

Folate/homocysteine metabolism and lung cancer risk among smokers

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Supporting information, S1 Table**S1 Table. Genotyping conditions**

Polymorphisms	Probes, primers, other reagents		Thermal profile
Taqman method			
rs1801133	T: 6FAM-ATGAAATCGACTCCCGC-MGBNFQ*	50 nM	95°C for 10 min
<i>MTHFR</i> c.665C>T	C: VIC-ATGAAATCGGCTCCCGC-MGBNFQ*	50 nM	95°C for 30 s
	F: GCAGGGAGCTTTGAGGCTGACC [1]	0.5 µM	59°C for 1 min
	R: TGGGGCAAGTGATGCCCATGT [1]	0.5 µM	repeat 50 times
rs1801131	C: 6FAM-AGACACTTGCTTCACT-MGBNFQ*	50 nM	95°C for 10 min
<i>MTHFR</i> c.1286A>C	A: VIC-CAAAGACACTTTCTTC-MGBNFQ*	50 nM	95°C for 30 s
	F: GAGGAGCTGCTG AAGATG T [1]	0.5 µM	56°C for 1 min
	R: CGAGAGGTAAAGAACGAAGA [1]	0.5 µM	repeat 50 times
rs1805087	G: 6FAM-ACAGGGCCATTATG-MGBNFQ*	50 nM	95°C-10 min
<i>MTR</i> c.2756A>G	A: VIC-ATTAGACAGGACCATTATG-MGBNFQ*	50 nM	95 °C -30 s
	F: AGTGTTCCCAGCTGTTAGATGA [1]	0.5 µM	62 °C -1 min
	R: TGTTTCTACCACTTA CCTTGAGAGACT*	0.5 µM	repeat 50 times
rs1801394	G: 6FAM-CTTGCTCACACATTT-MGBNFQ*	50 nM	95°C-10 min
<i>MTRR</i> c.66A>G	A: VIC-TGCTCACATATTTTC-MGBNFQ*	50 nM	92 °C -30 s
	F: CATGCCTTGAAGTGATGAGG*	0.5 µM	60 °C -1 min
	R: GATCTGCAGAAAATCCATGTACCA*	0.5 µM	repeat 50 times
rs1979277	C: 6FAM-CGCCTCTCTCTTC-MGBNFQ [2]	50 nM	95°C-10 min
<i>SHMT1</i> c.1420C>T	T: VIC-TCGCCTCTTTCTTC-MGBNFQ [3]	50 nM	95 °C -30 s
	F: AGGAGAGACTGGCAGGGGAT [3]	0.5 µM	60 °C -60 s
	R: CATCCATCTCTCAGGTGGGG [3]	0.5 µM	repeat 50 times
rs61510559	T: 6FAM-CGAGGTGCCGCCAG-MGBNFQ [4]	100 nM	95°C-10 min
<i>SLC19A1</i> c.80G>A	C: VIC-CACGAGGCGCCGC-MGBNFQ [4]	50 nM	95 °C -30 s
	F: GGCCTGACCCCGAGCT [4]	0.5 µM	63 °C -1 min
	R: AGCCGTAGAAGCAAAGGTAGCA [4]	0.5 µM	repeat 60 times
rs2236225	G: 6FAM-TGCAGACCGGATC-MGBNFQ [3]	50 nM	95°C-10 min
<i>MTHFD1</i> c.1958G>A	A: VIC-AGACCAGATCGCACT-MGBNFQ [3]	50 nM	95 °C -30 s
	F: GTTTGCCAACATCGCACAT [3]	0.5 µM	60 °C -1 min
	R: CATAAGTCTATTCCTGTTTGC [3]	0.5 µM	repeat 50 times

