Arg462Gln sequence variation in the prostate-cancer-susceptibility gene *RNASEL* and age of onset of hereditary non-polyposis colorectal cancer: a case-control study

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Summary

Background ***RNASEL*** **is thought to be a susceptibility gene for hereditary prostate cancer and encodes the endoribonuclease RNase L, which has a role in apoptosis and is a candidate tumour-suppressor protein. A common sequence variation in *RNASEL*, Arg462Gln, has been associated with hereditary and sporadic prostate cancer, and the Gln variant has about three-fold reduced RNase activity in vitro. In view of the association between the age of onset of hereditary non-polyposis colorectal cancer and functionally different variants of *P53*, which play a key part in the apoptotic pathway, we aimed to assess whether the Arg462Gln variation of *RNASEL* affects the age of onset of hereditary non-polyposis colorectal cancer.**

Methods **We screened 251 patients with hereditary non-polyposis colorectal cancer who were unrelated, had pathogenic germline mutations in *MSH2* (n=141) or *MLH1* (n=110), and had colorectal carcinoma as the first tumour, for variation at codon 462 of *RNASEL* and compared them with 439 healthy controls.**

Findings **The median age of onset was 40 years (range 17–75) for patients with an Arg/Arg genotype at codon 462, 37 years (13–69) for patients with an Arg/Gln genotype, and 34 years (20–49) for those with a Gln/Gln genotype (p=0·0198). Only the *RNASEL* genotype had a significant effect on age of onset (p=0·0062) in an additive mode of inheritance. Pair-wise comparisons between genotype groups showed that the two homozygous groups (ie, Arg/Arg *vs* Gln/Gln) differed significantly in age of disease onset (mean age difference 4·8 years [SD 1·7], p=0·0044).**

Interpretation **A sequence variation in the prostate-cancer-susceptibility gene *RNASEL* has a role in a different, unassociated malignant disease. Genotypes at *RNASEL* codon 462 are associated with age of onset of hereditary non-polyposis colorectal cancer in a dose-dependent way, and might have a role in preventive strategies for this disease.**

Introduction

RNase L, encoded by *RNASEL*,[1] is an ubiquitously expressed endoribonuclease that acts in the interferon-regulated 2′–5′-linked oligoadenylates (2–5A) system, which mediates antiviral and proapoptotic activities. Through the JAK-STAT signal transduction pathway, interferon induces a family of 2′–5′-oligoadenylate synthetases (OAS), which are themselves activated by double-stranded RNA (dsRNA). OAS converts ATP to inorganic pyrophosphate (PPi) and to a series of short 2′–5′-linked oligoadenylates collectively referred to as 2–5A. The only well established function of 2–5A is the activation of RNase L, which has a binding domain and a repressor domain in the N-terminal, and a kinase-like domain and a ribonuclease domain in the C-terminal. 2–5A binds to RNase L to convert it from an inactive, monomeric state to a dimeric endoribonuclease. Activated RNase L cleaves 28S and 18S ribosomal RNA (rRNA) at unique sites that are characteristic of RNase L.[2] Several lines of evidence suggest that RNase L has a role in the induction or regulation of apoptosis, or both.[2–11] The 2–5A/RNase L pathway is implicated in the mediation of apoptosis in response to either viral infections or several other external stimuli.[2–11] Furthermore, *RNASEL* is thought to be a tumour-suppressor gene.[2,3,12,13]

Two different germline mutations in *RNASEL* (Glu265X and Met1Ile) cosegregate in two types of families with hereditary prostate cancer, suggesting that *RNASEL* is the candidate gene on the previously mapped[14] susceptibility locus for hereditary prostate cancer on chromosome 1q24–25 called *HPC1*. Functional analyses have shown that heterozygous carriers of these mutations had half the level of RNase L activity in lymphoblasts compared with homozygous wildtype (Glu265 and Met1) *RNASEL* family members, suggesting that both mutations are inactivating. The role of *RNASEL* as a tumour-suppressor gene was further substantiated through the findings of loss of the wildtype *RNASEL* allele (ie, loss of heterozygosity) in tumour tissue from carriers of the Glu265X variant[13]—a mutation that has also been associated with risk of prostate cancer in other studies.[15,16] Another inactivating germline mutation in *RNASEL* (471_474delAAAG) has been identified in a Ashkenazi sibling pair who presented with prostate cancer; one brother was

homozygous whereas the other was heterozygous for the mutation. The tumour of the brother with the heterozygous mutation, but not a sample from a benign prostate hyperplasia from the same brother, showed loss of heterozygosity of the wildtype *RNASEL* allele.[17]

