

## Maternal risk factors in young Egyptian mothers of Down syndrome

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### ABSTRACT

**Introduction:** We investigated the possible maternal risk factors that may increase the incidence of Down syndrome (DS) in young Egyptian mothers (younger than 35 years) especially methylene tetrahydrofolate reductase (MTHFR) enzyme C677T polymorphism.

**Subjects and Methods:** The study included 200 mothers of karyotypically ascertained non-disjunction DS attending Genetics clinic, Children's hospital, Ain Shams University (100 mothers were < 35 years and 100 mothers ≥ 35 years). 50 mothers of none-DS children served as a control group.

For all cases, history was taken laying stress on: Parental ages at conception, maternal grandparent's ages at conception of mother, DS birth order, history of oral contraceptive use 6 months before conception, genital infection, vitamin supplementation and smoking or exposure to irradiation.

**Results:** MTHFR C677T mutational analysis was done to twenty DS mothers with ages ≤ 35 years revealed that 35% of young mothers had C677T mutation (10% had homozygous mutation and 25% had heterozygous mutation). MTHFR C677T polymorphism was found to be a possible maternal genetic risk factor for DS although statistically non-significant.

Other maternal risk factors included the use of oral contraceptive pills (OCP) 6 months before pregnancy which was significantly higher only in DS mothers ≥ 35 years. on the other hand, parental consanguinity, maternal grandparents' ages, the presence of genital infection and birth order did not show a significant difference between young and old mothers of DS.

**Conclusion:** MTHFR C677T could not be considered as a maternal risk factor in young Egyptian mothers of DS. The risk effect may depend on gene-environment interaction between the genotype and dietary intake in particular folic acid consumption which should be further studied on a larger scale population including other MTHFR polymorphisms and environmental factors. Other risk factors may include the use of OCP in older mothers. Parents consanguinity, paternal age and maternal grandparents' ages were not found to be risk factors in DS in this study.

#### Key Words:

Down syndrome, risk factors, mothers, MTHFR.

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## INTRODUCTION

Down syndrome, or trisomy 21, is a complex genetic disease resulting from the presence of 3 copies of chromosome 21. The origin of the extra chromosome is maternal in 95% of cases and is due to failure of normal chromosomal segregation during meiosis. Many risk factors are suggested to cause DS. The only well established risk factor is advanced maternal age and so age –specific rates have been documented<sup>1</sup>. Other factors include higher socioeconomic status, which is due in part to maternal age<sup>2</sup>, advanced paternal age (>49 years)<sup>3</sup> and birth order.<sup>4,5</sup>

In 1999, Jill James and colleagues reported that a particular variant (polymorphism) of MTHFR might be a maternal risk factor for having a child with DS and that a significant increase in plasma homocysteine levels exists in mothers of children with DS. This polymorphism is known to decrease the activity of MTHFR<sup>6</sup>. Since then, studies have been carried out on populations from different countries including Egypt.<sup>7</sup>

Because most Egyptian children with Down syndrome are born to mothers less than 35 years of age<sup>8</sup>, we aimed in this study to investigate possible maternal risk factors that may increase the incidence of DS in this Group especially MTHFR C677T polymorphism.

## SUBJECTS

The study included 200 mothers of karyotypically ascertained non-disjunction DS children attending Genetics clinic, Children's hospital, Ain Shams

University and another 50 mothers of non DS children who served as controls. They were classified into:

**Group I:** Included 100 young mothers of DS (age < 35 years at time of conception).

**Group II:** Included 100 older mothers of DS (age ≥ 35 years at time of conception).

**Group III:** Included 25 young mothers of normal children (age < 35 years).

**Group IV:** Included 25 older mothers of normal children (age ≥ 35 years).

## METHODS

For all cases the following was done:

Full history taking laying stress on: parental ages at conception, maternal grandparent's ages at conception of mother, DS birth order, history of oral contraceptive use 6 months before conception, history of genital infection (itching, secretions etc), history of vitamin supplementation before or during pregnancy and history of smoking or exposure to irradiation.

Twenty mothers from Group I were subjected to MTHFR C677T mutational analysis:

### A- DNA extraction:

Genomic DNA was extracted using QIAamp DNA extraction kit (Cat#51104).

