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Research Article

Distribution of MTHFD1 gene 1958G>A Polymorphism in the Jordanian Population and its Relation with Neural tube Defects

Helmi Y Alfarra*, and May F. Sadiq
Department of Biological Sciences, Yarmouk University, Irbid, Jordan
*Correspondence to Email: helmiyousif@gmail.com

ABSTRACT
The frequency of methylenetetrahydrofolate dehydrogenase (MTHFD1) 1958G>A polymorphism was determined using restriction fragment length polymorphism polymerase chain reaction analysis in a sample of 491 individuals from different regions of Jordan. The distribution of polymorphic alleles of MTHFD1 1958G>A in the Jordanian population were 65.2% for the G allele and 34.9% for the A allele. The genotype distributions were 48.7%, 32.8% and 18.5% for the GG, GA and AA genotypes respectively. Statistical analysis showed no significant correlation between the rates of alleles and genotypes across the different regions of Jordan. The association of MTHFD1 1958G>A polymorphism with the development of neural tube defects (NTDs) was examined for 17 mothers from the northern part of Jordan who gave birth to an NTD-affected child during the period of this study. The results showed no association between these 3 examined polymorphisms and maternal risk for an NTD-affected child.
***Keywords**: NTD, MTHFD1, Polymorphisms, Jordan*

INTRODUCTION

The human 5,10-methylenetetrahydrofolate dehydrogenase (MTHFD1) gene is located on the long (q) arm of chromosome 14 at position 24 and is composed of 28 exons [1, 2]. The MTHFD1 1958G>A variant is detected using the restriction fragment length polymorphism technique. MTHFD1 is a trifunctional nicotinamide adenine dinucleotide phosphate (NADP)-dependent cytoplasmic enzyme (often referred to as C1-THF synthase), which catalyses the conversion of tetrahydrofolate to the corresponding 10-formyl,5,10-methyenyl and 5,10 methylene derivatives. These derivatives are the donors of cofactors for de novo purine and pyrimidine biosynthesis and thus for the biosynthesis of DNA [3]. Several potential MTHFD1 single nucleotide polymorphisms (SNP) can be identified from the public database and other literature. However, studies in different populations showed that only MTHFD1 1958G>A polymorphism, which is located in exon 20 [4, 5], is a maternal risk for neural tube defects (NTDs) [3].

NTDs are the second most common severe disabling human congenital defect. They are very complex birth defects involving the interactions of multiple systems. Worldwide, the incidence of NTDs is approximately 1 per 1000 live births, with estimates varying between 0.78 and 12 per 1000 births in general populations. In Jordan, 3 studies performed during the years 1991 to 2003 showed that the average incidence of NTDs in the Jordanian population was about 3.8 per 1000 [5].

NTDs seem to be related to defects in the folate–methionine metabolic pathways, since preconception use of vitamin B12 supplemented with folic acid has been shown to substantially reduce the risk of NTDs. Different studies have reported the alteration in folate status of mothers with NTD-affected children [6, 7, 8]. In these mothers folate absorption, transport and metabolism are the focus of intense research to clarify the mechanisms underlying these observations. Yet the basis of this defect remains unknown. Many studies reported that folate deficiency is a contributor to the etiology of NTDs, and genes in this metabolic pathway have been the basis for many candidate gene studies [6, 7, 8].

The main aim of the present study was to examine the frequency of MTHFD1 1958G>A polymorphism in the Jordanian population and the correlation of this polymorphism with the incidence of NTDs at birth in the northern region of Jordan.

MATERIAL AND METHODS

Samples

Blood samples were collected from 491 adult individuals to be representative of the Jordanian population (controls). They were aged between 20 and 46 years and with no evident abnormalities of maternal origin. The samples were collected from different regions in Jordan according to the calculated sample sizes needed to represent the Jordanian population in the different regions. The minimal sample size to be representative of Jordan was estimated as 385 individuals. Most of the samples were taken from premarital testing centres, while some samples were collected from the Yarmouk University campus from the students in the campus by making campaign for blood sample collecting and from parents accompanying children to hospitals.

To study the correlation between MTHFD1 1958G>A polymorphism and a maternal risk for NTDs, the frequencies of these alleles in a group of mothers who gave birth to NTD-affected children in the northern part of Jordan were compared with frequencies of these alleles among the control samples collected from the same region over the same time period. Blood sample were collected from 17 mothers who had given birth to at least 1 child affected with an NTD (cases) at the 2 largest among the 8 hospitals with a department of paediatrics from the northern part of Jordan (King Abdullah and Princess Rahma hospitals) between the beginning of September 2007 until the end of November 2008. All the volunteers signed a written consent.

