploidies, preferentially Down syndrome [reviewed in Verma et al., 1982], there was consent that inv(Y) does not impede the production of normal sperm and does not predispose to nondisjunction of other chromosomes in the progeny [Verma et al., 1982; Schmid, 1985]. This con-

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cept was supported by Bernstein et al. [1986] reporting an inv(Y) at a very high frequency (30.5%) in the Gujerati Muslim Indian population of South Africa going back to a founder effect [Spurdle and Jenkins, 1992]. Apparently there was no indication that the inv(Y) chromosome is associated with any reproductive disadvantages. Furthermore, additional single cases with familial inv(Y) have been described since then [Pasantes et al., 1997; Acar et al., 1999; Peng et al., 2000; Rivera et al., 2002; Luo et al., 2009]. However, the inv(Y) has also been reported in association with fertility impairment [Madon et al., 2005; Marchina et al., 2007] and in fertility patients with either concomitant minute Yq11 deletions [Iwamoto et al., 1995; Tomomasa et al., 2000] or a breakpoint in the *DAZ* gene cluster region resulting in *DAZ* splitting [Causio et al., 2000]. These data are indicative of heterogeneity in the human inv(Y) chromosome.

Therefore, we re-investigated 9 cases of inv(Y) exhibiting unvaryingly metacentric morphology after a banding resolution at the 550- to 850-band levels [ISCN, 2009]. Two of the carriers were referred to our institute because of fertility impairment and 7 of the cases turned out to be familial. For a better definition and refinement of the inversion breakpoints we applied FISH with Y-specific DNA probes to all 9 cases. A further inv(Y) with almost acrocentric morphology was also included in our study for a closer definition of the inversion breakpoints with FISH.

## Material and Methods

*Case Reports*

Case 1: A karyotype of 46,X,inv(Y)(p11.2q11.23) was diagnosed for a healthy, fertile male. Cytogenetic investigation was indicated by the detection of an inv(Y) chromosome in a male fetus of his pregnant wife during routine amniocentesis. The same inv(Y) was also detected in a further healthy son of the couple.

Case 2: A karyotype of 46,X,inv(Y)(p11.2q11.23) was described for a healthy, fertile male. Cytogenetic investigation was performed after the detection of an inv(Y) chromosome in a male fetus during routine CVS prenatal diagnosis of his pregnant wife.

Case 3: A karyotype of 46,X,inv(Y)(p11.2q11.23) was diagnosed in a healthy, fertile male after the detection of a male fetus with inv(Y) during routine amniocentesis of his pregnant wife.

Case 4: A karyotype of 46,X,inv(Y)(p11.2q11.23) was diagnosed in a healthy, fertile male after detection of an inv(Y) chromosome in a male fetus during routine CVS.

Case 5: Because of suspected Down syndrome a newborn boy was cytogenetically diagnosed with the karyotype 47,X,inv(Y)(p11.2q11.23),+21. The same inv(Y) was detected in the father of this newborn boy.

Case 6: Because of suspected Down syndrome in an almost one-year-old boy, a conventional karyotype of 47,X,inv(Y)(p11.2q11.23),+21 was described. The same inv(Y) was then diagnosed for the father of this boy.

Case 7: Cytogenetic investigation of a male proband with oligoasthenoteratozoospermia (OAT III) before ICSI resulted in a karyotype of 46,X,inv(Y)(p11.2q11.23). PCR amplification of sY84 (AZFa), sY143 (AZFb), sY254, sY255, sY152, sY155, sY147 and sY149 (AZFc) did not point to deletions in any of these regions. The father of this proband was not available for cytogenetic investigation.

Case 8: After genetic counselling because of an unfulfilled wish to have children a couple was cytogenetically investigated. The constitutive karyotype of the male partner was 46,X,inv(Y)(p11.2q11.23) while that of the female partner was normal, 46,XX. The proband's inv(Y) turned out to be de novo since the father had a normal 46,XY karyotype. No paternity testing was performed in this case.

Case 9: A karyotype of 46,X,inv(Y)(p11.2q11.23) was described in a healthy, fertile male after an inv(Y) chromosome was detected in a male fetus during routine amniocentesis of his pregnant wife.