**B- Polymerase chain reaction (PCR):**

Polymerase chain reaction (PCR) was performed according to the method described by Frosst et al.<sup>9</sup>.

1µL of genomic DNA was amplified in a 50µL reaction volume containing 50 mM KCL, 10mM trisHCL pH=8.3, 1.5 mM MCL2, 20mM dNTPs, 0.5µM primers and one unit of Taq DNA polymerase enzyme.

PCR consisted of initial denaturation at 94°C for 150 seconds followed by 35 cycles at 94°C for 30 seconds, 57°C for 60 seconds and 72°C for 120 seconds and a final elongation step at 72°C for 3 minutes.

PCR product of 198bp was analyzed on 3% agarose gel electrophoresis.

**C- Enzyme digestion:**

Twenty microliter of PCR product was

mixed with 5 units of HinfI (promega, Madison) enzyme at 37°C overnight incubation. The substitution created a HinfI recognition sequence that is digested into 175-bp and 23-bp.

**RESULTS**

Parental and maternal grandparents' ages of both patients and controls are shown in (Table 1). Mean paternal age was significantly higher in Group II while mean maternal grandfathers and grandmothers' age did not show significance difference between the two Groups, (Table 2). When advanced paternal age was considered ≥ 49 years, 47% of DS fathers in Group I and only 3% of Group II had advanced age and this was highly significant (P value < 0.001) and when advanced maternal grandmothers' age was considered ≥ 35 years, 23% of grandmothers had advanced age in Group II compared to only 7% of Group I which was also statistically significant (P value < 0.001).

**Table 1:** Mean and slandered deviation of parents ages and maternal grandparents' ages of both patients and controls.

	<b>Patients Mean ± SD</b>	<b>Controls Mean ± SD</b>	<b>P value</b>
Maternal ages	33.25 ±7.69	31.62 ±8.76	0.19
Paternal ages	39.22 ±9.78	39.92 ±8.86	0.65
Maternal grandmothers' ages	26.51 ±6.49	27.44 ±6.34	0.36
Maternal grandfather's ages	26.35 ±6.64	27.1 ±6.7	0.48

**Table 2:** Comparison between Group I and II as regards paternal and maternal grandparents' ages.

	<b>Group I (mean ± SD)</b>	<b>Group II (mean ± SD)</b>	<b>P value</b>
Paternal ages	32.6± 7.53	45.84± 6.87	< 0.001
M. grandmothers' ages	25.99 ± 5.56	27.02 ± 7.29	0.845
M. grandfathers' ages	25.55 ± 6.91	27.15 ± 6.28	0.736

M: maternal

Parental consanguinity was present in 22% of patients compared to 28% in controls with no significant difference between young (Group I) and old (Group II) mothers. OCP were received by 37% of DS mothers which is significantly higher in old mothers of Group II compared to Group I. None specific

genital infection manifested itching or abnormal secretion was present in 34.5% of DS mothers with no significant difference between Group I and II. None of the DS mothers received vitamin supplementation before or during pregnancy, (Table 3).

**Table 3:** Comparison between Group I and II as regards consanguinity, use of oral contraceptives, genital infections and vitamin supplement during pregnancy.

	Group I (%) .No	Group II (%) .No	Odd ratio	CI	P value
Consanguinity	(21) 21	(23) 23	1.12	2.19 0.58-	0.43
Use of oral contraceptives	(25) 25	(49) 49	2.88	1.58-5.25	0.001 >
Genital infections	(37) 37	(32) 32	0.8	0.45-1.44	0.28
Vitamin supplement	0	0	-	-	-

None of the DS mothers took vitamin supplement before or during pregnancy. 37 % of DS mothers took oral contraceptive pills in the last 6 moths before pregnancy while 34.5% had genital infection. Use of oral contraceptive pills 6 months before pregnancy was significantly higher in Group II while parents'

consanguinity, the presence of genital infection and birth order did not significantly differ between Group I and II, (Table 3). Most of DS children were the first or second in order of birth and did not show significance difference between young and old mothers in Group I and II, respectively, (Table 4).

