

NAT2 Polymorphisms and Sporadic Colorectal Cancer Survival

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Abstract

Background and Aim: *NAT2* gene polymorphisms can influence colorectal cancer (CRC) risk. We aimed to determine the extent to which *NAT2* gene polymorphisms influence the survival of patients with sporadic colorectal cancer. **Methods:** Seventy patients with sporadic colorectal cancer that underwent surgery at the 3rd Surgical Department of Cluj-Napoca between October 2003–May 2005 were randomly selected. Correlations between *NAT2**5C(T341C), *NAT2**5A(C481T), *NAT2**6B(G590A), *NAT2**7B(G857A) polymorphisms and survival of patients with different Dukes-MAC stages of CRC were analyzed. We compared patients with a slow acetylator genotype with those having an intermediate or rapid acetylator genotype. **Results:** The slow acetylator *341CC* genotype is a negative prognostic factor, 20% vs. 30.8%, as compared to rapid acetylator *341TT/TC* genotypes ($p=0.02$) in the patients diagnosed with stage C CRC. For the same stage patients, the slow acetylator *481CC* was a positive prognostic factor, 33% vs. 25% ($p=0.03$). The slow acetylator *590AA* was a negative prognostic factor for the survival of patients with stages B and C, 0% vs. 31% ($p=0.02$). The slow acetylator *857AA* genotype was a negative prognostic factor for the patients in stage B, survival rate 0.69% vs. 50%, and positive for patients with stage C, survival rate 50% vs. 21% ($p=0.0101$). The rapid acetylator *341TT/TC* represented a good prognostic factor, while the slow *341CC* a negative one for stage D patients ($p=0.04$, survival of 18.9%) HR=0.30 with 95%, CI[0.025-0.9810]. **Conclusion:** The *NAT2* gene may be considered as a prognostic factor for the survival of patients with CRC.

Key words

NAT2 – acetylator – Dukes-MAC stages – sporadic colorectal cancer – survival.

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Introduction

Colorectal carcinogenesis has a genetic basis, referring to certain genes and their products which, in conjunction with environmental factors, suffer multiple mutations, eventually leading to the appearance of the cancerous cell. The majority of environmental factors involved in the pathogenesis of colorectal cancer (CRC) - high lipid intake, red fried meat, alcohol [1], smoking[2] - require activation before interacting with the DNA and altering it [3].

The *NAT2* gene codes N-acetyltransferase 2 and is located on the short branch of chromosome 8, site 22-8p22 [4, 5], together with the *NAT-1* gene that codes N-acetyltransferase 1 (with mainly extrahepatic location) and the *NATP* pseudogene.

N-acetyltransferase 2 is an isoenzyme of N-acetyltransferase, most frequently present in the liver and the intestinal mucosa [6, 7]. This enzyme catalyzes N-acetylation and O-acetylation of certain pro-carcinogens and heterocyclic amines. N-acetylation takes place in the liver and results in N-hydroxi-metabolites which are excreted into the gastrointestinal lumen and are O-acetylated at the level of the colorectal mucosa, forming covalent compounds that will bind to the DNA chain [8] and will induce gene alterations and then formation of cancerous cells. The substrate for the N-acetyltransferase 2 activity, involved in colorectal carcinogenesis, is represented mainly by heterocyclic amines and polycyclic aromatic hydrocarbon rings found in cooked or smoked meat [9, 10] and cigarette smoke [2, 6, 11, 12].

N-acetyltransferase 2 polymorphisms were discovered in 1954 by observation of the peripheral neuritis in patients with TB treated with isoniazide [13]. Subsequent studies have made it possible to define three population types: rapid, intermediate and slow acetylators [14], representing the acetylator phenotype. This variability was subsequently attributed to *NAT2* polymorphisms [15, 16], constituting the acetylator genotype.

