Young Mothers Produce More Chromosomal Syndrome Babies in Mysore, South India

Suttur S. Malini and Nallur B. Ramachandra

Human Genetics Laboratory, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysore 570 006, Karnataka, India

ABSTRACT Chromosomal syndromes contribute significantly to reproductive failure, birth defects, mental retardation, delayed puberty, and hermaphrodites in humans. It has been estimated that at least 5% of all human conceptions are aneuploids, most of them resulting in pregnancy loss. The well-established factor to produce babies with chromosomal syndromes is advanced age of mothers. However, in India, more of young mothers give birth to babies with chromosomal syndromes. The present study has been attempted to investigate the possible causes. A total of 175 children with chromosomal aneuploidy and 300 controls were screened for cytogenetic investigation from major hospitals of Mysore city. Genetic register was established, pedigree was constructed and degree of consanguinity was studied for the cases where parental consanguinity was evident. Cytogenetic and statistical analysis were carried out using logistic regression. Logistic regression of case-control study of babies with chromosomal aneuploidy revealed that the odds ratio was significant for advanced father and maternal grandmother's age when all the variables were used together. The effect of age of father and age of maternal grandmother were increased in odds by 16% and 46% per extra year respectively. Along with the established risk factors like advanced age of parents, maternal grandmother's age is also the potential possible risk factor for the manifestation of babies with chromosomal aneuploidy in young mothers.

INTRODUCTION

Chromosomal syndromes contribute significantly to reproductive failure, birth defects, mental retardation, delayed puberty and hermaproditism in humans (McKusick 1994). It has been estimated that at least 5% of all human conception are aneuploidy, most of them resulting in pregnancy losses (Hassold and Hunt 2001). There are not many epidemiological studies assessing the association between chromosomal syndromes and parental demographic factors in India. In the present study, we quantified the effects of parental age, grandparental age and consanguinity on the prevalence of live birth of babies in Mysore with chromosomal aneuploidies and examined possible interactions between them.

METHODOLOGY

Chromosomal Aneuploidy Cases

A total of 175 cases with suspected chromosomal aneuploidy referred for cytogenetic investigation for a period of five years from major hospitals of Mysore city. One hundred fifty Down syndrome and 25 cases with sex chromosomal aneuploidy were confirmed by cytogenetic analysis. An informed consent was obtained from the parents before including them in the study. The genetic register was maintained to collect the complete information of the proband and their parent's as well as control families. With this information, the pedigree of the families under study was constructed. Parental and grandparental consan-guinity was also recorded and the degree of consanguinity was analyzed.

Control Population

Randomly selected 300 healthy families belonging to different religions as well as different localities in and around Mysore city, South India were used as controls for chromosomal aneuploidy. Case-control data set was established was generally of same ethnic and socio-economic background. Pedigree of control families was constructed, consanguinity and its degree was analyzed.

Statistical Analysis

Logistic regression was performed using the software, SPSS version 10.0 to record the effect

Corresponding Author:
Prof. Nallur B. Ramachandra
Telephone: 08212419781 (O)
Fax: 91082125126056
E-mail: nallurbr@gmail.com

of the variables. Case-control status was used as dependent variable and parental, grand-parental age and consanguinity as covariates. Results were reported as odds ratios from model with one variable at a time as well as a model with multivariable.

RESULTS

A total of 175 suspected cases of chromosomal aneuploidy namely, Down syndrome, Turner syndrome and Klinefelter syndrome were karyo-typically analysed and their classical chromosomal constitution was confirmed. Table 1 presents the distribution of parental, maternal grandparental age and number of children born to 300 controls and 175 chromosomal aneuploidy families. The highest numbers of children were born to mothers and fathers of babies with chromosomal aneuploidy as well as controls aged between 18-24 years and 30-35 years respectively. Figure 1 illustrates the pedigree of chromosomal aneuploidy of 18 years young mother (a), 25 years young mother (b), 34 years advanced age mother (c) and 41 years advanced age mother

(d). Perusal of the pedigree indicates the relationship of age of mother, father and maternal grandmother in the family. The pedigrees show the order of birth of mother and father and also children with chromosomal aneuploidy status.

