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ORIGINAL ARTICLE

The methylenetetrahydrofolate reductase gene variant (C677T) in risk mothers with Down syndrome among Saudi population

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Abstract This unreeled study aimed to examine the relationship between the genetic polymorphisms C677T in MTHFR gene and mapped this figure with other ethnic populations. The present study examined 70 Saudi females (30 mothers with DS children plus 40 healthy mothers who gave birth only to healthy children) for C677T genotypes using restriction fragment length polymorphism (RFLP) of the amplified genomic DNA. The frequencies of the combined mutant genotypes CT and TT in the MTHFR gene were modestly represented in the case mothers compared to that in controls (33% vs. 35% and 13% vs. 10%) with no significance (OR 1.1, 95% CI, 0.41–2.77, $p = 0.91$). The frequency of the mutant 677T allele was 28% in the case mothers and plotted as a moderate value with different ethnic populations. The present study concluded that there was a null association between the common C677T polymorphism and the increased risk of Down syndrome, but the T allele slightly supported the increase of this maternal risk. The intermediacy to previous reports may probably be due to the small sample size, gene-nutritional-environmental factors, or the consequences of much social intermarriage between some Asian, Arab peoples and the Saudi community.

1. Introduction

Down syndrome (DS) or trisomy 21 is a genetic disease caused by abnormal chromosomal segregation [1]. Free trisomy 21 is found in 95% of DS cases and is due to chromosome 21 non-disjunction, mostly occurring during maternal meiosis [2]. A mechanism to explain chromosomal non-disjunction has been proposed and consists of defects in DNA methylation with consequent centromeric hypomethylation, causing abnormal kinetochore and microtubule formation [3–6]. A number of genetic and environmental factors have been suggested to play interactively a role in aneuploidization, among which dietary

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factors [7]. Folate acid (pteroiloglutamic acid) has an important role in the process of genetic material distribution during cell division, because of its part in the cellular methylation reactions, which, in turn, epigenetically regulate segregation and other processes [8].

Recent evidence shows that almost 92% of the DS children are born from young mothers, suggesting that other risk factors than advanced maternal age must be involved. Within this context, some studies suggested and even demonstrated a possible link between DS and maternal polymorphisms in genes involved in folate metabolism [9,10]. Methylenetetrahydrofolate reductase (MTHFR) has a crucial role in regulating cellular methylation, through the conversion of 5,10-methyltetrahydrofolate (THF) to 5-methyl THF, the methyl donor in the transformation of homocysteine to methionine [11]. The C–T common polymorphism at nucleotide 677 (C677T), which results into an alanine to valine substitution in the MTHFR protein, causes higher thermolability and reduced enzyme activity in lymphocyte extracts [12]. Increased folic acid intake is required to maintain homocysteine remethylation to methionine at normal levels [13]. Reduced MTHFR activity due to the C677T change has been associated with different disorders, including vascular diseases [12], neural tube defects [14–16], male infertility [17], Alzheimer's [18], and Parkinson diseases [19]. The C677T polymorphism has been related also to maternal risk for DS, because of a higher prevalence of the T allele among mothers of children with trisomy 21, compared to control mothers [9,10]. The identification of the C677T as a factor of maternal risk for DS might be of great importance in the genetic counseling of this disease, since in this case other female relatives of a carrier DS mother should be tested for the presence of this mutation. Here, this study investigated the prevalence of the C677T polymorphism in a sample of Saudi mothers of DS children.

2. Subjects and methods

2.1. Study population

The present study includes 70 women (ages 18–45 years old): 30 of them, that gave birth to DS children; cytogenetically confirmed as regular trisomy 21. The average ages ± SD of case mothers was 31.7 ± 7.5 years. The 40 controls were mothers who gave birth only to healthy children, without any history of miscarriages or abnormal pregnancies. All women in this study live in the same geographic area (Western region of Saudi Arabia). Informed consent was obtained from all the participants. There was no periconceptional use of folic acid.

2.2. DNA extraction

Peripheral blood samples were collected on EDTA from both DS and control mothers. Genomic DNA was isolated from whole blood (0.2 ml), using QIAamp DNA Blood kit (Qiagen, Hilden, GmbH, Germany) as recommended by the manufacturer's instructions.

