Advanced Maternal Grandmother Age and Maternal Age as Risk Factors for Down Syndrome in a Group of Jordanian Families

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ABSTRACT

Objectives: To study whether advanced maternal age and maternal grandmother age are associated with increased risk of Down syndrome siblings in a group of Jordanian families.

Methods: This study was conducted on 127 confirmed Down syndrome cases with the age range of 18 weeks gestation to 15 years old, which were referred between the period of 2005-2008 for cytogenetic analysis at the Cytogentics section, Princess Iman Research and Laboratory Sciences Center/King Hussein Medical Center. Maternal and grandmaternal mother ages were obtained directly from the study group when the samples were collected from siblings. The maternal age ranged between 19-45 years while the maternal grandmother's age ranged between 15-49 years. One hundred healthy families were randomly recruited from the hospital staff as a control group. Logistic regression was used for statistical analysis.

Results: One hundred seventeen down syndrome cases had free trisomy 21, 7 with translocation, 2 mosaic and one with double aneuploidy (47,XXY, +21). Fifteen cases were diagnosed prenatally while 112 were diagnosed postnatally. The effect of maternal age and maternal grandmother age were found to be significant using logistic regression statistics (P = 0.001; OR= 2.816; 95% CI, 1.48-5.33) for the mother's age and (P = 0.001; OR= 2.902; 95%CI, 1.521-5.53) for the grandmother's age.

Conclusion: Advanced maternal and maternal grandmother ages are risk factors for Down syndrome. More studies and investigations are needed for better understanding of the biological factors responsible for the proper meiotic segregation of germ cells during the fetal development of the embryo in advanced maternal and grandmother's age.

Key word: Advanced grand maternal age, Chromosomal aneuploidy, Down syndrome, Robertsonian translocation

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Introduction

Around 50% of spontaneous abortions before 15 weeks of gestation are chromosomally aneuploid, with trisomies accounting for 50% of abnormal abortions.[1] Trisomy 21, the chromosomal abnormality responsible for >95% of individuals with Down Syndrome (DS), is the most commonly identified cause of mental retardation, with an incidence of 1 in 600 live births.[2] While the incidence of fetal trisomies is directly related to advanced maternal age, no specific genetic factors had been identified thus far.[3] The mechanisms for maternal meiotic non-disjunctional events are under study. Generally, children with Down syndrome

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have "free" trisomy 21 (92-95%), mosaic trisomy 21 (2-4%), or trisomy 21 due to a Robertsonian translocation (3-4%).[4]

An association has been found between the risk of Down syndrome and the age of the maternal grandmother at the mother's birth.[5-7] Female meiosis starts in fetal life, and nondisjunction in the first meiotic division of a female might be induced during the fetal period, especially if her mother is older.

At least in 5% of all clinically recognized human pregnancies, meiotic segregation errors give rise to zygotes with the wrong number of chromosomes. The nondisjunction error is more frequent in first meiotic division (80%) rather than second meiotic division (20%).[8] The polymorphic microsatellites have revealed that Trisomy 21 is due to nondisjunction of 90% of the maternal and 10% of paternal chromosome.[9] These observations have led to the hypothesis that chromosome 21 nondisjunction requires two hits: The first hit, which occurs during fetal meiosis, establishes bivalents with 'susceptible' meiotic configurations. The second hit involves an age-related degradation of a meiotic process which increases the risk of improper segregation for these susceptible bivalents. Under normal meiotic conditions, the presence of a single chiasma-regardless of its location is sufficient for proper chromosome segregation. However, as the ovary ages, a decay or breakdown in the meiotic apparatus (e.g. a spindle component or sister chromatid cohesion protein) may occur, disturbing the meiotic process. At this point, certain exchange configurations may be more likely to undergo improper segregation and non-disjunction. In this manner, as the age of a woman increases, so too does her chance of a meiotic disturbance.[10,11]

This study was conducted to assess whether maternal and /or maternal grandmother's age is associated with increased risk of Down syndrome siblings in a group of Jordanian families.

