Available online at www.sciencedirect.com
SCIENCE DIRECT®
Cancer Letters 241 (2006) 150–157
CANCER Letters
www.elsevier.com/locate/canlet

N-acetyltransferase (NAT) 2 acetylator status and age of onset in patients with hereditary nonpolyposis colorectal cancer (HNPCC)

Steffen Pistorius [a,*], Heike Görgens [b], Stefan Krüger [b], Christoph Engel [c], Elisabeth Mangold [d], Constanze Pagenstecher [d], Elke Holinski-Feder [e], Gabriela Moeslein [f], Magnus von Knebel Doeberitz [g], Josef Rüschoff [h], Judith Karner-Hanusch [i], Hans-Detlev Saeger [a], Hans K. Schackert [a], The German HNPCC-Consortium

[a] *Department of Visceral, Thoracic and Vascular Surgery, University of Technology Dresden, Fetscherstr. 74, 01307 Dresden, Germany*
[b] *Department of Surgical Research, University of Technology Dresden, Fetscherstr. 74, 01307 Dresden, Germany*
[c] *Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Haertelstr. 16-18, 04107 Leipzig, Germany*
[d] *Institute of Human Genetics, University of Bonn, Wilhelmstr. 31, 53111 Bonn, Germany*
[e] *Department of Medical Genetics, University of Munich, Goethestr. 29, 80336 Munich, Germany*
[f] *Department of General and Visceral Surgery, Heinrich Heine University Düsseldorf, Moorenstr. 5, 40225 Düsseldorf, Germany*
[g] *Department of Molecular Pathology/Applied Tumor Biology, Institute of Pathology, University of Heidelberg, Im Neuenheimer Feld 220, 69120 Heidelberg, Germany*
[h] *Institute of Pathology, Klinikum Kassel, Mönchebergstr. 41-43, 34125 Kassel, Germany*
[i] *Department of Surgery, AKH, University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria*

Received 8 September 2005; received in revised form 11 October 2005; accepted 13 October 2005

Abstract

N-acetyltransferase (NAT) 2 is an essential polymorphic enzyme involved in the metabolism of various xenobiotics, including potential carcinogens. The individual differences in the *NAT2* metabolic capacity are caused by allelic variants of the *NAT2* gene which are determined by a pattern of single nucleotide polymorphisms (SNPs) resulting in slow (SA), intermediate (IA) or rapid acetylator (RA) phenotypes. Highly penetrant germline mutations in mismatch repair (MMR) genes are the cause of the disease in hereditary nonpolyposis colorectal cancer (HNPCC). There is no strict correlation between the type of germline mutation in MMR genes and the HNPCC phenotype, but age of tumor onset (AO) in HNPCC has been associated at least in part with different variants in apoptosis-related genes. To clarify the potential modifying role of the *NAT2* acetylator status in HNPCC, we performed a multicenter study in 226 individuals with colorectal cancer carrying exclusively pathogenic germline mutations in *MSH2* or *MLH1*. We did not observe any significant difference in the *NAT2* acetylator status frequency between HNPCC patients and 107 healthy controls ($P=0.156$), and between *MLH1* and *MSH2* mutation carriers ($P=0.198$). Multivariate Cox regression analysis revealed that male patients had a significantly increased risk to develop CRC compared to females during any interval ($P=0.043$), while the *NAT2* acetylator status ($P=0.447$) and the mutated gene (*MLH1* or *MSH2*) ($P=0.236$) were not risk factors for AO. The median AO in HNPCC patients was 39 years in patients with RA as well as with SA status ($P=0.347$). In *MLH1* mutation carriers, the median AO was 38 years in RA and 36 years in SA status patients ($P=0.901$), whereas in *MSH2* mutation carriers, the median AO was 39 years in RA and 42 years in SA status patients ($P=0.163$). Log-rank test revealed a significantly lower age of CRC onset in

* Corresponding author. Tel.: +49 351 458 6958; fax: +49 351 458 4350.
E-mail address: steffen.pistorius@uniklinikum-dresden.de (S. Pistorius).

0304-3835/$ - see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2005.10.018

male compared to female HNPCC patients ($P=0.0442$). These data do not support the hypothesis that the *NAT2* acetylatorship acts as a modifying factor on AO in HNPCC-associated CRC.
© 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: *NAT2*; Modifying genes; Colorectal cancer; HNPCC

1. Introduction

N-acetyltransferase (NAT) 2 is an essential polymorphic enzyme involved in the metabolism of various xenobiotics, including potential carcinogens like aromatic and heterocyclic amines [1]. The individual differences in the *NAT*2 metabolic capacity are caused by allelic variants of the *NAT*2 gene (chromosome 8p22), which are determined by a pattern of single nucleotide polymorphisms (SNPs). The allelic variants result in slow (SA), intermediate (IA) or rapid acetylator (RA) phenotypes. While the *NAT*2*4 allele (wild-type) encodes the RA phenotype, other alleles (*NAT*2*5–7 and rare alleles) encode the SA or IA phenotype [2]. Although a recent meta-analysis including 36 studies and more than 13,000 CRC cases had shown that the *NAT*2 RA phenotype is associated with an increased CRC risk [3], the data of numerous related studies and meta-analyses remain controversial.

