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A family case of fertile human 45,X,psu dic(15;Y) males

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Abstract. We report on a familial case including four male probands from three generations with a 45,X,psu dic(15;Y)(p11.2;q12) karyotype. 45,X is usually associated with a female phenotype and only rarely with maleness, due to translocation of small Y chromosomal fragments to autosomes. These male patients are commonly infertile because of missing azoospermia factor regions from the Y long arm. In our familial case we found a pseudodicentric translocation chromosome, that contains almost the entire chromosomes 15 and Y. The translocation took place in an unknown male ancestor of our probands and has no apparent effect on fertility and phenotype of the carrier. FISH analysis demonstrated the deletion of the pseudoautosomal region 2 (PAR2) from the Y chromosome and the loss of the

nucleolus organizing region (NOR) from chromosome 15. The formation of the psu dic(15;Y) chromosome is a reciprocal event to the formation of the satellited Y chromosome (Yqs). Statistically, the formation of 45,X,psu dic(15;Y)(p11.2;q12) is as likely as the formation of Yqs. Nevertheless, it has not been described yet. This can be explained by the dicentricity of this translocation chromosome that usually leads to mitotic instability and meiotic imbalances. A second event, a stable inactivation of one of the two centromeres is obligatory to enable the transmission of the translocation chromosome and thus a stably reduced chromosome number from father to every son in this family.

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The karyotype 45,X is usually associated with a female and only rarely with a male phenotype. Female 45,X patients (usually) show the phenotype of Ullrich-Turner-Syndrome including short stature and infertility. Male individuals with a 45,X karyotype carry small fragments of Y-chromosomal material, including the testis-determining gene

SRY, mostly due to translocations from the Y chromosome to an autosome (Schempp et al., 1985; Fracarro et al., 1987; Gal et al., 1987; Andersson et al., 1988). Although there are some familial cases of Y-autosomal translocations where fertility is maintained (Subrt and Blehova, 1974; Callen et al., 1987; White et al., 1998), most male patients with a 45,X karyotype are usually infertile because of missing azoospermia factor regions (AZFs) from the Y long arm. Some of these patients have additional clinical features depending on the type of translocation that had occurred (Maserati et al., 1986; Weber et al., 1987).

Here we report on a familial case of a Y-autosomal translocation that was passed in at least three generations from fathers to sons, without causing fertility problems. The index patient of this Indian family was at the time an 11-year-old boy (Fig. 1, III 1), who was referred to genetic counsel-

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ling because the parents worried about delayed puberty. His right testis was only partially descended and situated below the inguinal ring. His father, parents and grandparents were phenotypically normal. The paternal grandmother had two abortions because of unknown factors.

Chromosomal analysis of blood lymphocytes revealed a 45,X,psu dic(15;Y) condition with a translocation of almost the entire Y chromosome to the short arm of a chromosome 15. Also the proband's younger brother, his father and grandfather were found to have a 45,X constellation and to possess the same translocation chromosome.

Material and methods

Chromosome preparation

Standard chromosome preparation methods were applied to peripheral lymphocyte cultures according to Schempp et al. (1995). Conventional G-, C-, DAPI-banding and AgNO₃ staining of the nucleolus organizing regions (NORs) was performed. For FISH analysis a minimum of 20 metaphases was scored.

Fluorescence in situ hybridization (FISH)

FISH followed essentially the methods described by Schempp et al. (1995). Prior to FISH, the slides were treated with RNase and pepsin digestion as described by Ried et al. (1992). Chromosome in situ suppression (CISS) was applied to the cosmid KS3 containing PAR1-gene *SLC25A6* (formerly *ANT3*) (Schiebel et al., 1993), the PAR2-gene *SYBL1* containing cosmid Lly-cos130G4, as well as cos 37C9 from PAR2, including marker DXYS61 (G. Rappold, unpublished) and the *DAZ* cosmid 7A69M (Gläser et al., 1998). Classic satellite probes, digoxigenin-labeled DYZ1 and biotin-labeled DYZ3 (Oncor), were used to paint the human Y long-arm heterochromatin (Yqh) and the Y centromere, respectively. The SpectrumGreen-labeled classical satellite-III probe, D15Z1 (Abbott), served to analyse the short arm of chromosome 15. In addition to AgNO₃ staining, nucleolus organizer regions were detected with biotin-labelled plasmid pMr100, a mouse-derived rDNA containing most of 28S and a part of 18S (Tiemeier et al., 1977; Grummt et al., 1979). After FISH, the slides were counterstained with DAPI (0.14 mg/ml) and mounted in Vectashield (Vector Laboratories).

