The additive effect of *p53* Arg72Pro and *RNASEL* Arg462Gln genotypes on age of disease onset in hereditary nonpolyposis colorectal cancer (HNPCC) patients with pathogenic germline mutations in *MSH2*/*MLH1*

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Introduction Lynch syndrome, also named hereditary nonpolyposis colorectal cancer (HNPCC), is one of the most common cancer susceptibility syndromes, with an autosomal dominant mode of inheritance. In addition to an incomplete penetrance of about 80% for colorectal cancer (CRC) and to the broad tumor spectrum, a wide variety of age of onset (AO) (ranging from 16 to 90 years) has been described, suggesting the existence of modifying genetic or environmental factors. The tumour suppressor gene *p53* plays a key role in the apoptotic pathway, and the prostate-cancer-susceptibility gene *RNASEL* is a tumour suppressor also involved in apoptosis. Recently, we could show that common functionally different variants in both genes (Arg72Pro in *p53* and Arg462Gln in *RNASEL*) are significantly associated with the AO of CRC in HNPCC patients in a dose-dependent manner. The aim of this study was to assess the combined effect of both variants in these two genes on the phenotype of HNPCC.
Material and Methods We studied 246 unrelated HNPCC patients consecutively registered at the clinical centres Bonn, Dresden, Munich-Regensburg, Heidelberg, Duesseldorf, and Bochum of the German HNPCC Consortium. All patients gave written informed consent for study participation. 245 anonymous healthy blood donors from the Dresden Regional Blood Centre served as controls. This study was approved by the respective local ethics committee in each of the participating centres. All 246 individuals studied were the nominal probands in their respective family and their first tumour manifestation was a CRC. All patients were carriers of germline mutations in either *MSH2* (138 patients) or *MLH1* (108 patients), predicted to be pathogenic due to their nature as protein truncating small insertions/deletions, large genomic rearrangements, nonsense or splice site mutations. To control for the false inclusion of non-pathogenic missense mutations in *MSH2* and *MLH1*, we completely excluded carriers of missense mutations. Genotyping of *p53* p.Arg72Pro and *RNASEL* p.Arg462Gln sequence variations was performed using Sanger sequencing or real time PCR and melting point analysis on a LightCycler™. The age of onset of the first colorectal cancer was analysed by the Kaplan-Meier (product-limit) method. The log rank test was applied to compare the AO between genotype groups. A global comparison across all three groups in each single gene (i. e. *p53* and *RNASEL*) and across all nine possible combinations of genotypes from both genes was performed. Multivariate Cox regression analysis was used to assess whether age of onset was affected by mismatch repair gene or gender, whether the data can be explained assuming a dominant or an additive mode of inheritance for each single gene (i. e. *p53* and *RNASEL*), and whether there is an interaction between genotypes in both genes.
Results We did not observe any significant difference in frequency of genotypes among patients and controls neither in the *p53* Arg72Pro variation (p=0.991, $\chi 2$ test) nor in the *RNASEL* Arg462Gln variation (p=0.912, $\chi 2$ test). No significant deviations from the Hardy-Weinberg equilibrium were noted. The distribution on the nine possible combinations between the two variants analysed in patients and controls was in accordance with those previously expected from the frequencies of the both single genotypes in patients (p=0.574, Fisher's exact test) as well as in controls (p=0.157, Fisher's exact test). No significant differences in the combinations of both genotypes among patients and controls were observed (p=0.649, Fisher's exact test).