Hereditary nonpolyposis colorectal cancer (HNPCC) is caused by highly penetrant germline mutations, especially in the mismatch repair (MMR) genes *MLH*1, *MSH*2, *MSH*6 or *PMS*2 [4–12]. In HNPCC, there is an increasing cumulative risk of CRC with aging, an incomplete mutation penetrance and no strict correlation between the type of the mutation in MMR genes and the phenotype. This suggests that modifying genes acting as additional risk factors are involved in the carcinogenesis and the age of tumor onset [13–18]. However, only two studies to date have investigated the role of *NAT*2 acetylatorship and its potential association with AO in HNPCC, with controversial results. To clarify the role of the *NAT*2 acetylator status in HNPCC, we performed a multi-center study in 226 individuals with CRC carrying exclusively pathogenic mutations in *MLH*1 or *MSH*2.

2. Material and methods

2.1. Patients

Peripheral blood samples of 226 HNPCC patients with CRC who carried a pathogenic germline mutation in *MLH*1 or *MSH*2, consecutively registered at the university hospitals of the German HNPCC Consortium in Bonn, Dresden, Munich-Regensburg, Heidelberg, Duesseldorf, and the Austrian HNPCC centre in Vienna, were selected for *NAT*2 genotyping. These 226 HNPCC patients originated from 204 families (mean: 1.11 members, range 1–3 members). All patients described here were carriers of germline mutations in either *MSH*2 (108 patients) or *MLH*1 (118 patients), predicted to be pathogenic due to their nature as protein truncating small insertions/deletions, large genomic rearrangements, nonsense or splice-site mutations. All patients gave informed consent for study participation. 107 anonymous healthy blood donors from the Dresden Regional Blood Center served as controls. The scientific studies within the German HNPCC consortium were approved by the local ethics committee in each of the participating centers.

2.2. NAT2 Genotyping

We have employed an assay based on the LightCycler® (Roche Molecular Systems) technique to screen for the *NAT*2 SNPs, which determine *NAT*2 allelic variants. Template DNA amplification was performed with realtime PCR, and fluorescence resonance energy transfer (FRET) technology was applied to facilitate the online melting-curve analysis of oligonucleotide probes bound to the target SNPs. After LightCycler PCR, hybridization probes in combination with the LightCycler DNA Master

Table 1
Primers used for PCR

SNP	Primers
C282T	F: 5′-GTCACACGAGGAAATCAAATGC-3′
	R: 5′-TCCTTCCCAGAAATTAATTCTAG-3′
C341T	F: 5′-GTCACACGAGGAAATCAAATGC-3′
	R: 5′-TCCTTCCCAGAAATTAATTCTAG-3′
C481T	F: 5′-TGCATTTTCTGCTTGACA-3′
	R: 5′-GTTGGGTGATACATACACAA-3′
G590A	F: 5′-TGCATTTTCTGCTTGACA-3′
	R: 5′-GTTGGGTGATACATACACAA-3′
A803G	F: 5′-TGCATTTTCTGCTTGACA-3′
	R: 5′-GTTGGGTGATACATACACAA-3′
G857A	F: 5′-TGCATTTTCTGCTTGACA-3′
	R: 5′-GTTGGGTGATACATACACAA-3′

F, forward; R, reverse.

Table 2
Hybridizyation probes used for SNP detection

SNP	Hybridization probes
282	5′-GGTATTTTTACATCCCTCCAGTTAA-X-3′ 5′-LC 640-ATACAGCACTGGCATGGTTCACCTTCTC-p-3′
341	5′-CAGGTGACCACTGACGGCAGGAATTACAT-X-3′ 5′-LC705-TCGATGCTGGGTCTGGAAGCTCCTCCC-p3′
481	5′-GCATTTTCTGCTTGACAGAAGAGAGAGGA-X-3′ 5′-LC 640-TCTGGTACCTGGACCAAATCAGGA-p-3′
590	5′-GACGTCTGCAGGTATGTATTCATAGACT-CAAAAT-X-3′ 5′-LC 705-TCAATTGTTCGAGGTTCAAGCGT-p-3′
803	5′-AAGAGGTTGAAGAATTGCTGAAAAATATATT-TAA-X-3′ 5′-LC 640-TTCCTTGGGGAGAAATCTCGTGC-p-3′
857	5′-ACCTGGTGATGAATCCCTT-X-3′ 5′-LC 705-CTATTTAGAATAAGGAACAAAA-TAAACCCTTG-p-3′

X, fluorescein labelled; LC 640, LC Red640 labeled; LC 705, LC 705 labeled.