Laboratory techniques

A 3 mL sample of blood was collected from each subject into an EDTA tube, and then genomic DNA was isolated from lymphocytes using standard procedures (9). The 330 basepair (bp) fragment of the MTHFD1 gene in the 1958 region of the MTHFD1 gene was amplified. This was performed in a total volume of 25 μL reaction mixture, containing 12.5 μL of 2× Taq Master Mix (Promega), 5 pmol of the forward MTHFD1 5'-CACTCCAGTGTTTGTCCATG-3' and the reverse MTHFD1 5'-GCATCTTGAGAGCCCTGAC-3' (Midland) for each, and about 500 ng of genomic DNA template (2). The mixture was denatured initially at 95 °C for 3 min., and the polymerase chain reaction (PCR) (GeneAmp 9600, Perkin–Elmer) was carried out for 35 cycles under the following conditions: denaturation at 94 °C for 30 s, annealing at 58 °C for 1 min., extension at 72 °C for 1 min. and final extension cycle at 72 °C for 10 min [2].

Restriction enzyme digestion was performed on amplified DNA using the MspI restriction enzyme. Each restriction digestion reaction contained 10 μL of the PCR product, 10 units of MspI (Biolabs), 10 × NEBuffer 2 (Biolabs), 0.1 g/mL bovine serum albumin (Promega) and sterile double distilled H2O, to a final volume of 20 μL. The reaction tubes were incubated at 37 °C for 3 hours (2). Restriction fragment products were resolved on 2% agarose gel in 1× tris-borate EDTA, 10× 109 g/L tris-HCl, 55.6 g/L boric acid, 9.3 g/L Na2-EDTA (pH 8.0) containing 0.5 mg/mL ethidium bromide. Gels were exposed to 10 V/cm for 1.5–2 hours. The sizes of the digestion products were determined using the 100 bp ladder (Promega).

The digestion of the normal 1958G/G genotype resulted in 3 fragments of 196, 71 and 55 bp, while the homozygous mutant polymorphism 1958AA resulted in 2 fragments of 267 and 71 bp and the digestion of the heterozygous 1958A>G genotypes produced 4 fragments of 267, 196, 71 and 55 bp [2]. The 71 bp and 55 bp fragments were too difficult to resolve from primer dimers and therefore were ignored when identifying the genotypes.

Analysis

The data are presented as the number and percentages of individuals with various genotypes and the comparisons between regions were evaluated using the chi-squared and Fisher exact tests. P-value < 0.05 was considered significant and all analyses were done using the SPSS software, version 15.5.

RESULTS

The frequencies of the different alleles and genotypes of the MTHFD1 1958 position were examined in 491 individuals from the 3 regions of Jordan. The presence of the MTHFD1 1958G>A mutation was investigated in all samples from the population and the 17 mothers of NTD cases. All the genotypes GG, AG and AA were found in the samples.

Figure 1 represents the digestion products of the different genotypes found. The amplified fragment of the normal MTHFD1 1958G had 2 sites for the MspI enzyme and when digested with this enzyme gave 3 fragments of sizes 196, 71 and 55 bp. However, only large fragments can be seen in Figure 1 due to the small size of the other fragments. The amplified fragment containing the MTHFD1 A

polymorphism gave 2 fragments of sizes 267 and 71 bp. Figure 1 shows only the large fragment of 267 bp because the short fragment cannot be clearly seen on the gel, while the heterozygote with the MTHFD1 AG genotype showed only 267 and 196 bp fragments of the 4 fragments produced.

Table 1 summarizes the frequency distribution of MTHFD1 1958G and MTHFD1 1958A alleles in the different regions of Jordan. The frequency of the G allele among the population of the northern region was 62.0%, in the middle region was 66.8% and 72.8% in the southern region, while the frequencies of the A allele in the northern, middle and southern regions were 38.0%, 33.2% and 27.2% respectively. The overall frequencies of the G and A alleles in the whole Jordanian population were 65.1% and 34.9% respectively. A 2-way contingency table analysis showed that there was no statistically significant difference in the frequencies of the MTHFD1 1958G>A alleles across the regions of Jordan (P=0..082).

The genotype frequencies are shown in Table 2, which shows that the frequencies of the normal MTHFD1 1958GG genotype were 45.3% in the northern part of Jordan, 49.8% in the middle and 60.9% in the southern part. The frequencies of the heterozygous MTHFD1 1958G>A genotype was 33.3%, 34.1% and 23.9% in the northern, middle and the southern regions respectively. The frequencies of the mutant MTHFD1 1958 AA genotype in the northern area was 21.4%, while it was 16.1% in the middle and 15.2% in the southern part. A 2-way contingency table analysis showed that there was no statistically significant difference in the frequencies of the 3 MTHFD1 1958G>A genotypes across the regions of Jordan (P=0.267).