Table 2 provides the comparison of the mean age of fathers and maternal grandmothers of Down syndrome and sex chromosomal aneuploidy children with different age range of mothers in Mysore. Here the maternal and paternal ages are directly proportional to each other whereas, maternal and maternal grandmother ages are inversely proportional to each other to produce chromosomal aneuploidy children. The same thing holds good for sex chromosomal aneuploidy.

Figure 2 represents pedigree of consanguinity with different degrees like first cousin (a), second cousin (b), uncle-niece (c) and far relative consanguinity (d). Comparison between the degree of consanguinity in controls and families with chromosomal aneuploidy revealed that uncle- niece union was more followed by first cousin marriage (Fig. 3). Some of the representative metaphase plates and karyotypes

Table 1: Distribution of parental and maternal grandparental age, and number of children born to 300 control and 175 Chromosomal syndrome families.

Agerange (in years)	No. of children born to															
	Mother				Father				Maternal grandmother				Maternal grandfather			
	Controls		Chromosomal syndrome		Controls		Chromosomal syndrome		Controls		Chromosomal syndrome		Controls		Chromosomal syndrome	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
18-24	508	61.65	87	49.71	92	11.16	3	1.71	807	64.14	11	6.28	137	10.89	0	0
25-29	197	23.90	52	29.71	264	32.03	43	24.57	298	23.68	32	18.28	243	19.31	6	3.42
30-35	97	11.77	30	17.14	326	39.56	82	46.85	147	11.68	90	51.42	507	40.30	46	26.28
36-40	19	2.30	6	3.42	98	11.89	35	20	4	0.31	38	21.71	302	24.00	83	47.42
>= 41	3	0.36	0	00	44	5.33	12	6.85	2	0.15	4	2.28	69	5.48	40	22.85

Table 2: Comparison between the mean age of fathers and maternal grandmothers of Down syndrome (DS) children and Sex chromosomal aneuploidy (SA) children with different age range of mother in Mysore.

Age range of mothers with chromosomal aneuploidy babies (in years)	Mean age of fathers of DS children	Mean age of maternal grandmothers of DS children	Mean age of fathers of SA children	Mean age of maternal grandmothers of SA children
18-24	23.33 ± 0.33	32.93 ± 0.50	0.0	30.61 ± 0.88
25-29	27.02 ± 0.23	33.46 ± 0.74	27.35 ± 0.67	30.87 ± 1.36
30-35	32.57 ± 0.21	30.18 ± 0.82	33.25 ± 0.35	29.33 ± 3.8
36-40	38.16 ± 0.29	26.42 ± 1.3	38.4 ± 0.5	0.0
>= 41	42.50 ± 0.55	0.0	0.0	0.0
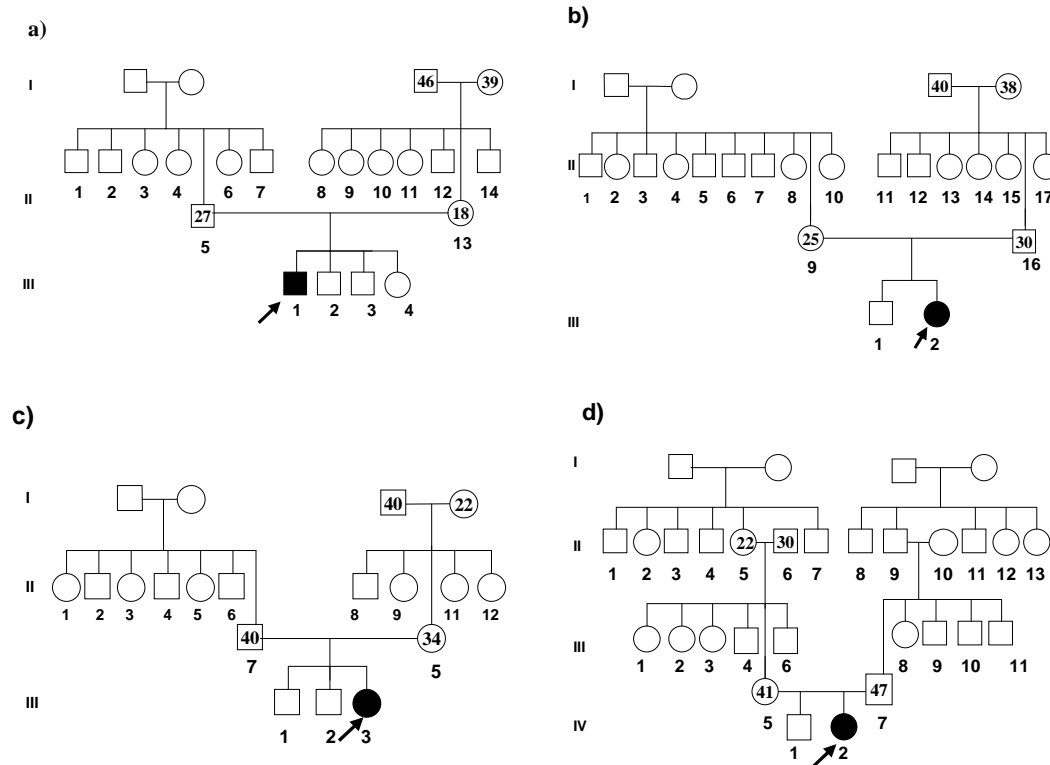
Fig. 1. Pedigrees of families of 18 years young mother (a), 25 years young mother (b), 34 years advanced age mother (c) and 41 years advanced age mother (d) with chromosomal syndromes. The Roman number in the left side of the figure indicates the number of generations. The Arabic number below the symbol denotes the number of individuals in that generation. The number inside the symbol of grandmother represents the age when she gave birth to the mother of chromosomal syndrome child. The number inside the symbol of father and mother in the 2nd generation indicates their age when they gave birth to chromosomal syndrome child. The arrow directed to the shaded symbol in the 3rd generation represents the child with chromosomal syndrome. These are the representative pedigrees out of 175 chromosomal syndrome families.