Hybridization Probes Kit (Roche Diagnostics) were used. PCR primers and hybridization probes were synthesized by TIB Molbiol (Berlin, Germany) and Metabion (Martinsried, Germany), respectively. Primer and hybridization probe sequences are shown in Tables 1 and 2. PCR was performed in a total volume of 20 μm in glass capillaries. The reaction mixture used in each PCR consisted of 50 ng of genomic DNA, 2 μl of each primer (5 μmol/l), 2 μl of the LightCycler FastStart DNA Master Hybridization Probes Kit reagent, 1.5 μl of each hybridization probe (1.5 μmol/l), and 1.6 μl of $MgCl_2$ (25 mmol/l). After 10 min of initial denaturation at 94 °C and 2 min at 95 °C, 45 PCR cycles were performed with 5 s of denaturation at 94 °C and 20 s (for SNPs 282, 341, 481, 857)/15 s (for SNPs 590, 803) of annealing at 50 °C (for SNPs 282, 341)/52 °C(for SNPs 481, 590, 803, 857) with a 25 s (for SNPs 282, 341, 481, 857)/20 s (for SNPs 590, 803) extension at 72 °C. The PCR and the melting procedures were detected online with the LightCycler. The generated melting curves enabled a clear-cut differentiation between homozygous wild-type (w/w), homozygous variant (v/v) and heterozygous wild-type/variant (w/v) samples, as previously described (Fig. 1) [19].

Although it has been shown that the determination of the SNPs at bp 282 and 341 is sufficient for prediction of the *NAT*2 phenotype in Caucasians [20], SNPs at bp 481, 590, 803 and 857 were included in the analysis. The resulting pattern of the SNPs allowed the distinction between the *NAT*2*4 allele (confering the RA phenotype) and the groups of *NAT*2*5 (including alleles A, B, and C), *NAT*2*6 and *NAT*2*7 alleles, all of which confer the SA phenotype. Homo- or heterozygous carriers of the *NAT*2*4 allele were phenotypically classified as RA [2].

2.3. Statistical analysis

*NAT*2 acetylator frequencies were compared between patients and controls and between *MLH*1 and *MSH*2 mutation carriers using the χ^2 test. The age of onset of CRC was analysed by the Kaplan–Meier (product-limit) method. The log-rank test was applied to compare the age of onset between different groups. Multivariate Cox regression analysis was used to evaluate the independent effect of gender, the location

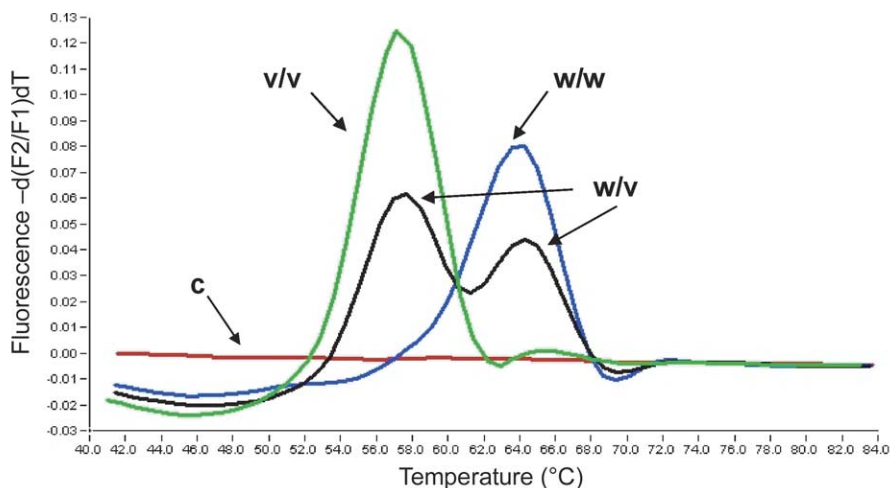

Fig. 1. Principle of genotyping of SNPs by LightCycler amplification with melting curve analysis with SNP-specific hybridization probes. w/w, homozygous wild-type; w/v, heterozygous wild-type/variant; v/v, homozygous variant; c, control.

of the MMR gene mutation (*MSH*2 or *MLH*1) and the *NAT*2 acetylator status on age of onset. *P*-values below 0.05 were considered significant. All statistical analyses were performed using SPSS 10.0.7 (SPSS Inc., Chicago, IL, USA).

3. Results

*NAT*2 allele genotype frequencies and frequencies of rapid (RA) and slow acetylators (SA) are shown in Tables 3 and 4, respectively. We did not observe any significant difference in the *NAT*2 acetylator status frequency among HNPCC patients and 107 healthy controls ($P=0.156$, Table 4), nor between MLH1 and MSH2 mutation carriers ($P=0.198$, Table 5).

