

# Molecular and cytogenetic characterization of two patients with recurrent miscarriages and X-autosome translocation

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**Aim:** To report two patients with recurrent miscarriages and unique reciprocal X-autosomal translocation. **Materials and Methods:** Cytogenetic analysis was performed using G-banding and Molecular cytogenetic analysis by Fluorescence *in situ* hybridization to confirm the breakpoint regions. **Results:** The chromosomal analysis of the two cases revealed a karyotype of 46,X,t(X;22)(p11.21;q13.3) in the first patient and 46,X,t(X;2)(q22;q13) in second patient. Both the cases were confirmed by using whole chromosome paint probes. **Conclusions:** This is the rare report of X-autosomal translocations with unique breakpoint regions and their association with recurrent miscarriages. The translocation breakpoint in case 2 on Xq22 and on Xp11.21 in case 1 might be a risk factor for recurrent miscarriages. Here the impact of the X-autosomal translocations is discussed.

**Key words:** Recurrent miscarriages, translocations, X-autosomal translocations

## INTRODUCTION

Recurrent miscarriage (RM) is defined as a condition of three or more consecutive pregnancy losses before 24 weeks of gestation.<sup>[1]</sup> The causes of RM are heterogeneous, But chromosomal abnormalities mainly balanced rearrangements are common in couples with RM.<sup>[2]</sup> Though autosome–autosome translocations are quite common, translocations involving X chromosome and an autosome are rather rare, occurring in about 1 in 30,000 live births, as most of the men and half of women with this condition are infertile.<sup>[3]</sup>

X-autosomal (X;A) translocations are generally of maternal origin or arise *de novo*.<sup>[4]</sup> Apparently, all *de novo* balanced X;A translocations studied so far have been of paternal origin<sup>[5]</sup> and are more likely to be associated with an abnormal outcome, suggesting that *de novo* status versus breakpoint location is the most important risk factor in defining the phenotype.<sup>[6]</sup> Female carriers of X;A translocations can be broadly classified into four classes; phenotypically normal with/without a history of RM, gonadal dysfunction with primary

amenorrhea of polycystic ovarian failure (POF), well-defined X-linked recessive or dominant disorders and congenital abnormalities with/without developmental delay (including learning disabilities).<sup>[6]</sup> Here, we report two cases with RM and X;A translocations. First case is a reciprocal translocation involving chromosomes X and 22 and the second case is a reciprocal translocation involving chromosomes X and 2. This is the first report of X;A translocations with unique breakpoints and their association with RM. The translocation breakpoints in both the cases on Xq22 and Xp11.21 might be a risk factor for RM. Here the impact of the X;A translocation is discussed.

## MATERIALS AND METHODS

### Patient history

Both the patients had come to our genetic clinic at the Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India with RM. In both the cases detailed reproductive histories were taken and chromosomal analyses were performed from the peripheral blood cultures. The first patient was 26 years old and had been married for 5 years with 3 recurrent miscarriages, whereas the second patient was 28 years old with 4 recurrent miscarriages, she had been married for 6 years. Her menarche was induced at 14 years of age and her FSH levels were 29.3 mIU/ml which was elevated.

### Chromosomal analyses

Metaphase chromosome preparations from the peripheral blood cultures were made according to

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the standard cytogenetic protocols. Cytogenetic analysis was performed by G-banding using Trypsin and Giemsa at approximately 400–450 band level. Fifty metaphases were analyzed and reported according to the International System for Human Cytogenetic Nomenclature (ISCN).

### Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization (FISH) was performed using commercially available whole chromosome paint probes (WCP) for chromosomes X, 2, and 22 (Kreatech). In parallel, in the first case, FISH with microdeletion probe on 22q11.2 and 22q13 region. Nothing, it was a repetition was also performed. Chromosome denaturation, hybridization and signal detection were done according to the published protocols.<sup>[7]</sup>

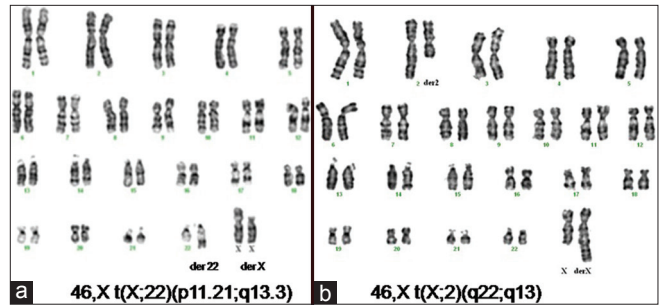
## RESULTS

Cytogenetic analysis of the two patients with recurrent miscarriages showed X-autosomal translocations. The chromosomal analysis of the first patient revealed a karyotype of 46,X,t(X;22)(p11.21;q13.3) and the second patient showed 46,X,t(X;2)(q22;q13) [Figure 1]. Whole chromosome paint with 22 in the first case (data not shown) and also X and 2 in the second case confirmed the translocation [Figure 2]. Subsequently, we confirmed the translocation event by using probes on 22q11.2 and 22q13 region in the first patient. The 22q11.2 region (red signals) was present on the derivative 22 whereas the 22q13 region (green signals) was present on the derivative X chromosome [Figure 3].