Of the total of 26 *NAT2* varieties identified until now, only 7 mutations in the coding exon of the gene influence the acetylator type. The (allele) variety *NAT2**4 does not present any of these mutations and is considered a wild variety of the gene, being most frequent in ethnic groups, including

Caucasians and Africans [5, 17]. *NAT2*4* is expressed by the rapid acetylator type [18, 19]. The presence of a single mutant allele determines the intermediate acetylator phenotype. The presence of mutations, in homozygotic form, determines the appearance of the slow acetylator phenotype [20-22], *NAT2*5* - *NAT2*18* varieties.

Numerous studies have shown an increase of CRC risk in individuals with *NAT2* rapid acetylator type. These individuals would have an increased risk because of a more rapid activation of procarcinogens [6]. Experimental studies have demonstrated that the rapid acetylator homozygotes exposed to heterocyclic amines in roasted meat have an increased risk of CRC [23]. Similarly, slow acetylators would have a lower risk of CRC [24, 25], especially patients with non-polyposic hereditary cancer [26, 27]. It is considered that due to the mutation of genes repairing replication defects, these patients present a greater susceptibility to by-products resulting from the action of mutant *NAT2*, as compared to the population without MMR mutations [26].

Starting from the theoretical premise that individuals with rapid acetylator phenotype are exposed to higher amounts of carcinogens resulting from the action of N-acetyltransferase 2 on environmental procarcinogens, that they are subjected to a higher risk of CRC, and that there is a documented correlation between the *NAT2* mutations – *NAT2*5C* (*T341C*), *NAT2*5A* (*C481T*), *NAT2*6B* (*G590A*), *NAT2*7B* (*G857A*) – and the risk of CRC, we studied the influence of these mutations on postoperative survival rates of CRC patients.

Material and methods

Patient selection

We studied a homogeneous group of Caucasian ethnics from the region of Transylvania, characteristically consuming a diet rich in roast red meat providing additional heterocyclic amines. Exposure to other environmental factors (smoking, alcohol) was homogeneous.

We studied 70 patients with sporadic CRC who were randomly selected and underwent surgery at the 3rd Surgical Clinic of Cluj-Napoca between October 2003 – May 2005. The selection criteria were the histological confirmation of CRC and the operation performed within the above-mentioned clinic. Exclusion criteria were the history of familial adenomatous polyposis, hereditary non-polyposic cancer, or inflammatory bowel disease. The data were collected prospectively and retrospectively by specially trained residents, who obtained the informed consent of the patients at the time of harvesting samples for the biological tests. Subsequent data such as histopathology results, staging, postoperative evolution, genetic typing etc. were filled in as they became available. All the procedures regarding patient selection and data completion were supervised by the Ethical Commission of the “Iuliu Hatieganu” University of Cluj Napoca. The study concluded on March 15, 2008, when survival of all the patients was assessed. We have confirmed deaths through the National Deaths Registry, and through telephone calls with the families. All the deaths were caused

by the CRC, as detailed in the death certificates emitted either by the General Practitioners or the hospital where the patients were admitted into at the moment of death.

Method

Colonoscopy was performed at the Department of Digestive Endoscopy, and the histopathological tests were performed in the Department of Pathology of the “Prof. Dr. Octavian Fodor” Emergency Hospital of Cluj-Napoca. The identification of *NAT2* polymorphisms ((*NAT2*5C* - *T341C*, *NAT2*5A* - *C481T*, *NAT2*6B* - *G590A*, *NAT2*7B* - *G857A*) was performed by the biology laboratory of the University College London and the Department of Biochemistry of the “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca.

Assessment of *NAT2* gene mutations. Peripheral blood was obtained from all the patients in vacuum tubes with EDTA, stored at -20° Celsius. DNA extraction was performed using Lahiri’s method [28] on peripheral blood leukocytes.

Identification of the *T341C* (*NAT2*5C*) mutation was based on the PCR enhanced 361pb fragment of *NAT2* and used the following primers: sense primer with sequence 5’-TGGTGTCTCCAGGTCAATCA-3’ and anti-sense primer with the sequence 5’-GGTGTCTCTCTTTGGCAGG-3’. An ABI377 sequencer was used for the DNA fragment sequencing.