Multivariate Cox regression analysis revealed that male patients had a significantly increased risk to develop CRC compared to females at any interval ($P=0.043$), while the *NAT*2 acetylator status ($P=0.447$) and germ-line mutations in *MLH*1 or *MSH*2 (P=0.236) were not risk factors. The median AO was 39 years in patients with RA (95% CI 37–41) as well as with SA status (95% CI 37–41) ($P=0.347$; Fig. 2). In *MLH*1 mutation carriers, the median AO was 38 years in RA (95% CI 36–40) and 36 years in SA status patients (95% CI 32–40; $P=0.901$), whereas in *MSH*2 mutation carriers, the median AO was 39 years in RA (95% CI 35–43) and 42 years in SA status patients (95% CI 40–44: $P=0.163$). Log-rank test revealed a significantly lower age of CRC onset in male compared to female HNPCC patients ($P=0.0442$) (Fig. 3). The median AO in male patients was 38 years (95% CI 35–41), and the median AO in female patients was 39 years (95% CI 37–41).

4. Discussion

We analyzed the role of the *NAT*2 acetylator status in HNPCC and performed a multicenter study in 226 individuals with CRC carrying exclusively pathogenic germline mutations in *MLH*1 or *MSH*2. We did not observe any significant difference in the *NAT*2 acetylator status frequency between 226 HNPCC patients and 107 healthy controls and between *MLH*1 and *MSH*2 mutation carriers. The median AO did not differ between carriers of RA (39 years) and SA (39 years). Our RA / SA frequency (36.7/63.3%) was not statistically different ($P=0.083$) from another large Caucasian population (42.6/57.4%) [21].

Published data regarding the influence of the *NAT*2 acetylatorship on the risk of CRC are controversial. Although a pooled analysis revealed an association between the risk for CRC and the RA status based on *NAT*2 phenotype, the analysis of single *NAT*2 genotype studies detected neither an association between CRC risk and presumed RA status overall, nor in subgroup analyses for ethnicitiy, gender, and tumor localisation [22]. The risk for colorectal cancer associated with RA status was increased in the majority of studies but was only statistically significant in three small studies based on phenotyping in a pooled analysis [23]. A meta-analysis published in 2002 did not support the hypothesis that *NAT*2 acetylatorship alone is an important risk factor for colon cancer [24]. In addition, two large case-control studies with more than 1500 cases with CRC each showed no significant association of *NAT*2 variants alone with colon cancer risk [25,26]. However, a recent meta-analysis published in 2005 which included 36 studies and more than 13,000 cases revealed a significantly increased risk for CRC in *NAT*2 RA phenotype [3]. Whereas only the *NAT*2 acetylatorship genotype and/or phenotype has been correlated in most studies with CRC risk, some others included an exposure assessment with mode and quantity of meat consumption, diet and smoking in the analyses [25–32]. In addition, combinations of the *NAT*2 acetylatorship with variants in other low penetrance or potentially

Table 3
Results of *NAT2* genotyping

NAT2 alleles	Patients *n* (%)	Controls *n* (%)
*4/*4	11 (4.9)	6 (5.6)
*4/*5A	0 (0)	1 (0.9)
*4/*5B	40 (17.7)	23 (21.5)
*4/*5C	1 (0.4)	0 (0)
*4/*6	24 (10.6)	17 (15.9)
*4/*7	7 (3.1)	1 (0.9)
RA	**83 (36.7)**	**48 (44.9)**
*5A/*5A	0 (0)	0 (0)
*5A/*5B	5 (2.2)	1 (0.9)
*5A/*5C	0 (0)	0 (0)
*5A/*6	3 (1.3)	0 (0)
*5A/*7	0 (0)	0 (0)
*5B/*5B	37 (16.4)	17 (15.9)
*5B/*5C	11 (4.9)	1 (0.9)
*5B/*6	57 (25.2)	29 (27.1)
*5B/*7	6 (2.7)	1 (0.9)
*5C/*5C	0 (0)	0 (0)
*5C/*6	2 (0.9)	1 (0.9)
*5C/*7	0 (0)	0 (0)
*6/*6	18 (8.0)	8 (7.5)
*6/*7	3 (1.3)	1 (0.9)
*7/*7	1 (0.4)	0 (0)
SA	**143 (63.3)**	**59 (55.1)**

RA, rapid acetylators; SA, slow acetylators.

Table 4
Data analysis and results of *NAT2* phenotyping in HNPCC patients and controls

	HNPCC patients	Healthy controls	*P*-value
All	226	107	
RA (%)	83 (36.7)	48 (44.9)	0.156
SA (%)	143 (63.3)	59 (55.1)	
Sex			
Male (%)	137 (60.6)	56 (52.3)	
Female (%)	89 (39.4)	51 (47.7)	
MMR gene mutated			
MLH1 (%)	118 (52.2)		
MSH2 (%)	108 (47.8)		
Median age (years) of CRC onset (range)	39 (13–67)	37 (19–68)[a]	

RA, rapid acetylators; SA, slow acetylators; MMR, mismatch repair; CRC, colorectal cancer.
[a] Age.

