

## Maternal grandmothers with advanced age reproduction are more likely to have Down syndrome grandchildren

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### Abstract

Down syndrome (DS), trisomy 21, is the most common chromosomal syndrome that affects one in 600-800 live births. The advanced maternal age is the only well known risk factor to cause DS. Our study revealed that many young mothers produced DS children than advanced age mothers in India. A total of 150 suspected DS cases were investigated cytogenetically. Randomly selected 200 healthy families in South India were used as controls. Logistic regression was performed on case-control dataset which was generated by randomly selecting the child from each of the control families.

Pedigree analyses indicated that the maternal grandmothers had advanced age during conception of their daughters who gave birth to DS child. Case-control status was used as dependent variable, whereas parental and grandparental age was used as covariates. Logistic regression was reported as odds ratios, univariate and multivariate. The age of maternal grandmother showed highly significant difference in odds ratio, indicating that the advanced age of maternal grandmother was the possible risk factor. Therefore, it is important to sort-out the effect of advanced age mothers vs grandmothers on increased frequency of DS reported in different populations.

**Key words:** Maternal grandmother; advanced age; Down syndrome

### INTRODUCTION

Down syndrome (DS) is the most common genetic disorder affecting one in 600-800 live births irrespective of gender, ethnic origin or racial group (1, 2). It is associated with mental retardation, immune system disorders, autoimmune problems, congenital heart diseases, premature aging and Alzheimer disease between the age of 30-40 years (3, 4). Though vast amount of work has been done on DS since 1959 on the etiological and demographic factors, the advanced maternal age is the only well known risk factor for DS (5-10). Studies on DS by Talukder and Sharma (11) in Indian population affecting one in 1139 live births has not provided convincing data to support the hypothesis that advanced maternal age is indeed the risk factor in the occurrence of DS (12-19). Despite the clinical importance of age dependent nondisjunction in human, the other mechanisms are yet to be identified. Present study

was undertaken in view of the controversial reports of the involvement of age of grandmothers (20, 21) or not (22, 23) in causing the DS. Here we report the increased frequency of DS in India is due to advanced maternal grandmother age and it is also possible that higher incidence of DS in other western countries could be due to both advanced age of mother and maternal grandmother.

### MATERIALS AND METHODS

A total of 150 suspected DS cases were investigated cytogenetically. An informed consent was obtained from the parents before including them in the study. Ethical clearance was obtained by the institutional ethical clearance committee of the University of Mysore. Randomly selected 200 healthy families in South India without any incidence of DS or any other genetic disorders were used as controls irrespective of caste, sub

caste, religion, region both from urban and rural areas. A genetic register was designed and used to collect the complete information about family history, medical history, presence or absence of consanguinity in the family and parental diseases among parents both in control and affected families.

Chromosomal analysis of the patient was carried out on peripheral blood leucocyte culture by using the standard protocol of Seabright (24) with slight modifications. G banded metaphase plates were analyzed by automated LEICA KARYO software and karyotyped according to the International System for Human Cytogenetic Nomenclature (2005).

Case-control dataset was generated by randomly selecting the child from each of the control families. Case-control groups were generally of the same ethnic and socio-economic backgrounds. Logistic regression was performed using the software, SPSS version 10.0 to record the effect of the variables. Case-control status was used as dependent variable, and parental as well as grandparental age as covariates. Results were reported as odds ratio from model with one variable at a time as well as a model with multivariables.

## RESULTS

For the present study, the age of parents and grandparents were classified into different age groups, 18-24, 25-29, 30-35, 36-40, and 41+ years. Of the 150 DS cases studied, 147 were found to be trisomy 21 with extra free 21<sup>st</sup> chromosome, two cases were mosaic and one was with translocation. Mothers of both control and DS families produced more children in their young age than in their advanced age (Fig.1). In controls, young age maternal grandmothers produced high number of normal grandchildren, while advanced age maternal grandmothers produced high number of DS grandchildren (Fig. 2).

Mean maternal age of control and DS was 22.30 and 25.57 years while mean paternal age of control and DS was 22.30 and 25.57 years respectively. A representative pedigree of DS family of young mothers (Fig. 3) also shows that her mother's age was advanced at the time of her conception. On the other hand, the highest numbers of children were produced by the fathers and maternal grandfathers in their advanced age in both control and DS families.