Fluorescence microscopy and imaging

Preparations were evaluated using a Zeiss Axiophot epifluorescence microscope equipped with single-bandpass filters for excitation of red, green, and blue (Chroma Technologies, Brattleboro, VT). During exposures, only excitation filters were changed allowing for pixel-shift-free image recording.

Results

Conventional chromosome banding

Chromosome analysis on G-banded metaphases from cultured lymphocytes of the four male family members revealed 45,X constellation. A pseudodicentric translocation chromosome *psu dic(15;Y)* was found. Each copy of this translocation chromosome (200 examined) had only one primary constriction at the chromosome 15 centromere giving the translocation chromosome an almost metacentric appearance (Fig. 2). Deletion of the Y centromere was excluded by FISH analysis with a DYZ3 probe, resulting in two clearly separable signals for each chromatid (not shown).

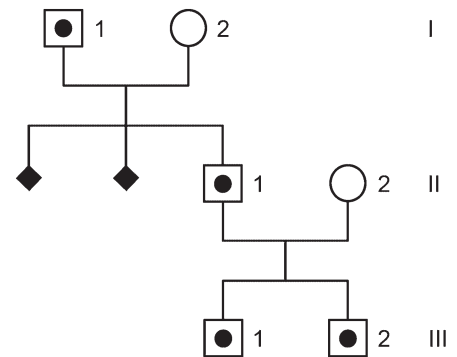


Fig. 1. Pedigree of the analysed Indian family. All the male family members have a 45,X,psu dic(15;Y) karyotype. The reasons that led to the abortions of the paternal grandmother (I 2) are not known.

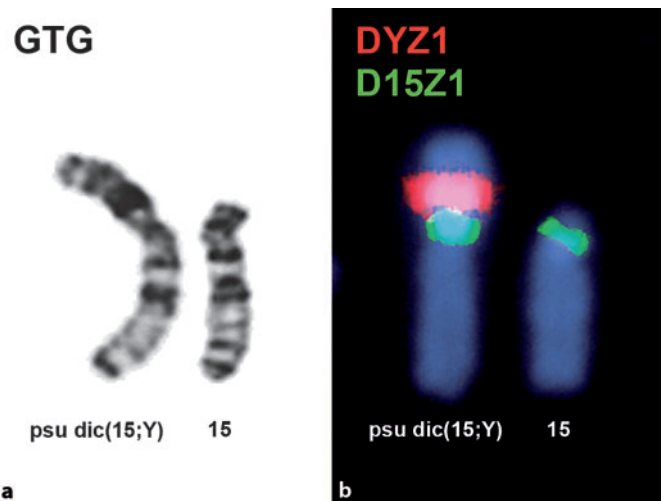


Fig. 2. G-banded *psu dic(15;Y)* (a, left) and chromosome 15 of the index patient (a, right) and FISH results using the Yqh-specific probe DYZ1 (red) and the 15ph-specific probe D15Z1 (green) for the respective heterochromatins (b).

Thus, defined by the localisation of the primary constriction, an inactivation of the Y centromere must have occurred. C- and DAPI-banding confirmed the presence of a large heterochromatic block on the short arm of the translocation chromosome. Following AgNO₃ staining, all nine remaining D and G group chromosomes revealed active NORs, but not the *psu dic(15;Y)* chromosome (not shown). This indicated that the breakpoint maps either proximal to the NOR or within the proximal rDNA segment with no further NOR activity. This would explain that in no case the translocation chromosome was observed to be in association with D and G group chromosomes.