of Down syndrome (females and males), Klinefelter syndrome and Turner syndrome are presented in Figures 4-7 .

In order to establish the associations of maternal age, paternal age, maternal grandmother age, maternal grandfather age, and consanguinity, a logistic regression test (Table 3) was applied using the case-control data sets. The analysis revealed that 95% confident interval (CI) for the effect of maternal, paternal and maternal grandfather age was lower than that for maternal grandmother age. When the age of father, mother, maternal grandmother and maternal grandfather were considered as covariate there was a significant difference in the odds ratios.

At multivariable level the odds ratios was significant for father and maternal grandmother's age when all the variables were used together. This analysis supports the fact that advanced age of father and maternal grandmother will add up the risk of giving birth to babies with chromosomal aneuploidy.

DISCUSSION

Understanding the mechanisms by which chromosomal syndromes are produced is a major challenge for human cytogeneticists. Though there are several factors, so far the only well-established risk factor for commonly occurring

Table 3: Logistic regression analysis of parental, maternal grandparental age and consanguinity of control and chromosomal aneuploidy families in Mysore (c.i = confidence intervals).

Variables	Univariate		Multiple		Multiple	
	Odds ratio (95% c.i.)	p value	Odds ratio (95% c.i.)	p value	Odds ratio (95% c.i.)	p value
Mother (per year)	1.136 (1.090; 1.185)	0.0001*	1.001 (0.900; 1.114)	0.980	0.994 (0.892; 1.108)	0.917
Father (per year)	1.144 (1.097; 1.192)	0.0001*	1.167 (1.044; 1.303)	0.006*	1.176 (1.051; 1.315)	0.05*
Maternal grandmother (per year)	1.419 (1.340; 1.502)	0.0001*	1.464 (1.320; 1.623)	0.0001*	1.466 (1.321; 1.626)	0.0001*
Maternalgrandfather (per year)	1.389 (1.310; 1.472)	0.0001*	0.973 (0.878; 1.079)	0.607	0.967 (0.871; 1.073)	0.528
Consanguinity	2.289 (1.501; 3.491)	0.0001*	-	-	1.393 (0.682; 2.843)	0.363