Case 10: Because of an unfulfilled wish to have children and before ICSI, the cytogenetic investigation of a couple was indicated. The karyotype of the male partner with azoospermia was 46,X,inv(Y)(p11.2q12) while that of the female partner was normal, 46,XX. In contrast to the metacentric appearance of the inv(Y) in cases 1–9, this inv(Y) of case 10 exhibited a rather acrocentric morphology and turned out to be familial. The father of the male proband showed the same aberrant Y chromosome but was fully fertile as he fathered 3 further children.

*Chromosome Preparations*

Standard human chromosome preparations were made from peripheral lymphocytes of a healthy, fertile male and of the male cases 1–10 as described previously [Schempp et al., 1995]. To obtain prometaphase chromosomes, the cultures were treated with methotrexate (0.05 μg/ml) after 48 h of incubation. BrdU (20 μg/ml) and FdU (1 μg/ml) were added 5.5 h before harvesting.

*Fluorescence in situ Hybridisation (FISH)*

Prior to FISH, the slides were treated with RNase and pepsin as described by Ried et al. [1992]. FISH followed essentially the methods described in Schempp et al. [1995]. Chromosome in situ suppression (CISS) was applied to the following DNA clones: *CDY* cosmid CDY-2A49 [Kühl et al., 2001], *DAZ* cosmid 6B7, *RBMY* cosmid A5F, *TSPY* cosmid 2.2133, *AMELY* cosmid C9E [Taylor et al., 1996], *PRKY* PAC 152F08 [Schiebel et al., 1997], *SHOX* cosmid 34F05 [Rao et al., 1997], *SLC25A6* (formerly *ANT3*) cosmid KS3 [Schiebel et al., 1993], and SYBL1 cosmid LLycos130G4 [G. Rappold, unpublished]. The biotinylated classical satellite probes, DYZ1 and DYZ2 (Qbiogene, formerly Oncor), served as markers for Yqh, and a Y-specific, biotinylated alphoid satellite DNA, DYZ3 (Qbiogene), served as a marker for Ycen. After CISS hybridisation, the slides were counterstained with DAPI (0.14 μg/ml) and mounted in Vectashield (Vector Laboratories).

*Fluorescence Microscopy and Imaging*

Two-colour FISH preparations were evaluated using a Zeiss Axiophot epifluorescence microscope equipped with single-band

pass filters for excitation of red, green, and blue signals (Chroma Technologies). Only excitation filters were changed during exposures, allowing for pixel shift-free image recording. Images of high magnification and resolution were obtained using a black-and white CCD camera (Photometrics, Kodak KAF 1400) connected to the microscope. Camera control and digital image acquisition made use of an Apple Macintosh Quadra 950 computer.

## Results

In a former study, we established a standard FISH signal pattern for Y chromosomal DNA probes on prometaphase Y chromosomes of healthy, fertile human males [Röttger et al., 2002]. With respect to our re-investigation of 9 cases of inv(Y) with seemingly unvarying inversion breakpoints, the male-specific ampliconic fertility genes *DAZ* and *CDY* turned out to be most informative for a closer definition by FISH of the inversion breakpoints in the Y chromosome long arm. Therefore, we first show the FISH signal pattern of *DAZ* and *CDY* together with its G-band diagram according to ISCN [2009] in figure 1. In 9 of the cases of inv(Y) investigated the short arm inversion breakpoints map proximal to the *TSPY* array in Yp11.2.

FISH experiments in 4 of our familial inv(Y) cases (cases 1–4) resulted in a uniform type of inverted Y chromosome, inv(Y)-type I. Inv(Y) chromosomes depicted in figure 2a–d demonstrate that almost the entire long arm euchromatin (Yq11) is inverted and falls onto the short arm. Only weak *RBMY* signals are still visible at the border to the Yq-heterochromatin in the long arm of the inv(Y) (fig. 2b). The long arm heterochromatin (Yq12) is not included in the inversion. Also not affected are both the pseudoautosomal segment PAR1 and the *SRY* gene in the short arm in Yp11.3 (not shown). The ideogrammatic presentation of the inv(Y)-type I with the *DAZ* and *CDY* signal pattern is shown in figure 3. Inversion breakpoints are in Yp11.2 in the short arm and, distal to *DAZ* and *CDY*, in Yq11.23 in the long arm.