FISH experiments

Various probes were selected from the functional different areas of the Y chromosome (PAR1, male specific region on the Y (MSY), Yqh and PAR2) to analyse its structural integrity, as well as the breakpoint regions on chromosome Y and 15.

FISH analysis of the derivative chromosomes showed the same results for all four probands (Fig. 2–4): The translocation chromosome consists of almost the entire chromosomes Y and 15. A huge block of DYZ1 repetitive DNA was found at its expected location, but attached to the heterochromatic part of chromosome 15 (Fig. 2). PAR1 (cosmid KS3) and the male-specific region on the Y (*DAZ*-gene cosmid 7A69M) were found unchanged. From the original Y chromosome only PAR2 (cosmids LLY-cos130G4 and cos 37C9) was missing (Fig. 3).

Also chromosome 15 appeared rather complete. FISH with a rDNA plasmid was performed to analyse if any rDNA copy was left at the fusion point. In accordance with the results of the AgNO₃ staining, all short arms of the remaining G and D group chromosomes showed FISH-signals with the rDNA clone, but not the translocation chromosome (Fig. 4).

The breakpoint on the Y chromosome localizes within the heterochromatic region of the distal long arm resulting in the deletion of pseudoautosomal region 2 (PAR2) (Fig. 3). The breakpoint on chromosome 15 resides within the heterochromatic region on the proximal short arm and resulted in the loss of the rDNA-containing nucleolus organizing region (NOR) (Fig. 4). The translocation chromosome is pseudodicentric, only the centromere of the former chromosome 15 remained active.

The karyotype of the four male family members is best described by 45,X,psu dic(15;Y)(15qter→15p11.2::Yq12→Ypter).

Discussion

The recombination (Fig. 5, on the left) that formed the psu dic(15;Y) in this family involved the heterochromatic parts of the chromosomes Y and 15 and generated a large almost metacentric translocation chromosome. It must have led also to a second small translocation product that contained the short arm rDNA copies from chromosome 15 as well as the PAR2 sequences from the distal long arm of the Y chromosome. Due to its acentric nature it was lost shortly after formation. The loss of these DNA sequences is not associated with any clinical features (Kühl et al., 2001). rDNA is highly repetitive and redundant on the acrocentric human chromosomes. PAR2 exists only on the human but not on the ape Y chromosomes, due to a translocation from the X chromosome during human specific evolution (Freije et al., 1992; Charchar et al., 2003).

The formation of the derivative chromosome 15 can be interpreted as a reciprocal event to the formation of the satellited Y chromosome (Yqs) (Fig. 5, on the right). The origin of Yqs goes back predominantly to a recombination between

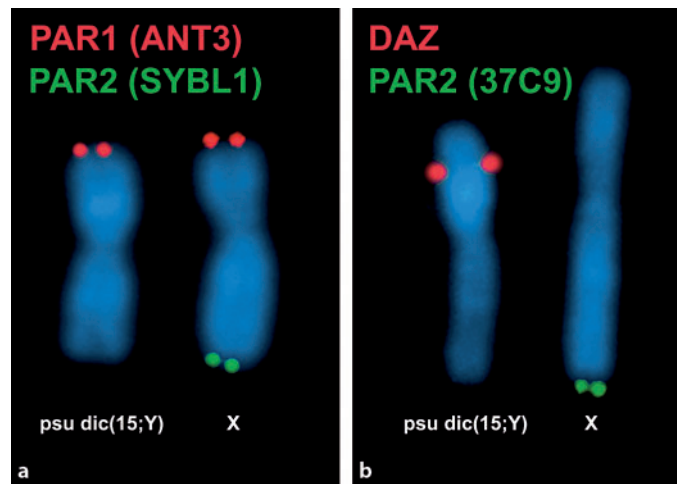


Fig. 3. FISH analyses with a PAR1 specific probe (a, red) and a MSY-specific probe for the gene family *DAZ* (b, red) show regular signals on the Y part of psu dic(15;Y), whereas two different PAR2 probes (a, b, green), both visible on the X chromosomes, are deleted on the psu dic(15;Y). Note, that on psu dic(15;Y) the primary constriction is the former centromere 15.

