DOI: 10.7860/JCDR/2015/8291.5612

Original Article

Genetics Section

Association of Parental Origin with Clinical Profile in Klinefelter Syndrome

RANGANATH VALLABHAJOSYULA[1], SAYEE RAJANGAM[2], LALITHA C.[3]

ABSTRACT

Introduction: Several genomic imprinting mechanisms have been postulated to report the parent-of-origin in Klinefelter syndrome. It was stated in the literature, parental origin has an effect on behavioral phenotype of Klinefelter individuals, but the association of the same on clinical profile was less reported. The detailed clinical phenotype when studied with the known origin of extra X may possibly explain the imprinting effect that may be helpful to derive diagnostic criteria in the syndrome. In the present study, we investigated the parental-of-origin of extra X chromosome in Klinefelter syndrome probands with an aim to report the association between the phenotype with that of its karyotype and the parental origin of supernumerary X.

Materials and Methods: Seventy two probands that were referred to division of Human Genetics, St.John's Medical College, Bangalore with variable complaints and phenotypic features were diagnosed with informed consent as Klinefelter syndrome with a confirmed karyotype. The Karyotype was prepared by peripheral lymphocyte culture and GTG banding method. The parental origin was studied in 9 families of Klinefelter probands with standard protocol for GENE SCAN using X-chromosome specific Short Tandem Repeat markers. The outcome was analyzed to determine the parental origin by GENE MAPPER.

Statistical analysis: Statistical analysis was conducted to ascertain the significance of parental origin of supernumerary X with the phenotypic profile with confirmed karyotype.

Results: Seven of nine probands had 47, XXY karyotype and 2 were mosaic with 47,XXY/46,XY karyotype. Five probands had their supernumerary X from maternal side and four were paternally derived. Sixteen features as framed proforma were tabulated against the originated X in Klinefelter probands. 55.56% of Klinefelter stigmata were seen in prob and who had maternally derived X and the rest were with paternal X.

Conclusion: The findings of the present study points on parent-of-origin effect on clinical profile and indicate that the imprinted X chromosome genes show differential effect general and systemic traits.

Keywords: Clinical phenotype, Karyotype, Probands, X chromosome

INTRODUCTION

Klinefelter syndrome (KFS) was the most common sex chromosome abnormality in male with a global incidence of 1 in 500 to 1000 male live births, with more prevalence in individuals with mental retardation [1]. The diagnosis of KFS happens mostly in late teens or adult age group, due to the indications of hypogonadism and/or infertility or with systemic abnormalities. The most commonly seen clinical manifestations include reduced or absence of secondary sexual characters, tall stature, gynaecomastia, small or infantile mesonephric derivatives and external genitalia can be ascertained to possible errors in steroidogenesis that may influence on the spermatogenetic pathway. Polymorphic research studies have reported remarkable variation of X chromosome trisomy's with regard to the parental origin of additional X [2], with higher preponderance of paternal origin of supernumerary X in Klinefelter syndrome, where the etiology is the non-disjunction mechanism that may happen during meiosis I. This mechanism may change with reference to maternal origin, which may happen either in meiosis I or II or during the post-mitotic division [3-5].

The association of clinical phenotype with the parental origin in KFS is open to discussion and limited studies exist to report the association. In continuation with our previously published observational study on data profile of phenotypic features in 72 KFS male [6], the authors have designed the objective to report the parental origin of extra X chromosome and the association of clinical profile of in 9 KFS male probands.

MATERIALS AND METHODS

In the first phase of cytogenetic study, we have a record of 72 male individuals referred to division of Human Genetics, Department of Anatomy, St John's Medical College, Bangalore with hypogonadism, Infertility sterlity, KFS (?) as the main chief complaints. At the time of referral, a detailed clinical proforma was documented to report the expressed phenotypic features of the probands. With the informed consent, cytogenetic analysis was performed by conventional karyotyping method and automated karyotyping system. Semen analysis was carried to know about the status of sperm production.