A second inversion type, inv(Y)-type II, with a different inversion breakpoint in the Y chromosome long arm was found in 2 further familial cases (cases 5 and 6). Yq-heterochromatin in Yq12, *CDY* in Yq11.23, *DAZ* in Yq11.223, and also the pseudoautosomal gene *SLC25A6* in Yp11.3 were not affected by the inversion (fig. 4). However, the main cluster of *RBMY* in Yq11.223 to Yq11.222 and the proximal *CDY* in Yq11.221 were included and inverted onto the short arm of the inv(Y)-type II. An ideogrammatic presentation of the inv(Y)-type II including the *DAZ* and *CDY* signal pattern is shown in figure 5. Inversion breakpoints are in Yp11.2

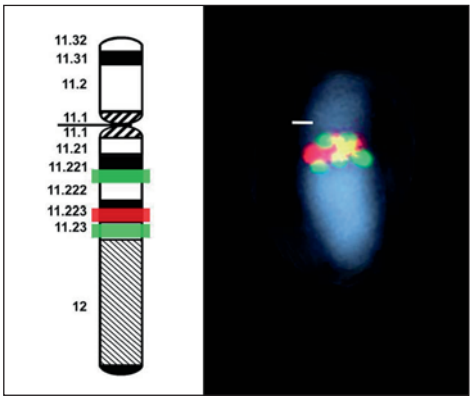


**Fig. 1.** FISH signal pattern of *DAZ* (red) and *CDY* (green) on the DAPI-stained normal human Y chromosome. Yellow colour results from overlapping of red and green signals. The centromere is marked by a horizontal line.

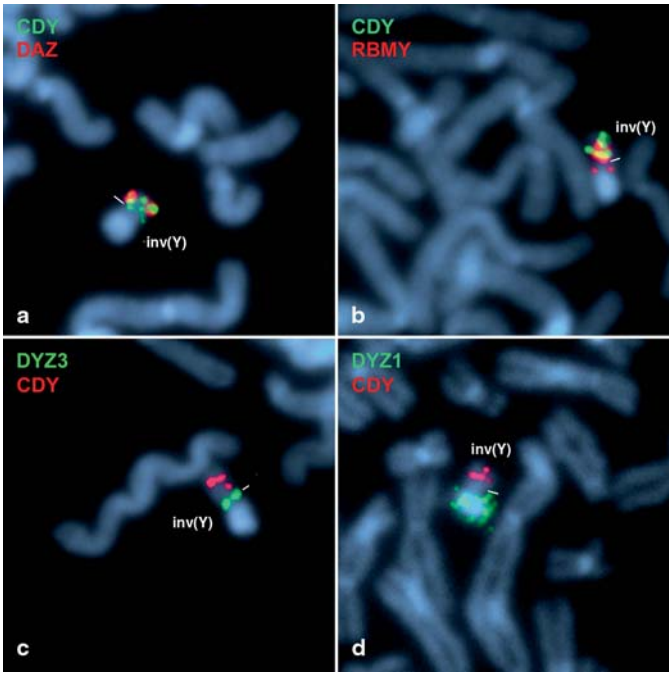


**Fig. 2.** FISH with Y-specific DNA probes on inv(Y) chromosomes of cases 1–4. **a** *DAZ* (red) and *CDY* (green) signals are visible in the short arm of the inv(Y). **b** *CDY* signals (green) and the main cluster of *RBMY* (red) are in the short arm of the inv(Y). Minor signals for *RBMY* (red) are located in the long arm of the inv(Y) in close vicinity to the DAPI-positive Yq-heterochromatin. **c** *CDY* (red) maps in the short arm of the inv(Y), the Y centromere marked by DYZ3 (green). **d** *CDY* (red) maps in the short arm of the inv(Y), the Yq-heterochromatin marked by DYZ1 (green) exclusively maps in the long arm. Centromeres are marked by horizontal lines.