Y and 15, which is facilitated by the association of the bivalent 15 and the sex vesicle during male meiosis (Schmid et al., 1984; Metzler-Guillemain et al., 1999).

Statistically, the appearance of a dicentric der(15)t(Yq12;15p11.2) is as likely as the appearance of Yqs or chromosomes 15 with heterochromatic short arms originating from Yqh. Indeed, there are a number of cases with brightly fluorescent heterochromatin of giant-satellited acrocentric chromosomes (mostly chromosomes 15 and 22), that consist of translocated Yq heterochromatin (Gal et al., 1987; Haaf et al., 1989).

Nevertheless, the translocation chromosome found in this family has not been described yet. This can be explained by its dicentricity, which usually leads to mitotic instability and meiotic imbalances. A second event, the stable inactivation of one of the two centromeres is obligatory to enable its transmission and therefore assures the stable integrity of the karyotype of the male carriers. Two male patients with de novo 45,X,dic(Y;13) or 45,X,dic(Y;14) karyotypes, respectively, are described as having similar breakpoints on the Y chromosome and on the acrocentric autosome, but miss stable inactivation, leading to male infertility by spermatogenic arrest (Siffroi et al., 2001; Buonadonna et al., 2002).

In our case, the translocation took place in an unknown male ancestor of our probands and has no apparent effect on fertility and phenotype of the carrier. FISH analysis demonstrated the deletion of PAR2 from the Y chromosome and the loss of the NOR from chromosome 15. As these losses are minimal and of no pathological consequences, the translocation chromosome and thus a stably reduced chromosome number is passed on from father to every son in this family. At least theoretically, one might expect an in-

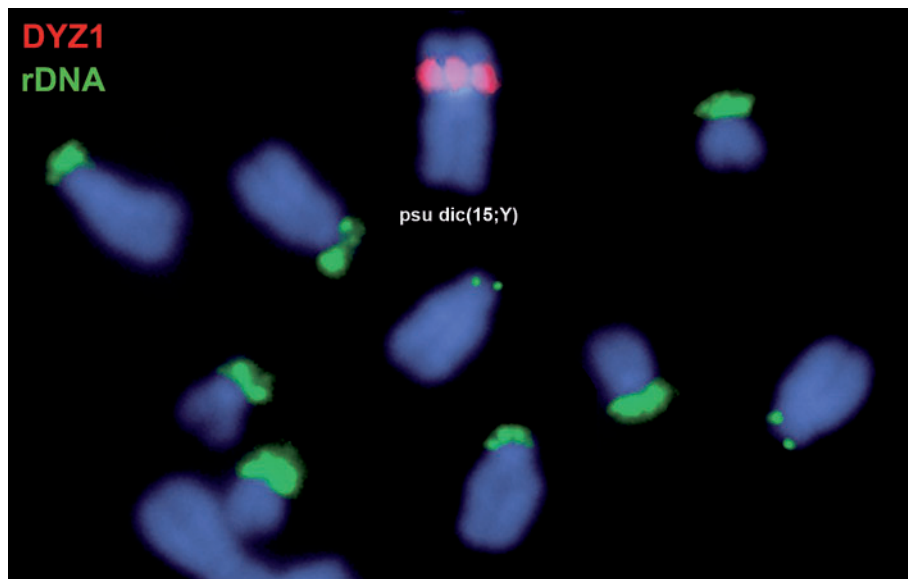


Fig. 4. Two-colour-FISH with an rDNA probe (green) and the Yqh-specific probe DYZ1 (red) produces regular signals on each acrocentric chromosome, but not on *psu dic(15;Y)*, which has lost its NOR.

