

# Polymorphisms of folate related genes and male infertility

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## INTRODUCTION

Folate deficiency and related hyperhomocystinemia are considered a risk factor for various diseases including infertility. Three folate metabolism enzymes, i.e. methyltetrahydrofolate reductase (MTHFR), methionone synthase (MTR) and methionine synthase reductase (MTRR) play a critical role in DNA synthesis and methylation reactions.

## AIM OF THE STUDY

The aim of this study was to investigate the possible association of four polymorphisms in the three folate-related genes (MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G) with male infertility.

## MATERIALS AND METHODS

We have studied a total of 129 infertile males (87 Macedonians, 32 Albanians and 10 of other ethnic origin) and 90 fertile controls (63 Macedonians, 25 Albanians and 2 of other ethnic origin). The infertile group consisted of 59 patients with azoospermia, 51 with severe oligozoospermia and 19 with mild oligozoospermia.

The methodology included multiplex PCR followed by single nucleotide extension reaction. The PCR products were cleaned up with ExoSAP-IT and multiplex single base extension reactions were performed using SNaPshot multiplex kit (Applied Biosystems). To remove the unincorporated ddNTPs, the SNaPshot reaction mixtures were incubated with shrimp alkaline phosphatase.

The SNaPshot products were run on ABI 310 Genetic Analyzer (Applied Biosystems). Analysis of electrophoregrams was performed using the GeneScan 3.1 software (Applied Biosystems), and the size of the fragments were determined based on GeneScan-120 LIZ size standard (Figure 1).

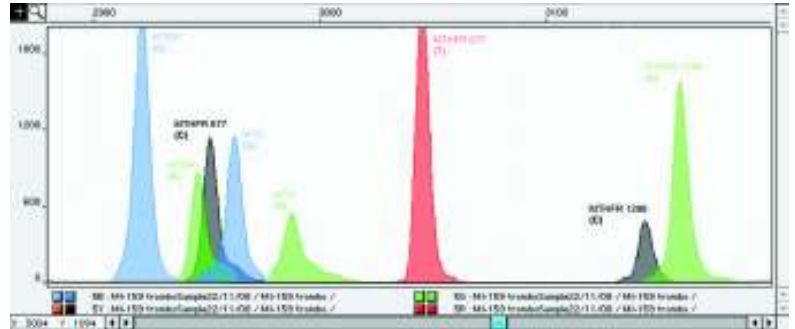


Figure 1. Electrophoregram of the multiplex SNaPshot analysis for the detection of four folate-related genes polymorphisms.

## RESULTS

Allele frequencies of four studied folate-related gene polymorphisms among Macedonians and Albanians are shown in Table 1. The MTHFR C677T and MTRR A66G polymorphisms were slightly more common among Albanians (51.8% and 58.8%, respectively) than Macedonians (40.0% and 48.3%, respectively). The allele frequencies of MTHFR A1298C and MTR A2756G were 29.3% and 22.3% in Macedonians, 21.9% and 20.2% in Albanians respectively.

The allele frequencies of the four studied folate-related gene polymorphisms among infertile men and controls are presented in Figure 2, while the genotype frequencies are shown in Table 2. There was no significant difference in the allele and genotype frequencies of the studied polymorphisms between the infertile males and fertile controls. The lower frequency of MTHFR677 TT genotype among infertile men with mild oligozoospermia, that showed a borderline significance, was due to the lower percentage of Albanians in this group (3/19 or 15.8%) in comparison to the control group (25/90 or 27.8%). No significant difference was observed also when infertile males were grouped according to their sperm parameters (Figure 3).

Table 1. Allele frequencies of four folate-related gene polymorphisms among Macedonians (Mac) and Albanians (Alb).

	MTRR A66G		MTHFR C677T	
	Mac	Alb	Mac	Alb
n				
%				
A	155	47	180	55
%	51.7	41.2	60	48.2
G	145	67	120	59
%	48.3	58.8	40	51.8
Total	300	114	300	114

	MTR A2756G		MTHFR A1298C	
	Mac	Alb	Mac	Alb
n				
%				
A	233	91	212	89
%	77.7	79.8	70.7	78.1
G	67	23	88	25
%	22.3	20.2	29.3	21.9
Total	300	114	300	114

Table 2. Genotype frequencies of four folate-related gene polymorphisms among infertile men and controls.

