Less common genotype variants of *TP53* polymorphisms are associated with poor outcome in adult patients with adrenocortical carcinoma

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Abstract

Context: The Li-Fraumeni tumor syndrome is strongly associated with adrenocortical carcinoma (ACC) and is caused by germline mutations in *TP53* in 70% of cases. Also, *TP53* polymorphisms have been shown to influence both cancer risk and clinical outcome in several tumor entities. We, therefore, investigated *TP53* polymorphisms in a cohort of adult patients with ACC.

Objective: Evaluation of the role of *TP53* polymorphisms in adult patients with ACC.

Subjects and methods: Peripheral blood for DNA extraction was collected from 72 ACC patients. Polymorphism analysis was carried out by amplification and sequencing of exons and adjacent intron sections of *TP53*. Results were correlated with clinical data and the distribution of the polymorphisms was compared with published Caucasian control groups.

Results: Compared with control groups, genotype frequencies of analyzed *TP53* polymorphisms among ACC patients were significantly different in three out of four polymorphisms: IVS2+38G>C (G/G, $P=0.0248$), IVS3ins16 (NoIns/NoIns, $P<0.0001$; NoIns/Ins, $P<0.0001$), and IVS6+62A>G (G/G, $P<0.0001$; G/A, $P<0.0001$). Overall, the survival of ACC patients, which harbored at least one of the less frequent genotype variants of four analyzed polymorphisms ($n=23$), was significantly inferior (median survival: 81.0 months in patients with the common homozygous genotypes vs 20.0 months in patients with the less frequent genotypes, HR 2.56, 95% CI 1.66–7.07; $P=0.001$). These results were confirmed by multivariable regression analysis (HR 2.84, 95% CI 1.52–7.17; $P=0.037$).

Conclusion: Some *TP53* polymorphisms seem to influence overall survival in ACC patients. This effect was observed for a combination of polymorphic changes rather than for single polymorphisms.

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Introduction

TP53 is a well-known tumor suppressor gene located on chromosome 17p13 with mutations frequently observed in many types of human cancers (1, 2). The corresponding protein p53 is a transcription factor that regulates the

expression of stress-response genes and mediates a variety of antiproliferative processes (3). Polymorphisms of *TP53* alleles occurring in the same population are often defined as single-nucleotide polymorphism (SNP) in coding and

noncoding regions but also comprise deletions or insertions of one or more nucleotides.

More than 90% of the polymorphisms identified so far are localized in intronic regions (1, 2), with a 16 bp insertion as the most frequently described polymorphism in intron 3 (c.96+41_96+56del16, SNP no. 17878362, named IVS3ins16, Fig. 1). IVS3ins16 is correlated with an increased risk of various cancers, including ovary (4), lung (5), colon (6), and breast cancer (7). Another well-characterized intron polymorphism is located in intron 6 (c.672+62A>G, SNP no. 1625895, named IVS6+62A>G, Fig. 1), which is associated with lower apoptotic indices and DNA repair capacity (5). To our knowledge, no functional information has yet been published concerning the polymorphism described in intron 2 (c.74+38G>C, SNP no. 1642785, named IVS2+38G>C, Fig. 1) (8, 9).

The most frequently investigated exonic polymorphism is located in exon 4 of *TP53*, codon 72 (R72P, SNP no. 1042522, Fig. 1) and has been mainly discussed as a susceptibility factor for different malignancies. The R72P polymorphism leads to an arginine (Arg) to proline (Pro) substitution, which results in a structural alteration of the protein (10). Geographical differences in distribution have been observed, which depend mainly on latitude (11, 12). So far, the role of the proline-rich domain in the regulation and function of p53 is, however, unclear (2). Several studies have reported that the arginine variant of R72P induces apoptosis more potently and protects cells

better from neoplastic transformation than the proline variant (2, 10, 13, 14). Up to now variable results about the association of R72P with cancer risk have been published (2, 7, 15, 16, 17, 18). Murata *et al.* (18) reported lung cancer to be associated with the arginine variant of the R72P polymorphism, whereas others observed a higher prevalence of the proline variant compared with controls in such patients (17). In the only previous study on *TP53* polymorphisms in ACC patients, a threefold higher prevalence of the proline variant of the R72P polymorphism compared with controls was observed (19).