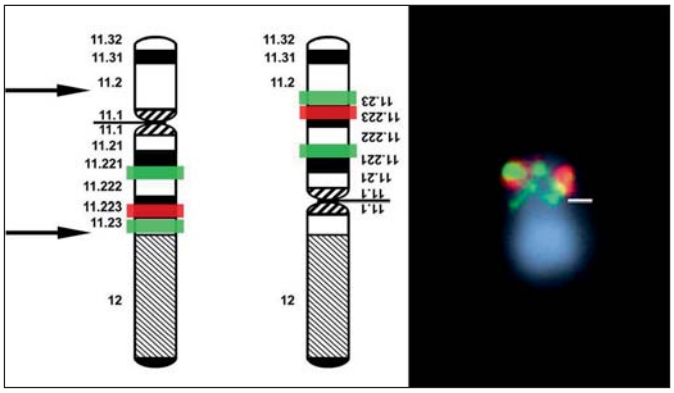


**Fig. 3.** Ideogrammatic representation of the inv(Y)-type I of cases 1–4. Arrows point to inversion breakpoints in the short arm in Yp11.2 and in the long arm in Yq11.23 distal to *DAZ* (red) and *CDY* (green) in the euchromatin/heterochromatin transition region. The FISH signal pattern for *DAZ* and *CDY* of the inv(Y) on the right is taken from figure 2a.

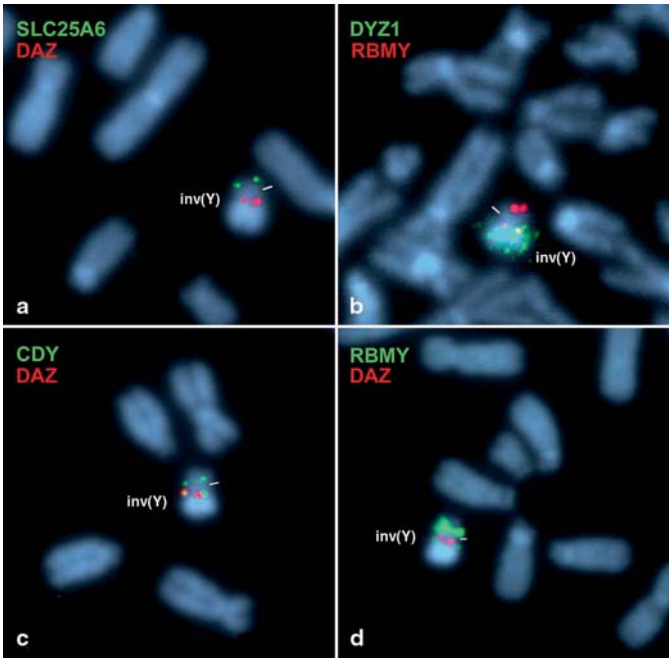


**Fig. 4.** FISH with Y-specific DNA probes on inv(Y) chromosomes of cases 5 and 6. **a** *DAZ* (red) and the pseudoautosomal (PAR1) gene *SLC25A6* (green) are not affected by the inversion. **b** The main signal cluster of *RBMY* from Yq11.223 is inverted to the short arm of the inv(Y). Only minor signals of *RBMY* sequences from distal Yq11.23 are still visible at the border of the Yq-heterochromatin that is marked by DYZ1 (green) and is not affected by the inversion. **c** *DAZ* (red) and *CDY* (green) signals in proximity of the DAPI-positive Yq-heterochromatin remain in their original position, while the second *CDY* signal from Yq11.221 is inverted to the short arm of the inv(Y). **d** *DAZ* (red) and minor signals of *RBMY* (green) sequences in distal Yq11.23 are still visible in the long arm of inv(Y), while the main signal cluster of *RBMY* is inverted to the short arm of inv(Y). Centromeres are marked by horizontal bars.

in the short arm and proximal to *DAZ* in Yq11.223 in the long arm.