Table 1: Logistic regression analysis of parental and maternal grandparental age of control and Down syndrome families in Mysore population (c.i. = confidence intervals). \*= significant

Variables	Univariate		Multiple	
	Odds ratio (95% c.i.)	<i>p</i> value	Odds ratio (95% c.i.)	<i>p</i> value
<b>Mother (per year)</b>	1.163 (1.105;1.223)	0.001*	0.984 (0.841;1.151)	0.838
<b>Father (per year)</b>	1.163 (1.108;1.221)	0.001*	1.198 (1.017;1.412)	0.031*
<b>Maternal grandmother (per year)</b>	1.762 (1.569;1.98)	0.001*	1.854 (1.554;2.221)	0.001*

<b>Maternal grandfather (per year)</b>	1.568 (1.434;1.715)	0.001*	0.946 (0.814;1.099)	0.468
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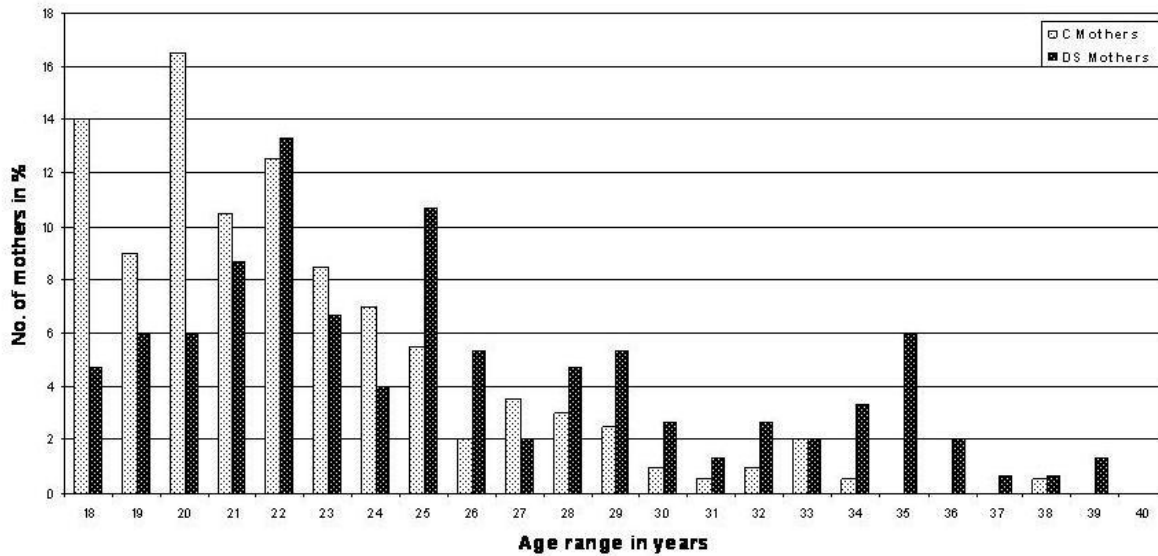


Fig.1. Age distribution of mothers in control and Down syndrome families (C= Control, DS= Down syndrome)

Logistic regression analysis of parental and maternal grandparental age of control and DS families (Table 1) was done at all combinations to establish specific relations of grandmother's age with other variables. The 95% confidence intervals for the effect of the age of mother and age of father were lower than the age of maternal grandfather and maternal grandmother. The odds ratios were significant when all the four variables were used one at a time. When the age of mother and father were considered as covariates, there

was no significant difference in odds ratio. At the four variable levels, maternal grandmother showed highly significant (85%) difference in odds ratio, indicating that the maternal grandmother age was the possible risk factor. Similarly, at the four variable levels, advanced paternal age was also showed 19% difference in odds ratio, indicating that the advanced paternal age was also the possible risk factor, however, it is not effective as maternal grandmother age.