So far, most studies concentrate on a single polymorphism and only a few studies have considered two or more polymorphisms in the same series (7, 15, 20, 21). Hrstka *et al.* (15) showed a positive correlation of lymph node metastases in breast cancer patients with IVS3ins16 and IVS6+62A>G polymorphisms. Another study described a prognostic effect of the multivariant alleles from R72P and IVS3ins16, resulting in a significantly poorer prognosis in non-small cell lung cancer (21). Costa *et al.* (7) described that *TP53* polymorphisms (IVS3ins16 and R72P) are associated with a higher incidence of lymph node metastases. Analyzing the three polymorphisms R72P, IVS3ins16, and IVS6+62A>G in 107 patients, Wang-Gohrke *et al.* (16) concluded that the rare alleles of IVS3ins16 and R72P of p53 may modify the risk of breast cancer in German women. All these data suggest a role of multiple *TP53* polymorphisms to predict incidence and survival in different cancers.

Adrenocortical carcinoma (ACC) is a rare malignancy of the adrenal cortex with an incidence of 1–2/1 000 000 population (22, 23) and a poor prognosis with a median OS of only 3.5 years (24, 25, 26, 27, 28, 29). It is part of the Li–Fraumeni tumor syndrome and thus specifically linked to germline mutations in *TP53* (30, 31). Accordingly, *TP53* polymorphisms and their impact on p53 function may be particularly relevant in ACC. This assumption is supported by a small study from Poland that determined the sequence variants of the R72P polymorphism in a cohort ($n=17$) of patients with ACC in comparison with a healthy control group and identified a threefold higher prevalence of the proline allele in codon 72 in patients with ACC. They concluded that the proline genotype may represent a possible risk factor for the development of ACC (19).

To further elucidate a possible role of *TP53* germline polymorphisms in ACC, we investigated a larger cohort of adult patients with ACC derived from the German ACC registry (24, 32) and included in our analysis not only

Figure 1

Scheme of three *TP53* intron polymorphisms and one exon polymorphism. The polymorphism (intron 2 (c.74+38G>C, SNP no. 1642785, named IVS2+38G>C), intron 3 (c.96+41_96+56del16, SNP no. 17878362, named IVS3ins16), intron 6 (c.672+62A>G, SNP no. 1625895, named IVS6+62A>G) and one exon polymorphism: exon 4 of *TP53*, codon 72 (R72P) discussed in the text) are shown in the figure as well as the localization in the corresponding protein domain.

the exon 4 polymorphism but also the three described intron polymorphisms.

Subjects and methods

Patients and samples

We studied 72 patients with ACC registered with the German ACC Registry (33, 34), in whom germline DNA and consent for genetic analyses were available (www.nebennierenkarzinom.de). This registry was established in 2003 and at the time of the last analysis (March 2012) comprised >700 patients. All data sets were collected by trained medical personnel as described previously (24). In addition histories of patients who had recorded malignancies before ACC diagnosis were also considered. There was no difference in prior malignancies between the common and the rare variants of *TP53* polymorphism. Contact to the registry is made either by the local attending physician or by the patient or his/her relatives. Patients had been recruited to the German ACC Registry from all over Germany; all patients were Caucasians. The patients had undergone clinical, radiological, and hormonal examination followed by surgery. Histopathological evaluation of adrenal tissue including assessment of Weiss score (35) was used to establish the diagnosis of ACC. Clinical stage was determined according to ENSAT classification (24). Ethical approval (No. 93/02) was obtained from the local Ethics Committee at the University of Wuerzburg and the participating centers in Germany, and patients gave written informed consent. Information from hospital records and blood samples was provided by the respective supervising clinics. The distribution of *TP53* polymorphisms in Caucasian reference populations was taken from the literature (9, 36, 37): for R72P and IVS3ins16, 563 German healthy controls (327 (58.1%) males and 236 (41.9%) females, with a mean age of 66.9 years) were available (37); for IVS6+62A>G controls were Caucasians born in Italy, 177 (60%) were male, and 118 were female (40%), the mean age was 63.5 years (36); and for IVS2+38G>C we used 72 healthy Danish women with a median age of 61 years as controls (9). The observed *TP53* polymorphisms were also checked against the database <http://gvs.gs.washington.edu/GVS137/>. Here the global minor allele frequency (MAF) for the various SNPs are as follows: intron 2 SNP no. 1642785: MAF=26%; intron 3 (SNP no. 17878362): MAF=50%; intron 6 SNP no. 1625895: MAF=18%; R72P exon 4, codon 72 SNP no. 1042522: MAF=23%.