Identification of the *C481T* (*NAT2*5A*) mutation by sequencing was based on the same protocol, but the primers sequence was different. The sense primer had the sequence 5’-AAGGATCAGCCTCAGGTGCCTT-3’, while the anti-sense primer had 5’-CTGCTCTCTCCTGATTTGGTCC-3’.

Identification of the *G590A* (*NAT2*6B*) mutation involved the same steps. The primers used in order to amplify the fragment of interest had the following sequences: the sense primer 5’-GGACCAAATCAGGAGAGAGCAG-3’ and the anti-sense primer 5’-GTTGGAGACGTCTGCAGGTATG-3’.

For the *A845C* and *G857A* (*NAT2*7B*) mutations a 547pb fragment was amplified from *NAT2* using the following primers: the sense primer had the sequence 5’-GCTGGGTCTGGAAGCTCCTC-3’, while the anti-sense primer had 5’-TTGGGTGATACATACACAAGGG-3’.

These methods were presented in detail in our previous study [29].

Survival was evaluated by following up the patients’ evolution. Conclusive results regarding postoperative evolution after discharge from the hospital were obtained from 56 patients, 54 respectively in the case of the *T341C* mutation. In case of death, data were confirmed by phone call. All the data were re-confirmed by the National Population Register.

The *NAT2* genotype was classified into two groups: rapid acetylator (homozygotes for the normal allele, or patients with one normal and one mutant allele), and slow acetylator (homozygotes for the mutant allele). We compared the survival of patients with the wild allele, with that of

patients that were negative homozygotes or heterozygotes for the *NAT2*5C (T341C)*, *NAT2*5A (C481T)* and *NAT2*6B (G590A)* mutations or positive homozygotes for *NAT2*7B (G857A)*.

Statistical analysis

Survival analysis was performed using the actuarial method (scale-like survival curves of Kaplan-Meier type) and taking into account the median survival time. Comparison between the categories was performed using the Logrank test, with a threshold value of $p \leq 0.05$. In the case of a comparison between two groups of patients, the analysis was completed with a proportional hazards model with HR (CI95%) estimation. Standard HR interpretation, as an estimated relative risk of the event of interest occurring in one group compared to the other group was used. (HR(CI95%)>1 - risk effect, HR(CI95%)<1 - protective effect).

Results

The general characteristics of the studied groups are presented in Table I. The relationship between *NAT2* polymorphisms and Dukes-Mac stages is presented in Table II. The results regarding the relation genotype/overall mortality were inconclusive and could not lead to statistical relevance.

The survival analysis was done separately for each acetylator type and each stage, as well as separately for each mutation.

Stages B and C

Survival curves of the patients with *NAT2*5C (T341C)* mutation and stages B and C are presented in Fig. 1. Overall survival rates by stages was: 64.2% for the rapid acetylator TT/TC patients diagnosed with stage B, 100% for slow acetylators CC and stage B, 30.8% for rapid acetylators TT/TC in stage C, and 20% for the slow CC in stage C. Median survival rates for the patients in stage C were 4 months for CC slow acetylators, and 11 months for the rapid TT/TC acetylators. Statistical analysis confirmed the poorer survival rate of slow acetylators *342CC* diagnosed with stage C, $p=0.02$ (Table III).