Table 5
NAT2 phenotyping in mismatch repair gene mutation carriers

	RA *n* (%)	SA *n* (%)	*P*-value
MLH1 mutation carriers	48 (40.7)	70 (59.3)	0.198
MSH2 mutation carriers	35 (32.4)	73 (67.6)	

RA, rapid acetylators; SA, slow acetylators.

modifying genes, such as *NAT*1, *GSTM*-1, *GSTT*-1, *CYP*1*A*1 have been investigated [25,26,28,29,33–36].

Concerning AO, one study showed a significant association between the *NAT*2 SA status and early AO in 275 colon cancer patients [37]. Details about the family history or the molecular cause of CRC were available solely in a few studies [33–35,38], suggesting that patients with sporadic CRC were investigated in the majority of those. Concerning familial CRC, neither age at diagnosis of colorectal adenomas nor occurrence of extra-intestinal tumors differed significantly between genotypes at the *NAT*2 and *GSTM*1 loci in 411 patients with familial adenomatous polyposis (FAP) [39], whereas another study of 151 FAP patients showed a significant association between more severe disease and the presence of *NAT*2 RA alleles [40].

The first study related to modifying genes in HNPCC was published in 1998 and analysed the role of *NAT*1, *GSTM*1 and *GSTT*1 alleles in 37 Finish HNPCC kindreds comprising 114 mutation-carriers with HNPCC-associated tumors, 90 of them presenting with CRC. The genotype frequencies were similar in mutation carriers and noncarriers. In a subset of patients, *NAT*1*10$^+$ as well as *GSTM*1 and *GSTT*1 null genotypes were associated with younger AO. The combined null genotype of *GSTM*1 and *GSTT*1 was associated with proximal colon tumors [13]. *NAT*2 acetylatorship in HNPCC was analysed in a Swiss study with 52 *MLH*1 and *MSH*2 carriers with different types of cancer including CRC and 26 unaffected mutation carriers. The SA status was found significantly more prevalent in the group of affected mutation carriers when compared to the group of unaffected mutation carriers. The mean age at diagnosis did not significantly

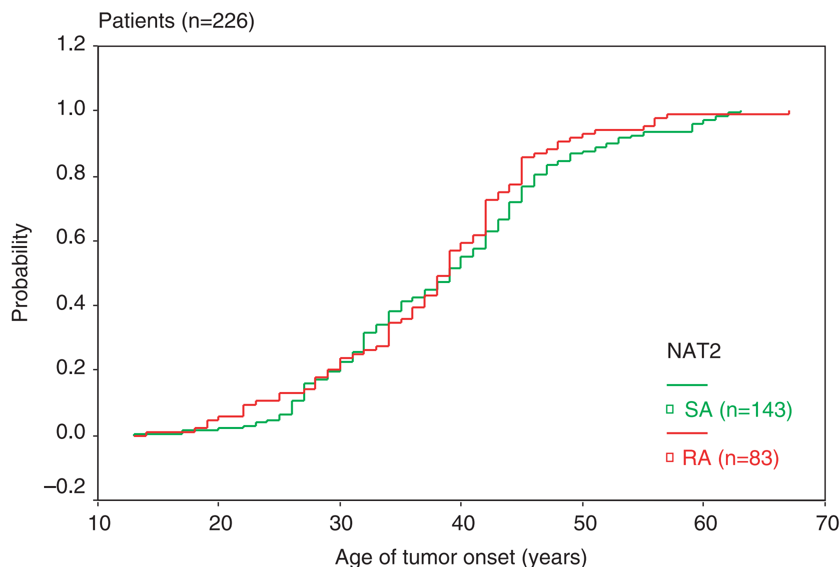

Fig. 2. Kaplan–Meier analysis: Age of CRC onset in HNPCC patients with rapid (RA) and slow acetylator (SA) status in NAT2 (P=0.347). SA, slow acetylators; RA, rapid acetylators.

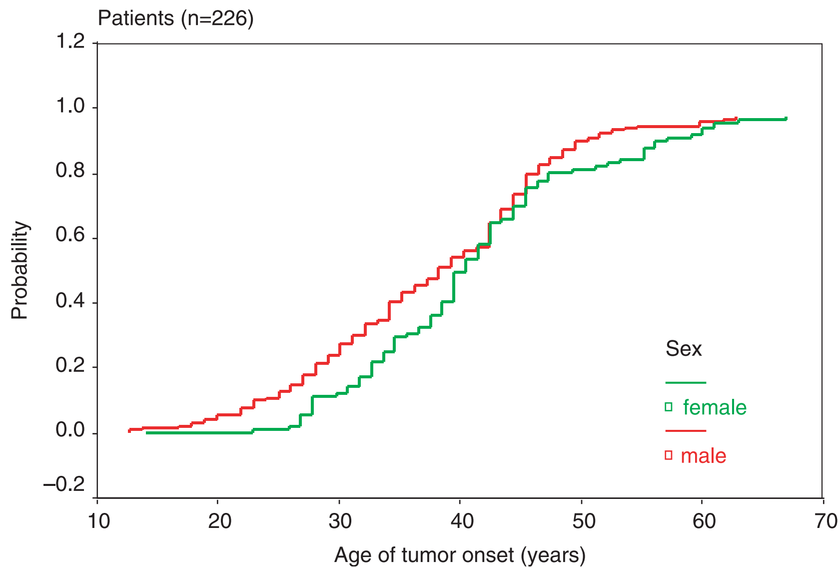

Fig. 3. Kaplan–Meier analysis: age of CRC onset in male and female HNPCC patients (P=0.0442).