DNA extraction

Peripheral blood (1–5 ml) was collected from all patients and genomic DNA was extracted from blood samples using the FlexiGene DNA Extraction Kit (Qiagen).

PCR and sequence analysis

For sequence analysis of the *TP53* exons and introns from genomic DNA, the samples were amplified by PCR using primers according to the design of BioGlobe GmbH, Hamburg, Germany (Metabion International AG, Planegg-Martinsried, Germany) with 1× Hotstar Buffer (Qiagen), 1.5 mM MgCl₂, 200 μM of each dNTPs (Fermentas Life Sciences, Schwerte, Germany), 2 U HotStar DNA polymerase (Qiagen), and 0.4 μM of each primer. PCR comprised 35 cycles (94 °C for 60 s, 63 °C for 60 s, and 72 °C for 60 s) with 25 ng of genomic DNA; final extension was at 72 °C for 10 min. Before cycle sequencing, PCR products were purified using Agencourt AMPure (Beckman-Coulter, Krefeld, Germany). For cycle sequencing of *TP53* exon PCR products, BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) was used. Each 10-μl reaction contained 1 μl BigDye reaction mix and 5 pmol of forward or reverse primer (according to Bioglobe GmbH) respectively. Cycle sequencing comprised 35 cycles (96 °C for 10 s, 55 °C for 5 s, and 60 °C for 4 min). Sequence reactions were purified using Agencourt CleanSEQ (Beckman-Coulter). Samples were subjected to capillary electrophoresis on a MegaBACE 1000 DNA Analyzer. Electrokinetic injection was 2 kV for 12 s and electrophoresis was continued at 8 kV for 110 min. All sequence data were analyzed using GENTle (Manske M, University of Cologne, Germany, <http://gentle.magnus-manske.de/>) and 4 peaks (Mekentosj, <http://mekentosj.com/4peaks>).

The four most often described *TP53* polymorphisms were analyzed: one exonic polymorphism located in exon 4 (R72P) and three intronic *TP53* polymorphisms: IVS2+38G>C (c.74+38G>C, SNP no. 1642785), IVS3ins16 (c.96+41_96+56del16, SNP no. 17878362), and IVS6+62A>G (c.672+62G>A, SNP no. 1625895). Exemplary chromatograms for each of the four analyzed polymorphisms are shown in Fig. 2.

Statistical analyses

Clinical parameters, such as age at diagnosis, tumor size, tumor stage (ENSAT) between groups with the common and rare variants of *TP53* polymorphisms, were compared by χ^2 test. Fisher's (F) exact test was used to investigate

Figure 2
Exemplary chromatograms for each of the four analysed polymorphisms. R72P, IVS2 + 38G > C, IVS3ins16 ((A) sequence with no insertion, (B) heterozygous insertion, (C) homozygous insertion) and IVS6 + 62A > G.

