RESEARCH NOTE

Two further triple-X/rea(X) females in an inv(X)(p22q22) family

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Introduction

Recently two reports published in this journal described the exceptional concurrence of triple-X aneuploidy with a rearranged X chromosome, namely a maternal Xq+ transmitted to two 47,XX,add(X)(q26) sisters (Ramachandram *et al.* 2013) and a *de novo* Xp deletion in a 47,XX,del(X)(p21) patient (Malla *et al.* 2014). In addition, two sporadic and unrelated 45,X/46,X,rea(X)/47,X,rea(X),rea(X) females are also known (Daly *et al.* 1977; Reinehr *et al.* 2001). These observations prompt us to describe here a comparable concurrence in a family with an inv(X)(p22q22) that was first diagnosed in a 14-year-old girl with a height of 135 cm (<3rd centile) and delayed milestones. The inverted X was inherited from her 46-chromosome mother but the same condition was also found in her sister and maternal grandmother who were triple-X females and had an unremarkable clinical phenotype.

Subjects and methods

Karyotyping was carried out on at least 16 G-banded metaphases from lymphocyte cultures of four maternally related (proband, mother, sister and maternal grandmother) females and from proband's father. Moreover, X-chromosome inactivation was visualized by late replication after a BrdU terminal pulse in 50 metaphases of each of all four females. Finally, fluorescence *in situ* hybridization (FISH) with Xp/Yp subtelomeric, Kallmann/alphoid, DXZ1, and Quint-Essential DMD probes (the former two from Vysis, Downers Grove, USA; the latter two from Oncor, Gaithersburg, USA) probes were carried out on proband's chromosomes (at least five scored metaphases per assay).

Results

The proband and her mother had a 46,X,inv(X)(p22q22) karyotype whereas the proband's sister and maternal grandmother were triple-X females with a single copy of the inverted X chromosome (figure 1a). In addition, the grandmother was a 46,X,inv(X)[36]/47,XX,inv(X)[13] mosaic (a single cell was 45,X); the proband's father was 46,XY. The inv(X) was early-replicating in 18 out of 50 metaphases in the proband, 21 out of 50 metaphases in the mother, 6 out of 50 metaphases in the sister, and 29 out of 50 metaphases in the grandmother (figure 1b). In FISH results, the inversion did not displace the Xp/Yp subtelomeric repeats but relocated both Kallmann and DMD loci to the other arm; for the alphoid signal, it mapped at the primary constriction in the inverted chromosome. The normal homologue exhibited the expected signals (figure 1c).

Discussion

Even though the frequency of the XXX aneuploidy is one in 1000 newborn girls (Jacobs 1979; Tartaglia *et al.* 2010), its concurrence with a familial or sporadic rearranged X chromosome, other than the relatively common i(Xq) (Melaragno *et al.* 1993), in five instances, it is intriguing and analogous to three cytogenetic variants found in a minority of Klinefelter syndrome patients: 47,X,i(Xq),Y; 47,X,rea(X),Y; and 47,XX,rea(Y) karyotypes disclosed in 25, 5 and 11 individuals, respectively (for review, see Frühmesser and Kotzot 2011).

Whether the extra X chromosome and the rea(X) in a given XXX patient came from the same parent is unclear. In the family described here, it can intuitively be argued that both the extra and the rearranged chromosomes came from the mother carrying the inv(X), even if the karyotype of the proband's maternal great-grandmother is unknown.

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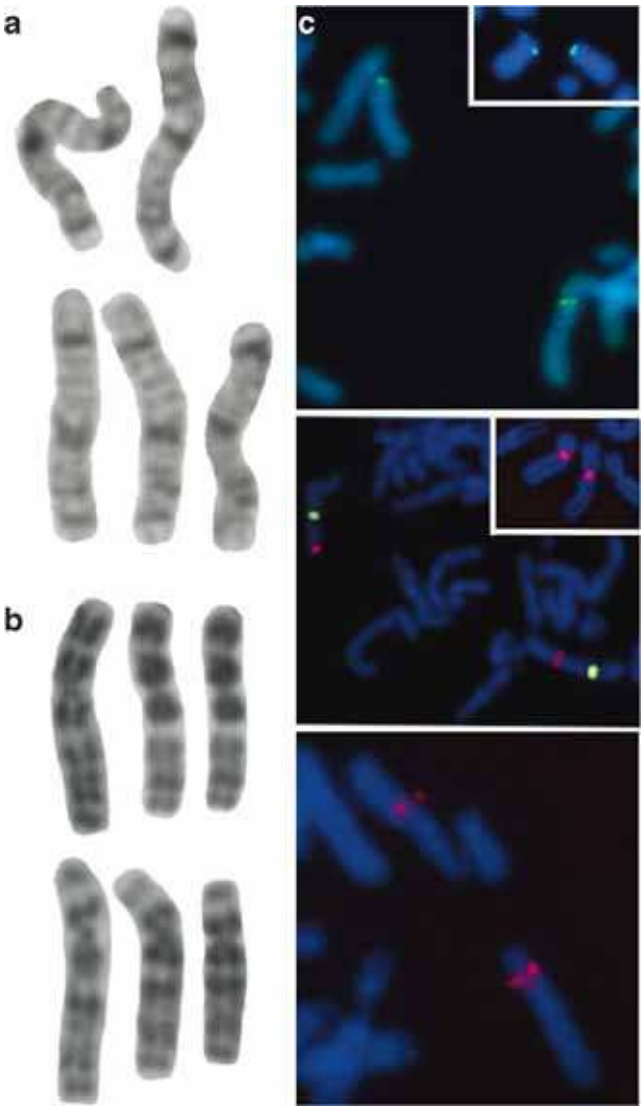