Table I. Characteristics of the studied patients

Characteristics	Patients (N=70)
Tumor location	
Right colon, n (%)	13 (18.57)
Left colon, n (%)	33 (47.14%)
Rectum, n (%)	24 (34.28)
Age (years)	
50- 59, n (%)	25 (34.28%)
60- 69, n (%)	21 (30%)
> 70 ani, n (%)	25 (35.71)
Age (years) (mean ± SD)	65.78 ± 9.55
Median	64.5
Gender	
Women, n (%)	37 (52.85%)
Men, n (%)	33 (47.14%)

Table II. Relationship between the *NAT2* genotype / phenotype and colorectal cancer stage (Dukes- MAC)

<i>NAT2</i> gene mutations (phenotype)	Dukes MAC Stage			OR [CI95%]	p [^]
	B	C	D		
<i>NAT2*5C (T341C)</i>					
TT/TC (rapid)	29 (52.72%)	15 (27.27%)	5 (9.09%)	15.5 [1.7-135.5]	0.008
CC (slow)	1 (6.67%)	8 (53.33%)	6 (40%)		
<i>NAT2*5A (C481T)</i>					
CC/CT (rapid)	27 (46.55%)	17 (29.31%)	8 (13.8%)	0.3 [0.1-1.4]	0.239
TT (slow)	3 (25%)	6 (50%)	3 (25%)		
<i>NAT2*6B (G590A)</i>					
GG/GA (rapid)	24 (46.15%)	20 (38.46%)	6 (11.54%)	0.12 [0.01-0.85]	0.764
AA (slow)	6 (33.33%)	3 (16.66%)	5 (27.78%)		
<i>NAT2*7B (G857A)</i>					
GG/GA (rapid)	19 (47.5%)	9 (22.5%)	8 (20%)	2.7 [0.8-8.2]	0.141
AA (slow)	11 (36.67%)	14 (46.66%)	3 (10%)		

[^]- p calculated for B vs. C, significant if $p < 0.05$

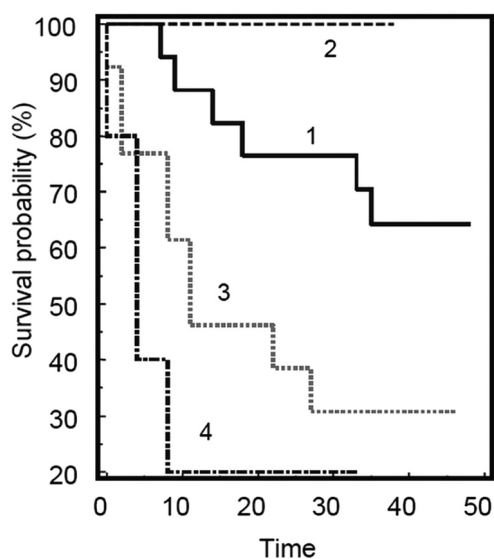


Fig 1. Comparative survival curves of Dukes-MAC B and C stages for *NAT2*5C (T341C)* genotype. 1 – B stage, rapid phenotype, TT/TC mutation; 2 – B stage, slow phenotype, CC mutation; 3 – C stage, rapid phenotype, TT/TC mutation; 4 – C stage, slow phenotype, CC mutation.

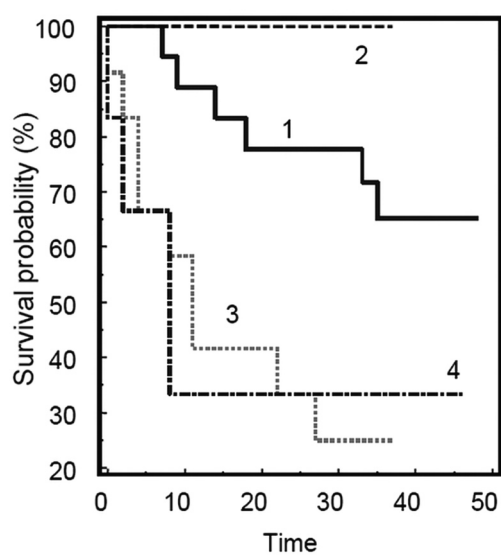


Fig 2. Comparative survival curves of Dukes-MAC B and C stages for *NAT2*5A (C481T)* genotype. 1 – B stage, rapid phenotype, CC/CT mutation; 2 – B stage, slow phenotype, TT mutation; 3 – C stage, rapid phenotype, CC/CT mutation; 4 – C stage, slow phenotype, TT mutation.