dichotomous variables, such as differences in the frequency of genotype distribution in case and control groups (i.e. two groups and three conditions). To assess whether the presence of a less frequent *TP53* allele influences OS and could be used as a predictive marker, Kaplan–Meier survival analysis was carried out for calculation of median duration and 95% CI. OS was defined as the time from the date of primary diagnosis to death or last follow-up. Genotype distribution was investigated for the presence of Hardy–Weinberg equilibrium. Multivariable regression analysis was carried out by the Cox proportional hazards regression model to identify factors (*TP53* polymorphisms, ENSAT stage, age, Ki67 index, involvement of lymph nodes, and vessel infiltration) that might have influenced survival. For all parameters showing significance in univariable analysis, multivariable regression analysis was carried out. The statistical analysis was carried out with SPSS software package (version 15.0.) and using GraphPad Prism (version 5.0, La Jolla, CA, USA). Results are given as hazard ratios (HR) including 95% CI and a significance level of $P < 0.05$ was considered to be statistically significant for each analysis.

Results

Patients

The clinical data of the 72 Caucasian patients from the German ACC registry are given in Table 1. Median age at primary diagnosis was 48 years (range 18–79 years), 30 (41.7%) were male and 42 (58.3%) were female. Follow-up information was available for all patients, with a median follow-up of 34.2 months (Table 1).

Distribution of four *TP53* polymorphisms in ACC patients

To assess a possible deviation of the distribution of the four analyzed polymorphic genotypes we compared ACC patients with Caucasian reference populations (9, 36, 37). The only previously described polymorphism in ACC patients is located in exon 4, codon 72 (R72P) (19). The more frequent genotype (G/G) was found in our collective in 51 patients (70.8%) and the less frequent genotypes (G/C $n = 17$, C/C $n = 4$) in 21 patients (29.2%). The genotype distribution of R72P between cases and controls (G/G 61% ($n = 343$), G/C ($n = 193$), and C/C ($n = 27$) 39%) did not differ significantly (Fig. 3). The observed *TP53* polymorphisms were also checked against the database <http://gvs.gs.washington.edu/GVS137/> (see also the section Patients and samples). For R72P we observed a good

Table 1 Clinical data of patients with ACC ($n=72$).

	$n=72$
Sex	
Male	30
Female	42
Age at PD (years)	
Median (range)	48 (18–78)
Tumor size (cm)	
Median (range)	10.5 (3–30)
ENSAT stage at diagnosis (%)	
I	7.0
II	35.2
III	26.8
IV	31.0
Hormone activity (%)	
Yes ^a	56.9
No	29.2
ND	13.9
Alive patients	26
Time of follow up ^b (months)	
Median (range)	49 (9.4–339)
Overall survival ^c	
Median (range)	33.3 (4.2–339)

ND, not done.

^aIncluding aldosterone, cortisol and androgen-producing tumors.^bSince first diagnosis to last follow-up in alive patients.^cSince first diagnosis to last follow-up or death.

correlation of our control groups with the large database (R72P exon 4, codon 72 SNP no. 1042522: global MAF=23%). The genotype distribution for insertion of the 16-bp duplication polymorphism (IVS3ins16) was 90.3% ($n=65$) for homozygotes (NoIns), 5.6% ($n=4$) for heterozygotes, and 4.2% ($n=3$) for homozygotes (Ins) in ACC patients. In the Caucasian reference populations the genotype distribution was 68.4% ($n=385$), 29.5% ($n=166$), and 2.1% ($n=12$) for homozygotes (NoIns), heterozygotes, and homozygotes (Ins) respectively (NoIns/NoIns, $P<0.0001$; NoIns/Ins, $P<0.0001$). For the IVS3ins16 polymorphism a deviation from the database <http://gvs.gs.washington.edu/GVS137/> is shown (intron 3 (SNP no. 17878362): MAF=50%). A significantly altered distribution in comparison with the selected Caucasian control group was also found in relation to IVS2+38G>C (cases: G/G 72.2% (52 patients), G/C 22.2% ($n=16$), C/C 5.6% ($n=4$); controls: G/G 52.8% ($n=38$), G/C 37.5% ($n=27$), C/C 9.7% ($n=7$); cases/controls G/G, $P=0.0248$) and IVS6+62A>G polymorphisms (cases: G/G 88.9% ($n=64$), G/A 4.1% ($n=3$), A/A 7.0% ($n=5$); controls: G/G 65.1% ($n=192$), G/A 29.5% ($n=87$), A/A 5.4% ($n=16$); cases/controls: G/G, $P<0.0001$; G/A, $P<0.0001$). For IVS2+38G>C and for IVS6+62A>G we observed a good correlation to the GVS database (intron 2 SNP no. 1642785: MAF=26%; intron 6 SNP no. 1625895: MAF=18%).