A third inversion type, inv(Y)-type III, is representative for our inv(Y) cases 7–9. Two of these cases (7 and 8) had been referred to chromosome diagnosis because of fertility disturbances, while the inv(Y) in case 9 was first detected in a fetus during routine amniocentesis and thus is familial. Interestingly, in all 3 cases the inversion breakpoints in the long arm of the inv(Y) map in the *DAZ* and *CDY* gene cluster in Yq11.223 proximal to the *RBMY* cluster. This inversion breakpoint resulted in a splitting of the *DAZ* and *CDY* signals representing the ampliconic gene clusters that are interspersed in Yq11.223 in the AZFc region [Vogt et al., 1996; Skaletsky et al., 2003], and in the inversion of the main cluster of *RBMY* onto the short arm and that of the *TSPY* onto the long arm of the inverted Y chromosome (fig. 6a, b). Our interpretation of the *DAZ* and *CDY* signal pattern of inv(Y)-type III is given in an ideogrammatic representation in figure 7. Inversion breakpoints in Yq11.223 and in Yp11.2 resulted in the already described split signal of *DAZ* and *CDY*. The third signal for *CDY*, seen in the short arm of the inv(Y) chromosome, can be traced back to the proximal *CDY* genes in Yq11.221 of the normal Y chromosome.

A totally different inv(Y) is described for case 10, a familial inversion of the Y chromosome again, here defined as inv(Y)-type IV. The acrocentric morphology of the Y chromosome resulted from inversion breakpoints in the distal part of the heterochromatin in the long arm in Yq12 and in the short arm in Yp11.2. The inversion breakpoint in Yp11.2 caused a splitting of both the main cluster of the *TSPY* array and the minor *RBMY* cluster (fig. 8a, b). A splitting of the FISH signals for the heterochromatin classes DYZ1 and DYZ2 is also seen as a result of the inversion breakpoint in the distal part of the heterochromatin in Yq12 (fig. 8c, d). Not affected by the inversion breakpoints are the Y chromosomal genes *DAZ*, *CDY*, *PRKY* and *AMELY*, inside the inverted segment, as well as the PAR1 gene *SHOX* and the PAR2 gene *SYBL1*, outside the inverted segment (fig. 8a–f). The ideogrammatic representation of the inv(Y)-type IV labelled with all Y chromosomal sequences used for FISH is shown in figure 9.

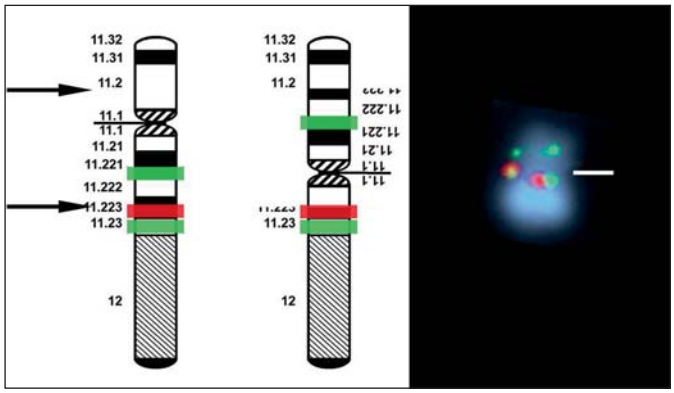


**Fig. 5.** Ideogrammatic representation of the inv(Y)-type II of cases 5 and 6. Arrows point to inversion breakpoints in the short arm in Yp11.2 and in the long arm in Yq11.223 proximal to *DAZ* (red) in Yq11.223 and *CDY* (green) in Yq11.23. The FISH signal pattern for *DAZ* and *CDY* of the inv(Y) on the right is taken from figure 4c.

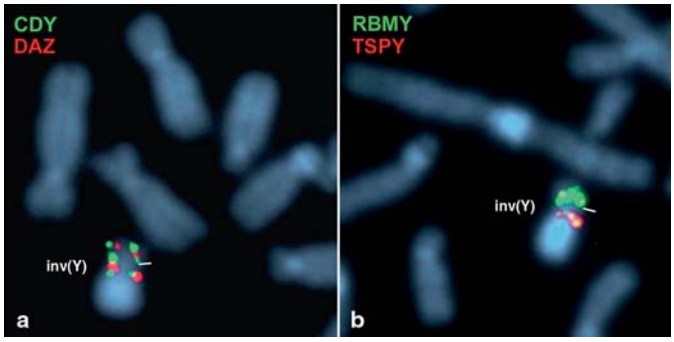


**Fig. 6.** FISH with Y-specific DNA probes on inv(Y) chromosomes of cases 7–9. **a** *DAZ* (red) shows split signals visible in the long and the short arm of inv(Y). *CDY* (green) shows 3 signals, one in the long arm and 2 in the short arm. **b** Main clusters of *RBMY* (green) and *TSPY* (red) are included in the inversion of this inv(Y)-type. Centromeres are marked by horizontal lines.