Methods

A total of 127 confirmed DS cases with the age range of 18 weeks (prenatal) gestation to 15 (postnatal) years, were referred between the period of 2005-2008 for cytogenetic analysis at Cytogenetics section, Princess Iman Research and Laboratory Sciences Center/King Hussein Medical Center to confirm the clinical diagnosis of Down Syndrome postnatally or referred with abnormal ultrasound findings prenatally. (Table I and Table II)

One hundred healthy families were randomly recruited from the hospital staff as a control group (Table I).

Statistical analysis with logistic regression was performed using the SPSS version 10 to record the effect of the variables (Table III).

Lab procedures

Chromosomal preparations obtained from phytohemagglutinin (PHA)-stimulated peripheral blood cultures and Amniomax (Gibco-USA) special media was used for culturing the amniotic fluid and chorionic villi. All samples were subjected to Giemsa Trypsin Gurr (GTG) banding and karyotype analysis according to ISCN 1995.

Fluorescence-in-situ Hybridization (FISH) was performed using AneuVysion Assay Kit (Abbot-Vysis, USA). Two sets of combination probes (chromosomes 13, 21 and 18, X, and Y) were used..

Results

One hundred seventeen DS were found as free trisomy 21, 7 with translocation (Table IV), two mosaic, and one with non-classical type (47,XXY, +21). Fifteen cases were diagnosed prenatally and 112 postnatally.

Figure 1 shows the Pedigree of four families out of the seven families, whom were found to have translocations. Two families were found to have inherited pattern, one family had t (14; 21) and the other family diagnosed prenatally had t (13; 21). The paternal karyotype was normal in five cases suggesting de-novo origin, interestingly one family has two Children with DS and the parental karyotype was normal. Both mosaic cases were diagnosed prenatally.

Table III presents the logistic regression of this study. Logistic regression indicated that the mothers (58%) had advanced age during their conception of their DS siblings, and grandmothers (60%) had advanced age during conception of their daughters who gave birth to a DS child.

The effect of maternal age and maternal grandmother age was found significant, where the logistic regression statistics were *P*=0.001; OR= 2.816; 95%CI, 1.48-5.33 for the mother's age and *P*=0.001; OR=2.902; 95%CI, 1.521-5.53 for grandmother's age.

Table I. Study and control age groups

ategoryC	Study group Age	Control group age
Min maternal's age	19	18
Max maternal 's age	45	46
Min grandmother's age	15	17
Max grandmother's age	49	45
DS Patients Age range	18wks gestation-15years	-
Average maternal age	32	32
Average grandmother's age	31	29

Table II. Study group numbers and their corresponding Down syndrome subtypes

Category	No	%
Postnatal diagnosis	112	88
Prenatal diagnosis	15	12
Male	76	59.8
Female	51	40.2
Translocations	7	5.5
Mosaic	2	1.57
Free	117	92.1
Double aneuploidy 46, XXY, +21	1	0.78

Table III. Logistic regression for the mother's age and grandmother's age

	OR Odd ratios	95% Confidence interval	P value
Mother's age	2.816	1.48-5.33	0.001
Grandmother's age	2.902	1.521-5.53	0.001

Table IV. Karyotype analysis of seven families with Down syndrome cases with Robertsonian translocation

Karyotype translocations	Family Member	Mother age	Grandmother age
Family 1 (C)			
45,XX,rob(13;21)(q10;q10)	mother		
46,XX,rob(13;21)(q10;q10)+21	sibling	23	34
Family 2 (B)			
46,XX,rob(21;21)(q10;q10)+21	sibling	23	28
47,XY,+21	sibling		
Family 3 (A)			
45,XY,rob(13;21)(q10;q10)	father		
45,XY,rob(13;21)(q10;q10)	sibling	30	32
46,XY,rob(13;21)(q10;q10)+21	sibling	22	32
45,XY,rob(13;21)(q10;q10)	father		
46,XY,rob(13;21)(q10;q10)+21	sibling	33	38
Family 4(D)			
46,XX,rob(14;21)(q10;q10)+211	sibling	30	35
Family 5			
46,XX,rob(21,21)(q10;q10)+21	sibling	25	38
Family 6			
46,XX,rob(21,21)(q10;q10)+21	sibling	23	28
Family 7			
46,XX,rob(14,21)(q10;q10)+21	sibling	35	40