Figure 3

Distribution of R72P, IVS2+38G>C, IVS3ins16 and IVS6+62A>G polymorphisms in 72 adult ACC patients compared with healthy control groups available in references (9, 36, 37). * P value <0.05. Data are shown as % but statistical analysis was performed with absolute values as described in the text (see the Results section).

The distribution of the genotype frequencies in R72P ($P=0.982$), IVS2+38G>C ($P=0.501$), IVS3ins16 ($P=0.226$), and IVS6+62A>G ($P=0.148$) polymorphisms among the control groups is in agreement with those expected under the Hardy–Weinberg equilibrium. The distribution of the genotype frequencies in R72P ($P=0.1329$) and IVS2+38G>C ($P=0.089$) among our ACC patients is also in agreement with Hardy–Weinberg equilibrium. We found a deviation from Hardy–Weinberg equilibrium in ACC patients for the IVS3ins16 and IVS6+62A>G polymorphisms most likely due to the small sample size. For all investigated intronic TP53 polymorphisms, a higher incidence of the more frequent allele and a lower incidence of the less frequent allele were found in ACC patients compared with the control groups (Fig. 3).

TP53 polymorphisms and impact on prognosis

Survival analysis was conducted for all described polymorphisms. Patients with the less frequent genotype of R72P polymorphism (G/C (Arg/Pro) and C/C (Pro/Pro)) showed a significantly shorter OS than patients with the common genotype (G/G (Arg/Arg): median, 19.4 vs 73.5 months; HR, 0.47; 95% CI, 0.20–0.82; $P=0.012$; Fig. 4 and Table 2). Similarly, patients with the less frequent genotype of IVS2+38G>C polymorphism (G/C, C/C) showed a significantly shorter OS in contrast to the common genotype (G/G) (median, 18.7 vs 73.5 months; HR, 0.50; 95% CI, 0.22–0.88; $P=0.021$; Fig. 4 and Table 2).

In our cohort, 49 patients (68.1%) were homozygous carriers of the common allele of each of the investigated polymorphisms. The remaining 23 patients (31.9%) were carriers of one or more polymorphisms with the less common allele, with a high percentage in these patients (82.6%) having two or more of the less common TP53 polymorphisms (Fig. 5). Twenty-one of these patients (91.3%) were carriers of the less common allele of the R72P polymorphism and 20 patients (87.0%) were carriers of the less common allele of the IVS2+38G>C polymorphism. Taking a closer look, our study showed that about 78.3% of patients with the less common allele of the R72P polymorphism were also carriers of the less common allele of the IVS2+38G>C polymorphism (Fig. 5), precluding a reliable analysis of the individual contribution of the respective polymorphism. Moreover, 34.8% of the patients who were carriers of these two polymorphisms additionally showed one or two of the rare variants of the other two analyzed polymorphisms (Fig. 5), further hindering the analysis of the impact of individual

polymorphisms. Therefore, we combined all patients with at least one less common allele of the respective polymorphism for analysis of the clinical data. This univariable analysis showed a significant impact of the

Figure 4

Impact of the less common alleles of R72P, IVS2+38G>C, and the combination of the common allele of each of the investigated polymorphism vs the less common allele on overall survival of 72 ACC patients. Common variant: patients were carriers of the common allele of each of the four investigated polymorphisms (R72P: G/G (Arg/Arg), IVS2+38G>C (G/G), IVS3ins16 (NoIns/NoIns), IVS6+62A>G (G/G)); rare variant: patients were carriers of one or more polymorphisms with the less common allele R72P: (G/C, C/C), IVS2+38G>C (G/C, C/C), IVS3ins16 (NoIns/Ins, Ins/Ins), IVS6+62A>G (G/A, A/A).

