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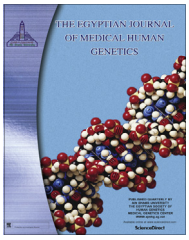

ORIGINAL ARTICLE

Methionine synthase A2756G and reduced folate carrier1 A80G gene polymorphisms as maternal risk factors for Down syndrome in Egypt

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KEYWORDS

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Abstract *Background:* Polymorphisms of genes encoding enzymes involved in folate metabolism have long been hypothesized to be maternal risk factors for Down syndrome, however, results are conflicting and inconclusive.

Aim of the study: To analyze the effect of methionine synthase (*MTR*) A2756G, and reduced folate carrier (*RFC1*) A80G gene polymorphisms on the maternal risk for DS.

Patients: This study was conducted in the Medical Genetics Center, Ain-Shams University hospitals, on a total of 170 mothers of children, diagnosed with Down syndrome, who were attending the center. Eighty-five control mothers were also enrolled in the study.

Methods: Genotype analyses were performed using PCR-RFLP to detect *RFC*1A80G and *MTR*A2756G gene polymorphisms in all case and control mothers.

Results: Comparing *RFC*1A80G genotype frequency between both groups revealed, that the frequency of the AA genotype in case mothers (94.11%) is highly significantly ($p < 0.001$) greater than its frequency in control mothers (74.11%), with no significant difference between the two groups regarding GG genotype. Comparing *RFC1* A80G allele frequency between the two groups revealed a high frequency of the A allele among case mothers (94.11%), which showed a highly statistically significant difference ($p < 0.001$) from the control group (55.29%), meanwhile the G allele showed a low frequency of 5.88% in DS mothers compared to 22.35% in the control mothers, with a highly statistically significant difference ($p < 0.001$) between the two groups. Regarding *MTR*A2756G polymorphism, it was found that the AA genotype predominated in the control group (65.88%) with a highly statistically significant difference ($p < 0.001$) from case mothers group (5.88%). Comparing MTR allele frequency between the two groups revealed predominance of the G allele among mothers of DS children (76.47%).

Abbreviations: DS, Down syndrome; MTR, methionine synthase; RFC, reduced folate carrier

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Conclusion: Current results provide strong evidence that the *MTR*A2756G, and *RFC1* 80 A genotypes could be considered as maternal risk factors for DS in Egyptian mothers.

1. Introduction

Down syndrome (DS), the most frequent cause of mental retardation in live-born infants, is mostly caused by the presence of three copies of chromosome 21, due to failure of chromosomal segregation during maternal meiosis (meiotic nondisjunction) [1].

The incidence of DS in Egypt varies between 1:555 and 1:770 [2], with an estimated risk of 2285 DS births annually [3]. The well-established risk factor, advanced maternal age, was not found in many of the Down syndrome cases in Egypt, while other possible risk factors still have to be elucidated [4]. Studies suggest that chromosomal instability and abnormal segregation may be caused by genomic DNA hypomethylation [5,6].

Folate is the general term for a water-soluble B vitamin (vitamin B9) which is naturally found in foods such as green leafy vegetables, liver, beans, egg yolks, cereals, some citric fruits, kiwis, and strawberries [7]. 5-Methyltetrahydrofolate (5-methylTHF) is the main form of circulating folate in the plasma and can be transported into the cells by means of folate carriers and receptors, the best characterized being the ubiquitously expressed reduced folate carrier (RFC), coded by the *SLC19A1* gene (commonly known as *RFC1* gene) [8]. Intracellularly, 5-methylTHF functions as a methyl donor for homocysteine (hcy) remethylation, a reaction catalyzed by the methionine synthase (MTR) enzyme that transfers a methyl group from 5-methylTHF to hcy, forming tetrahydrofolate (THF) and methionine. Methionine is then converted to S-adenosyl methionine (SAM) by methionine adenosyltransferase, and most of the SAM generated is used in transmethylation reactions whereby it is converted to *S*-adenosylhomocysteine (SAH) by transferring the methyl group to diverse biological acceptors, including proteins and DNA [9]. Therefore, a folate restriction results in aberrant cell growth, impaired DNA methylation, and increases the rate of point mutations, chromosome damage, and aneuploidy [7,10,11].