After obtaining a confirmed Klinefelter karyotype either in pure form or mosaic form with Klinefelter karyotype more than 10% or Klinefelter variant, consent was requested from the proband and the parents to determine the parental origin.

Eighteen KFS male and families gave their consent for the parental origin detection of the X. DNA was extracted from the proband and parents by modified phenol-chloroform method (Thangraj et al., [7] Quantification of DNA was carried by gel electrophoresis method. X chromosome specific STR (Short Tandem Repeat) markers were used to amplify the DNA of the probands and parents under specific PCR settings (BIORAD). The amplified product was subjected to GENE SCAN (Applied Biosystems 3730) for parental origin determination. The results of GENE SCAN was analyzed and interpreted in GENEMAPPER (ABI Prism Linkage mapping sets v2.5 user's manual).

The listed phenotypic features that were documented are correlated with karyotype and the parental origin of the X. A percentage analysis was carried out to report the entire documented and observed data.

RESULTS

The parental origin could be determined in nine families with KFS probands. Maternal origin of the X was confirmed in five and the paternal origin in four. 47, XXY karyotype was observed in seven cases; out of which three showed maternally derived X (Xm) and four paternally derived X (Xp). Maternal origin of X chromosome (Xm) was observed in two cases with 46,XY/47,XXY mosaic Klinefelter karyotype.

Sixteen cardinal features were listed and tabulated in the proforma. These features are listed against the determined parental origin in the nine KFS cases in [Table/Fig-1].

Sl.No	Features	Xm n 5	Xp n 4
1	Chief complaint at the time of referral		
	? KFS (05)	02	03
	Hypogonadism (01)	01	-
	Sterility (02)	02	-
	Infertility (01)	-	01
2	Age at the time of referral		
	10-20 years (03)	01	02
	21-30 years (03)	02	01
	31-40 years (03)	02	01
3	Birth Order		
	1 (04)	03	01
	2 (01)	-	01
	4 (01)	-	01
	5 (02)	02	-
	NK (01)	-	01
4.	Maternal age at conception		
	15-20 years (01)	01	-
	21-30 years (03)	02	01
	31-40 years (04)	02	02
	NK (01)	-	01
5	Paternal age at conception		
	15-20 years (01)	01	-
	21-30 years (02)	02	-
	31-40 years (02)	-	02
	41-50 years (03)	02	01
	NK (01)	-	01
6	Consanguinity		
	1st cousin (02)	01	01
	Non-consanguineous (07)	04	03
7	Education		
	Schooling (04)	02	02
	PUC (01)	01	
	Graduation (04)	02	02
8	Height		
	< 160 cms (01)	01	-
	> 160 cms (04)	02	02
	NK (04)	02	02
9	Build		
	Tall (03)	01	02
	Normal (06)	04	02
10	Gynaecomastia		
	Present (04)	02	02
	Absent (05)	03	02
11	Axillary Hair Growth		
	Normal (06)	02	04
	Scanty (01)	01	-
	Absent (02)	02	-
12	Pubic Hair Growth		
	Normal (06)	02	04
	Scanty (03)	03	
13	Size of Male external genitalia		
	Small (02)	01	01
	Normal (07)	04	03
14	Size of Gonads		
	Small (05)	01	04
	Normal (04)	04	
15	Semen analysis		
	Azoospermia (03)	02	01
	Normal (06)	03	03
16	Karyotype		
	47,XXY (07)	03	04
	46,XY/47,XXY (02)	02	
	Total (144)	80	64
	Normal	24	23
	abnormal	56	41

[Table/Fig-1]: KFS: Clinical phenotype Vs Parental origin (n 9)

The documented KFS stigmata such as height, build, gynecomastia, axillary hair growth, pubic hair growth, size of gonads, size of external genitalia were observed to be at associated with maternally derived X (Xm). Semen analysis revealed azoospermia in three KFS male, two with Xm and one with Xp.