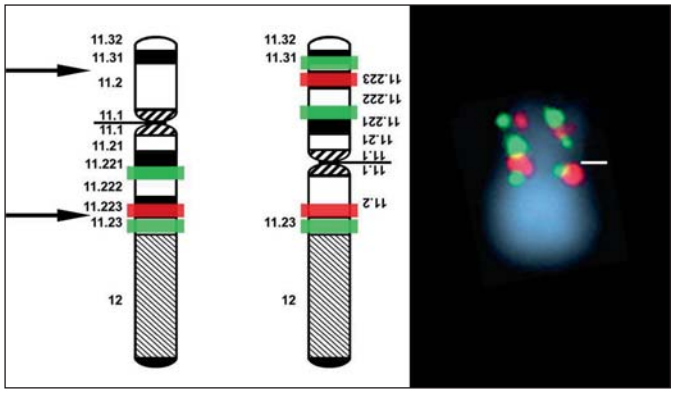


**Fig. 7.** Ideogrammatic representation of the inv(Y)-type III of cases 7–9. Arrows point to inversion breakpoints in the short arm in Yp11.2 and in the long arm in Yq11.223. The inversion breakpoint in Yq11.223 resulted in split signals for *DAZ* (red) and *CDY* (green) (see text in Results). The FISH signal pattern for *DAZ* and *CDY* of the inv(Y) is taken from figure 6a.

## Discussion

Employing FISH for a better definition and refinement of the inversion breakpoints of our cases of inv(Y) chromosomes, we intended to answer 2 questions: (i) do the seemingly unvarying metacentric inv(Y) chromosomes exhibit different inversion breakpoints?, and (ii) are specific inversion breakpoints indicative of a clinical manifestation, e.g. impaired fertility, in the inv(Y) cases?

The human Y chromosome can be subdivided into 2 regions: the male-specific region (MSY) that does not ordinarily recombine with the X chromosome, and the pseudoautosomal regions (PARs) that flank the MSY on both sides. The PAR1 at the tip of the short arm, where X-Y pairing and crossing over is obligatory to ensure the proper segregation of the X and Y chromosomes in male meiosis, and the PAR2 in the distal part of the long arm end, where pairing and crossing over may occur. Among other genes, the MSY contains the sex-determining region on the Y *(SRY)* and several ampliconic fertility genes with testis-specific expression. These ampliconic fertility genes are preferentially organised in palindromes that comprise 25% of MSY euchromatin [Skaletsky et al., 2003]. Sequence homogenisation in MSY palindromes indicates that gene conversion there remains a frequent process [Rozen et al., 2003]. Recently, Lange et al. [2009] reported idic(Y) chromosomes formed by homologous crossing over between opposing arms of palindromes on sister chromatids. The authors propose that intrapalindrome sequence identity is maintained via noncrossover pathways of homologous recombination. DNA double-strand breaks that initiate these pathways can be alternatively resolved by crossing over between sister chromatids to form idic(Y) chromosomes, with clinical consequences ranging from spermatogenic failure to sex reversal and Turner syndrome.

Here, we paid special attention to the question whether the integrity of the PAR1, the *SRY* that is in close proximity to PAR1, and the testis-specific expressed ampli-