Table 3 shows that the frequencies of MTHFD1 G and A alleles of position 1958 among the mothers of NTD cases were 58.8% and 41.2% respectively, while the frequencies in the controls in the same (northern) region of Jordan were 62.0% and 38.0% respectively. The chi-squared test showed that there was no difference between the frequency of the 2 MTHFD1 (P=0.716) 1958 alleles in the NTD cases and the controls. Table 3 also shows that the frequencies of MTHFD1 1958 genotypes GG, AG and AA in the NTD cases in the northern region were 29.4%, 58.8% and 11.8% respectively, while in the control group from the northern region they were 45.3%, 33.3% and 21.4% respectively. The 2-way contingency table analysis showed that there was no significant difference (0.103) between the rates of the 3 MTHFD1 1958G>A genotypes in NTD cases and controls in the northern part of Jordan.

DISCUSSION

This is the first study to examine the frequencies and distribution of the polymorphisms of MTHFD1 1958G>A in the Jordanian population or other Arab countries. This study has also evaluated the correlation between the polymorphisms MTHFD1 1958G>A and maternal risk of delivering an NTD-affected infant in the northern area of Jordan. This polymorphism was examined due to its role in the folate metabolic pathway, which is essential for the methylation and regulation of developmental processes in embryos [3]. The aetiology of NTDs is poorly understood, but it is now suggested that there is a complex interplay between environmental and genetic factors [5].

Table 1: Frequencies of methylenetetrahydrofolate dehydrogenase 1958G>A alleles in the 3 regions of Jordan

Region	Allele G		Allele A	
	No.	%	No.	%
Northern region (*n* = 468)	290	62.0	178	38.0
Middle region (*n* = 422)	282	66.8	140	33.2
South region (*n* = 92)	67	72.8	25	27.2
All Jordan (*n* = 982)	639	65.1	343	34.9

n = number of alleles.

Table 2: Frequencies of methylenetetrahydrofolate dehydrogenase 1958G>A genotypes in the 3 regions of Jordan

Region	% GG genotype		% GA genotype		% AA genotype	
	No.	%	No.	%	No.	%
Northern region (*n* = 234)	106	45.3	78	33.3	50	21.4
Middle region (*n* = 211)	105	49.8	72	34.1	34	16.1
South region (*n* = 46)	28	60.9	11	23.9	7	15.2
All Jordan (*n* = 491)	239	48.7	161	32.8	91	18.5

n = number of genotypes.

Table 3: Frequencies of genotypes and alleles of methylenetetrahydrofolate dehydrogenase 1958G>A in the mothers with children affected by neural tube defects and the controls from the general population of the northern region of Jordan

Variable	Neural tube defects		Controls	
	No.	%	No.	%
Genotype	(*n* = 17)		(*n* = 234)	
GG	5	29.4	106	45.3
GA	10	58.8	78	33.3
AA	2	11.8	50	21.4
Allele	(*n* = 34)		(*n* = 468)	
G	20	58.8	290	62.0
A	14	41.2	178	38.0

n = number of subjects.