Almost 15 years ago, it was hypothesized that polymorphisms of genes encoding enzymes involved in folate metabolism could lead to aberrant methylation of peri-centromeric regions of chromosome 21, favoring its abnormal segregation during maternal meiosis. Subsequently, more than 50 small case–control studies investigated whether or not maternal polymorphisms of folate pathway genes could be risk factors for the birth of a child with DS, yielding conflicting and inconclusive results [12].

2. Aim of the study

The aim of this study is to analyze the effect of two genetic polymorphisms, *MTR* A2756G, and *RFC1* A80G on the maternal risk for DS.

3. Patients

This study was conducted in the Medical Genetics Center, Ain-Shams University hospitals, on a total of one hundred seventy mothers of children, diagnosed with Down syndrome, who were attending the center. Eighty-five control mothers of healthy children were also enrolled in the study.

The work was done after taking acceptance of all participants and taking an informed consent to share in the study as well as acceptance of ethics committee of the University. The works have been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

4. Methods

4.1. DNA extraction

DNA was extracted from whole blood using a QIAamp Blood mini-prep Kit (QIAGEN, Germany) according to manufacturer's instructions Genomic DNA (300 ng) was amplified in a final volume of 50 ll, containing 10 mM TRIS pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 200 mM each dNTP, 1 lM of each primer and 2U Taq polymerase (all reagents from MBI Fermentas, St. Leon-Rot, Germany).

4.2. RFC1A80G genotype detection

Genotype analyses were performed using PCR-RFLP. The primers for amplification were: forward: 5′-AG TGT CAC CTT CGT CCC-3′ and reverse 5′-TCC CGC GTG AAG TTC TTG-3′. PCR conditions were 44 cycles of 30 s at 94 °C, 30 s at 52 °C, and 45 s at 72 °C, preceded by an initial denaturation of 2 min at 94 °C, and followed by a final extension of 7 min at 72 °C. Three hours digestion with CfoI (Sigma) resulted in three fragments of 125, 68, and 37 bp, in the presence of the 80G allele, while the 80A allele produced two fragments of 162 and 68 bp.

4.3. MTR A2756G genotype detection

Genotyping for the MTR A2756G polymorphism was carried out using specific primers (forward: 5′-CCA GGG TGC CAG GTA TAC AG-3′, and reverse: 5′-GCC TTT TAC ACT CCT CAA AAC C-3′) to amplify a 498-bp fragment spanning the polymorphism, followed by digestion with enzyme HaeIII, that digests the fragment in the presence of the mutated G allele giving three fragments of 300, 130 and 68 bp, while in the presence of the normal A allele, a whole uncut fragment of 498 bp appears.

4.4. Statistical analysis

Results were analyzed using the Statistical Package of Social Sciences (SPSS) computer software program, version 21.0 (Chicago, IL, USA). Data were presented in the form of frequencies and percentages. Differences among groups were tested using Pearson's chi-square test (v2). A p value less than 0.05 was considered statistically significant.

5. Results

In the present study, two gene polymorphisms, namely, *RFC*1A80G and *MTR*A2756G were studied in 170 case mothers along with 85 control mothers.

In the current study, comparing RFC1A80G genotype frequency in DS and control mothers **(**Table 1**)** revealed, surprisingly, that the frequency of the AA genotype in mothers of DS children (94.11%) is highly significantly ($p < 0.001$) greater than its frequency in control mothers (74.11%), meanwhile there was no significant difference between the two groups regarding GG genotype. Comparing *RFC1* A80G allele frequency between the two groups **(**Table 2**)** revealed a high frequency of the A allele among case mothers (94.11%), which showed a highly statistically significant difference from the control group (55.29%), meanwhile the G allele showed a low frequency of 5.88% in DS mothers compared to 22.35% in the control mothers, with a highly statistically significant difference between them ($p < 0.001$).