In the case of the *NAT2*5A (C481T)* mutation, at the end of the study, survival was 65.30% for stage B rapid acetylators CC/CT, 100% B slow acetylators TT, 25% for C rapid acetylators CC/CT, and 33% for the stage C slow acetylators TT. The diagram (Fig. 2) evidences a shorter survival rate of rapid acetylators CC/CT in stage C, including stage C slow acetylators TT. Patients in stage C had median survival rates of 8 months for slow TT acetylators, and 11 months for rapid CC/CT acetylators respectively. Statistical analysis confirmed a better survival rate of slow acetylators *481TT* diagnosed as stage C, as compared to rapid *481 CC/CT*, $p=0.03$ (Table III).

For the *NAT2*6B (G590A)* mutation, median survival was 9.5 months, both for slow *590AA* acetylators and the rapid *GG/GA* in stage C. Fig. 3 evidences a poorer survival of slow *590AA* as compared to rapid *590GG/GA* acetylators in both B and C stages. The detailed statistical analysis is presented in Table III, indicating that slow *590AA* acetylators had a shorter survival than the rapid *590GG/GA* ones, statistical significance $p=0.02$.

In the case of the *NAT2*7B (G857A)* mutation, Fig. 4 shows that in stage B slow acetylators *587AA* had a lower survival than the rapid *587GG/GA* ones. In stage C the survival of slow acetylators *587AA* was better than that of the rapid *587GG/GA* ones. Median survival of rapid acetylators in stage C was 8 months (Table III). Genotype *NAT2*7B* influenced survival in stage B and C significantly. Thus, slow acetylators in stage B had a lower survival than the rapid ones, 50% vs. 69%. In stage C rapid acetylators survived less than the slow ones, 21% vs. 50%. In both cases $p=0.0101$.

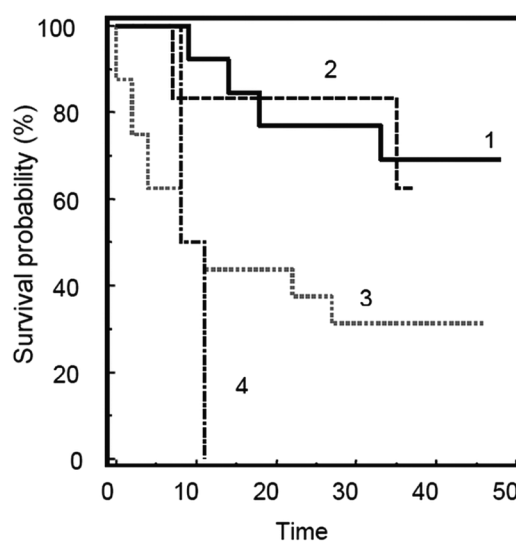


Fig 3. Comparative survival curves of Dukes-MAC B and C stages for *NAT2*6B (G590A)* genotype. 1 – B stage, rapid phenotype, GG/GA mutation; 2 – B stage, slow phenotype, AA mutation; 3 – C stage, rapid phenotype, GG/GA mutation; 4 – C stage, slow phenotype, AA mutation.

Stage D

The influence of *NAT2* polymorphisms on patients diagnosed with colorectal cancer in stage D was analyzed separately. Fig. 5 presents the survival curves for the *NAT2*5C* mutation. The rapid acetylator *341TT/TC*

Table III. Survival of patients with *NAT2* mutations diagnosed in the B and C stages