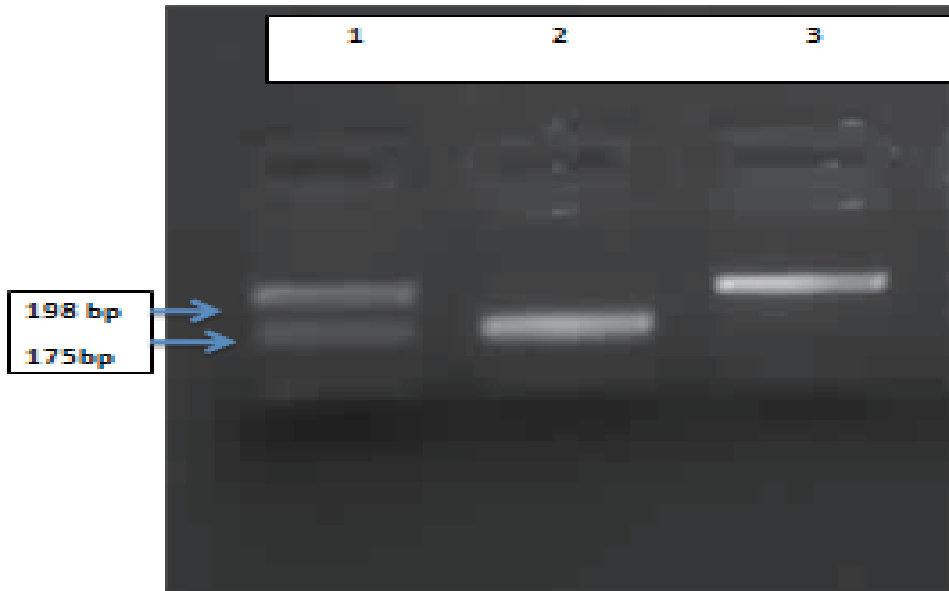
**Table 4:** Comparison between Group I and II as regards birth order.

DS birth order	Group I No. (%)	Group II No. (%)
First	35 (35)	30 (30)
Second	31 (31)	30 (30)
Third	18 (18)	27 (27)
Fourth	12 (12)	8 (8)
Fifth	4 (4)	5 (5)

P value: 0.54.

Molecular analysis for C677T polymorphism was done to 20 young mothers of DS children; 35% of them had C677T

mutation (10% had homozygous mutation and 25% had heterozygous mutation), (Fig. 1).



**Fig. 1:** Agarose gel showing: 1: Heterozygous MTHFR C677C/C677T genotype, 2: Homozygous MTHFR C677T/C677T genotype and 3: Wild alleles C677C/C677C genotype.

When compared to Egyptian controls<sup>7</sup>, our study showed higher risk (OR: 1.308) but with no significance difference (Table 5). Comparison of

our results to previous studies done on young mothers of DS is shown in (Table 6).

**Table 5:** MTHFR C677T mutation analysis in our study compared to Egyptian controls. C/C: Normal, C/T: Heterozygous, T/T: Homozygous mutation, CT/TT homozygous plus heterozygous mutations. \*Abdelmeguid et al.<sup>7</sup>.

Genotype	Cases (%) N	*Controls (%) N	P-value	Odd ratio (CI 95%)
C/C	(65)13	(68.8)33		
C/T	(10)2	(6.2)3	0.462	(0.2-10.8) 1.6
T/T	(25)5	(25)12	0.628	(0.3-3.3) 1
CT/TT	(35)7	(31.2)15	0.420	(0.4-3.9) 1.3
Total	(100)20	(100)48		

The first three studies were done on white population while the fourth was on Indians.

**Table 6:** Comparison of our study and previous studies as regards MTHFR C667T mutation.

	TT		CT		CC		Results of MTHFR C667T
	cases	controls	cases	controls	cases	controls	
James et al. <sup>6</sup>	4 (8)	8 (14)	22 (44)	34 (59)	24 (48)	15 (26)	Significant higher risk
Coppede et al.	16 (20)	18 (16)	43 (54)	54 (48)	20 (25)	39 (35)	Risk is not increased
Scala et al. <sup>16</sup>	24 (25)	57 (22)	39 (41)	125 (48)	31 (32)	74 (28)	Significant higher risk
Rai et al. <sup>15</sup>	12 (8)	2 (1)	40 (26)	39 (23)	97 (65)	124 (75)	Significant higher risk in homozygous only
Our study	2 (10)	-	5 (25)	-	13 (65)	-	Non-significant higher risk