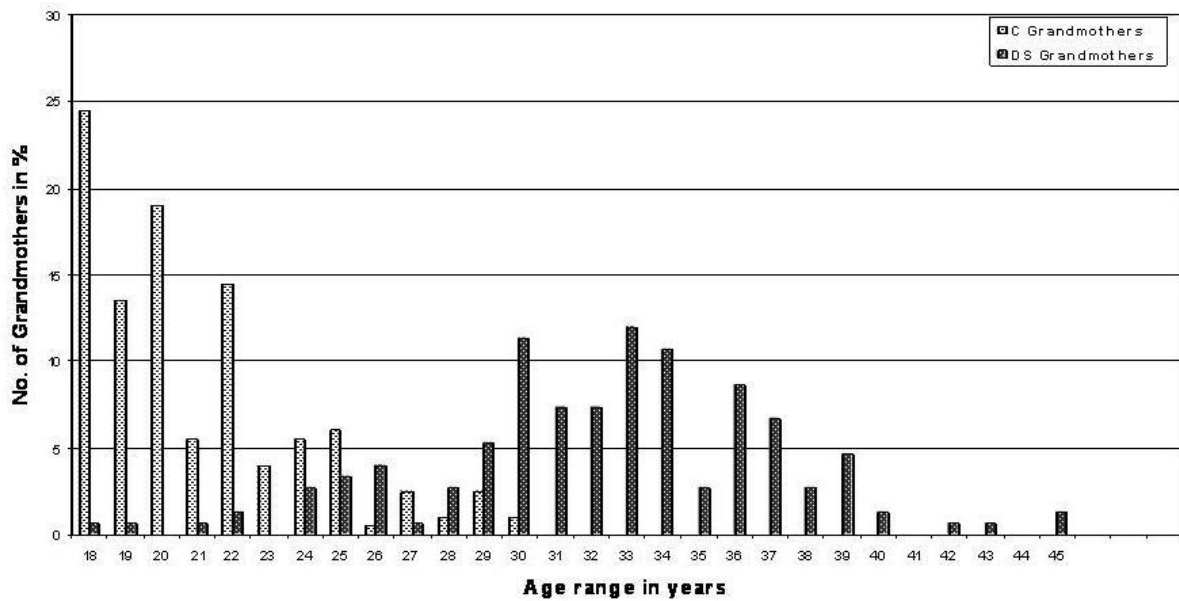


Fig.2. Age distribution of maternal grandmothers in control and Down syndrome families (C= Control, DS= Down syndrome)

**DISCUSSION**

In India majority of the marriages are performed around 20 and 25-30 years of age for women and men respectively. The age difference between husband and wife normally varies from 1 to 10 years due to cultural and socio-economic status (25, 26). Generally, in India, women plan to have babies in the early age of their marriage. This could be the possible reason wherein the mother and the grandmother produced more children in their young age. Incidence of DS children in

different parts of India shows the mean age of mother with DS children is 27.6 years in Punjab (18), 26.8 years in Mumbai (16, 19) and 30.2 years in Hyderabad (15, 17). Surprisingly, our study also revealed that in many cases more DS children are born to young mothers. This clearly indicates that more DS children are born to young mothers than to mothers with advanced age in India. This also brings out that the maternal age is not responsible for nondisjunction of chromosome 21.

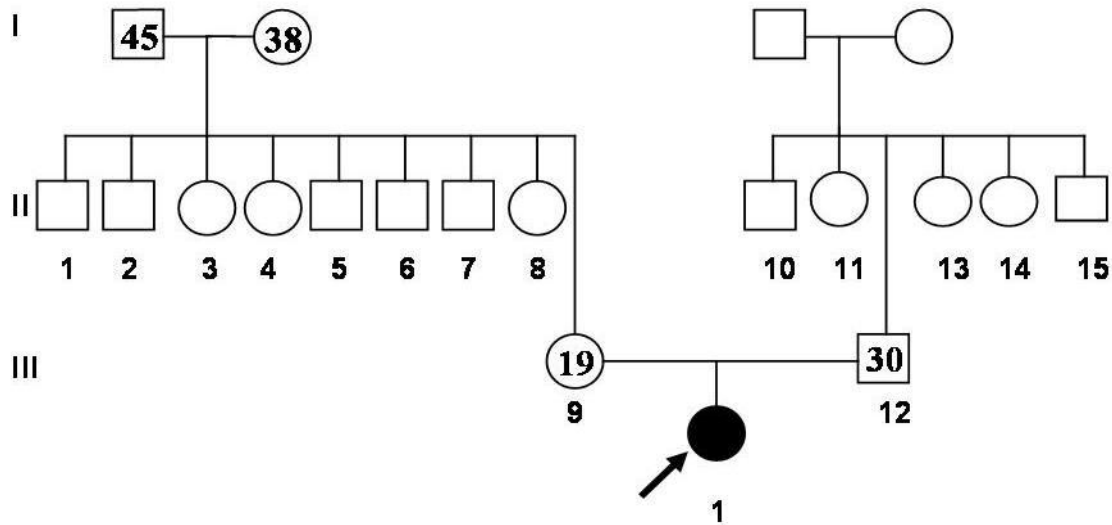


Fig. 3: Pedigree of Down syndrome children of young age mother. The Roman number in the left side of the figure indicates the number of generation. The Arabic number below the symbol denotes the number of individual in the generation. The number inside the symbol of grandmother represents the age when she gave birth to the mother of Down syndrome. The number inside the symbol of father and mother in the 2<sup>nd</sup> generation indicates their age when they gave birth to Down syndrome child. This is the representative pedigree out of 150 Down syndrome families.