In addition to inactivating mutations, several missense variations in *RNASEL* have been identified.[13,15] Of these mutations, only one—1385G→A (ie, Arg462Gln)—is associated with both hereditary and sporadic prostate cancer. In a large Finnish series,[15] homozygosity for the Gln variant was more frequent in patients with hereditary prostate cancer (15 [23%] of 66) than in controls (23 [13%] of 176, p=0·07), but was not more frequent in unselected patients with prostate cancer (24 [14%] of 167) compared with controls (p=0·73). Homozygosity for Gln was highest (six [29%] of 21) in families with four or more affected members.[15] Casey and colleagues[18] analysed 423 unselected patients with prostate cancer and 454 unaffected sibling controls from the USA, and found that the Arg462Gln variant was significantly associated with those who had prostate cancer (p=0·011; odds ratio for Arg/Gln heterozygotes 1·46 [95% CI 1·09–1·95] and for Gln/Gln homozygotes 2·12 [1·19–3·78]). The findings of Casey and colleagues[18] suggest that about 13% of prostate cancers are attributable to this missense mutation. By contrast, Wang and co-workers[19] found that the Arg/Arg variant was more frequent in 433 patients with familial prostate cancer than in 493 controls (p=0·02), and noted no significant differences between controls and 499 sporadic cases from the USA in the frequency of codon 462 variants (p=0·92). However, patients with familial prostate cancer who were homozygous for the Gln variant commonly had node-positive, advanced-stage, and high-grade disease. Therefore, the Gln variant was associated with a more aggressive disease pattern in familial prostate cancer but not in sporadic prostate cancer.[19] Although three large studies[15,18,19] found that this variation was associated with risk of sporadic or familial prostate cancer or with progressive disease, studies from Sweden[20] and Germany[21] found no association between Arg462Gln and prostate cancer. The role of the Arg462Gln sequence variation in any of the common neoplasias remains unclear.[2]

The variants at codon 462 are functionally distinct: in-vitro studies have shown that the Gln variant had a three-fold decreased enzyme activity compared with the Arg variant,[9,18] and was deficient in causing apoptosis in response to 2–5A in a mouse cell line that was homozygous negative for RNase L. The researchers[9] suggested that this variant allowed tumour cells to escape a potent apoptotic pathway. Furthermore, SIFT (sorting intolerant from tolerant aminoacid substitutions) analysis, which compares protein sequences of the same gene product from various species and assigns a probability of substitution that ranges from 0 to 1 for all possible aminoacid changes. Substitutions with a probability of less than 0·05 are regarded deleterious, whereas those of 0·05 or higher are deemed to be tolerated substitutions. The procedure is based on the assumption that important aminoacids tend to be conserved across species,[22] and predicts that Arg462Gln would be an intolerant (ie, functionally significant) aminoacid substitution.[23]

Hereditary non-polyposis colorectal cancer is one of the most common cancer-susceptibility syndromes, with an autosomal dominant mode of inheritance and incomplete penetrance. In most people, the disease is caused by germline mutations in the DNA mismatch-repair genes *MSH2*, *MLH1*, *MSH6,* and *PMS2*, and the most common mutations arise in *MSH2* and *MLH1*.[24] Mutation carriers have an increased risk of developing colorectal carcinoma and extracolonic neoplasias such as endometrial, small-bowel, ureter, renal, pelvis, stomach, ovary, and hepatobiliary cancer.[25,26] Therefore, a specific surveillance programme for the early detection of tumours is recommended for these individuals. A feature of hereditary non-polyposis colorectal cancer is the contraction and expansion of simple DNA sequence motifs—ie, microsatellite instability.[27] The revised Bethesda guidelines,[28] which include family history and number and age of onset of tumours associated with hereditary non-polyposis colorectal cancer, are recommended for the identification of patients who have tumours with high microsatellite instability.[29] In addition to an incomplete penetrance of about 80% for colorectal cancers and susceptibility to a wide range of tumours, the age of onset of disease varies widely, ranging from 16 years to 90 years.[30] Until 2004, the only genetic factors reported to be associated with the age of onset of hereditary non-polyposis colorectal cancer were the mutant status of *NAT2*, one of several isozymes of *N*-acetyltransferase,[31] and a common variant in the cyclin D1 gene,[32] although this association remains controversial.[33]