Discussion

Although the effect of maternal age as a risk factor for Down syndrome (DS) is well known, there are very few reports indicating the influence of grandmaternal age, on the risk of their grandchild being born with DS.[12,13] An interesting finding in our study, is that out of the 127 DS cases studied, only 18 (14.1%) cases have both mother and grandmother age < 30 years old, where the other 109 (85.9%) cases either or both mother and

grandmother have advanced age during conception of their DS sibling or daughters, respectively. As shown in the logistic regression (Table III), the present study demonstrates both maternal and maternal grandmother advanced age (>30) as risk factors for Down syndrome. If we compare this study with Suttur *et al.*'s study,[14] where logistic regression analysis using the four covariates of maternal age, grandmother age, father age, and consanguineous marriages together showed that the effect of maternal age, father's age and consanguineous marriage were diluted but still of clinical relevance, albeit not statistically significant. However, the effect of age of the maternal grandmother was not diluted, showing an increase in odds by 30% per extra year. In our study we found both maternal age and grandmother advanced age to be significant. Looking at family pedigrees in his study, it is clear that whenever the daughter was born to aged mother the chance of this daughter giving birth to DS children is increased.

How the advanced age of grandmother is responsible to bring disturbance in the meiosis of her daughter when the grandmother conceived is explained by Antonarakis.[15] At the advanced age, the grandmother's reproductive system may fail to produce the essential proteins like spindle associated proteins, factors responsible for resting of oocyte, chiasma-binding proteins, DNA repair enzymes, etc. which are needed for proper meiotic segregation in the germ cells of her daughter. The non-availability or non-functioning of proteins leads to impairment in the meiotic process, which in turn results in nondisjunction of chromosome 21 in the oocyte of the daughter. This event takes place during the embryogenesis of the mothers of the DS 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 chromosome 21. Therefore, DS not only depends on the maternal age but also on the age of the maternal grandmother which results in nondisjunction of chromosome 21.[16]

The frequency of free trisomy 21 observed in this study is most common and is seen in 93% of cases, where 4.7% had translocation, 1.5% had mosaic, and non-classical (47,XXY, +21) in 0.75%.[17] All these figures are close to those figures reported in the literature.[4]

Robertsonian Translocation (RT)

In the present study seven cases were found with translocation, we screened some members of the families while others we were unable to contact (Table IV). RT is the second most common translocation and comprises 4.7% of the cases. Familial inheritance in Robertsonian translocation is seen in one quarter whereas in the remaining it is de-novo.[18] In this study two families (Fig. 1 A, C) out of seven were found with inherited translocation t(13;21). One showed maternal inheritance (C) and the other one paternal inheritance (A), in the latter, the mother underwent amniocenthesis and the fetus had balanced translocation, and after studying the family, we found that his brother is DS case where the father had unbalanced translocation which he transmitted to the offspring. The recurrence risk is <1% if the translocation is *de-novo*. In case of familial RT DS, the genetic risk for female carrier to have a live born child with translocation DS is about 10%, which increases to 15% at amniocentesis. For male carriers the recurrence risk to have a child with translocation DS is about 1%.[19]

Mosaicism

In the present study two cases (1.5%) were diagnosed prenatally with mosaicism. Both grandmothers were 40, and 45 years old respectively and both mothers were young. This figure is similar to that reported in the literature.[4] Mosaicism arises after the egg and sperm have fused at conception. As the cells divide and multiply by ordinary cell division, a chromosome goes astray and a single cell with an extra chromosome 21 is formed. This cell continues to divide by ordinary cell division together with the non-trisomic cells and a mixture is produced.[20]

As with the other two types of Down's syndrome (apart from when a parent is a carrier) there is no known reason why mosaic Down's syndrome occurs. It happens equally often in parents of all ages.