Clinical Study	B Heinze and others	TP53 polymorphisms in adult adrenal cancer	170:5	713
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Table 2 Univariable regression analyses of *TP53* polymorphisms in patients.

<i>TP53</i> polymorphisms	<i>n</i>	Median survival (months)	<i>P</i> *	Frequent allele/less frequent allele	<i>n</i>	Median survival (months)	HR	95% CI	<i>P</i> *
R72P			0.021				0.47	0.20–0.82	0.012
G/G (Arg/Arg)	51	73.5		G/G (Arg/Arg)	51	73.5			
G/C (Arg/Pro)	17	19.4		G/C (Arg/Pro)					
C/C (Pro/Pro)	4	28.3		C/C (Pro/Pro)	21	19.4			
IVS2+38G>C			0.037				0.50	0.22–0.88	0.021
G/G	52	73.5		G/G	52	73.5			
G/C	16	18.7		G/C					
C/C	4	28.3		C/C	20	18.7			
IVS3ins16			0.877				0.92	0.38–2.24	0.863
NoIns/NoIns	65	40.0		NoIns/NoIns	65	40.0			
NoIns/Ins	4	33.1		NoIns/Ins					
Ins/Ins	3	43.6		Ins/Ins	7	38.3			
IVS6+62A>G			0.859				0.79	0.33–1.89	0.597
G/G	64	40		G/G	64	40			
G/A	3	59.9		G/A					
A/A	5	33.1		A/A	8	33.1			

HR, hazard ratio. **P*<0.05 was considered significant.

examined parameters on survival concerning *TP53* polymorphisms (HR, 2.56; 95% CI, 1.66–7.07; *P*=0.0010). In addition, age at primary diagnosis, ENSAT stage, Ki67, involvement of lymph nodes, and vessel infiltration also affected the outcome. Of note, the median survival was 20 months for ACC patients with one or more polymorphisms with the less common allele compared with a median survival of 81 months in patients with the common allele in all examined polymorphisms (Table 3 and Fig. 4). The follow-up time of patients alive with one or more polymorphisms of the rare variant was 30 months (9–258) and the follow-up time of patients with the common variant was 53.5 months (28.8–339).

To evaluate whether the worse survival of the less common variant was influenced by confounding factors (e.g. higher Ki67, older age, and all rare variants are ENSAT stage IV) we analyzed the relationship between the common and the rare variants of *TP53* polymorphisms and the baseline clinical features of adult ACC patients (Table 4). There were no significant correlations between the less common variants and all tested prognostic parameters (age at PD, ENSAT stage, sex, tumor size, Ki67, involvement of lymph nodes, capsule infiltration, and vessel infiltration).

As the full set of selected prognostic parameters was not available for all the 72 patients, the multivariable regression analysis was carried out for only 41 patients. Despite the low number of affected cases, the presence of at least one of the less common *TP53* polymorphisms remained a significant predictor of survival (HR, 2.84; 95% CI, 1.52–7.17; *P*=0.037) after multivariable adjustment (Table 3).

Discussion

Our study in patients with ACC led to two major findings: significant differences in the prevalence of three out of four *TP53* polymorphisms and a prognostic role of *TP53* alleles on OS. Intriguingly, the prevalence of the common alleles was increased in ACC patients compared with

Patient	Sex	Age at PD	Survival (in months)	R72P	IVS2+38 G>C	IVS3 ins16	IVS6+62 A>G
1	m	36	30				
2	f	44	12				
3	f	40	21				
4	m	18	9				
5	f	51	16				
6	f	24	29				
7	m	44	19				
8	f	49	13				
9	f	38	111				
10	f	31	95				
11	m	60	81				
12	m	69	7				
13	m	58	15				
14	f	65	18				
15	f	77	14				
16	f	60	14				
17	m	42	101				
18	f	35	43				
19	f	32	8				
20	m	55	12				
21	m	61	33				
22	m	49	18				
23	m	40	258				

Figure 5

Distribution of the less frequent genotype polymorphism variants in 23 adult ACC patients. Age at PD in years; m, male; f, female; white rectangle, common variant homozygous; light grey rectangle, heterozygous; dark grey rectangle, rare variant homozygous.