* = significant
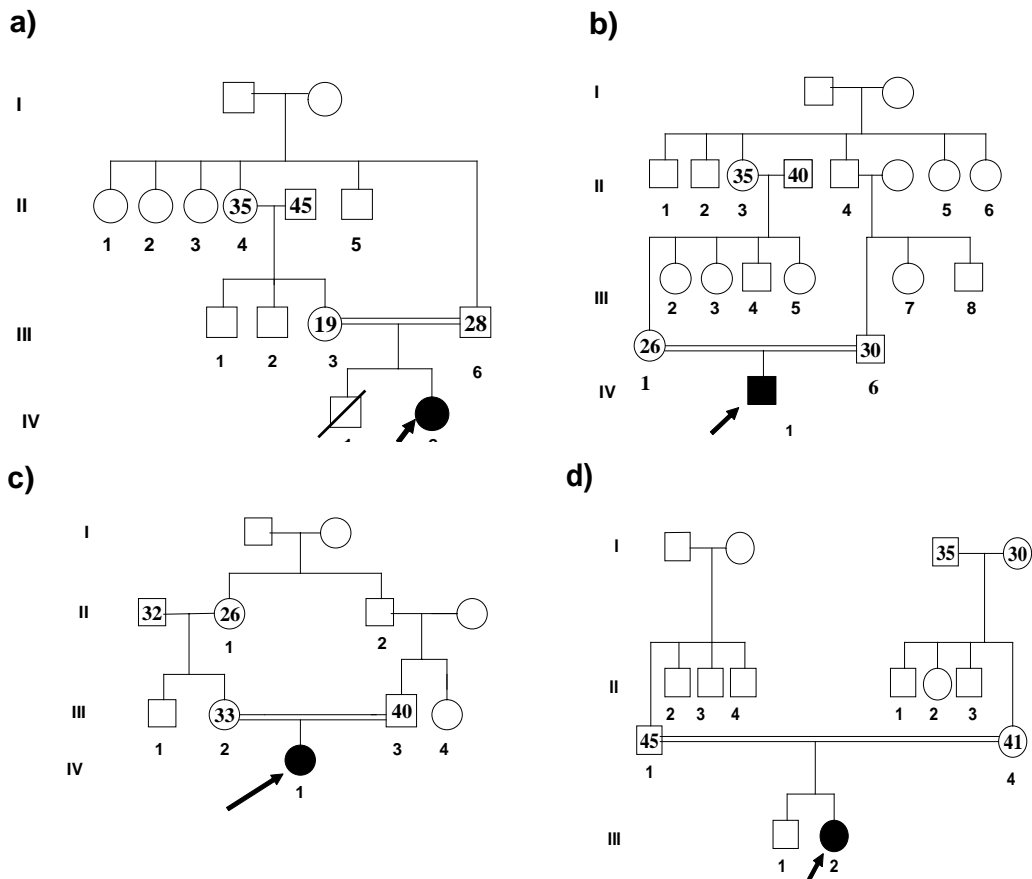
Fig. 2. Pedigrees of consanguinity with different degrees, first cousin (a), second cousin (b), uncle-niece (c) and far relative consanguinity (d). The Arabic number below the symbol denotes the number of individuals in that generation. The number inside the symbol of grandmother represents the age when she gave birth to the mother of chromosomal syndrome child. The number inside the symbol of father and mother in the 2nd generation indicates their age when they gave birth to chromosomal syndrome child. The arrow directed to the shaded symbol in the 3rd generation represents the child with chromosomal syndrome. These are the representative pedigrees of showing consanguinity out of 58 families.
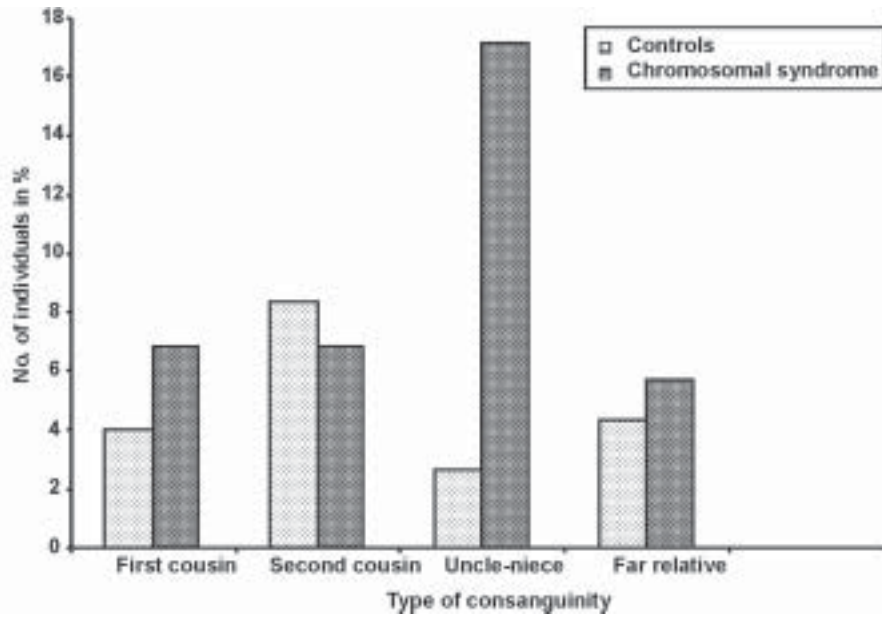
Fig. 3. Distribution of types of consanguinity in 300 controls and 175 chromosomal syndrome families.
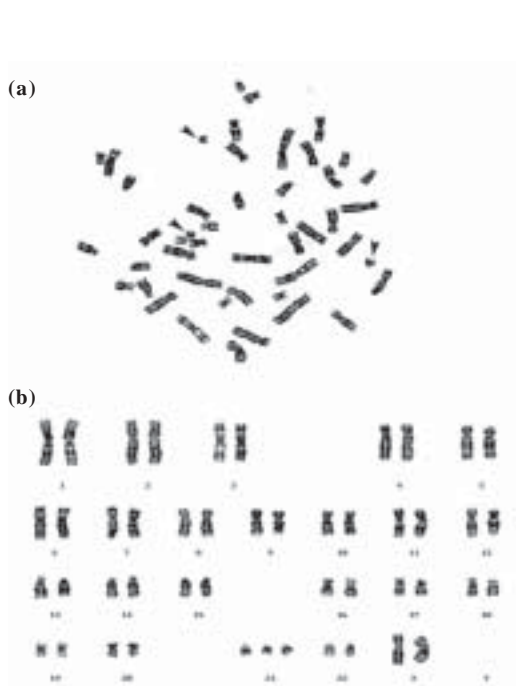