Mutation	Stage, phenotype and genotype	Survival				Chi ²	HR	95% CI	Significance
		6 months	12 months	24 months	48 months				
<i>NAT2*5C (C341T)</i>	B rapid TT/TC	0.941 (0.057)	0.882 (0.078)	0.765 (0.103)	0.642 (0.118)	9.7135	1.8	1.2-2.7	P = 0.0212
	B slow CC ^B	-	-	-	-				
	C rapid TT/TC	0.769 (0.117)	0.462 (0.138)	0.385 (0.135)	0.308 (0.128)				
	C slow CC	0.800 (0.179)	0.400 (0.219)	0.200 (0.179)	0.200 (0.179)				
<i>NAT2*5A (C481T)</i>	B rapid CC/CT	0.944 (0.054)	0.889 (0.074)	0.778 (0.098)	0.653 (0.116)	8.4799	1.6	1.1-2.4	P = 0.037
	B slow TT ^B	-	-	-	-				
	C rapid CC/CT	0.667 (0.136)	0.417 (0.142)	0.333 (0.136)	0.250 (0.125)				
	C slow TT	0.833 (0.152)	0.667 (0.192)	0.333 (0.192)	0.333 (0.192)				
<i>NAT2*6B (G590A)</i>	B rapid GG/GA	0.923 (0.074)	0.846 (0.100)	0.769 (0.117)	0.692 (0.128)	8.9539	2.0	1.2-3.3	P = 0.0299
	B slow AA	0.833 (0.152)	0.833 (0.152)	0.625 (0.213)	0.625 (0.213)				
	C rapid GG/GA	0.625 (0.121)	0.437 (0.124)	0.375 (0.121)	0.312 (0.116)				
	C slow AA	1.000 (0.354)	0.500 (0.354)	0.000 (0.000)	0.000 (0.000)				
<i>NAT2*7B (G857A)</i>	B rapid GG/GA	0.941 (0.057)	0.882 (0.078)	0.760 (0.105)	0.697 (0.114)	11.3190	1.6	1.1-2.3	P = 0.0101
	B slow AA	1.000 (0.354)	0.500 (0.354)	0.500 (0.354)	0.500 (0.354)				
	C rapid GG/GA	0.571 (0.132)	0.286 (0.121)	0.214 (0.110)	0.214 (0.110)				
	C slow AA	0.750 (0.217)	0.750 (0.217)	0.500 (0.250)	0.500 (0.250)				

^B – Survival data was obtained in these cases for just 1 patient in each group. Both patients had survived.

represented a favorable prognostic factor for the patients in this stage, who had a much lower mortality rate than slow acetylators *341CC*, HR=0,30 with 95%, IC [0.025- 0.9810] and p= 0,04 (Table IV).

The analysis of the *NAT2*5A (C481T)* mutation did not evidence statistically significant relations. Though slow acetylators *481TT* died earlier, the rapid acetylators *CC/CT* had a higher mortality rate. Median survival was 15 months for the rapid acetylators and only 3 months for the slow ones. There was no statistical significance (Table IV).

In the case of *NAT2*6B (G590A)*, the rapid acetylators diagnosed in stage D had a higher mortality rate than the slow ones. The statistical analysis did not provide a significance for the relationship between the genotype and survival (Table IV).

All the patients with the *NAT2*7B (G857A)* mutation diagnosed in stage D were rapid acetylators *857GG/GA* and had a median survival of 13.5 months, with an average of 19.6 months (SD ± 5.19).

Discussion

To our knowledge, this is the first study focusing on the impact of *NAT2* polymorphisms and acetylator genotype on the survival of patients with sporadic colorectal cancer.

The impact of the *NAT2* mutations as risk factors for sporadic CRC is well studied and discussed, and as such there are very few uncharted areas in this field. This study aims to propose a new view on the way the metabolic profile, identified through genetic mutations such as that of the *NAT2* gene - which ultimately affecting the acetylation capacity of the organism, influences the evolution and response to therapy of the patients with CRC.