Figure 1. (a) The familial inv(X)(p22q22) after G-banding: X chromosomes from a 46,X,inv(X) carrier (upper row) and from a 47,XX,inv(X) carrier (lower row). (b) Random X inactivation: three active normal X chromosomes (upper row) and three active inv(X) chromosomes (lower row) from different metaphases. (c) FISH assays with four probes: subtelomeric Xp/Yp, Kallmann/alphoid, DXZ1 and DMD. The Xp subtelomeric repeats (two metaphases in the upper panel) remained in place whereas the Kallmann (middle panel) and DMD (lower panel) loci were relocated onto the other arm. The inset in the middle panel highlights the centromere of both the inverted and normal X chromosomes after FISH with the DXZ1, probe.

This assumption also applies to a pair of 47,XX,add(X)(q26) sisters (Ramachandram *et al.* 2013) and all three sporadic patients with a triple-X/rea(X) concurrence (Daly *et al.* 1977; Reinehr *et al.* 2001; Malla *et al.* 2014) in whom the parental origin of the X chromosomes was not determined. Moreover, it is supported by the observation of a high proportion (~80%) of typical XXX females arising from a maternal nondisjunction at either meiosis I or meiosis II; the remaining fraction is ascribed to a postzygotic error involving either homologue (Tartaglia *et al.* 2010). Likewise, a maternal meiosis II nondisjunction coupled with a postzygotic formation of an idic(X) likely accounted for a Klinefelter 47,X,idic(X)(p11.1),Y patient (Höckner *et al.* 2008). Yet, the indistinct parental origin of Xq isochromosomes (for review see Melaragno *et al.* 1993) and the extra X chromosome in XXY males (Frühmesser and Kotzot 2011) as well as the preponderant paternal descent of other *de novo* rearrangements of the X chromosome (Gardner and Sutherland 2004; Rivera *et al.* 2013) should be kept in mind.

The 45,X and 47,X,rea(X),rea(X) clones observed in two sporadic patients (Daly *et al.* 1977; Reinehr *et al.* 2001) are consistent with the respective zygotes being 47,X, rea(X),rea(X) and resulting from a nondisjunction in a secondary oocyte with a *de novo* rea(X). The 45,X/46,X,rea(X) or 46,X,rea(X)/47,XX,rea(X) mosaicism found in the one aged female from each of two families (Ramachandram *et al.* 2013; present case) suggests that the respective zygote was 46,X,rea(X) in the first subject and 47,XX,rea(X) in the second one, the latter being secondary to a meiosis I nondisjunction in her supposed rea(X) carrier mother. A subsequent age-related centromere dysfunction (Fitzgerald *et al.* 1975) might confer mitotic instability and account for the observed mosaicism in all the three aged females (the fourth patient was a 16-year-old girl). Alternatively, the 45,X/46,X, rea(X)/47,X,rea(X),rea(X) mosaicism found in both sporadic females could result from a nondisjunction of the rea(X) in a 46,X,rea(X) zygote. Likewise, the 46,X,rea(X)/47,XX, rea(X) mosaicism in the present family's grandmother can be ascribed to a mitotic nondisjunction of the normal homologue provided there was a lost or undetected 45,rea(X) clone; in contrast, a 45,X clone would exclude such a mitotic error because three normal Xs would then be required to get both aneuploid clones.

Genetic counselling in the present family should consider unfavourable reproductive outcomes because a similar or identical inv(X)(p22q22) in another Mexican family gave rise to two sisters with the rec(X)dup-p/del-q and primary amenorrhoea (Madariaga and Rivera 1997). Although their different surnames point to both families being unrelated, they come from the same region and may share a common ancestor. Indeed, the apparent absence of this specific breakpoint combination, notwithstanding the fact that among ~40 X-chromosome inversions ascertained in other countries, the more frequent breakpoints were p22 and p11 (18 times) and q22 (11 times), suggests that it is confined to the Mexican Mestizo population.

Finally, the G-banding pattern and positivity to the X-specific whole chromosome painting of an Xq+ chromosome transmitted from a mosaic 45,X/46,X,add(X)(q26) mother to two 47,XX,add(X)(q26)mat daughters (Ramachandram *et al.* 2013) allow us to visualize such an Xq+ as a recombinant-like dup-p/del-q chromosome (Rivera *et al.* 2013), namely Xpter→q26::p11.4→pter. That the segment added onto Xq26 actually corresponds to a large Xp duplication can better be appreciated by comparing both ends of the Xq+ in figures 1a and 1b

(Ramachandram *et al.* 2013). Altogether, 19 rearranged X chromosomes of *de novo* or untraced origin and entailing a dup-p/del-q double imbalance are on record (Marozzi *et al.* 2000; Nakamura *et al.* 2001; Ramachandram *et al.* 2013; 16 patients compiled in Ramírez-Velasco and Rivera 2014).

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