Fig. 4. G- Banded Metaphase plate (a) and Karyotype (b) of Downs syndrome Female (2n=47,XX+21). Arrow indicates the trisomy 21.

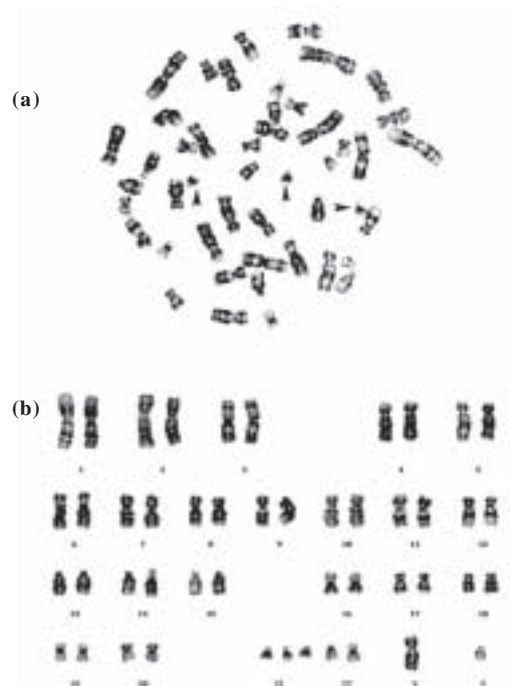**Fig. 5. G- Banded Metaphase plate (a) and Karyotype (b) of Down syndrome Male (2n=47,XY+21). Arrow indicates the trisomy 21.**

chromosomal syndrome like Down syndrome is advanced maternal age although the magnitude varies (Mikkelsen 1985; Risch et al. 1986; Morton et al. 1988; Hecht and Hook 1996).