2.3. Genotyping of MTHFR gene

To identify the MTHFR 677C > T polymorphism, genomic DNA was amplified by polymerase chain reaction (PCR) using specific primers sequences of MTHFR gene; 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ and 5′-AGG ACG GTG CGG TCA GAG TG-3′ [12]. A mixture of 25-μl reaction volume of 0.5 μM of each primer, 200 μM of each dNTP, 67 mg Tris–HCl, 16 mM $(NH_4)_2SO_4$, 0.01% Tween-20, 1 mM $MgCl_2$ and 0.15 units *Taq* DNA polymerase. The samples were then subjected to 35 cycles on PCR Engine Dyad (Bio-Rad Laboratories, USA) with annealing at 58 °C for 30 s. The PCR product was incubated overnight with the *Hinf*I enzyme at 37 °C for complete digestion, and the fragments were separated on 3% MetaPhor agarose gel (BMA, Rockland, ME, USA). The product was then identified by ethidium bromide staining and photographed using a gel-documentation system (G-Box, SynGene, Frederick, MD, USA). The absence of *Hinf*I recognition site revealed a 198-bp fragment due to the homozygous wild-type 677C/C genotype, while the presence of the *Hinf*I cutting site due to the homozygous mutant 677T/T genotype gave the 175-bp main fragment. The 677C/T heterozygous status is expressed as two fragments of 175-bp and 198-bp sizes.

2.4. Data analysis

Statistical analysis was performed using the SPSS 16.0 (SPSS, Chicago, IL, USA). The data are presented as mean ± standard deviation (SD). Nonparametric measures were used to calculate chi-square values. A probability < 0.05 was considered statistically significant. Odds ratios with 95% confidence intervals were calculated.

3. Results

In the mothers of DS children, the C/C wild homozygous genotype was found in 53.3%, the C/T heterozygous genotype in 33.3% and the T/T mutant homozygous genotype in 13.3% (Table 1). In the control mothers, the C/C genotype frequency was 55%, C/T 35% and T/T 10%. There were no significant differences in genotype frequencies between the two groups (OR 1.1, 95% CI, 0.41–2.77, $p = 0.91$). Overall, the frequency percentage of the C allele was 70% in DS mothers and 73% in control mothers. There was no difference in the prevalence of the MTHFR variant T allele in the same groups (30.5% and 31.25%, respectively).

4. Discussion

Progressions have been achieved to clarify the important role of the MTHFR enzyme in metabolic pathway of folate and nucleotide methylation. Presence of 677T allele leads to reduction of MTHFR activity and DNA hypomethylation. It has been understood that hypomethylation of DNA has been related to chromosomal changes and instability. Studies on the role of polymorphisms in folate metabolism as a risk factor for DS have obtained controversial results [13,20]. Most of these studies have investigated polymorphisms in the MTHFR gene.

The hypothetical effect of 677T onto maternal nondisjunction was linked to an altered DNA methylation pattern on the oocyte, secondary to reduced MTHFR activity. Hassold et al. [21] analyzed maternal polymorphism at

Table 1 The distribution of *MTHFR* genotype and allele frequencies in case and control population.

Population	*n*	Genotype distribution (%)			*Allele frequency*	
		C/C	C/T	T/T	C	T
Case	30	16 (53.3)	10 (33.3)	4 (13.3)	0.70	0.30
Control	40	22 (55.0)	14 (35.0)	4 (10.0)	0.73	0.28

Table 2 Distribution of a common C677T MTHFR polymorphism among different ethnic populations.

Population	Total subjects	Heterozygous, C/T *n* (%)	Homozygous mutant, T/T	T allele frequency (%)	References
Australia	85	8 (9.4)	0 (0.0)	4.7	[33]
Africa[a,b]	301	38 (12.6)	0 (0.0)	6.3	[36]
US Blacks[b]	496	127 (25.6)	6 (1.2)	14.0	[36]
Middle east					
Egypt	48	12 (25)	3 (6.2)	18.8	[26]
Yemen	46	14 (30.4)	1 (2.2)	17.4	[33]
Saudi Arabia	40	14 (35)	4 (10)	28	Present study
Turkey	93	39 (41.9)	7 (7.5)	28.5	[37]
Asia[c]	279	92 (33.0)	12 (4.3)	20.8	[33]
US Whites[b]	1147	512 (44.6)	136 (11.9)	34.2	[36]
Japan[b]	2472	1171 (47.4)	284 (11.5)	35.2	[36]
Britain[b]	1046	465 (44.5)	138 (13.2)	35.4	[36]
Canada (Quebec)[b]	414	183 (44.2)	59 (14.3)	36.3	[36]

[a] Including Sub-Saharan Africa, Zaire, Cameroon.
[b] Pooled data.
[c] Including Hong Kong, Mongolia, Indones.