A common sequence variation in the tumour-suppressor gene *P53* (215G→C [Arg72Pro]) in its heterozygous state has been associated with the age of onset of hereditary non-polyposis colorectal cancer in 92 carriers of truncating (n=62) or missense (n=30) mutations in *MSH2* or *MLH1*, 47 of whom had colorectal cancer. Patients who were heterozygous developed colorectal cancer 13 years earlier than did those who were homozygous for the wildtype allele (p=0·04).[34] Furthermore, this polymorphic variant at codon 72 of *P53* is functionally distinct in vitro, in that the wildtype allele induces apoptosis more efficiently than does the Pro variant.[35]

We aimed to assess whether the functionally different sequence variation 1385G→A (ie, Arg462Gln) of *RNASEL*, which acts in the apoptotic pathway, affects the phenotype of patients with hereditary non-polyposis colorectal cancer.

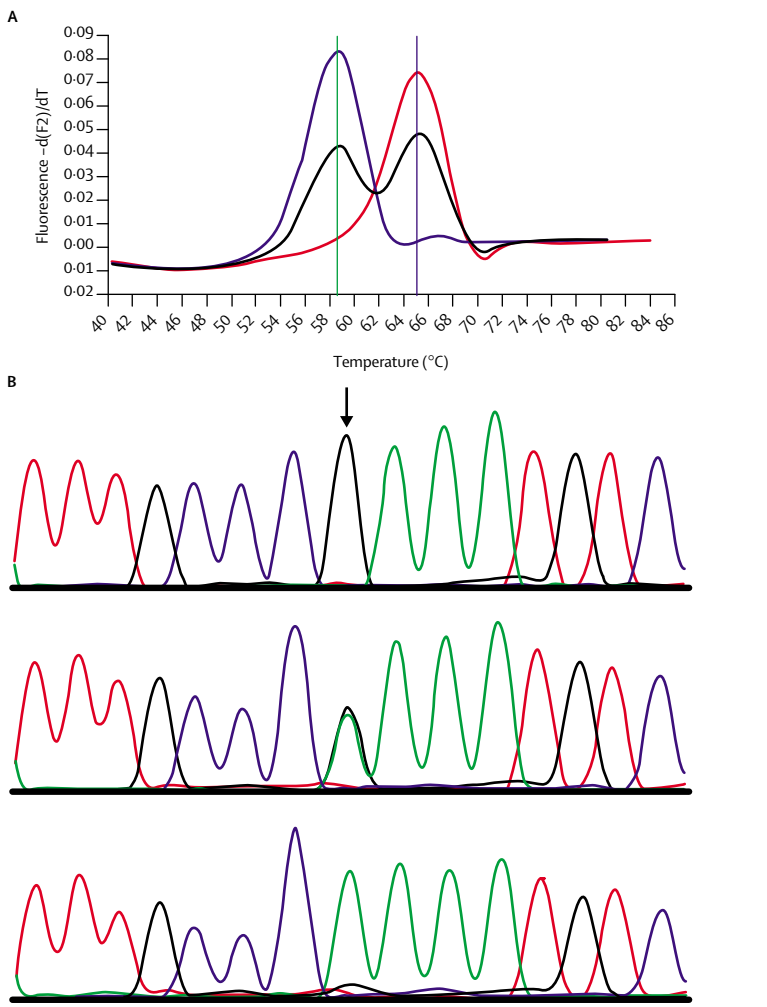


Figure 1: **Genotyping of *RNASEL* Arg462Gln sequence variation**
(A) Derivative melting curve plots –dF/dT *vs* temperature. Red=GG. Black=GA. Blue=AA. (B) Confirmation of sequencing results by further analysis of 74 samples chosen at random. Green=A. Blue=C. Black=G. Red=T. Arrow shows position of nucleotide 1385 of *RNASEL*: GG (top), GA (middle), and AA (lower). Vertical bars are specific melting temperature for alleles.