In the present study, majority of women belonged to the age group of 18-24 years gave birth to maximum number of children in controls (85.55%) as well as chromosomal aneuploidy families (79.42%). The least production of the children was observed in advanced age group and no production was seen in 41 and above years. Thus, the age distribution between the mother of chromosomal aneuploidy cases and controls indicate that maternal age has no deci-sive influence for the manifestation of the syndrome. But for the association of paternal age is controversial (Erickson and Bjerkedal 1981; Murdoch et al. 1984; Petersen et al. 1993; McIntosh et al. 1995; Savage et al. 1998; Buwe et al. 2005; Dzurova and Pikhart 2005). In the present study, both in controls and chromosomal

aneuploidy families, majority of the fathers belonging to the age group 30-35 years had maximum number of children because, usually in Indian families fathers age is higher than mothers age. Therefore, fathers gave birth to more number of children at the age range of 30-41 years. Whereas more chromosomal aneuploidy children are produced when the father age is 36-40 years and in controls in the age range of 30-35 years indicating the advance age of the father will be a possible risk factor for chromosomal aneuploidy.

There are few reports indicate the association of maternal grandmothers age and Down syndrome (Aagesen et al. 1984; Mikkelsen 1985). In the present study, about 12.14% of normal and 75.41% of children with chromosomal aneup-loidy was born when the maternal grandmothers belonging to the age group of 30 and above years. Even by comparing the mean age of mother, father and maternal grandmother it is evident that age of mothers and grandmothers are inversely
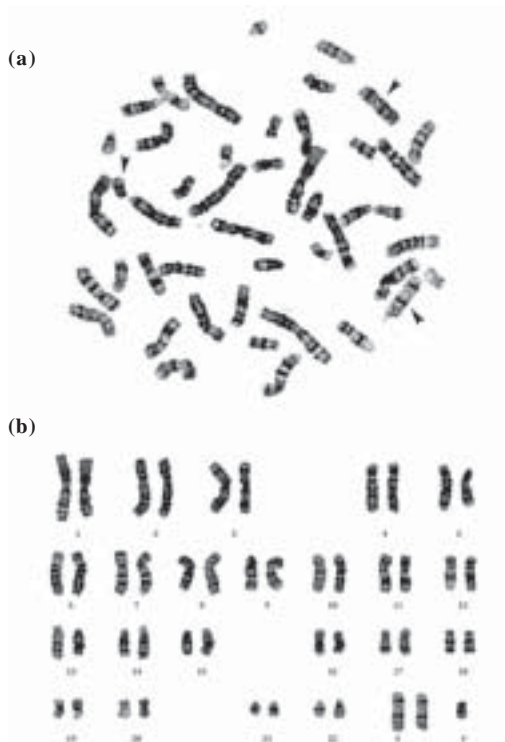
(a)

(b)

Fig. 6. G- Banded Metaphase plate (a) and Karyotype (b) of Klinefelter syndrome (2n=47,XXY). Arrow indicates the sex chromsomes.
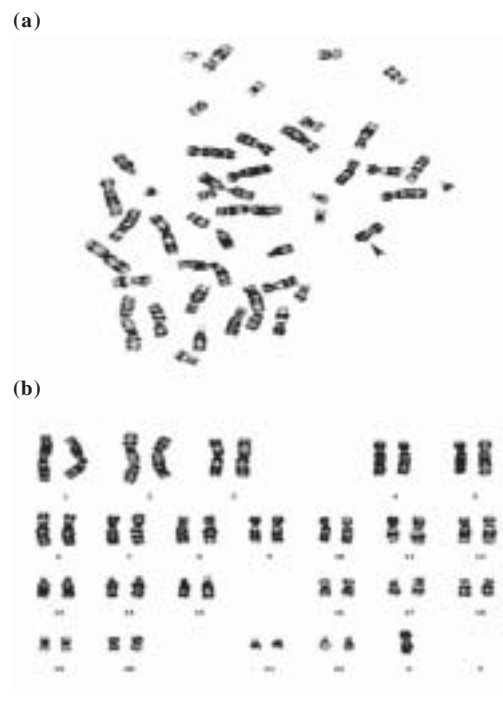
(a)

(b)

Fig. 7. G- Banded Metaphase plate (a) and Karyotype (b) of Turner syndrome (2n=45,XO). Arrow indicates only one X chromosome.

proportional. This clearly indicates the age of the maternal grandmother is a risk factor to cause chromosomal syndromes. This kind of situation is not found in majority of western population studied so far. An association between the influence of advanced age of maternal grandfather and the risk of causing chromosomal aneuploidiy has not been found in any literature and also in the present study.