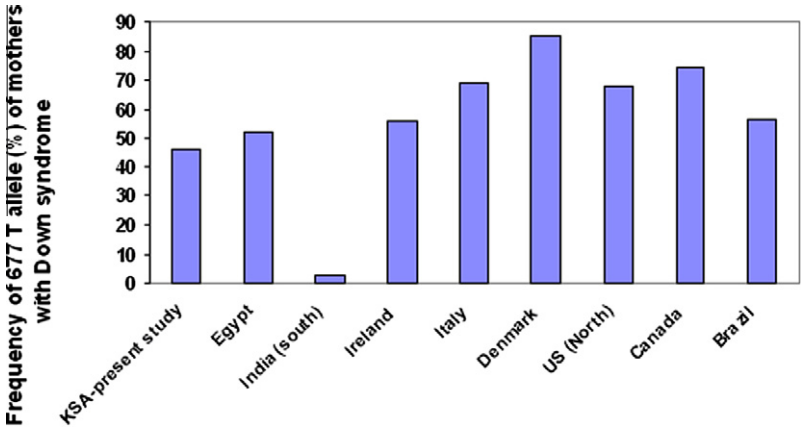

Figure 1 Frequency of 677T mutant allele in mothers with DS children in comparison with that in different populations.

MTHFR in cases of trisomies of other chromosomes, detecting significant association only with trisomy 18. Reports of the 677C > T nucleotide substitution in relation to oral clefts, Down syndrome, and fetal anticonvulsant syndrome either have yielded conflicting results or have not been yet replicated [22–24].

To the best of our knowledge, this is the first study that investigated the prevalence of MTHFR C677T alleles and genotype distribution in the Saudi females. Data obtained in our study does support a little increase of DS mothers with the 677T allele compared to controls in Saudi samples (Table 1). The C/T or T/T genotypes results do not rule out an effect of the 677T allele on maternal non-disjunction, but were nearly being equal in both controls and mothers with DS children. The present study suggests that in populations with adequate folate dietary intake the reduced enzymatic activity of the MTHFR in the carriers of the T allele is balanced by folates, thus reducing the risk of cellular demethylation and non-disjunction.

It is a point of interest since the genotypes in the mothers of DS children were comparable to those reported in Table 2, while controls disclosed a lower frequency of the T allele (0.28), and the C/T and T/T genotypes accounting for 46% of the analyzed controls. The difference between case and controls was not significant ($p > 0.05$). These data confirm previous studies by showing a lower prevalence of the T allele in the Saudi population [9,10,25–27].

The distribution of 677T allele indicated that the rate of combined mutant 677 (C/T + T/T) genotypes reported mod-

Table 3 Prevalence of the 677C/T MTHFR genotypes in DS mothers and controls.

Population (references)	*Cases controls*	DS mothers				Controls			
		CC (%)	CT (%)	TT (%)	CT + TT (%)	CC (%)	CT (%)	TT (%)	CT + TT (%)
This study	30	53	33	13	46	55	35	10	45
(Saudi Arabia)	40								
Egypt [26]	42	47.6	40.5	11.9	52.4	68.8	25	6.2	31.2
	48								
South India [32]	36	91.7	8.3	0	3	60	0	0	0
	60								
Irish [28]	48	44	51	5	56	47	44	9	53
	192								
Italy [25]	64	31	50.7	18.3	69	24.1	55.4	20.5	75.9
	112								
Danish [23]	177	52	40.7	7.3	85	50.3	41.4	8.3	49.7
	1084								
North America [10]	157	32	54	14	68	48	42	10	52
	77								
Canada [9]	57	26	60	14	74	48	44	8	52
	50								
Brazil [30]	154	43.5	46.8	9.7	56.5	53.2	42.4	4.4	46.8
	158								