DISCUSSION

The first objective of the study was to know the origin of extra X chromosome in a sample of nine confirmed KFS cases. The observations derived from the study five with maternal X and four with paternal X correlate with the few reports in the literature. Linden et al., [8] reported 40 percentile range of X of maternal origin in their study of sex chromosomal tetrasomy and pentasomy. Eskenazi et al., [9] highlighted in his study about 72.2% of maternal origin of additional X and paternally derived X 27.8% with a marginal significant difference from the study of Harvey et al., [10] which report parental origin was about 44%. These observations indicate the trend of maternal derived X in KFS.

Technical approach has pivotal role in identifying the parental origin of additional X. Linden et al., [8] with Restricted Fragment Length Polymorphism reported 40% of Xm and 60% of Xp in 5 KFS cases. Eskenzi et al., [9] reported a higher percentage of Xm using microsatellite markers. Froland et al., [11] based on Xg blood grouping, determined the parental origin of extra X in 10 KFS male and reported 60% with Xm and 40% with Xp origin. Large sample of KFS was investigated Jacobs [12] and Harvey et al., [10] used X centromeric probe. The reports from their metacentric study show Xm and Xp in 111 and 61 KFS male respectively CAG repeat length of AR gene was reported by Zinn et al., [13] and Stemkens et al., [14] with equal percentage Xm or Xp.

The percentage analysis of the parental origin of X in KFS, in the present study was within the reported range for each category. The observations of the present study for maternally derived X

(Xm) (55.56%, 5) and paternally derived X (Xp) (44.44%, 4) agreed with that of the study by Harvey et al., [10] and Zinn et al., [13] (56% Xm; 44% Xp). The determined percentage of the parental origin of X in KFS, in the present study, could be interpreted that more or less the non-disjunction of X during gametogenesis might be in line with proportional distribution reported in the literature. The second objective of the study is to establish the association between parentally derived X with the manifested phenotypic features confirmed KFS male. The results indicate that trend of KFS stigmata was more towards the maternal X (Xm) and there was no significant relation between the general features such as parental age at conception, age at the time of referral and parental consanguinity.

Zinn et al., [13] reported no significant difference in any mean anthropometric measure or in penile length or testicular volume irrespective of the parental origin, in KFS.

Stemkens et al., [14] reported motor impairment and speech and language delay, low verbal IQ in KFS male with Xp than Xm; however, the observations were found to be statistically non-siginificant. Anthropometric parameters such as occipital frontal circumference, sitting height and penile length, biacromian distance and middle finger length were associated with Xp, but statistically not significant.

The above authors also reported short penile length and lesser testicular volume; but parental origin effects on these two features were not mentioned. In the present study, in Xp there was hypogonadism in one KFS and short penis in another.

In the present study, features were categorized into 16 groups. The calculated total numbers of 16 features are multiplied for the sample of 9 was 144. Irrespective of the parental origin, the KFS males manifested 67.36% of the abnormal KFS features. Out of which, with Xm (5), the manifestation of phenotypic features was 57.7% and with Xp (4), it was 42.26%.

The observation of the 67.36% of the manifestation of the KFS features in the present study could be interpreted that in Indian KFS men the severity of the KFS features are associated with Xm and Xp in more or less equal percentage. From, the findings it could be opined, that out of the listed features, in case scoring is carried out, any suspected KFS with >50% scoring and above the chance of receiving either Xm or Xp is equal.

CONCLUSION

In KFS, out of nine, seven had 47, XXY karyotype and two were with X-mosaicism (46,XY/47,XXY) Parental origin of X was determined in these nine cases. The maternal origin in KFS was seven and paternal in two. On correlation, Irrespective of the parental origin, the KFS males manifested 67.36% of the abnormal KFS features. Out of which, with Xm (5), the manifestation of phenotypic features was 57.7% and with Xp (4), it was 42.26%. From, the findings it could be opined, that out of the listed features, in case scoring is carried out, any suspected KFS with >50% scoring and above the chance of receiving either Xm or Xp is equal.