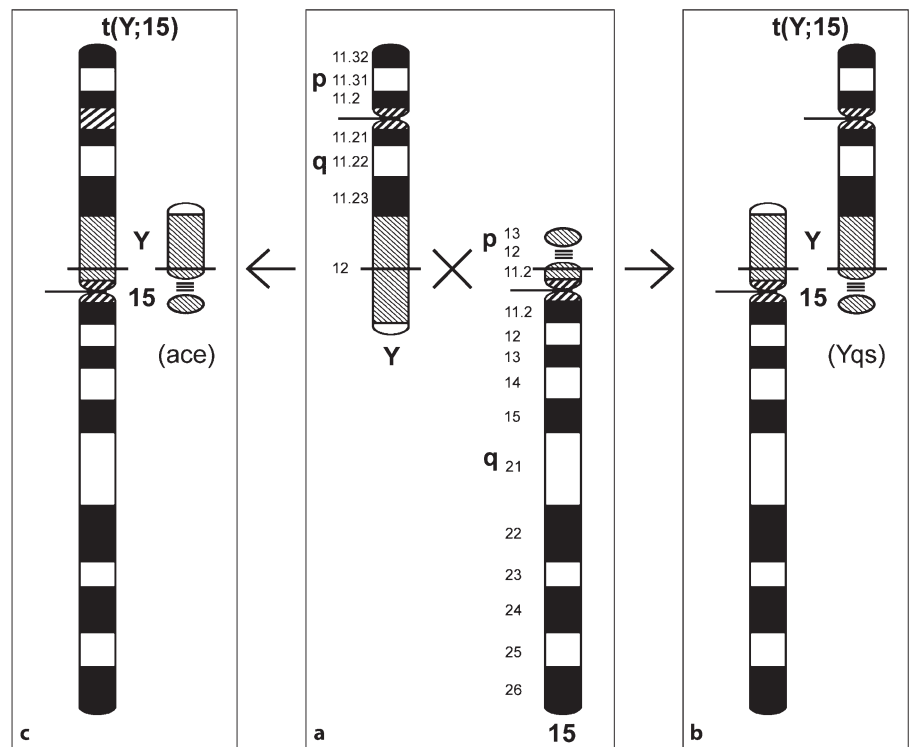


Fig. 5. Two possible outcomes (**b, c**) of the same reciprocal translocation event (**a**). This event may either result in the well known translocation products Yqs and the brightly fluorescent chromosome 15 (**b**), or in the translocation chromosome found in this family (**c**), where an additional event stably inactivated the Y centromere.

creased risk for 46,XX,*psu dic(15;Y)* males with Klinefelter syndrome, 45,X females with Turner syndrome (or reproductive failure) and progeny with uniparental disomy 15. Unfortunately, no further details about the family history are available to prove this assumption.

Interestingly, this translocation leads to a stable reduction of chromosome number in the male sex without impairing fertility and further reproduction. Even though the great majority of mammals have an XX/XY sex determina-

tion system, some species with X0 males have been observed. The most famous example is a rodent, the mole vole *Ellobius lutescens*. However, in this species we have the exceptional situation in that a 17,X0 chromosome constitution exists in both sexes (Matthey, 1953), and the male sex is constitutively determined without the *SRY* gene (Baumstark et al., 2005). An XX female and X0 male sex determining system has been described in the new world monkey clade. There are several examples reported in the literature: Goel-

di's marmoset (*Callimico goeldii*, Hsu and Hampton, 1970), Black-headed Uakari (*Cacajao melanocephalus*, Koiffmann and Saldanha, 1981), one owl monkey species (*Aotus*, Ma et al., 1976, 1989) and four of six Howler monkey species (*Alouatta*, de Oliveira et al., 2002) reproduce without an independent Y chromosome. In these species the difference in chromosome number between the female and the male sex is a product of ancestral translocation events.

The human family described here shows an early stage of such a disappearance of a separate Y chromosome without consequences for male sex determination and fertility.

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