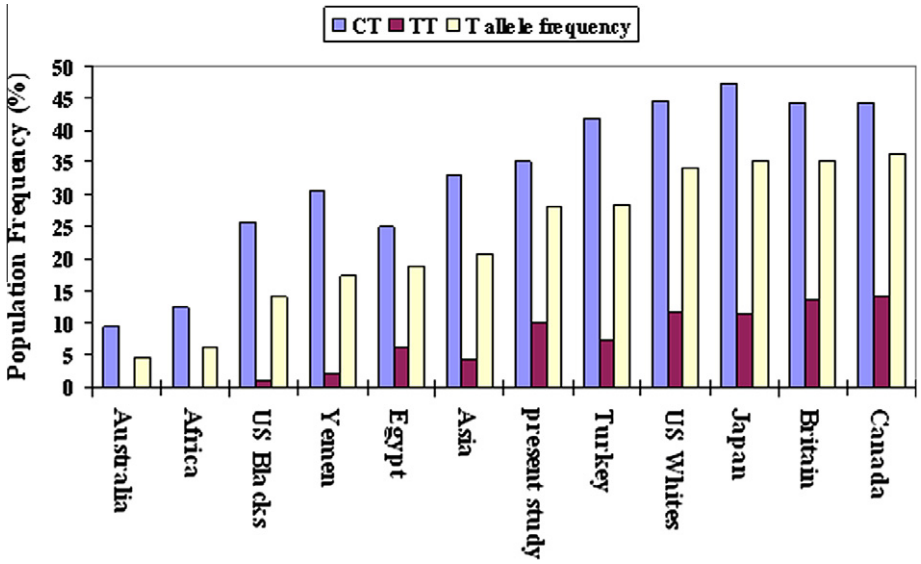

Figure 2 The heterozygous, homozygous mutants and T allele frequencies at position 677 of MTHFR gene in different populations and present study.

erate frequencies in the Saudi females with DS children (Fig. 1). Several reports reviewed in Table 2 imply that the T allele frequency are substantially lower in our cases compared to that of North American, Canadian, Irish and Italian populations [9,10,25,28]. The Egyptian report of Meguid et al. [26] and Shawky et al. [27] might closely agree our genotypic data. Acacio et al. [29] found that the frequency of joint heterozygotic polymorphism 677C/T of *MTHFR* gene was significantly higher in 70 Brazilian DS females, but in contrast da Silva et al. [31] showed it was near borderline significance among 154 Brazilian DS females ($p = 0.08$) (Table 3). In an study from India, where 677T allele frequency is quite low in the population Rai et al. [31] have shown a strong association of this SNP, especially the T homozygote, with <30 year-DS mothers. Interestingly, the T homozygous case mothers were all younger than 30 years of age. In this regard, Fig. 1 displayed the two extremes of the T allele frequency in both Danish and Indian populations [23,32].

The ethnic population frequency of C677T homozygosity ranges from 1% or less among Blacks from Africa and the United States to 20% or more among Italians and US Hispanics [33]. The CT, TT and T allele frequencies in Saudi mothers with DS children were compared with different populations (Table 2 and Fig. 2).

The issue of the intermediacy of our outcomes in Saudi females in comparison with other ethnic populations might be reasonably subjected to (1) the hypothesis that the interaction between different polymorphisms within the human genome

may totally modify their individual effects [34], (2) the consequences of much social intermarriage with some Asian, Arab people and the Saudi community toughened the heterogeneity, the pleiotropy, and variable expressivity of the disease [35], and (3) the adequate folate dietary intake reduces the enzymatic activity of the MTHFR in the individuals with the 677T allele, thus reducing the risk of cellular demethylation and non-disjunction.

In conclusion, though conflictions have been come up among different ethnic populations, here, the unreeled study described null association between the C677T genetic variant and the mothers with DS children among the Saudi community. This finding does support a modest risk increase of the T allele in the cases compared to controls. Our observations were in agreement with other ethnic populations, but sustain the need for replication of a cohort study of the 677C > T polymorphism among DS mothers.

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