Methods

Patients

We investigated 251 patients with hereditary non-polyposis colorectal cancer who were unrelated; were *MLH1* or *MSH2* mutation carriers; and were consecutively registered at the clinical centres in Bonn, Dresden, Munich-Regensburg, Heidelberg, Düsseldorf, and Bochum of the German HNPCC (Hereditary Non-Polyposis Colorectal Cancer) Consortium. Every centre served its local region and extended surroundings. To exclude additional unknown exogenous and genetic factors that might modify penetrance, we investigated only patients who had developed colorectal cancer as the first presented tumour. Almost all (about 99%) patients were white, and the ethnic origin of patients did not differ between centres. Bethesda criteria[28] were applied as inclusion criteria. In addition, six patients with young age of onset (median 48 years [range 45–62]) of colorectal cancer or with a family history suspicious of hereditary non-polyposis colorectal cancer who did not meet any of the Bethesda criteria were included; five met the revised Bethesda criteria,[29] and one had colon cancer aged 62 years and a brother with colon cancer at age 53 years and had a germline mutation in *MSH2*. To avoid familial or genetic factors inducing a correlation in family members (besides the *RNASEL* genotype), we included only one member per family. All 251 individuals assessed were the nominal probands in their respective family.

Microsatellite analyses and immunohistochemical analyses of the expression of mismatch-repair proteins MSH2 and MLH1 were done as the screening method if tumour material was available. For analysis of microsatellite instability, at least five markers according to the reference panel of the international guidelines for assessment of microsatellite instability in colorectal cancer were applied.[36] High microsatellite instability was classified as presence of instability in at least 30% of markers. Aberrant findings such as high microsatellite instability or lost or reduced expression of at least one mismatch-repair protein on immunohistochemical analysis led to mutation screening of *MSH2* and *MLH1*. In patients who fulfilled Amsterdam I/II criteria[37] but who did not have tumour material available, mutation screening in *MSH2* and *MLH1* was done without findings from microsatellite or immunohistochemical analyses. Inclusion criteria, clinical and molecular data for our registry, microsatellite analysis, immunohistochemical analysis, and mutation screening are described in detail elsewhere.[38–40] 141 patients had a germline mutation in *MSH2* and 110 patients in *MLH1*, all of which were predicted to be pathogenic because of their nature as protein-truncating small insertions or deletions, large genomic rearrangements, or non-sense or splice-site mutations. To control for the false inclusion of non-pathogenic missense mutations in *MSH2* and *MLH1*, we excluded carriers of missense mutations.

Diagnosis of colorectal cancer as first tumour manifestation in all 251 patients was confirmed by

***Panel*: Primers and probes for PCR amplification**

PCR amplification

Forward
5′-TGTGTGTGTCACCCTC-3′

Reverse
5′-TGGGGACTCACCTATTAAG-3′

Anchor probe
5′-GGACAAGTGTAGTTCTTGAACAGCCTTAAAT X-3′

Sensor probe
5′-LCRed 640-AGATGACAGGACATTTCGGGC P-3′

medical history and histopathological examination. Age of onset of disease was defined as the time of histological tumour diagnosis. All patients gave written informed consent for study participation. 439 anonymous healthy blood donors from the Dresden Regional Blood Centre, Germany, served as controls. The study was approved by the local ethics committee of every participating centre.

Genotyping of Arg462Gln sequence variation

Genotyping of the *RNASEL* codon 462 of all 692 samples was done on genomic DNA isolated from peripheral-blood leucocytes by use of a LightCycler™ (Roche Diagnostics, Mannheim, Germany) as described elsewhere.[41] The panel shows the primers for PCR amplification, and the sequences of the anchor probe (labelled with fluorescein) and sensor probe (labelled with LC-Red 640). Primers, anchor probe, and sensor probe were from Metabion, Planegg-Martinsried, Germany. For PCR the LightCycler-FastStart DNA Master Hybridization Probes Kit (Roche Diagnostics) was used. After initial denaturation at 95°C for 10 min, amplification was done with 32 cycles of denaturation (94°C for 5 s), annealing (58°C for 20 s), and extension (72°C for 15 s). After amplification, a final melting curve was recorded. First, the probe was cooled to 40°C at 20°C/s and then slowly heated (0·2°C/s) to 90°C (figure 1A). In two samples we did not generate a PCR product because of poor DNA quality. 74 of the 690 genotyped probes were chosen randomly for confirmation of LightCycler results with direct Sanger sequencing on an ABI 3730 DNA Analyser (Applied Biosystems, Foster City, CA, USA). PCR was done with the same primers (panel). Sequencing was done with the reverse primer (panel) by use of the ABI PRISM® BigDye® Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase v3.0 and v3.1 (Applied Biosystems). After initial denaturation at 95°C for 1 min, amplification was done with 30 cycles of denaturation (96°C for 10 s), annealing (52°C for 5 s), and extension (60°C for 3 min). Sequences were analysed by use of the ABI PRISM® AB DNA Sequencing Analysis Software version 5.1.1 (Applied Biosystems, figure 1B).

