Modulation of sporadic colorectal cancer risk by polymorphisms and haplotypes of mismatch repair genes

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Introduction:

➢**The DNA mismatch repair (MMR) system** plays a key role in maintenance of genomic stability, cell cycle arrest and induction of apoptosis in response to DNA damage. Inactivation of MMR leads to microsatellite instability (MSI) [1] and is associated with hereditary and sporadic human cancers (Fig. 1).

➢**Colorectal cancer (CRC)** is one of the most common cancers, accounting annually for 1.200.000 newly diagnosed cases and over 525.000 deaths worldwide. Incidence rate of CRC in the Czech Republic is one of the highest in the world [2].

➢The GWA scans provide evidence for the role of low penetrance variants in "common disease - common variant" model of CRC predisposition on a population level [3].

➢The functional relevance of majority of single nucleotide polymorphisms (SNPs) in the MMR genes is not known. SNPs may influence biochemical interactions between components of the MMR pathway (Fig. 2) or their epigenetic regulation [4,5].

Aim of the study:

➢ **to assess the tentative risk of sporadic CRC associated with polymorphic variants and haplotypes of MMR genes *hMLH1, hMSH2, hMSH3, hMSH6,* and *hEXO1* using a hospital-based study in population from the Czech Republic.**

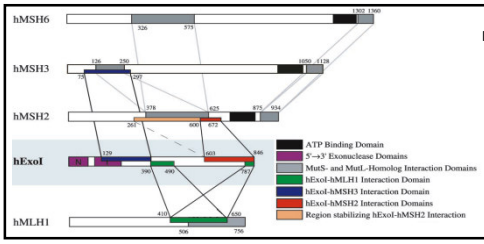
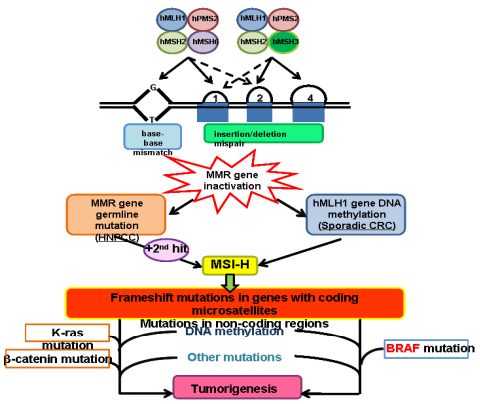


Figure 2. Interaction regions between MMR proteins (adapted from G. Villani and N. Tanguy Le Gac, 2001)

Figure 1. Role of MMR in carcinogenesis



Study population:

➢ 614 colorectal cancer cases from the Czech Republic

➢614 controls undergoing colonoscopy for various gastrointestinal complaints matched with cases by sex and age

Methods:

- ***Polymorphisms studied:*** ten SNPs located in coding and non-coding regions of genes ***hMLH1, hMSH2, hMSH3, hMSH6*** and ***hEXO1*** with a possible functional effect according to association and/or *in vitro* studies (Table 1).
- ***Genotyping:*** using Taqman assays and PCR-RFLP on DNA from peripheral blood lymphocytes of CRC patients and controls.
- ***Statistical analysis:*** multivariate logistic regressions for estimation of the association between each genotype and risk of CRC (gender and age as covariates). Separate analyses were carried out following the stratifications for smoking and for tumor localization.
- ***Haplotype analysis:*** SAS/Genetics software module.
- ***Linkage disequilibrium calculation:*** Haploview software (www.broad.mit.edu/mpg/haploview/documentation.php).

Table 1. Studied SNPs with minor allele frequencies (MAF) in different populations

•Utah residents with European ancestry
•(http:// www.hapmap.org)
**data for SNP500 CAUC1 population of individuals with self-described Caucasian heritage (http://snp500cancer.nci.nih.gov)

Gene	Position and nucleotide change	db number	Amino acid change	Taqman assay ID	MAF (HAPMAP Ceu population*)/MAF in the CZ population
hMLH1	-93G>A	rs1800734	No change	C_7535141_1_	0.200/0.232
	IVS9-1406C>T	rs4647269	-	C_29968609_10	0.475/0.456
hMSH2	IVS12-6 T>C	rs2303428		C_11804019_1	0.108/0.078
	Ex6+23 G>A	rs4987188	Gly322Asp	not available	0.025/0.018
hMSH3	Ex4-100G>A	rs1805355	Pro231Pro	C_11434406_10	0.058/0.070
	Ex23+3 G>A	rs26279	Ala1045Thr	C_800002_1_	0.217/0.287
hMSH6	-556G>T	rs3136228	-	C_28985526_10	0.405/0.362
	Ex1-145 G>A	rs1042821	Gly39Glu	not available	0.207*/0.196
	IVS4-101G>C	rs2072447	-	C_22273199_10	0.237/0.291
EXO1	Ex12+49C>T	rs4149963	Thr439Met	C_25762095_10	0.075/0.098