Genotype		Controls (n = 245)	Patients (n = 246)	Median AO
p53	RNASEL	n (%)	n (%)	years (range; 95% CI)
WW [a]	WW	49 (20.0)	60 (24.4)	42 (22 – 75; 39 – 45)
WW	Wp	76 (31.0)	74 (30.1)	37 (13 – 69; 34 – 40)
WW	Pp	25 (10.2)	18 (7.3)	36 (21 – 48; 32 – 40)
Wp	WW	35 (14.3)	25 (10.2)	39 (17 – 59; 32 – 46)
Wp	Wp	35 (14.3)	41 (16.6)	35 (18 – 67; 32 – 38)
Wp	Pp	8 (3.2)	11 (4.5)	32 (20 – 49; 29 – 35)
pp	WW	3 (1.2)	5 (2.0)	32 (27 – 34; 21 – 43)
pp	Wp	11 (4.5)	8 (3.3)	33 (26 – 48; 21 – 45)
pp	Pp	3 (1.3)	4 (1.6)	30 (26 – 47; 23 – 37)

[a] W, wild-type (i. e. *p53* Arg72, *RNASEL* Arg462); p, polymorphic variant (i. e. *p53* Pro72, *RNASEL* Gln462); AO, median age of onset of colorectal cancer in patients (years); CI, confidence interval

Table 1. Distribution of the combination of *p53* Arg72Pro and *RNASEL* Arg462Gln genotypes among cases and controls and median age of onset of the patients with range and 95% confidence intervals

The difference in median AO among homozygous carriers of the wild-type in both genes (42 years) and homozygous carriers of the polymorphic variant in both genes (30 years) was twelve years. In the global comparison, the AO was significantly associated with the combination of genotypes of both genes (p=0.0174, log rank) (Table 1). However, the number of Gln alleles at codon 462 of *RNASEL* was a significant predictor of age of disease onset (hazard ratio 1.855 [95% confidence interval 1.133 – 3.038], p=0.014) as well as the number of Pro alleles at codon 72 of *p53* (hazard ratio 2.247 [95% confidence interval 1.166 – 4.328], p=0.016), but no significant deviation from additivity was noted in both genes (for *RNASEL*, hazard ratio 0.883 [95% confidence interval 0.674 – 1.157] p=0.368, and for *p53*, hazard ratio 0.911 [95% confidence interval 0.647 – 1.282], p=0.592). Analysis for a possible interaction between the two genes was not significant (hazard ratio 0.770 [95% confidence interval 0.566 – 1.047], p=0.095). Thus, the sequence variants in both genes were significantly associated with age of disease onset in a dose-dependent manner and the combined effect of both variants is purely additive.
Discussion Here we show that the combined effect of two common functionally different sequence variants (i. e. Arg72Pro in the tumour suppressor gene *p53* and Arg462Gln in the prostate cancer susceptibility gene *RNASEL*) on the age of onset of colorectal cancer in HNPCC patients who have pathogenic *MSH2* and *MLH1* germline mutations is purely additive. We had hypothesised that different apoptotic potentials of the *p53* and *RNASEL*

variants modify the disease phenotype, in which the wild-type variants (i. e. Arg72 in *p53* and Arg462 in *RNASEL*) mediates the destruction of tumours more efficiently than the polymorphic variants. Thus, more tumour-initiating events may be required in carriers of the wild-type alleles than in those who carry the polymorphic alleles. Because patients with HNPCC have a life-long high probability of developing synchronous and/or metachronous tumours, or both, more tumour-initiating events would result in a later clinical manifestation. We did not record any significant differences in genotype frequencies between patients and controls neither in the *p53* Arg72Pro variant nor in the *RNASEL* Arg462Gln variant nor in the combination of both genotypes, suggesting that both variants have no role in tumour initiation. In conclusion, *p53* Arg72Gln and *RNASEL* Arg462Gln sequence variation, in conjunction with other modifying factors and additional exogenous and genetic factors may contribute to a more detailed tumour risk assessment in MMR gene mutation carriers. The knowledge of the age of onset of disease in individual carriers of pathogenic MMR germline mutations may have an impact on preventive strategies, including the age at first surveillance, surveillance intervals, and age at preventive surgery.

Acknowledgement This work was supported by the Verbundprojekt “Familiärer Darmkrebs” of the Deutsche Krebshilfe (DKH, German Cancer Aid, 70-3032).