Statistical analysis

The age of onset of the first colorectal cancer was analysed by use of the Kaplan-Meier (product-limit) method. The log-rank test was used to compare age of onset between genotype groups. First, we did an overall comparison of all three genotype groups (ie, Arg/Arg, Arg/Gln, and Gln/Gln). Second, pair-wise posthoc comparisons between genotype groups were done only after the overall test showed a significant difference. The χ^2 test was used to assess the homogeneity in the distribution of genotype frequency in the three groups. Genotype frequencies in all groups were cross-checked with the Hardy-Weinberg expectations, and by use of the χ^2 test.

	Patients (n=251*)	Controls (n=439)
Sex		
Male	155 (62%)	190 (43%)
Female	96 (38%)	249 (57%)
Mutated mismatch-repair gene		
MSH2	141 (56%)	..
MLH1	110 (44%)	..
Age (years)		
Median (range)	37 (13–75)†	38 (18–70)
Criterion		
Amsterdam I/II‡	142 (57%)	..
Bethesda 2	6 (2%)	..
Bethesda 2 and 4	11 (4%)	..
Bethesda 2–4	37 (15%)	..
Bethesda 2, 3, and 7	1 (<1%)	..
Bethesda 2–4, and 7	2 (<1%)	..
Bethesda 2, 4, and 7	3 (1%)	..
Bethesda 3	1 (<1%)	..
Bethesda 3 and 4	18 (7%)	..
Bethesda 3, 4, and 7	2 (<1%)	..
Bethesda 4	22 (9%)	..
None	6 (2%)§	..

*Excludes two patients for whom a PCR product could not be generated because of poor DNA quality. †Age at diagnosis of colorectal cancer. ‡Criterion 1 of Bethesda criteria. §Five carriers met revised Bethesda criteria.

Table 1: **Characteristics of patients and controls**

Multivariate Cox regression analysis was used to assess whether age of onset was affected by sex or mismatch-repair gene, and whether the data can be explained assuming a dominant or an additive mode of inheritance. The hazard function in this model was defined as: $h(t)=h_0(t)\exp(\beta_1 A+\beta_2 D+\beta_3 S+\beta_4 G)$, on which A is the number of Gln alleles (ie, 0 for Gln/Gln, 1 for Arg/Gln, and 2 for Gln/Gln) and is a measure of the additivity of alleles; D is the dominance (ie, 1 for Arg/Gln and 0 for Arg/Arg and Gln/Gln) and is a

	Immunohistochemistry		Mutation (number of carriers)	
	MLH1	MSH2	*MLH1*	*MSH2*
MSI-H				
69	Negative	Positive	69	0
1	Negative	Negative	0	1
1	Negative	NA	1	0
96	Positive	Negative	0	96
14	Positive	Positive	10	4
3	Positive	NA	0	3
1	NA	Negative	0	1
1	NA	Positive	1	0
25	NA	NA	11	14
MSS				
1	Positive	Positive	1	0
1	NA	NA	1	0
NA				
4	Negative	Positive	4	0
3	Positive	Negative	0	3
31	NA	NA	12	19

MSI-H=high microsatellite instability. MSS=microsatellite stable. NA=not available because tumour material unavailable (90%) or investigations without analysable results (10%).

Table 2: **Microsatellite analyses of patients in relation to mutated mismatch-repair gene**

	Patients (n=251)	Controls (n=439)
Arg/Arg	91 (36%)	163 (37%)
Arg/Gln	126 (50%)	212 (48%)
Gln/Gln	34 (14%)	64 (15%)

Table 3: **Distribution of *RNASEL* Arg462Gln sequence variation in patients and controls**

measure of departure from additivity; S is an indicator variable for sex (ie, 1 for men and 2 for women); G is an indicator variable for the location of the defect in the mismatch-repair gene (ie, 1 for *MLH1* and 2 for *MSH2*). p values of less than 0·05 were regarded as significant. We used SPSS version 10.0.7 for all statistical analyses.

Role of the funding source

The funding source had no role in study design; in the collection, analysis, and interpretation of data; or in the writing of the report. The corresponding author had full access to all data and had final responsibility to submit the paper for publication.