Results:

Figure 3. SNPs in MMR genes in CRC patients stratified for tumor location and in controls

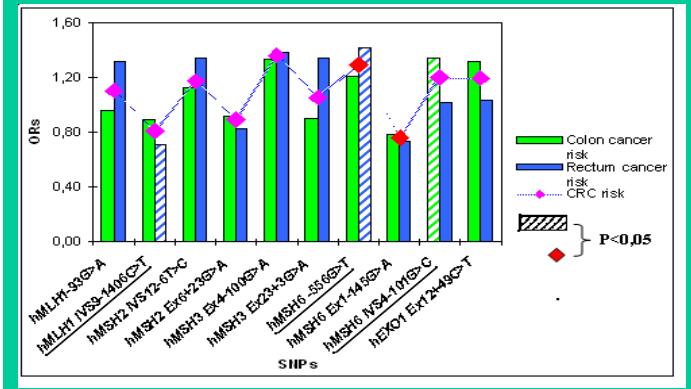


Figure 4. Linkage disequilibrium structure of the *hMSH6* gene region (www.hapmap.org)

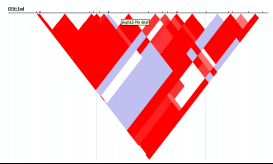


Figure 5. Linkage disequilibrium |D'| (R2) between polymorphisms in the *hMSH6* gene

-556G>T	Ex1-145G>A	IVS4-101G>C	
1	0.92 (0.11)	0.89 (0.22)	-556G>T
	1	0.65 (0.04)	Ex1-145G>A
		1	IVS4-101G>C

Table 2. Haplotypes of *hMSH6* (-556G>T - Ex1-145G>A - IVS4-101G>C) in CRC patients stratified for tumor location and in controls

Haplotypes	Controls	Colon	Univariate ORs (95% CI)	Rectum	Univariate ORs (95% CI)
TGG	218	114	1.03 (0.80-1.33)	66	1.35 (0.97-1.85)
TGC	322	217	1.17 (0.95-1.45)	132	1.06 (0.83-1.36)
TAG	217	101	**0.74 (0.51-0.96)**	64	**0.73 (0.53-1.00)**
TAC	16	13	1.36 (0.61-3.00)	4	0.65 (0.18-2.08)
GGG	413	264	1.10 (0.90-1.34)	188	**1.32 (1.05-1.65)**
GGC	13	9	1.15 (0.45-2.90)	3	0.60 (0.14-2.25)
GAG	3	4	2.23 (0.42-12.52)	4	3.50 (0.66-19.69)

➢The T-allele of the ***hMSH6* -556G>T** polymorphism was associated with ↑ risk of CRC (OR 1.29; 95% CI 1.02-1.62), confined to **rectal** cancer in particular (OR 1.42; 95% CI 1.03-1.95).

➢ The A-allele of the ***hMSH6* Ex1-145G>A** polymorphism was associated with ↓ risk of CRC (OR 0.76; 95% CI 0.60-0.98).

➢The C-allele of the ***hMSH6* IVS4-101G>C** polymorphism was associated with ↑ risk of **colon** cancer (OR 1.34; 95% CI 1.03-1.74).

➢The variant allele for the polymorphism ***hMLH1* IVS9-1406C>T** exhibited ↓ risk of **rectal** cancer (OR 0.71; 95% CI 0.51-0.98).

➢The **haplotype TAG** based on three ***hMSH6*** polymorphisms **(-556G>T - Ex1-145G>A - IVS4-101G>C)** in was associated with ↓ risk of **CRC** (OR 0.74; 95% CI 0.59-0.92; global P=0.02).

➢The most frequent **haplotype GGG** was associated with ↑ risk of **rectal** cancer (OR 1.32; 95% CI 1.05-1.65).

Conclusions:

➢After correction for **multiple hypotheses testing** our results cannot be considered as statistically significant.

➢In general, our data suggest a limited role for the investigated individual variants in MMR genes for the susceptibility to **CRC**. The **haplotypes** covering ***hMSH6*** gene may be involved in risk modulation in studied population.

➢Considering the importance of the **MMR genes** in the aetiology of CRC further studies with pooled data may determine if the common variants in the genes *per se* or in combination with other variants play any role in the disease pre-disposition.

References:

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