This is the point of view of the physician treating the disease, obviously the most important aspect of the pathology. It is evident that patients with an apparently identical tumor stage have a completely different evolution, which raises the question of the factors that influence this difference. We believe that mutations such as that of *NAT2*

Table IV. Survival of patients with *NAT2* mutations diagnosed in stage D

Mutation	Stage, phenotype and genotype	Survival				Chi ²	HR	95% CI	Significance
		6 months	12 months	24 months	48 months				
<i>NAT2*5C (T341C)</i>	D rapid TT/TC	0.714 (0.171)	0.714 (0.171)	0.571 (0.187)	0.429 (0.187)	3.9227	0.3073	0.0252 to 0.9810	P = 0.0476
	D slow CC	0.750 (0.217)	0.250 (0.217)	0.000 (0.000)	0.000 (0.000)				
<i>NAT2*5A (C481T)</i>	D rapid CC/CT	0.889 (0.105)	0.556 (0.166)	0.333 (0.157)	0.222 (0.139)	0.0374	0.8612	0.1541 to 4.6365	P = 0.8466
	D slow TT	0.333 (0.272)	0.333 (0.272)	0.333 (0.272)	0.333 (0.272)				
<i>NAT2*6B (G590A)</i>	D rapid GG/GA	0.667 (0.192)	0.333 (0.192)	0.167 (0.152)	0.167 (0.152)	0.6154	1.6521	0.4401 to 6.7970	P = 0.4327
	D slow AA	0.833 (0.152)	0.500 (0.204)	0.333 (0.192)	0.333 (0.192)				

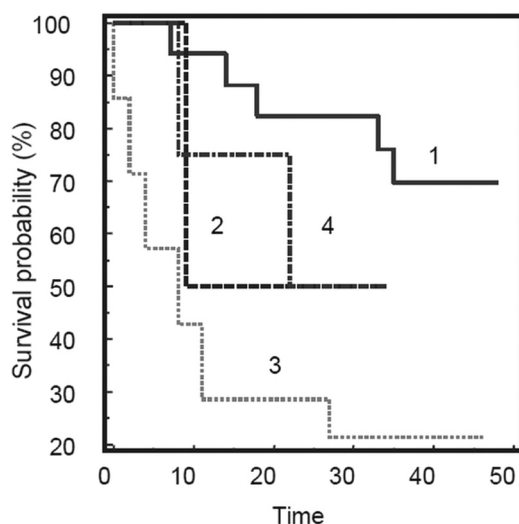


Fig 4. Comparative survival curves between Dukes-MAC B and C stages for *NAT2*7B (G857A)* genotype. 1 – B stage, rapid phenotype, GG/GA mutation; 2 – B stage, slow phenotype, AA mutation; 3 – C stage, rapid phenotype, GG/GA mutation; 4 – C stage, slow phenotype, AA mutation.

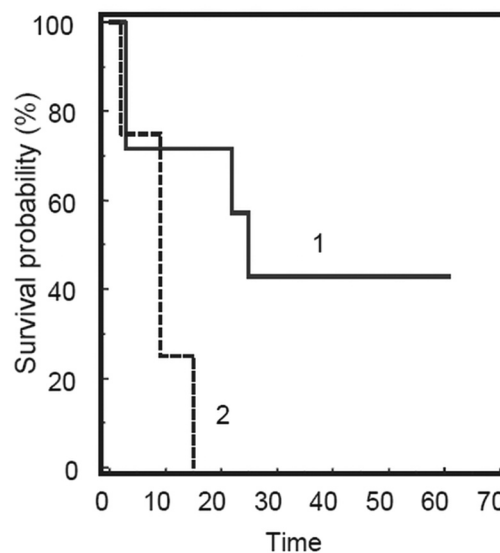


Fig 5. Survival curves for patients diagnosed in the Dukes-MAC D stage with *NAT2*5C (T341C)* genotype. 1 – D stage, rapid phenotype, TT/TC mutation; 2 – D stage, slow phenotype, CC mutation.

may constitute one of the causes that finally lead to these differences in evolution. We consider this concept to be entirely new and we believe that this study will prompt to define a new approach to evaluating patients with CRC - from a metabolic and genetic point of view, thus finally leading to the individualization and optimization of treatment.