Clinical Study	B Heinze and others	<i>TP53</i> polymorphisms in adult adrenal cancer	170:5	714
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Table 3 Univariable and multivariable Cox regression analyses for risk of death in the adult ACC patients. Common variant: patients were carriers of the common allele of each of the investigated polymorphisms (R72P: G/G (Arg/Arg), IVS2 + 38G > C (G/G), IVS3ins16 (NoIns/NoIns), IVS6 + 62A > G (G/G); rare variant: patients were carriers of one or more polymorphisms with the less common allele R72P: (G/C, C/C), IVS2 + 38G > C (G/C, C/C), IVS3ins16 (NoIns/Ins, Ins/Ins), IVS6 + 62A > G (G/A, A/A). As the full set of selected prognostic parameters was not available for all 72 patients, the multivariable regression analysis was performed for only 41 patients.

	Univariable analysis					Multivariable analysis			
	<i>n</i>	Median survival (months)	HR	95% CI	<i>P</i> *	<i>n</i>	HR	95% CI	<i>P</i> *
<i>TP53</i> polymorphisms									
Common variant	49	81.0	2.56	1.66–7.07	0.0010	30	2.84	1.52–7.17	0.037
Rare variant	23	20.0				11			
Age at PD									
≤ 46 years	35	81.8	1.93	1.05–3.52	0.0325	24	2.22	0.92–5.32	0.073
> 46 years	37	35.4				17			
ENSAT stage									
I–III	49	81.8	5.26	2.48–11.10	<0.0001	33	5.71	1.78–17.40	0.002
IV	23	18.0				8			
Sex									
Male	31	48.2	1.03	0.56–1.85	0.9364				
Female	41	49.2							
Tumor size									
≤ 10.5 cm	36	36.6	1.33	0.73–2.40	0.3417				
> 10.5 cm	34	43.6							
Ki67									
< 10	18	88.5	3.69	1.29–5.34	0.0079	22	0.89	0.37–2.12	0.788
≥ 10	38	31.3				19			
Involvement of lymph nodes									
None	46	81.0	8.16	2.34–28.4	0.0010	35	1.54	0.38–6.22	0.542
Yes	8	22.8				6			
Capsule infiltration									
None	32	73.5	1.29	0.66–2.54	0.4460				
Yes	26	37.5							
Vessel infiltration									
None	21	95.9	2.43	1.25–4.72	0.0087	23	0.59	0.22–1.61	0.307
Yes	40	33.1				18			

PD, primary diagnosis; HR, hazard ratio. **P* < 0.05 was considered significant.

controls, whereas poor prognosis was associated with the less common alleles.

The prevalence of the alleles of the different *TP53* polymorphisms varies widely both in reference populations and in cancer cohorts. Furthermore, highly variable results have been reported concerning the association of specific polymorphisms with cancer risk (2, 7, 15, 16, 17, 18). For example, while Murata *et al.* (18) reported lung cancer to be associated with the arginine variant of the R72P polymorphism, others observed a higher prevalence of the proline variant compared with controls in such patients (17). Similarly, in the only previous study on *TP53* polymorphisms in ACC patients, a threefold higher prevalence of the proline variant of the R72P polymorphism compared with controls was observed (19), although in our study no such difference of *TP53* R72P alleles was observed using both a larger patient sample and a larger control

group. However, although our patient sample is large for a rare cancer like ACC, it is still small in comparison with studies in other malignancies (7, 16, 37, 38). As the less common alleles have been shown to negatively affect p53 function (2, 10, 13, 14, 39), a higher prevalence of the more common alleles in ACC appears at first glance counterintuitive. However, such association may be indirect by affecting certain risk factors for ACC. For example, meta-analysis of *TP53* polymorphisms in hepatocellular carcinoma indicated that the R72P polymorphism may be associated with liver cancer and that difference in genotype distribution may be associated with race, sex, and also with chronic hepatitis virus as a possible risk factor (39). Due to the rarity of ACC, no risk factors have been established up to now for this malignancy. However, the recent development of a European patient registry holds the potential to change this picture.