differ between patients with RA (43.9 years) and SA (43.3 years) status [14]. One study published in 2001 by Frazier et al. investigated the age-associated risk of cancer among individuals with *NAT*2 mutations and germline mutations in DNA MMR genes including 86 mutation carriers in *MLH*1, *MSH*2 and *PMS*1, 48 of them with CRC [18]. A significant difference between RA and SA carriers was observed neither for CRC risk nor for the time of CRC onset. However, when individuals were stratified by single *NAT*2 polymorphisms, a significantly increased risk for CRC and all cancers was found among *NAT*2*5 heterozygotes (22 out of 48 CRC patients) and *NAT*2*7 heterozygotes (6 out of 48 CRC patients) in multivariate analysis. In our study, which comprised 226 carriers of a pathogenic *MSH*2 or *MLH*1 germline mutation and CRC, we have found neither a significant difference of RA and SA status frequencies in HNPCC patients and controls nor in *MLH*1 and *MSH*2 mutation carriers. We did find that 15 out of 226 (6.6%) CRC patients were heterozygous and one patient was homozygous mutant at the *NAT*2*7 locus compared to 3 out of 107 (2.8%) controls, albeit this difference was not statistically significant ($P=0.149$). In the study of Frazier et al., the median AO for the six *NAT*2*7 heterozygotes with CRC was decreased compared to 42 homozygous *NAT*2*7 wild-type patients. We found no significant correlation between the *NAT*2*7 genotype and AO ($P=0.453$). However, analysis of one single SNP in *NAT*2 does not allow a clear cut assignment of the *NAT*2 phenotype, hence the interpretation of these results on the functional level is difficult. Nevertheless, an acetylator-phenotype independent effect of the *NAT*2*7 variant as a risk factor for CRC and early age of tumor onset cannot be excluded.

In summary, we conclude that the *NAT*2 acetylator status is neither a risk factor nor a genetic modifier of age of CRC onset in Caucasians who carry pathogenic *MLH*1 or *MSH*2 germline mutations. We deem necessary that further genotype-phenotype correlations include numerous genetic variants as well as dietary factors in multivariate analyses in extended, molecularly and clinically well-characterised population of patients, which may lead to a more detailed risk assessment.

Acknowledgements

We thank Ms M. Krenz for excellent technical assistance and Dr Alexandre Serra for preparation of the manuscript. This work was supported by the Deutsche Krebshilfe grant ‘Familiärer Dickdarmkrebs’ (70-3032-Scha-4)

Appendix

The German HNPCC-Consortium consists of the following centers (in alphabetic order): clinical centres in Bochum (F. Brasch, J.T. Epplen, S. Hahn, E. Kunstmann, C. Pox, W. Schmiegel, K. Schulmann, J. Willert), Bonn (in addition to authors: R. Büttner, W. Friedl, A. Hirner, C. Lamberti, N. Friedrichs, P. Propping, T. Sauerbruch), Duesseldorf (in addition to author: T. O. Goecke, A. Hansmann, C. Poremba,

B. Royer-Pokora, A. Unger, T. Vogel, C. Wieland), Dresden (in addition to authors: D.E. Aust, F. Balck, G. Baretton, A. Bier, R. Höhl, F.R. Kreuz, J. Plaschke), Heidelberg (in addition to author: A. Buckowitz, J. Gebert, P. Kienle, M. Kloor, H.P. Knäbel, U. Mazitschek, M. Taraverdian), Munich-Regensburg (in addition to authors: W. Dietmaier, M. Grabowski, M. Gross, G. Keller, R. Kopp, P. Rümmele, C. Tympner, H. Vogelsang), center for reference pathology Kassel (in addition to author: T. Brodegger, A. Müller) and center for documentation and biometry in Leipzig (in addition to author: U. Enders, M. Herold, M. Loeffler).

References

[1] D.W. Hein, Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis, Mutat. Res. 506–507 (2002) 65–77.

[2] H.J. Lin, C.Y. Han, B.K. Lin, S. Hardy, Slow acetylator mutations in the human polymorphic N-acetyltransferase gene in 786 Asians, blacks, hispanics, and whites: Application to metabolic epidemiology, Am. J. Hum. Genet. 52 (1993) 827–834.