## DISCUSSION

Down syndrome (DS) or trisomy 21 is a genetic disorder attributed to the presence and expression of three copies of genes located on chromosome 21, which is mainly due to abnormal segregation during meiosis (maternal non-disjunction in 90% of cases)<sup>11</sup>. The mechanism underlying the meiotic non-disjunction is poorly understood and is thought to have a multifactorial aetiology, being influenced by both genetic and acquired factors<sup>6</sup>. One of these factors is abnormal folate metabolism theory in which a deficiency in dietary folate and methyle donor may result in DNA strand breaks and abnormal chromosome segregation.<sup>12</sup>

In our work, MTHFR C677T polymorphism was found to be a possible maternal genetic risk factor for DS in young mothers, although statistically insignificant. This is consistent with Abdelmeguid et al.<sup>7</sup> study on Egyptian population<sup>7</sup> and confirms the meta-analysis done by Zintzaras, 2007 who concluded

that the accumulated evidence indicated a lack of association between MTHFR C677T, MTHFR A1298C and MTRR A66G gene polymorphisms and maternal DS<sup>13</sup>. Actually, results of similar studies done on young mothers of DS were controversial, while James et al.<sup>6</sup>, Scala et al.<sup>14</sup> and Rai et al.<sup>15</sup> reported a significant increased risk, Coppede et al. did not report a significance difference.<sup>6,14-16</sup>

It was suggested that this lack of association may be due to other gene polymorphisms involved in the homocysteine and folate metabolic pathway that affect the risk of developing DS<sup>6,17</sup>. The risk effect may also depend on gene-environment interaction between the genotype and dietary intake in particular folic acid consumption which could not be accurately studied in this work because none of the mothers had any vitamin supplement before or during pregnancy.<sup>18</sup>

In this study, parental consanguinity was found in 22% of patients with DS with

insignificant difference between young and old mothers of DS. The percentage in the same range detected in the Egyptian population (29-50%)<sup>19</sup>. It could be suggested that consanguinity has a little association with the incidence of DS and that the process of non-disjunction during oogenesis and spermatogenesis is rather a consequence of other causes particularly increased maternal age.<sup>20</sup>

Other possible risk factors studied in young mothers included increased paternal age (>49 years) which was found in only 3% of fathers in Group I compared to 47% in Group II. This is consistent with Stoll et al.<sup>25</sup> and Cross and Hook<sup>10</sup>, studies and disagrees with few studies stating the strong association between advanced paternal age and increased risk of DS births<sup>3,10,21</sup>. In the latter, this risk was diminished with the appropriate adjustment for maternal age confirming the insignificant association.

On the other hand, maternal grandmothers' age may have a significant effect only if the DS mother is above 35 years. This is in contrast to the study done by Malini and Ramachandra, 2006 who recorded that young age mothers (18 to 29 years) born to their mothers at the age 30 years and above produced as high as 91.3% of children with DS. The difference may be attributed to the small number of their study Group (69), compared to ours (200) and to the very high percentage of young mothers in Indian population (91.3%).<sup>22</sup>

The same finding was observed in the oral contraceptive use which had a significant effect only if maternal age was above 35 years. This is in agreement of Lejeune and Prieur<sup>13</sup>, who found an excess of pill-taking by DS mothers over 35 years<sup>23</sup>. This excess was significant

when the delay between the cessation of pill-taking and the conception of the child is six months or less; when the duration of pill-taking has been longer than one year and when those two factors are present simultaneously. Other studies found no association but did not compare the use of oral contraceptive pills between different age Groups.<sup>24</sup>

Regarding birth order, most of DS were the first or second in order of birth with no significance difference between Groups I and II. This is consistent with Hay and Barbano, who suggested that the first-born infant is at higher risk independent on the maternal age and disagrees with Stoll et al. 1990, who reported firstborns to be at lower risk of DS.<sup>5,25</sup>

## CONCLUSION

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In conclusion, MTHFR C677T could not be considered as a maternal risk factor in young Egyptian mothers of DS. The risk effect may depend on gene-environment interaction between the genotype and dietary intake in particular folic acid consumption which should be further studied on a larger scale population including other MTHFR polymorphisms and environmental factors. Other risk factors may include the use of OCP in older mothers. Parents' consanguinity, paternal age and maternal grandparents' ages were not found to be risk factors in DS in this study.

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