Studies in literature disagree regarding the relationship between the *NAT2* varieties and CRC, a number of results depending on the associated exposure to untoward environmental factors: roast meat, smoking etc. Many studies evidenced an increased risk of CRC in patients with *NAT2* polymorphisms (rapid acetylators) [26, 27, 30-32]. Other studies, which included a higher number of patients,

focused more on environmental factors acting through procarcinogenic compounds activated by *NAT2*: meat, especially red roast meat, and smoking, did not confirm an increased cancer risk in patients with *NAT2* polymorphisms (rapid acetylators) [6, 33-36]. No systematic studies exist concerning the relationship between the *NAT2* genotype and the histological type or stage of CRC. Hardingham et al [37], in a group of 100 patients, and Hubbard et al [38] in 275 patients did not evidence links with the Dukes stage.

Data in the literature are thus controversial, so the issue of the relationship between *NAT2* genotype and colorectal cancer remains open. Our previous study [29] showed that rapid acetylator patients - negative homozygotes (wild

variant) and heterozygotes for the *NAT2*5C* (*T341C*), *NAT2*5A* (*C481T*) and *NAT2*6B* (*G590A*) mutations - presented an increased risk of colorectal cancer as compared to the slow acetylators positive homozygotes. Positive homozygotes *NAT2*7B* (*G857A*), although slow acetylators, presented a higher risk of developing CRC.

We have evidenced a number of statistically significant relations regarding survival that allow us to consider that the *NAT2* genotype may be a prognostic factor for the evolution of CRC. Moreover, the genotypes influence survival within the same Dukes-MAC stage.

Thus, slow acetylators *341CC* in stage C had a poorer survival than the rapid ones *341TT/TC* ($p=0.02$). This indicates the *341CC* slow acetylators genotype to be a negative prognostic factor in colorectal cancer, especially for the patients in stage C.

The statistical analysis confirmed a better survival of slow acetylators *481TT* in stage C as compared to the rapid *481CC/CT*, the *481CC* slow acetylators thus representing a positive prognostic factor for the disease, especially for the patients in stage C.

Slow acetylators *590AA* had a lower survival rate than the rapid *590GG/GA*, both in stage B and C. The slow acetylators *590AA* genotype seems to be a major negative prognostic factor for CRC for patients in stages B and C.

The *NAT2*7B* genotype influenced significantly the survival in stages B and C, but in different ways. In stage B, slow acetylators had a lower survival rate than the rapid ones, 50% vs. 69%. In stage C rapid acetylators had lower survival rates than the slow ones, 21% vs. 50%, significant in both cases ($p=0.0101$). Accordingly, the *857AA* slow acetylators genotype represents a negative prognostic factor for the patients with CCR stage B, but a positive one for the patients in stage C.

The patients in stage D had a good (18.9%) survival rate. The rapid acetylators *341TT/TC* genotype represented a favorable prognostic factor, in contrast with the *341CC* slow acetylators ($p=0.04$).

No systematic studies have been published regarding the association between the *NAT2* genotype and the TNM and Dukes-Mac stages. Hubbard et al published a study on 275 cases of CRC in the three Dukes stages [36]. In all stages the slow acetylators were predominant, representing 62-66% of the patients, findings different from ours. The relations between the genotype and the Dukes stages were determined in this context, along with other aspects of colorectal cancer.

We are aware of the limitations of our study, mainly concerning the small hospital sample of patients that has suffered a number of subgroup comparisons. The size of the study group may induce partial minimization of the statistical strength. This is why no further criteria of differentiation were applied, which would have diminished the size of the subgroups even more.

In **conclusion**, the *NAT2* gene may be considered as a prognostic factor for the survival of patients with CRC. In order to further analyze the relationship between *NAT2*

polymorphisms and colorectal cancer, large population studies are required that would take into account all the variables mentioned above: association with other mutations, exposure to environmental factors, ethnic and demographic particular features.

Conflicts of interest

None to declare.

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