rs1801198	G: 6FAM-CCCCACGCATGGG-MGBNFQ*	50 nM	96°C 5 min
<i>TCN2</i> c.776C>G	C: VIC-CCCCAGGCATGGG-MGBNFQ*	100 nM	96°C 10 s
	F: TCACCAGTTCCTCATGACTT	0.5 µM	60°C 10 s
	R: GAGCATTCTGGAAGGCT	0.5 µM	72°C 20 s
			repeat 50 times
rs202243265	ins: 6FAM-TGGTTATGAACTTTATAGTTG-MGBNFQ [4]	50 nM	95°C-10 min
rs11280056	del: VIC-TGGTTATGAACTTTAAAGTT-MGBNFQ [4]	50 nM	92 °C -30 s
rs16430	F: AGCTGAGTAACACCATCGATCATG [4]	0.5 µM	60 °C -1 min
<i>TYMS</i> 3'UTR ins/del	R: GGACGAATGCAGAACACTTCTTTA [4]	0.5 µM	repeat 50 times
PCR method			
<i>TYMS</i> 5'UTR 2R/3R	F: GTGGCTCCTGCGTTTCCCCC [5]	0.5 µM	96°C-5 min
	R: CCGCGCCATGCCTGTGGCCGGCTCGGAGC	0.5 µM	96 °C -1 min
	dNTPs	800 µM	64 °C -1 min
	10x conc. PCR buffer (Thermo Scientific)	2 µl	72 °C -1 min
	MgCl ₂	1.5 mM	repeat 35 times
	Maxima Hot Start Taq DNA polymerase (Thermo Scientific)	0.35 U	72 °C -5 min
	DMSO	0.5 µl	
	Genomic DNA	2 µl	
	Total volume	20 µl	
<i>DHFR</i> c.86+60_78ins22	F :CCACGGTCGGGGTACCTGGG [6]	0.5 µM	94°C-4 min
	F: ACGGTCGGGGTGGCCGACTC [6]	0.5 µM	94 °C -55 s
	R: AAAAGGGGAATCCAGTCGG [6]	1 µM	65 °C - 55 s
	dNTPs	800 µM	72 °C - 55 s
	10x conc. PCR buffer (Thermo Scientific)	2.5 µl	repeat 40 times
	MgCl ₂	2.5 mM	72 °C -12 min
	Taq DNA polymerase (Thermo Scientific)	0.25 U	
	Genomic DNA	5 µl	
	Total volume	25 µl	
<i>CBS</i> c.844_845ins68	F: TATTGGCCACTCCCATAATAGA [7]	0.5 µM	94°C-5 min
	R: CGGCTCTGCGAGGATGGACCCTT [7]	0.5 µM	94 °C -1 min
	dNTPs	800 µM	59 °C -1 min
	10x conc. PCR buffer (Thermo Scientific)	2 µl	72 °C -1 min
	MgCl ₂	1.5 mM	repeat 35 times
	Taq DNA polymerase (Thermo Scientific)	0.25 U	72 °C -5 min
	Genomic DNA	2 µl	
	Total volume	20 µl	

*primers / probes sequences were obtained from SNP500 Cancer website (<http://snp500cancer.nci.nih.gov/cgfseq/pages/snp500.do>).

References

1. Summers CM, Cucchiara AJ, Nackos E, Hammons AL, Mohr E, Whitehead AS, et al. Functional polymorphisms of folate-metabolizing enzymes in relation to homocysteine concentrations in systemic lupus erythematosus. *J Rheumatol.* 2008;35(11):2179-86.
2. Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. *Proc Natl Acad Sci U S A.* 1999;96(22):12810-5.
3. Summers CM, Mitchell LE, Stanislawski-Sachadyn A, Baido SF, Blair IA, Von Feldt JM, et al. Genetic and lifestyle variables associated with homocysteine concentrations and the distribution of folate derivatives in healthy premenopausal women. *Birth Defects Res A Clin Mol Teratol.* 2010;88(8):679-88.
4. Skibola CF, Forrest MS, Coppede F, Agana L, Hubbard A, Smith MT, et al. Polymorphisms and haplotypes in folate-metabolizing genes and risk of non-Hodgkin lymphoma. *Blood.* 2004;104(7):2155-62.
5. Brown KS, Kluijtmans LA, Young IS, McNulty H, Mitchell LE, Yarnell JW, et al. The thymidylate synthase tandem repeat polymorphism is not associated with homocysteine concentrations in healthy young subjects. *Hum Genet.* 2004;114(2):182-5.
6. Johnson WG, Stenroos ES, Szychala JR, Chatkupt S, Ming SX, Buyske S. New 19 bp deletion polymorphism in intron-1 of dihydrofolate reductase (DHFR): a risk factor for spina bifida acting in mothers during pregnancy? *Am J Med Genet A.* 2004;124A(4):339-45.
7. Barbaux S, Kluijtmans LA, Whitehead AS. Accurate and rapid "multiplex heteroduplexing" method for genotyping key enzymes involved in folate/homocysteine metabolism. *Clin Chem.* 2000;46(7):907-12.