ACKNOWLEDGEMENTS

We sincerely thank DrThangaraj CCMB Hyderabad and DrRajender Singh CDRI Lucknow for their immense help and support in carrying out parental origin study.

REFERENCES

[1] http://emedicine.medscape.com/article/945649.
[2] Thomas SN, Hassold TJ. Aberrant recombination and origin of Klinefelter syndrome. *Hum Reprod update*. 2003;9(4):309-17.
[3] Jacobs PA, Hassold TJ, Whittington E, Butler G, Collyer S, Keston M, et al. Klinefelter's syndrome: an analysis of the origin of the additional sex chromosome using molecular probes. *Ann of Hum Genet*. 1988;52:93-109.
[4] Hassold TJ, Sherman SL, Pettay D, Page DC, Jacobs PA. XY-chromosome nondisjunction in man is associated with diminished recombination in the pseudoautosomal region. *Amer J Hum Genet*. 1991;49:253-60.
[5] Lorda-Sanchez I, Binkert F, Maechler M, Robinson WP, Schinzel A. Reduced recombination and paternal age effect in Klinefelter syndrome. *Human Genetics*. 1992; 89:524-30.
[6] Ranganath V, Sayee R. A data profile of phenotypic features in 72 Klinefelter syndrome (KFS) males. *Int J Hum Genet*. 2012;12(3):139-43.
[7] Thangaraj K, Joshi MB, Reddy AG, Chakravarty B, Singh L. CAG repeat expansion in the androgen receptor gene is not associated with male infertility in Indian populations. *J* Androl. 2002;23:815-18.
[8] Linden MG, Bender BG, Robinson A. Sex chromosomal tetrasomy and pentasomy. *Pedia*. 1995;96(4):672-82.
[9] Eskenazi B, Wyrobek AJ, Kidd SA, Lowe X, Moore II D, Weisigner K et al. Sperm aneuploidy in fathers of children with paternally and maternally inherited Klinefelter syndrome. *Hum Reprod*. 2002;17(3):576-83.
[10] Harvey J, Jacobs PA, Hassold T, Pettay D. The parental origin of 47,XXY males. *BirdefOri Art Ser*. 1990;26(4):286-96.
[11] Froland A, Sanger R, Race RR. Xg blood groups of 78 patients with Klinefelter's syndrome and some of their parents. *J of Med Genet*. 1968;5:161-64.
[12] Jacobs PA. The role of chromosome abnormalities in reproductive failure. *Reprod Nut Devel*. 1990;1:63-74.
[13] Zinn AR, Ramos P, Elder FF, Kowal K, Sprouse CS, Ross JL. Androgen receptor CAGn repeat length influence phenotype of 47,XXY (Klinefelter) syndrome. *J of Clin Endocrinol and Metab*. 2005;90(9):5041-46.
[14] Stemkens D, Roza T, Verrij L, Swaab H, van Werkhoven MK, Alizadeh BZ et al. Is there an influence of X-chromosomal imprinting on the phenotype in Klinefelter syndrome? A clinical and molecular genetic study of 61 cases. *Clin Genet*. 2006;70:43-48.

PARTICULARS OF CONTRIBUTORS:
1. Senior Lecturer, Newcastle University Medicine Malaysia, Johor, Malaysia.
2. Professor, Department of Anatomy, International Medical School, Bangalore, India.
3. Professor, Department of Anatomy, Kempagowda Institute of Medical Sciences, Bangalore, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Vallabhajosyula Ranganath,
Senior Lecturer, Newcastle University Medicine Malaysia (NUMED), No.1, JalanSarjana 1, Kotallmu, Educity@Iskandar,
79200 Nusajaya, Johor, Malaysia.
E-mail: ranganath.vallabhajosyula@ncl.edu.my

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Dec 24, 2013**
Date of Peer Review: **Dec 19, 2014**
Date of Acceptance: **Jan 08, 2015**
Date of Publishing: **Mar 01, 2015**