Logistic regression revealed that when all the covariates considered together, the effects of mother's age, grandfather's age and consanguinity are diluted and not statistically significant. However, the effect of age of father and age of maternal grandmother are not diluted, showing an increase in odds by 16% and 46% per extra year respectively. When the pedigrees are carefully analyzed it is clear that wherever the daughter is born to aged mother the chances of the daughter giving birth to children with chromosomal aneuploidy is increased. This supports the birth of affected babies to advanced aged mothers. Similarly, the advanced age of the father will also influence the manifestation of aneuploidy of chromosomes.

The prevailing concept of human reproductive ageing assumes that the age dependent loss of female fertility is dictated by the decline of both the quality and quantity of the oocyte/ follicle pool. During fetal life, the ovaries are endowed with the entire stock of follicles that has to serve a woman's reproductive needs for the rest of her life. Thereafter, the number of follicles decline exponentially, with a marked increase in the rate of disappearance from age 37-38 years onwards. Below a critical number of some thousands reached at a mean age of 45-46 years, the menstrual bleeding pattern becomes irregular (Richardson et al. 1987) and when the menopause is reached at a mean age of 51 years, the supply is reduced to a thousand or less follicles, a number insufficient to sustain the cyclic hormonal process necessary for menstruation (Faddy et al. 1992). The process spermatogenesis compared to oogenesis begins at puberty when cells entering meiosis move from one stage to the other without delay, whereas in oogenesis meiosis is initiated in oocytes during fetal life. After homologous chromosomes synapse and initiate recombination, meiosis is arrested. Meiosis I resume in the woman adult life just before the ovulation of oocytes. At this point, Meiosis I is completed and the first polar

body is extruded. Meiosis II is initiated but goes through a short arrest as it travels down the fallopian tubes. Meiosis II is completed after fertilization and the second polar body is extruded. Thus, meiosis in a woman extends over a 10-50 years period with the oocytes being arrested in Meiosis I during most of its lifetime (Lamb et al. 2005).

As a woman ages her meiotic machinery accumulates the effects of years of environmental and age related insults, becoming less efficient more error prone. The proportion of nondisjunction occurring among oocytes with normal exchange configuration increases over a time as age dependent risk factors exert their influence. As a result, the most prevalent exchange profile of nondisjoined oocytes shifts from susceptible to nonsusceptible patterns (Lamb et al. 2005).

Lamb et al. (1997) and Jeffery et al. (2003) two hit model and *Drosophila* oocytes model proposed significant age–dependent meiotic nondisjunction. Taking into cognizance of this information, we propose that advanced age of grandmother is responsible to bring disturbance in the meiosis of her daughter when the grandmother conceived. At the advanced age, the grandmothers reproductive system may fail to make the essential proteins like spindle associated proteins, factors responsible for resting of oocytes, chiasma-binding proteins, DNA repair enzymes, resulting in an accumu-lation of toxic effects of the environment during the arrested state of the oocytes. This leads to a suboptimal resumption of Meiosis I and Meiosis II, a change in ovarian functioning due to suboptimal degradation of uterine environment, that are needed for proper meiotic segregation in the germ cells of her daughter. The nonfunc-tioning of proteins leads to impairment in the meiotic process, which in turn results in nondisjunction of chromosomes in the oocytes of the daughter. This event takes place during the embryogenesis of the mothers of chromo-somal syndrome children when she was in grandmother's womb. It is also possible that recombination is reduced in the oocytes, which brings about the nondisjunction of chromosomes (Malini and Ramachandra 2006). Therefore, we propose that irrespective of chromosomal aneu-ploidy the process of nondisjunction remains same. Chromosomal aneuploidy not only depends on the age of the mother but also on the age of the maternal grandmother that results in nondisjunction of chromosomes. Even

in the present study advanced paternal age shows increased chromosomal aneuploidy as reported in the earlier literature (Antonarakis 1991; Sherman et al. 1991).

CONCLUSION

The population screening and investigation of etiological factors for chromosomal aneuploidy will be useful to prevent disability and death by early intervention. One can prevent this genetic burden by providing proper education to all the parents, prenatal diagnosis of all the pregnancies and subjecting the suspected families for genetic counseling. Treatment and cure of these patients can be possible from the out come of functional genomics in the near future.

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