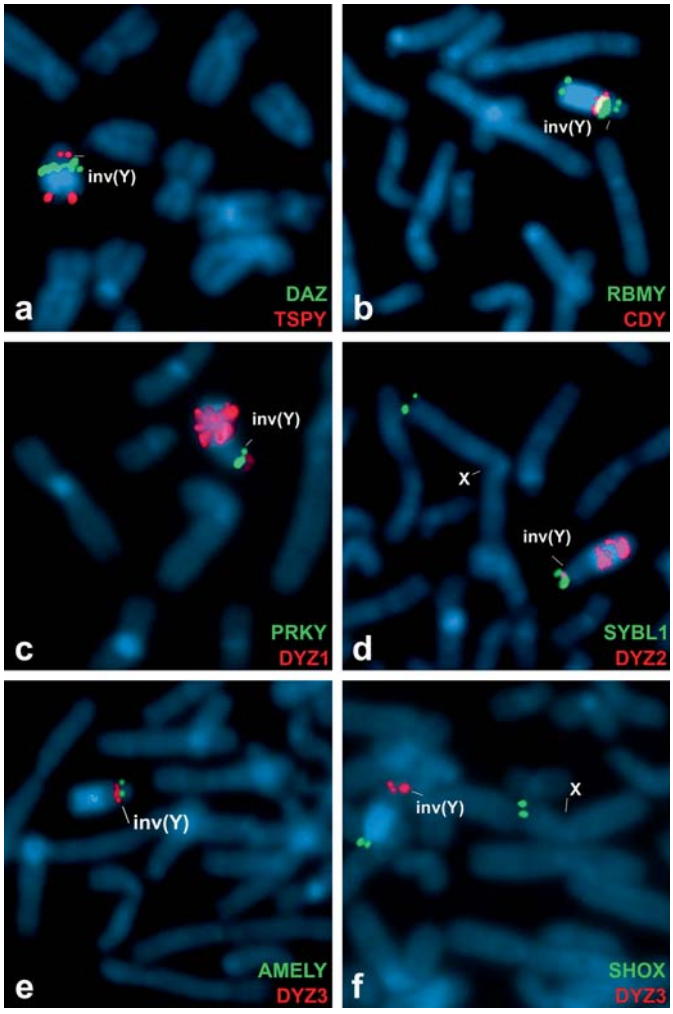


**Fig. 8.** FISH with Y-specific DNA probes on the inv(Y) chromosome of case 10. **a** *DAZ* (green) is not affected by the inversion; *TSPY* signals (red) are split by the inversion breakpoint in the short arm in Yp11.2. **b** *CDY* (red) and *RBMY* (green) in the long arm are not affected by the inversion breakpoint; *RBMY* signals (green) on the short arm are split by the inversion breakpoint in the short arm in Yp11.2. **c** *PRKY* (green) in the short arm in Yp11.2 is not affected by the inversion breakpoint; the Yq-heterochromatin marked by DYZ1 (red) is split by the inversion breakpoint in distal Yq12 resulting in minor signals for DYZ1 distal to *PRKY* on the distal short arm. **d** Signals for the PAR2 gene *SYBL1* (green) and a minor signal for the Yq12-heterochromatin (red) are seen on the short arm of the inv(Y). Note the correct position of *SYBL1* on telomeric Xq. **e** *AMELY* (green) in the short arm in Yp11.2 together with the centromere marked by DYZ3 (red) are not affected by the inversion breakpoint. **f** Signals for the PAR1 gene *SHOX* (green) map distally from the DAPI-positive heterochromatin in Yq12. Note the correct position of SHOX on telomeric Xp. The centromeres are marked by horizontal lines.

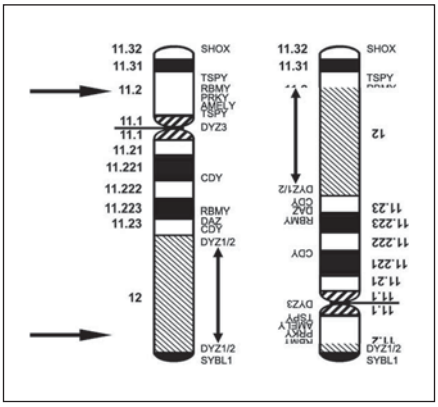


**Fig. 9.** Ideogrammatic representation of the inv(Y)-type IV of case 10. Arrows point to inversion breakpoints in the short arm in Yp11.2 and in the long arm in distal Yq12-heterochromatin. Note the almost acrocentric morphology of the inv(Y) on the right with PAR1 *(SHOX)* at the telomeric long arm and PAR2 *(SYBL1)* at the telomeric short arm.

conic fertility genes *CDY*, *DAZ*, *RBMY* and *TSPY* is affected by the inversion breakpoints in our inv(Y) cases.