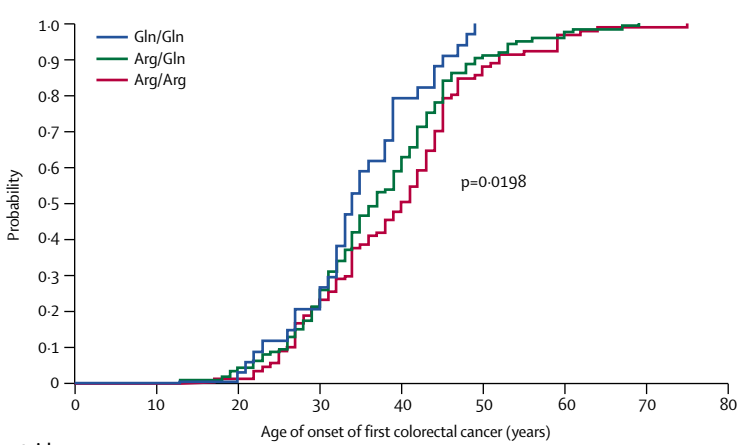
Results

254 (37%) people were homozygous Arg/Arg, 338 (49%) were heterozygous Arg/Gln, and 98 (14%) were homozygous Gln/Gln. Table 1 shows the characteristics of patients and controls. 211 patients had high microsatellite instability in tumour samples, two had microsatellite stability, and in 38 tumour material was unavailable or could not be assessed. Immunohistochemical analysis showed loss of MLH1 protein expression alone in the tumour cells of 74 patients, loss of MSH2 expression alone in 100, and loss of both proteins in one. In tumours of 19 patients, no loss of expression of mismatch-repair proteins could be detected, and in 57 patients tumour material was unavailable or could not be assessed (table 2).

Table 3 shows the distribution of *RNASEL* Arg462Gln genotypes in patients and controls. Genotype frequency did not differ between patients and controls (p=0·8729), and were consistent with those previously reported in white people.[15,18–21] No significant deviations from the Hardy-Weinberg equilibrium were noted.

The median age of disease onset was 40 years (range 17–75) for patients with Arg/Arg genotype, 37 years (13–69) for patients with Arg/Gln genotype, and 34 years (20–49) for those with Gln/Gln genotype. Thus, the median age of disease onset in patients who were homozygous for the polymorphic variant (ie, Gln/Gln) was 6 years earlier, and in those heterozygous (ie, Arg/Gln) was 3 years earlier, than in patients who were homozygous for the wildtype allele (ie, Arg/Arg; log-rank for overall comparison p=0·0198; figure 2). Pairwise comparisons between genotype groups showed that the two homozygous groups (ie, Arg/Arg *vs* Gln/Gln) differed significantly in age of disease onset (mean age difference 4·8 years [SD 1·7], p=0·0044). Mean age of disease onset in Arg/Gln heterozygotes did not differ significantly from that for Arg/Arg homozygotes (2·1 years [1·4], p=0·1548) and for Gln/Gln homozygotes (2·7 years [1·6], p=0·0663).

Regression analysis showed that sex and location of the defect in the mismatch-repair gene were not significant predictors of age of disease onset (β3 result for sex –0·102 [95% CI –0·361 to 0·158], p=0·4421; β4 for gene defect –0·024 [–0·278 to 0·230], p=0·8536). However, the number of Gln alleles was a significant predictor of age at disease onset (β1 result 0·282 [0·080 to 0·484], p=0·0062), although no significant deviation from additivity was noted (β2 result –0·080 [–0·344 to 0·185], p=0·5559). Thus, carriers of only one Gln allele were more likely to have colorectal cancer during any time than were homozygous carriers of the wildtype allele (hazard ratio 1·326 [1·083–1·623]), as were carriers of two Gln alleles (1·759 [1·174–2·636]). Findings from linear regression analysis were consistent with those from Cox regression analysis—ie, only *RNASEL* genotype had a significant effect on age of disease onset (p=0·0094) in an additive mode of inheritance.