Table 4 Relationship between common and rare variants of *TP53* polymorphisms and baseline clinical features of adult ACC patients. Data is presented as *n*(%). Common variant: patients were carriers of the common allele of each of the investigated polymorphisms (R72P: G/G (Arg/Arg), IVS2+38G>C (G/G), IVS3ins16 (Nolns/Nolns), IVS6+62A>G (G/G); rare variant: patients were carriers of one or more polymorphisms with the less common allele R72P: (G/C, C/C), IVS2+38G>C (G/C, C/C), IVS3ins16 (Nolns/Ins, Ins/Ins), IVS6+62A>G (G/A, A/A). Unfortunately, not all data were available in all patients. For this reason, the number of patients varied.

	Common variants	Rare variants	P*
<i>TP53</i> polymorphisms	49 (68.1)	23 (31.9)	
Sex			
Male	19 (38.8)	11 (47.8)	0.64
Female	30 (61.2)	12 (52.2)	
Age at PD (years)			
<46	26 (53.0)	12 (52.2)	0.94
>46	23 (47.0)	11 (47.8)	
ENSAT stage			
I	3 (6.1)	2 (8.7)	0.08
II	21 (42.8)	4 (17.4)	
III	9 (18.4)	10 (43.5)	
IV	16 (32.7)	7 (30.4)	
Tumor size (cm)			
≤10.5	25 (52.1)	11 (50)	0.87
>10.5	23 (47.9)	11 (50)	
Ki67 – <i>n</i> (%)			
<10	14 (35.9)	4 (23.5)	0.55
≥10	25 (64.1)	13 (76.5)	
Involvement of lymph nodes			
None	30 (83.3)	16 (88.8)	0.89
Yes	6 (16.7)	2 (11.2)	
Capsule infiltration			
None	24 (60.0)	8 (44.4)	0.41
Yes	16 (40.0)	10 (55.6)	
Vessel infiltration			
None	17 (40.5)	4 (21.1)	0.23
Yes	25 (59.5)	15 (78.9)	

PD, primary diagnosis; NS, not significant. *P* value by χ^2 test (**P*<0.05 was considered significant).

The probably clinically more relevant observation derived from this study is the prognostic impact of some less common *TP53* alleles on survival. As the majority of patients exhibited more than one of the less common alleles, it was not possible to dissect the role of individual polymorphisms. In particular, the less common genotypes of R72P and IVS2+38C>G often occurred together. Both polymorphisms have been described to belong to the same haplotype block, which may explain the similar pattern with regard to patient outcome. Importantly, the association between these genotypes and OS remained significant in multivariable analysis, together with tumor stage.

Recently it has been confirmed that p53 alterations are associated with an aggressive phenotype in ACC and poor prognosis (40). Thus one might speculate that even minor impairment of p53 function caused by certain *TP53* polymorphisms might amplify additional somatic alterations (e.g. loss of the other allele).

TP53 mutations caused by various carcinogens are often discussed in the literature to be associated with a less aggressive behavior (41). Two recent studies have now shown that also in adults with ACC, *TP53* germline mutations occur in a relevant percentage of patients (31, 42). Accordingly, analysis of the *TP53* gene has been suggested in adult patients with ACC, as it may impact on patient care and surveillance of affected relatives (43). Such screening for *TP53* mutations would hold the potential to also characterize *TP53* polymorphisms thereby generating possibly important prognostic information.

In summary, analysis of *TP53* polymorphisms may confer relevant prognostic information in ACC. Additional ACC cohorts are required to confirm that certain *TP53* polymorphisms should be considered as a risk factor for ACC.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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