On the other hand, the current results concerning MTRA2756G polymorphism, revealed that the AA genotype predominated in the control group (65.88%) with a statistically highly significant difference ($p < 0.001$) from the DS mothers group (5.88%), meanwhile, the GG genotype predominated in the DS mothers group (58.82%), with a statistically high significant difference ($p < 0.001$) from the control group (23.52%) **(**Table 3**)**. Comparing MTR allele frequency between the two groups revealed predominance of the G allele among mothers of DS children (76.47%) with a statistically high significant difference ($p < 0.001$) from the control mothers (22.35%) **(**Table 4**)**. There was no significant difference between the frequency of MTRA2756A allele in DS (23.53%) and control mothers (43.52%).

6. Discussion

DS is the most common chromosomal abnormality in live births. Many studies have assessed the association between maternal gene polymorphisms involved in folate metabolism

Table 1 *RFC1* A80G genotype frequency in DS and control mothers.

SNP Gene ID	Genotype	DS mothers *N* (%)	Control mothers *N* (%)	*P*-value	OR (95% CI)*
rs1051266 6573	AA	160 (94.11)[a]	63 (74.11)[a]	0.000[a]	5.587 (2.505–12.464)
	AG	0 (0)[b]	15 (17.64)[b]	0.000[b]	−(2.815–4.176)
	GG	10 (5.88)[c]	7 (8.23)[c]	0.478[c]	0.696 (0.255–1.899)

* OR = odds ratio; CI = confidence interval.
[a] A statistically significant difference in the frequency of AA genotype between DS mothers and control mothers ($p < 0.001$).
[b] A statistically significant difference in the frequency of AG genotype between DS mothers and control mothers ($p < 0.001$).
[c] No significant difference between the frequency of GG genotype in DS and control mothers ($p > 0.05$).

Table 2 *RFC1* A80G allele frequency in DS and control mothers.

SNP gene ID	Allele	DS mothers *N* (%)	Control mothers *N* (%)	*P* value	OR (95% CI)*
rs1051266 6573	A	160 (94.11)[a]	47 (55.29)[a]	0.000[a]	12.936 (5.997–27.904)
	G	10 (5.88)[b]	19 (22.35)[b]	0.000[b]	0.217 (0.096–0.492)

* OR = odds ratio; CI = confidence interval.
[a] A statistically significant difference in the frequency of A allele between DS mothers and control mothers ($p < 0.001$).
[b] A statistically significant difference in the frequency of G allele between DS mothers and control mothers ($p < 0.001$).

Table 3 *MTR* A2756G genotype frequency in DS and control mothers.

SNP (gene ID)	Genotype	DS mothers *N* (%)	Control mothers *N* (%)	*P* value	OR (95% CI)*
rs1805087 (4548)	AA	10 (5.88)[a]	56 (65.88) [a]	0.000 [a]	0.032 (0.015–.071)
	AG	60 (35.29)[b]	9 (10.58) [b]	0.000 [b]	4.606 (2.156–9.841)
	GG	100 (58.82)[c]	20 (23.52) [c]	0.000 [c]	4.643 (2.582–8.35)

* OR = odds ratio; CI = confidence interval.
[a] A statistically significant difference in the frequency of AA genotype between DS mothers and control mothers ($p > 0.001$).
[b] A statistically significant difference in the frequency of AG genotype between DS mothers and control mothers ($p > 0.001$).
[c] A statistically difference between the frequency of GG genotype in DS and control mothers ($p > 0.001$).

Table 4 *MTR* A2756 G allele frequency in DS and control mothers.