In all 9 familial and non-familial cases of inv(Y) appearing metacentric the inversion breakpoints in the short arm map in Yp11.2 proximal to PAR1 and *SRY* and distal to the ampliconic *RBMY* and *TSPY* sequences. The inversion breakpoint itself falls in a gene-poor region of X-transposed sequences harboring 2 genes only [Skaletsky et al., 2003]. As this inversion breakpoint in Yp11.2 seems to be common to all our familial and non-familial inv(Y) cases, any clinical impact is very unlikely. Our 7 familial inv(Y) cases, which can be further subdivided into inv(Y)-type I and II, are characterized by means of their different inversion breakpoints in the Y chromosome long arm. In 4 cases (cases 1–4) this breakpoint maps in the euchromatin/heterochromatin transition region in Yq11.23, distal to the ampliconic testis-specific expressed fertility genes *CDY* and *DAZ*. These 4 familial cases are represented by inv(Y)-type I. A similar familial inv(Y) case has been published by us earlier [Pasantes et al., 1997]. In 2 other familial cases (cases 5 and 6) the long arm inversion breakpoint maps proximal to these fertility genes *CDY* and *DAZ* in Yq11.223 resulting in our familial inv(Y)-type II. Although for both cases the cytogenetically confirmed suspicion was Down syndrome, trisomy 21, we consider that the association between aneuploidy and inv(Y) in these aberrant cases is random.

A differing third inversion breakpoint in the long arm of the Y chromosome is characteristic for our inv(Y)-type

III. This inversion breakpoint in Yq11.223 causes a splitting of the FISH signals for *DAZ* and *CDY* in 3 of our inv(Y) probands (cases 7–9). Whether this disruption of the integrity of the *DAZ* and *CDY* fertility gene cluster in Yq11.223 is causative for the fertility impairment in our cases 7 and 8 is debatable because the same inversion breakpoint is described for the inv(Y) chromosome in case 9 that is clearly familial. Interestingly, a similar case of inv(Y) has been described earlier for an infertile patient showing oligozoospermia. Here, the inv(Y) was de novo since the father had a normal 46,XY karyotype. This inversion causes the disruption of the *DAZ* gene area in the Y long arm as demonstrated by FISH [Causio et al., 2000].

It should be mentioned that FISH does not allow a precise definition of the breakpoint on the DNA level and, even in the case of a de novo situation, the pericentric inversion must not necessarily be causative for a given fertility impairment. Therefore, further cases of such inv(Y), with and without fertility disturbances, and the precise definition of the inversion breakpoints on the gene level are needed to draw any conclusions with regard to the genotype-phenotype relationship.

A special case is described by our inv(Y)-type IV showing an almost acrocentric Y chromosome (case 10). Here, the inversion breakpoints in Yp11.2 and in the long arm heterochromatin (Yq12) resulted in a splitting of the ampliconic fertility genes *RBMY* and *TSPY*, thus coming in close proximity to the Yq-heterochromatin. Whether the azoospermia of this proband is caused by this Y chromosome inversion cannot be decided as his fully fertile father has the same inv(Y) chromosome.

In conclusion, our FISH studies show that there is heterogeneity of the pericentric inversions of the human Y chromosome. In all described cases of inv(Y) chromosomes that appear metacentric after banding analysis the inversion breakpoints in the short arm in Yp11.2 fall in a gene-poor region of X-transposed sequences proximal to PAR1 and *SRY*. However, there are unequivocally 3 different breakpoints in the long arm of the Y chromosome. Two differing inversion breakpoints, characteristic for our inv(Y)-types I and II cases, map outside of the ampliconic fertility genes in AZFc and have no effect on normal spermatogenesis. Indeed, these are all familial cases. A further differing third inversion breakpoint that is characteristic of our inv(Y)-type III maps inside the ampliconic fertility genes in AZFc. This inv(Y)-type III represents both familial cases and cases with spermatogenetic impairment. It should be mentioned that although a distant relationship cannot be totally excluded, the cases with identical breakpoints show different regional provenience and even different ethnic background. Finally, our second question, whether specific inversion breakpoints are indicative for impaired fertility, remains open because there is no clear genotype-phenotype correlation in the investigated cases up to now.

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