Interestingly, we found that 78% of DS grandchildren were born when the maternal grandmothers age was 30 and above years. This is not the scenario in majority of western population studied so far. A few earlier reports suggest the influence of grandmaternal age, on the risk of their grandchild being born with DS (20, 21). Our careful observations of the pedigrees of DS children revealed that wherever the daughter was born to an aged mother the chances of that daughter giving birth to DS children are high. Logistic regression analysis using all the four covariates have shown that when they were considered together, the effect of age of father and the maternal grandmother were not diluted, showing an increase in odds by 19% and 85% per extra year respectively. This indicates the birth of a female child to a mother with advanced age has an increased effect for the birth of DS subjects as their grandchildren, while the age of the father seems to be of lesser importance in this context.

Golubovsky and Manton (27) have explained a three-generation approach in biodemographic studies on the developmental and the epigenetic profiles of female gametes. Each primordial germ cell formed in the 8-12 weeks old embryo

becomes an oogonium and enters into meiosis I, giving rise to the primary oocyte. At birth, meiosis I is arrested in females in the diplotene stage until puberty. A few hours before ovulation, the first meiotic block is removed. Subsequently, the oocyte blocked at meiosis II metaphase, completes meiosis only after sperm penetration (27). Every individual develops from the mother's egg, which originated as a primary oocyte during the grandmother's pregnancy. Therefore, every egg physically and genetically links three female generations. If diverse environmental factors influence the epigenetic dynamics of the oocyte in  $F(n-2)$  and  $F(n-1)$ , they can cause genotype/phenotype changes in the  $F(n)$  cohorts. Epigenetic maternalization continues in the  $F(n-1)$  generation during maturation of growing oocytes. Maternally inherited oocyte proteins are accumulated and used to demethylate and activate the paternal genome after fertilization (28). Dramard *et al.*, (29) demonstrated that the natural insertional repetitive elements (I-REs) could have a key regulatory role in the silencing of I-like sequences in the ovaries of ageing of *Drosophila melanogaster*. These variations arising in germ cells within the ovary would be inherited and

could thus play a role in the process of adaptive evolution. It has been reported that the promoter of *SALL4* was hypermethylated in aneuploidy tumor cells, which is one of the key players that act as caretakers for chromosome stability (30).

Meiosis in a woman extends over 10-50 years period with the oocytes being arrested in Meiosis I during most of its lifetime (30). This contrasts with spermatogenesis, which begins at puberty when cells entering meiosis move from one stage to the other without delay. Lamb *et al.* (32) proposed that altered recombination pattern along with nondisjoined chromosome, and advanced maternal age effect in meiotic disturbance are the causes of nondisjunction of chromosome 21. Jeffery *et al.* (33) demonstrated that *Drosophila* oocytes exhibit significant age-dependent meiotic nondisjunction wherein achiasmate chromosomes become vulnerable to nondisjunction as *Drosophila* oocytes age. Maternal primordial germ cells contain both parental genomic imprints. Transition from primordial germ cell to oocyte is accompanied by two genome reprogramming events: Erasure of parental imprints and subsequent epigenetic maternalization starts after primordial germ cell entry into the genital ridge and consists of rapid genome-wide demethylation (27, 34, 35). A deficit of oocyte proteins prevents normal development. The reproductive system of the grandmother in her advanced age fails to make essential proteins which in turn leads to changes in meiosis I and meiosis II, resulting in improper meiotic segregation of chromosomes in the germ cells of her daughter (27). With this background, we put forth the hypothesis that advanced age of maternal grandmother is involved in bringing about changes in the meiosis of her daughter at the time of conception. This cascade takes place during the embryogenesis of the mothers of DS children when she was in grandmother's womb. Therefore, DS not only depends on the age of the mother but also on the age of the maternal grandmother, which results in nondisjunction of chromosome 21. Based on this, one can surmise that the increased frequency of Down syndrome in

western studies could be due to advanced age of both mother and grandmother.

**Conclusion:** Apart from advanced maternal and paternal age, advanced maternal grandmother age is also a possible risk factor in causing DS in young mothers. Thus, the need of the day for India and elsewhere is implementation of prenatal screening of genetic disorders as a preventive public health programme on a priority basis as immunization program on hand.

#### ACKNOWLEDGEMENTS:

We are extremely grateful to the families who participated in this work and to the clinicians of Cheluvamba hospital, J.S.S hospital and HoldsWorth memorial hospital, Mysore who diagnosed and referred these cases to us. Written consent was obtained from the patient or their relatives for publication of the study. We thank Chairman of our department and Prof. H. A. Ranganath, Prof. S. R. Ramesh, Dr. G. Mahesh, Dr. N. Upendra, and our genetics research group for their support and help during the course of the preparation of the manuscript. This work was done with the self finance without financial support from any funding agencies.

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