SNP (gene ID)	Allele	DS mothers *N* (%)	Control mothers *N* (%)	*P* value	OR (95% CI)*
rs1805087 (4548)	A	40 (23.53)[a]	37 (43.52)[a]	0.416[a]	0.803 (0.473–1.363)
	G	130 (76.47)[b]	19 (22.35)[b]	0.000[b]	11.289 (6.065–21.013)

* OR = odds ratio; CI = confidence interval.
[a] No significant difference in the frequency of A allele between DS mothers and control mothers ($p > 0.05$).
[b] A statistically significant difference in the frequency of G allele between DS mothers and control mothers ($p < 0.001$).

and the risk of having a DS offspring, but data are conflicting [13]. RFC1, which is located in the intestinal mucosal membrane, is responsible for the amount of folate available in the cells through transport of 5-methyltetrahydrofolate [14].

To our knowledge, studies concerning the frequency of *RFC*1 A80G or *MTR*A2756G gene polymorphisms as maternal risk factor for DS in Egypt, are still lacking. However, our results are in contrast to Coppedè et al. [15], who showed a role for the 80GG genotype, but not for the 80AA genotype, combined with the *MTHFR* 1298AA genotype in the maternal risk for DS in an Italian population.

Another study performed in Southern Italy suggested that the 80G allele might increase DS risk in mothers aged more than 34 years at conception [16]. In addition, Yang et al. [14], has shown that *RFC*1 80G allele, is associated with increased risk of having a child with DS. However, Chango et al. and Neagos et al. [17,18], have found no significant association between *RFC*1 A80G gene polymorphism and increased risk for a mother to have a DS child.

Also conflicting with our results are those results of Wang et al. [19], who concluded that women with *RFC*-1 G80G genotype have increased risk of delivering a child with Down syndrome in China. Subsequent studies evaluating the possible contribution of this polymorphism to the maternal risk of having a DS child were conflicting, however, two meta-analyses, by Yang et al. and Coppedè et al. [14,20] suggested that it could represent an independent maternal DS risk factor. Moreover, despite that its functional role is still controversial, the *RFC1* 80G > A polymorphism has been associated with reduced red cell folate concentrations among healthy women [21], and with reduced serum folate concentrations in mothers of down syndrome children (MDS) [22]. However, less than 1.000 MDS were available for those meta-analyses, and subgroup stratification yielded inconclusive results likely because of the small case–control cohorts in each ethnic group [14,20]. However, *RFC1* 80G allele frequency was higher in Caucasian and Brazilian MDS (ranging between 49.0% and 54.0%) than in Asian ones (36.0–36.5%) [23]. Therefore, further studies with larger sample size are required to clarify the contribution of this polymorphism to the maternal risk of a DS birth.

Another gene polymorphism, the *MTR* 2756A > G polymorphism (rs1805087), leading to the Asp919Gly substitution, was the third variant of the folate pathway to be associated with the maternal risk of having a birth with DS [24].

The contribution of the *MTR* 2756G allele to the maternal risk of birth of a child with DS was investigated mainly in European and mixed Brazilian populations, with smaller reported allele frequencies of about 18–21% [23], while the study by Liao et al. [25], showed G allele frequencies of less than 10% in Asians. The functional role of *MTR* 2756A > G with regard to its possible contribution to circulating hcy, folate, or vitamin B12 levels, revealed conflicting results [26]. In this regard, Coppedè et al. [12], recently screened a large cohort of MDS and matched control mothers observing that the *MTR* 2756A > G, was not associated with increased serum folate levels in GG carriers [20,27].

Our results are in agreement with Bisellie et al. [28], who found that the MTR 2756GG genotype is a maternal risk factor for DS. However, other case–control studies failed to confirm this association where some recent meta- analyses confirmed that the *MTR* 2756A > G polymorphism is not an independent maternal risk factor for a DS offspring [12,14,20,29].

7. Conclusion

The current results provide strong evidence that the *MTR*A2756G, as well as the *RFC1* 80 A genotypes could be considered as maternal risk factors for DS in Egyptian mothers.

However, more studies, on larger samples, are needed to confirm the current results.

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