Numbers at risk

Arg/Arg	91	91	89	72	48	13	3	1	0
Arg/Gln	126	126	122	98	52	12	5	0	0
Gln/Gln	34	34	34	27	7	0	0	0	0

Figure 2: **Age of onset of first colorectal cancer in patients**

Discussion

We have shown a significant, dose-dependent association between sequence variation in Arg462Gln of the prostate-cancer-susceptibility gene *RNASEL* and age of onset of colorectal cancers in patients with hereditary non-polyposis colorectal cancer who have pathogenic *MSH2* and *MLH1* germline mutations. Our finding is of clinical importance not only because of the age of onset of this disease, but also because young age of onset is associated with poor 5-year survival in patients with highly unstable colorectal cancer.[42]

Because *RNASEL* is thought to be a tumour-suppressor gene,[2,3,12,13] researchers have postulated that it will be

associated with malignant diseases other than prostate cancer.[2] The only study[43] to investigate *RNASEL* in a different tumour type did not show any association between Arg462Gln sequence variations and occurrence of breast cancer in a large series of patients and controls. Notably, RNase L levels are increased in colorectal tumours, which seems to be an early event in colorectal tumorigenesis. Thus, control of RNA turnover might be an important step in tumour progression.[44] Furthermore, in the human colon-carcinoma cell line HT-29 the cytocidal effect of treatment with interferon gamma and tumour necrosis factor alfa was related to the impaired processing of rRNA, increased 2–5A synthetase, and possibly 2–5A-dependent activation of RNase L.[45]

We did not record significant differences in genotype frequencies between patients and controls, suggesting that the *RNASEL* Arg462Gln sequence variation has no role in tumour initiation. However, we noted a linear trend in the age of onset across genotype groups in patients. We therefore postulate that different apoptotic potentials of the *RNASEL* Arg462Gln sequence variations modify the disease phenotype—ie, wildtype Arg mediates tumour destruction more effectively than does the polymorphic variant Gln, which has reduced or undetectable apoptotic activity in response to 2–5A activation.[9] Thus, more tumour-initiating events might be needed in carriers of the wildtype allele than in those who carry the Gln allele. Because patients with hereditary non-polyposis colorectal cancer have a life-long high probability of developing synchronous or metachronous tumours, or both,[26] more tumour-initiating events would result in a later clinical manifestation. The role of RNase L in colorectal cancer is still unknown,[44] and its other possible mechanisms should be investigated (eg, whether RNase L acts on the immune system to contribute to tumour rejection).[2] Indeed, delayed rejection of skin allografts in mice homozygous deficient for RNase L suggests a role for RNase L in cellular immunity.[46]

The effect of *RNASEL* Arg462Gln sequence variation on the age of onset in hereditary non-polyposis colorectal cancer should be robust in populations with different allele frequencies. Therefore, the age of onset of this disease should correlate with the allele frequency of *RNASEL* Arg462Gln sequence variation in the general population. Accordingly, in Japan, where the *RNASEL* codon 462 wildtype allele is more frequent in the general population (80%)[47] than in white people (about 60%), the mean age of onset of hereditary non-polyposis colorectal cancer is about 4 years higher (41·9 years)[48] than in our series (37·7 years).

In conclusion, we report an association between a sequence variation in the prostate-cancer-susceptibility gene *RNASEL* and a different, unrelated type of malignant disease—ie, hereditary non-polyposis colorectal cancer. *RNASEL* Arg462Gln sequence variation, combined with other modifying factors and additional exogenous and genetic factors, might contribute to a more detailed tumour risk assessment in carriers of mutations in mismatch-repair genes: knowledge of the age of onset of disease in carriers of pathogenic germline mutations in mismatch-repair genes might affect preventive strategies, including age at first surveillance, surveillance intervals, and age at preventive surgery.

Contributors

S Krüger had the original idea and, together with H K Schackert, did the study; analysed and interpreted data; did sequence analyses of mismatch-repair genes, microsatellite analyses, and immunohistochemical analyses of patients from Dresden; and wrote the first draft of the paper. S A Silber generated and analysed genotype data and reviewed the manuscript. C Engel did all biostatistical data analyses, was responsible for central documentation and quality management of clinical data of the German HNPCC consortium, and reviewed the paper; H Görgens supervised generation and analysis of genotype data and reviewed the paper. E Mangold, C Pagenstecher, S Stemmler, and W Friedl contributed patient data and samples, did sequence analyses of mismatch-repair genes, and reviewed the paper. E Holinski-Feder, M von Knebel Doeberitz, G Moeslein, and W Dietmaier contributed patient data and samples; did immunohistochemical, microsatellite, and sequence analyses of mismatch-repair genes; and reviewed the paper. J Rüschoff provided comprehensive data on tumour pathology and reviewed the paper.

Conflict of interest

We declare no conflicts of interest.

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