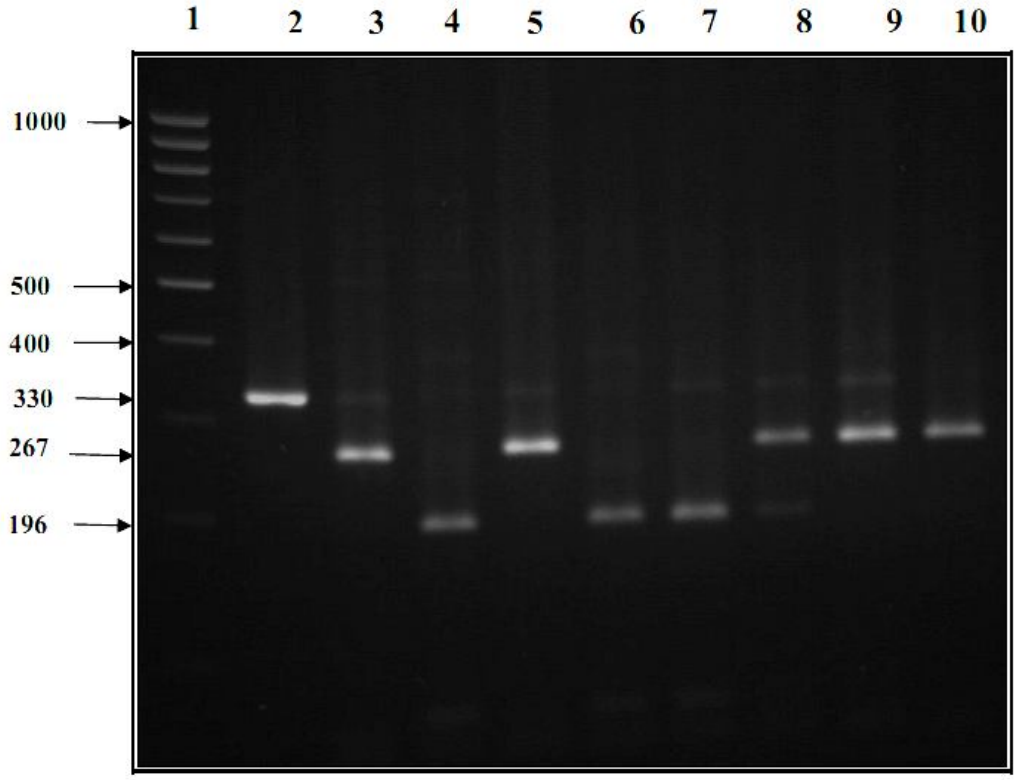

Figure 1: Representative digestion products of the 330 bp of polymerase chain reaction by MspI separated on 3% agarose gel. Lane 1 shows 100 bp DNA ladder; lanes 2 to 10 are of samples 19 to 26 respectively. Lane 2 shows amplified fragment of 330 bp. Lanes 3, 5, 9 and 10 represent digestion products of the homozygous methylenetetrahydrofolate dehydrogenase (MTHFD1) 1958AA genotypes showing only the large 267 bp fragment since the smaller fragments 71 and 55 were not clearly visible. Lanes 4, 6 and 7 show normal MTHFD1 1958GG genotype with the 196 bp fragment. Lane 8 shows heterozygous MTHFD1 1958AG genotype with the 2 large bands of 267 and 196 bp

Knowledge of the association between NTDs and various genetic markers related to development may help increase our understanding of the genetics of the pathogenesis of NTDs. Based on the links between MTHFD1 polymorphism and the folate metabolism pathway and the results of previous studies we examined the frequencies of the MTHFD1 1958G>A polymorphism in the Jordanian population and its relation to NTDs in the northerm region of the country. The results of this study showed that the distribution of all genotypes MTHFD1 1958G>A in the sample did not deviate from the Hardy–Weinberg equilibrium, which indicates that there were no genetic differences between the residents of the different major regions of Jordan in terms of MTHFD1 1958G>A genotypes.

The results of this study showed that in the northern part of Jordan there no association between MTHFD1 1958G>A polymorphism with maternal risk for having an NTD-affected infant. This is in agreement with the absence of association found between methionine synthase (MTR) 2756A>G polymorphism and NTDs in studies in the USA (Linden et al., 2007), in Ireland [8] and in the Netherlands [10]. The results of the present study are also similar to another study in the Dutch population [11], where there was no association between MTHFD1 1958G>A polymorphism and omphalocele cases (a form of NTD). These results are also similar to those of Hol et al., who reported an absence of evidence for the MTHFD1 gene in the etiology of NTDs. Contrary to our results and those discussed earlier, some other reports have demonstrated an association between NTDs and MTHFD1 1958G>A polymorphism [2, 5, 12].

One limitation of this study was that the period of study was only 1 year (as part of the requirements for a Masters of Science), so only a small sample of case mothers was found (the incidence of NTDs is 1.5 per 1000 live births in the northern part of Jordan) [5] and this may have affected the results. Testing a larger sample of mothers of NTD-affected children in the future would be essential to confirm the results.

Another explanation for the difference between these results and others may be explained by the different geographic background of the study populations that were studied previously. The MTHFD1 1958G>A SNP may only be associated with a risk of NTD in specific populations, as is the case for the methylenetetrahydrofolate reductase (MTHFR) 677C>T SNP (Botto and Yang, 2000). The MTHFD1 1958G>A SNP results in the substitution of an arginine by a glutamine residue at amino acid position 653 located within the synthetase domain of the MTHFD1 enzyme that is well conserved among species and has therefore been suggested to affect 10-formyl THF-dependent purine synthesis (3). However, glutamine and arginine are both large and hydrophilic amino acids, and therefore, substitution of arginine by glutamine might not affect MTHFD1 enzyme function or activity, which may explain our observation that the MTHFD1 1958AA genotype is not associated with a risk of spina bifida [10]. However, because of the relatively small study population, an association between the MTHFD1 1958G>A SNP and spina bifida risk cannot be ruled out completely, and more studies are warranted.

The results of this study suggest that further studies should done on larger populations to confirm the conclusion and also more studies on gene–gene interactions should be done to examine their association with NTDs.

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