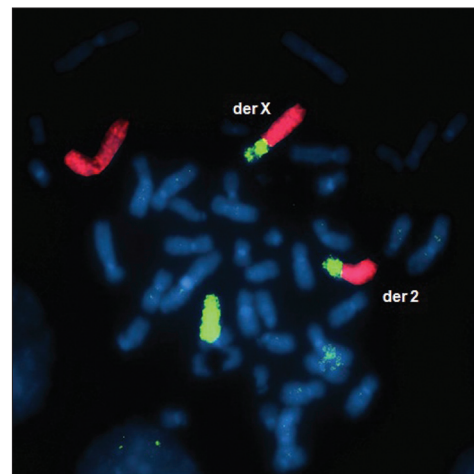
## DISCUSSION

X;A translocations are rare due to associated infertility in men and sub fertility in women. In balanced X;A translocation, the variation in the fertility status depends on the sex of the carrier, the position of the translocation breakpoints<sup>[8]</sup> and the pattern of X-inactivation.<sup>[6]</sup> Most carriers of an X;A translocations are phenotypically normal.<sup>[6,8]</sup> But in female carriers, gonadal dysgenesis may also occur, and about 9% may have multiple anomalies and/or mental retardation.<sup>[9]</sup> Also, infertility because of gonadal disgenesis is common among those women in whom the breakpoint in the derivative X chromosome involves the critical region Xq13-q26. However, X;A translocations may often lead to either primary or secondary ovarian failure or sometimes Turner-like features if it occurs within the region of Xq13-q26.<sup>[10]</sup>

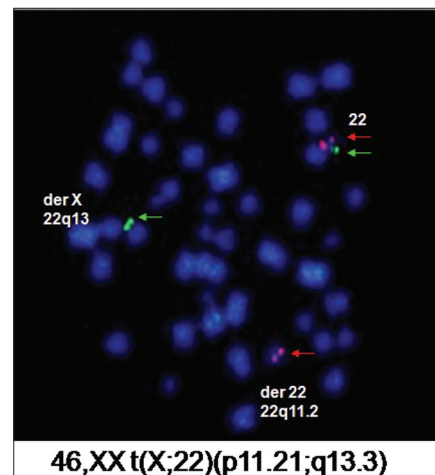
We are aware that X-chromosome inactivation is a mechanism of dosage compensation, which results in silencing of the majority of genes on one of the two X chromosomes in somatic cells of females, but in cases of balanced X;A



**Figure 1:** GTG banded karyotypes showing X;A translocations in the two patients. (a) The karyogram showing 46,X,t(X;22)(p11.21;q13.3) in the first patient. (b) The karyogram showing 46,X,t(X;2)(q22;q13) in the second patient



**Figure 2:** WCP FISH on the metaphase spreads in the second case. Normal X is shown in green color, normal chromosome 2 is shown in red color and both the derivative chromosomes are shown in both red and green colors



**Figure 3:** FISH on the metaphase chromosomes on the first case using the microdeletion probe. The red signals on 22q11.2 are shown on derivative 22 whereas the green signals on 22q13 are shown on derivative X chromosome

translocation in female carriers, the normal X chromosome is usually inactivated, leaving the derivative X-chromosome in the active state. Hence, there would not be a big phenotypic effect in X;A translocation patients but fertility variations are possible.