In the present study, one case (0.78%) had non-classical DS karyotype. These cases have been reported in major DS studies with a frequency ranging from 0-1.2% and our figure is consistent with those studies.[21,22]

As the incidence of fetal trisomies is directly related to maternal age factors, rather than genetic

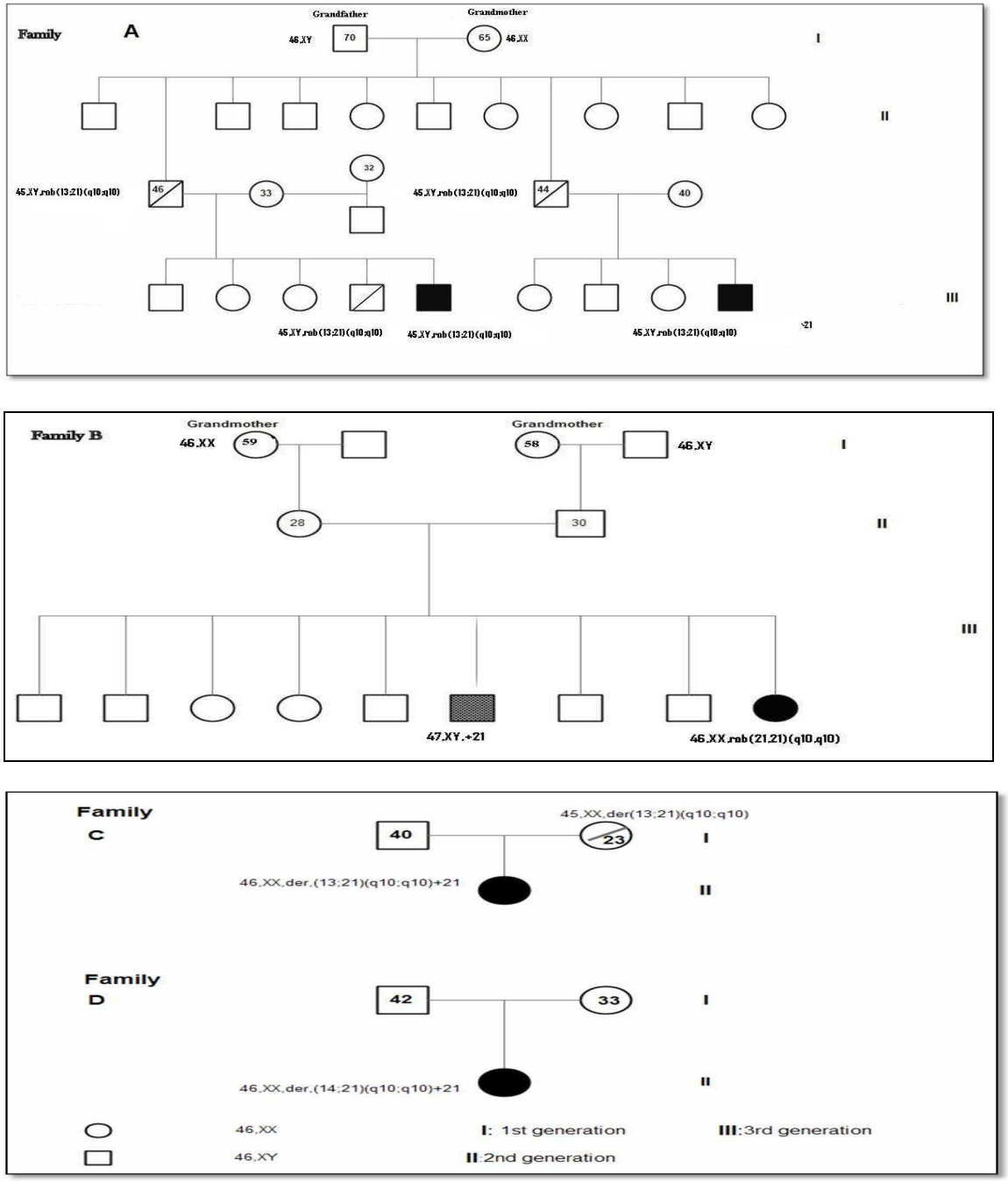

Fig. 1. Pedigrees of 4 families with Robertsonian Translocation (RT)
(Families A and C as shown, have inherited pattern)

predisposition, such factors may play a more important role in the etiology of the most common double anioploidy 48, XXY, +21, as evident in the present case (mother's age was 43 years).[23] The risk of having a child with Down syndrome increases in a linear fashion until about age 30 and then increases exponentially thereafter.[4]

Conclusion

Advanced maternal and maternal grandmother ages are risk factors for Down syndrome. More studies and investigations are needed for better understanding of the factors responsible for the proper meiotic segregation of germ cells during the

development of the embryo in advanced maternal and grandmother's age.

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