	Controls (n=90)	Infertile (n=129)	OR(95%CI)	P
<b>MTRR</b>				
A/A	23.3% (n=21)	24.1% (n=31)	(0.55-1.96)	0.905
A/G	52.2% (n=47)	47.2% (n=61)	(0.48-1.41)	0.472
G/G	24.4% (n=22)	28.6% (n=37)	(0.67-2.30)	0.486
A/G+G/G	76.6% (n=69)	76% (n=98)	(0.51-1.81)	0.904
<b>MTHFR677</b>				
C/C	34.4% (n=31)	36.4% (n=47)	(0.62-1.92)	0.762
C/T	40% (n=36)	48.8% (n=63)	(0.83-2.47)	0.196
T/T	25.6% (n=23)	14.7% (n=19)	(0.25-0.99)	0.045
C/T+T/T	65.5% (n=59)	63.5% (n=82)	(0.52-1.61)	0.762
<b>MTR</b>				
A/A	60% (n=54)	64.3% (n=83)	(0.69-2.09)	0.513
A/G	33.3% (n=30)	31% (n=40)	(0.50-1.60)	0.716
G/G	6.6% (n=6)	4.6% (n=6)	(0.21-2.19)	0.519
A/G+G/G	40% (n=36)	35.6% (n=46)	(0.48-1.45)	0.513
<b>MTHFR1298</b>				
A/A	52.2% (n=47)	48.1% (n=62)	(0.49-1.45)	0.544
A/C	42.2% (n=38)	44.1% (n=57)	(0.63-1.87)	0.772
C/C	5.5% (n=5)	7.7% (n=10)	(0.47-4.33)	0.526
A/C+C/C	47.7% (n=43)	51.9% (n=67)	(0.69-2.02)	0.544

## CONCLUSIONS

In conclusion, our study showed no association between the MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G polymorphisms and male infertility.

## ACKNOWLEDGMENT

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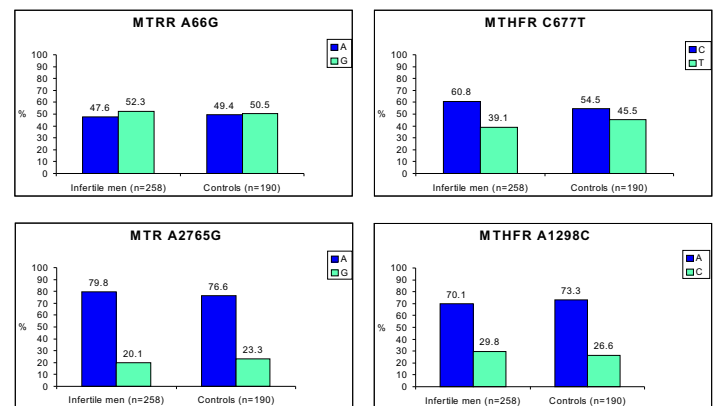


Figure 2. Allele frequencies of four folate-related gene polymorphisms among infertile men and controls.

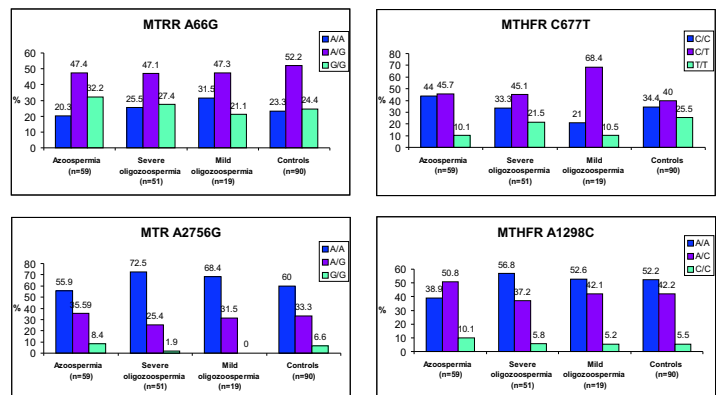


Figure 3. Genotype frequencies of four folate-related gene polymorphisms among infertile men with azoospermia, severe oligozoospermia, mild oligozoospermia and fertile controls.