[3] K. Chen, Q.T. Jiang, H.Q. He, Relationship between metabolic enzyme polymorphism and colorectal cancer, World J. Gastroenterol. 11 (2005) 331–335.

[4] C.E. Bronner, S.M. Baker, P.T. Morrison, G. Warren, L.G. Smith, M.K. Lescoe, et al., Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer, Nature 368 (1994) 258–261.

[5] R.D. Kolodner, N.R. Hall, J. Lipford, M.F. Kane, M.R. Rao, P. Morrison, et al., Structure of the human MSH2 locus and analysis of two Muir-Torre kindreds for msh2 mutations, Genomics 24 (1994) 516–526.

[6] R. Fishel, M.K. Lescoe, M.R. Rao, N.G. Copeland, N.A. Jenkins, J. Garber, et al., The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer, Cell 75 (1993) 1027–1038.

[7] F.S. Leach, N.C. Nicolaides, N. Papadopoulos, B. Liu, J. Jen, R. Parsons, et al., Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer, Cell 75 (1993) 1215–1225.

[8] B. Liu, R. Parsons, N. Papadopoulos, N.C. Nicolaides, H.T. Lynch, P. Watson, et al., Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients, Nat. Med. 2 (1996) 169–174.

[9] M. Miyaki, M. Konishi, K. Tanaka, R. Kikuchi-Yanoshita, M. Muraoka, M. Yasuno, et al., Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer, Nat. Genet. 17 (1997) 271–272.

[10] N. Papadopoulos, N.C. Nicolaides, Y.F. Wei, S.M. Ruben, K.C. Carter, C.A. Rosen, et al., Mutation of a mutL homolog in hereditary colon cancer, Science 263 (1994) 1625–1629.

[11] R.D. Kolodner, N.R. Hall, J. Lipford, M.F. Kane, P.T. Morrison, P.J. Finan, et al., Structure of the human MLH1 locus and analysis of a large hereditary nonpolyposis colorectal carcinoma kindred for mlh1 mutations, Cancer Res. 55 (1995) 242–248.

[12] N.C. Nicolaides, N. Papadopoulos, B. Liu, Y.F. Wei, K.C. Carter, S.M. Ruben, et al., Mutations of two PMS homologues in hereditary nonpolyposis colon cancer, Nature 371 (1994) 75–80.

[13] A.L. Moisio, P. Sistonen, J.P. Mecklin, H. Jarvinen, P. Peltomaki, Genetic polymorphisms in carcinogen metabolism and their association to hereditary nonpolyposis colon cancer, Gastroenterology 115 (1998) 1387–1394.

[14] K. Heinimann, R.J. Scott, P. Chappuis, W. Weber, H. Muller, Z. Dobbie, et al., N-Acetyltransferase 2 influences cancer prevalence in hMLH1/hMSH2 mutation carriers, Cancer Res. 59 (1999) 3038–3040.

[15] S. Kong, C.I. Amos, R. Luthra, P.M. Lynch, B. Levin, M.L. Frazier, Effects of cyclin D1 polymorphism on age of onset of hereditary nonpolyposis colorectal cancer, Cancer Res. 60 (2000) 249–252.

[16] S. Bala, P. Peltomaki, CYCLIN D1 as a genetic modifier in hereditary nonpolyposis colorectal cancer, Cancer Res. 61 (2001) 6042–6045.

[17] P. Maillet, P.O. Chappuis, G. Vaudan, Z. Dobbie, H. Muller, P. Hutter, et al., A polymorphism in the ATM gene modulates the penetrance of hereditary non-polyposis colorectal cancer, Int. J. Cancer 88 (2000) 928–931.

[18] M.L. Frazier, F.T. O'Donnell, S. Kong, X. Gu, I. Campos, R. Luthra, et al., Age-associated risk of cancer among individuals with N-acetyltransferase 2 (NAT2) mutations and mutations in DNA mismatch repair genes, Cancer Res. 61 (2001) 1269–1271.

[19] H. Gorgens, P. Schwarz, J. Schulze, H.K. Schackert, LightCycler assay in the analysis of haplotypes of the type 2 diabetes susceptibility gene CAPN10, Clin. Chem. 49 (2003) 1405–1408.

[20] I. Cascorbi, I. Roots, pitfalls in N-acetyltransferase 2 genotyping, Pharmacogenetics 9 (1999) 123–127.

[21] S. Garte, L. Gaspari, A.K. Alexandrie, C. Ambrosone, H. Autrup, J.L. Autrup, et al., Metabolic gene polymorphism frequencies in control populations, Cancer Epidemiol. Biomarkers Prev. 10 (2001) 1239–1248.

[22] M.M. De Jong, I.M. Nolte, G.J. Te Meerman, W.T. Van Der Graaf, E.G. De Vries, R.H. Sijmons, et al., Low-penetrance genes and their involvement in colorectal cancer susceptibility, Cancer Epidemiol. Biomarkers Prev. 11 (2002) 1332–1352.