When the breakpoint occurs within a gene, classic X-linked disorder may be phenotypically expressed.<sup>[11]</sup> Female carriers are at 20%–40% risk to have a live born with structural and/or functional aneuploidy with a spectrum of phenotypic manifestations ranging from mild effects to severe mental retardation, sub fertility/Infertility and birth defects.<sup>[12]</sup> For instance ovulation was successfully induced in a woman with t(X;16)(q26;qter) having POF, thus offering hope to these women through opting for assisted reproductive techniques.<sup>[13]</sup> However, fertility can never be restored if POF is diagnosed after complete follicular depletion. The only solution presently available for the women with absent follicular reserve is represented by ovum donation. Hence, there is a need for early diagnosis of genetic defects. Several candidate genes in X have been proposed by mapping genes interrupted by the breakpoints in X;A translocations.<sup>[14]</sup> In patients with X;A translocations with POF there exists 3 critical regions. One on Xp and others on Xq13-Xq21 and Xq23-Xq27 but patients with Xq22 escaped POF.<sup>[8]</sup> In our study we analyzed two patients with RM and X;A translocation. The OMIM gene search on Xp11.21 breakpoint region in the first case showed a PAGE5 (P antigen family, member 5) gene which is a member of GAGE family. This gene is expressed in a variety of tumors and in some fetal and reproductive tissues. In our second case the breakpoint was on Xq22 and the OMIM gene search showed that there is a FSHPRH1 (FSH primary response, rat, homolog of, 1) or LRPR1 (Leucine-rich primary response gene 1) gene which is transcriptionally activated in response to follicle-stimulating hormone and the response of LRPR1 was specific to FSH. Roberts *et al.*<sup>[15]</sup> hypothesized that the specificity of the response of LRPR1 to FSH makes LRPR1 a potential; candidate for human X-linked disorders of gonadal development. Thus the breakpoint region at Xq22 might have disrupted the gene causing RM. Probably elevated FSH supports our view. Thus occurrence of the X;A translocations confirms in our study the role of Xp11.21 and Xq22 regions in the RM.

## CONCLUSION

Identification of chromosomal abnormalities with X;A translocations in patients with RM helps in the identification of the genes directly or indirectly involved in the disorder. This in turn would help the patients in assisted reproductive technologies. Further fine mapping and precise characterization of the breakpoint region helps in elucidating the mechanism involved in the translocation and also in the identification of new genes in RM which helps in the field of reproductive genetics.

## REFERENCES

1. Royal College of Obstetricians and Gynecologists. The management of recurrent miscarriage. London, United Kingdom. Royal College of Obstetricians and Gynecologists; 1998.
2. Dutta UR, Rajitha P, Pidugu VK, Dalal AB. Cytogenetic abnormalities in 1162 couples with recurrent miscarriages in Southern region of India: Report and review. *J Assist Reprod Genet* 2011;28:145-9.
3. Layman LC. The genetic basis of female infertility. In: Rimoin DI, Connor JM, Pyeritz RE, Korf BR, editors. Principles and practice of medical genetics. London: Churchill Livingstone; 2002. p. 947-60.
4. Kalz-Fueller B, Slegers E, Schwanitz G, Schubert R. Characterization, phenotypic manifestations and X-inactivation pattern in 14 patients with X-autosomal translocations. *Clin Genet* 1999;55:362-6.
5. Powell CM, Taggart RT, Drumheller TC, Wangsa D, Qian C, Nelson LM, *et al.* Molecular and cytogenetic studies of an X;autosome translocation in a patient with premature ovarian failure and review of literature. *Am J Med Genet* 1994;52:19-26.
6. Waters JJ, Campbell PL, Crocker AJM, Campbell CM. Phenotypic effects of balanced X-autosome translocations in females: A retrospective survey of 104 cases reported from UK laboratories. *Hum Genet* 2001;108:318-27.
7. Licher P, Cremer T, Borden J, Manuelidis L, Ward DC. Delineation of individual human chromosomes in metaphase and interphase cells by *in situ* suppression hybridization using recombinant DNA libraries. *Hum Genet* 1988;80:224-34.
8. Madan K. Balanced structural changes involving the human X: Effect on sexual phenotype. *Hum Genet* 1983;63:216-21.
9. Schmidt M, Du Sart D. Functional disomies of the X chromosome influence the cell selection and hence the X inactivation pattern in females with balanced X-autosome translocations: A review of 122 cases. *Am J Med Genet* 1992;42:161-9.
10. Therman E, Laxova R, Susman B. The critical region on the human Xq. *Hum Genet* 1990;85:455-61.
11. Scala E, Ariani F, Mari F, Caselli R, Pescucci C, Longo I, *et al.* CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms. *J Med Genet* 2005;42:103-7.
12. Gardener RJ, Sutherland GR. Chromosome abnormalities and genetic counseling. New York: Oxford University Press; 2004. p. 119.
13. Causio F, Fischetto R, Leonetti T, Schonauer LM. Ovarian stimulation in a woman with premature ovarian failure and X;autosome translocation-a case report. *J Reprod Med* 2000;45:235-9.
14. Vitek WS, Pagidas K, Pepperell JR, Simpson JL, Tantravahi U, Plante BJ. Xq;autosome translocation in POF. Xq27.2 deletion resulting in haploinsufficiency for SPANX. *J Assist Reprod Genet* 2011;29:63-6.
15. Roberts RG, Kendall E, Vetrie D, Bobrow M. Sequence and chromosomal location of a human homologous of LRPR1, an FSH primary response gene. *Genomics* 1996;37:122-4.

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