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

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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

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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.

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 multicenter 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.