[23] R.S. Houlston, I.P. Tomlinson, Polymorphisms and colorectal tumor risk, Gastroenterology 121 (2001) 282–301.

[24] Z. Ye, J.M. Parry, Meta-analysis of 20 case-control studies on the N-acetyltransferase 2 acetylation status and colorectal cancer risk, Med. Sci. Monit. 8 (2002) CR558–CR565.

[25] M.L. Slattery, J.D. Potter, W. Samowitz, J. Bigler, B. Caan, M. Leppert, NAT2, GSTM-1, cigarette smoking, and risk of colon cancer, Cancer Epidemiol. Biomarkers Prev. 7 (1998) 1079–1084.

[26] E. Kampman, M.L. Slattery, J. Bigler, M. Leppert, W. Samowitz, B.J. Caan, et al., Meat consumption, genetic susceptibility, and colon cancer risk: a United States multicenter case-control study, Cancer Epidemiol. Biomarkers Prev. 8 (1999) 15–24.

[27] I.C. Roberts-Thomson, P. Ryan, K.K. Khoo, W.J. Hart, A.J. McMichael, R.N. Butler, Diet, acetylator phenotype, and risk of colorectal neoplasia, Lancet 347 (1996) 1372–1374.

[28] E.W. Tiemersma, E. Kampman, H.B. Bueno de Mesquita, A. Bunschoten, E.M. van Schothorst, F.J. Kok, et al., Meat

consumption, cigarette smoking, and genetic susceptibility in the etiology of colorectal cancer: results from a Dutch prospective study, Cancer Causes Control 13 (2002) 383–393.
[29] M.L. Slattery, S. Edwards, K. Curtin, D. Schaffer, S. Neuhausen, Associations between smoking, passive smoking, GSTM-1, NAT2, and rectal cancer, Cancer Epidemiol. Biomarkers Prev. 12 (2003) 882–889.
[30] J.H. Barrett, G. Smith, R. Waxman, N. Gooderham, T. Lightfoot, R.C. Garner, et al., Investigation of interaction between N-acetyltransferase 2 and heterocyclic amines as potential risk factors for colorectal cancer, Carcinogenesis 24 (2003) 275–282.
[31] A.T. Chan, G.J. Tranah, E.L. Giovannucci, W.C. Willett, D.J. Hunter, C.S. Fuchs, Prospective study of N-acetyltransferase-2 genotypes, meat intake, smoking and risk of colorectal cancer, Int. J. Cancer (2005).
[32] L. Tamer, B. Ercan, N.A. Ates, U. Degirmenci, A. Unlu, C. Ates, et al., N-Acetyltransferase 2 gene polymorphism in patients with colorectal carcinoma, Cell Biochem. Funct. (2004).
[33] M.L. Slattery, J.D. Potter, K.N. Ma, B.J. Caan, M. Leppert, W. Samowitz, Western diet, family history of colorectal cancer, NAT2, GSTM-1 and risk of colon cancer, Cancer Causes Control 11 (2000) 1–8.
[34] W.J. Butler, P. Ryan, I.C. Roberts-Thomson, Metabolic genotypes and risk for colorectal cancer, J. Gastroenterol. Hepatol. 16 (2001) 631–635.
[35] M.L. Slattery, K. Curtin, K. Ma, D. Schaffer, J. Potter, W. Samowitz, GSTM-1 and NAT2 and genetic alterations in colon tumors, Cancer Causes Control 13 (2002) 527–534.
[36] M.A. Murtaugh, K.N. Ma, C. Sweeney, B.J. Caan, M.L. Slattery, Meat consumption patterns and preparation, genetic variants of metabolic enzymes, and their association with rectal cancer in men and women, J. Nutr. 134 (2004) 776–784.
[37] A.L. Hubbard, D.J. Harrison, C. Moyes, A.H. Wyllie, C. Cunningham, E. Mannion, et al., N-Acetyltransferase 2 genotype in colorectal cancer and selective gene retention in cancers with chromosome 8p deletions, Gut 41 (1997) 229–234.
[38] M.L. Slattery, S.L. Edwards, W. Samowitz, J. Potter, Associations between family history of cancer and genes coding for metabolizing enzymes (United States), Cancer Causes Control 11 (2000) 799–803.
[39] C. Lamberti, M. Jungck, M. Laarmann, M. Knapp, R. Caspari, W. Friedl, et al., Arylamine N-acetyltransferase type 2 and glutathione S-transferases M1 and T1 polymorphisms in familial adenomatous polyposis, Pharmacogenetics 12 (2002) 49–54.
[40] M.D. Crabtree, C. Fletcher, M. Churchman, S.V. Hodgson, K. Neale, R.K. Phillips, et al., Analysis of candidate modifier loci for the severity of colonic familial adenomatous polyposis, with evidence for the importance of the N-acetyl transferases, Gut 53 (2004) 271–276.