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Original article

Association of 3′ nearby gene *BTLA* polymorphisms with the risk of renal cell carcinoma in the Polish population

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Abstract

Objective: T cells play an important role in antitumor immunity, and molecules regulating T-cell activity could influence cancer susceptibility. The distinct role of coinhibitory receptors in immunosurveillance has been considered. B- and T-lymphocyte attenuator (BTLA) is one of these receptors, which negatively regulate immune responses. The aim of this study was to investigate the association between *BTLA* gene polymorphisms and susceptibility to renal cell carcinoma (RCC) in the Polish population.

Methods: Altogether 282 patients with RCC and 480 healthy subjects were genotyped for the following polymorphisms: rs2705511, rs1982809, rs9288952, rs16859633, rs9288953, rs2705535, and rs1844089 using the TaqManSNP Genotyping Assays.

Results: Here, we found that the presence of rs1982809G allele (genotype GG + AG) is associated with increased risk of RCC (odds ratio = 1.38; 95% CI: 1.03–1.86; $P = 0.03$). In patients with clear-cell RCC (ccRCC) with high-grade (3 and 4) tumors, the frequency of rs1982809[GG] genotype was significantly higher as compared to those with low-grade (1 and 2) tumors and to the controls (0.14 vs. 0.06, $P = 0.05$ and 0.14 vs. 0.06, $P = 0.04$, respectively). Moreover, we have noticed the trend for overrepresentation of carriers of rs2705511C allele in patients with RCC as compared with the controls (0.51 vs. 0.44, $P = 0.08$)

Haplotype rs2705511C/rs1982809G/rs9288952A/rs9288953T/rs2705535C/rs1844089G (CGATCG) increased the risk of RCC of 46% (odds ratio = 1.46; 95% CI: 1.08–1.96; $P_{corrected} = 0.05$).

Conclusion: Our results indicate that polymorphisms rs1982809 situated in 3′ UTR nearby region of *BTLA* gene might be considered as low-penetrating risk factor for RCC, but results have to be confirmed in further studies.

Keywords: BTLA; Coinhibitory molecule; Gene polymorphisms; Renal cancer

1. Introduction

It is now well established that the immune system can control neoplastic development and growth in a process termed immunosurveillance. This process is controlled through soluble and membrane-bound regulators. Antitumor responses may be disturbed by regulatory mechanisms, which normally act to limit T-cell responses following chronic exposure to antigen [1]. Cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) are well-known coinhibitory molecules belonging to the CD28/B7 superfamily, which act as negative regulators of T-cell activation. B-and T-lymphocyte attenuator (BTLA) is the third immunoinhibitory receptor that belongs to this family, but in contrast to PD-1 and CTLA-4, BTLA binds to the tumor necrosis factor receptor

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family member—herpes virus entry mediator (HVEM) [2]. Ligation of BTLA by HVEM induces tyrosine phosphorylation of immunoreceptor tyrosine-based inhibitory motif and association with the src homology domain 2–containing protein tyrosine phosphatase (SHP)-1or SHP-2 which causes inhibitory signaling [3]. BTLA is expressed on $CD4^+$ and $CD8^+$ T cells, B cells, NK T cells, NK cells, DCs, and macrophages [4].

Growing evidence supports the importance of the immune checkpoints such as CTLA-4, PD-1, and BTLA in RCC immunotherapy [1,5,6]. To enhance T-cell functions and consequently antitumor activity, so far monoclonal antibodies blockading PD-1/PD-L1 and CTLA-4/B7 pathways have been developed. The new therapeutic agents such as ipilimumab, tremelimumab in case of CTLA-4 and nivolumab, and pembrolizumab and atezolizumab in case of PD-1, have been tested in clinical trials with promising results [7]. Those first 2 molecules have become standard treatment according to NCCN Guidelines for Melanoma v.3.2015. It is also postulated that BTLA/HVEM interaction might be considered as another target in clinical immunotherapy [6]. Dual blockade of BTLA and PD-1 revealed encouraging results intensifying immune antitumor response [8].

Taking into account the importance of coinhibitory molecules in cancer immunology, we hypothesized that variation in genes encoding coinhibitory molecules may be associated with susceptibility to renal cell carcinoma (RCC). We have shown previously that CT60 polymorphism in *CTLA-4* gene was associated with clear-cell RCC (ccRCC) risk, especially with necrosis and advanced stages of the disease [9]. Consequently, we undertook a prospective study to investigate the association between *BTLA* gene polymorphisms and the risk of RCC in a Polish population.

2. Materials and methods

2.1. Subjects

This study was approved by Local Bioethics Committee (Medical University of Wroclaw—KB—55/2010) and all individuals involved in this study have signed informed consent.

A total of 282 patients with RCC were prospectively recruited from the Department of Urology and Oncologic Urology of Wroclaw Medical University, a tertiary urologic cancer center. Detailed characteristic of the group of patients is shown in Table 1. The stage of the disease was determined according to the 2009 TNM system, and grading was conducted according to Fuhrman classification [10]. Control subjects were 480 (210 women/270 men) unrelated healthy volunteers from the same geographic area.

2.2. Selection of single nucleotide polymorphisms

For this study, we have selected the following single nucleotide polymorphisms (SNPs) described previously in literature: rs1844089, rs2705535, rs9288953, rs9288952, and rs16859633 [11–14]. Additionally, we included in the study 2 tag dSNPs—rs1982809 and rs2705511 of *BTLA* gene, chosen with use of SNPinfo [15] on the basis of the criteria described [16].

Table 1
Patient and disease characteristics

N	282
Male	179 (63.5%)
Female	103 (36.5%)
Age	
Mean	62.4 (range: 20–85)
Median	62
Histopathology	
RCC	282 (100%)
ccRCC	236/282 (83.7%)
Others and unknown	46/282 (16.3%)
Stage at presentation (ccRCC only)	236
I	108 (45.8%)
II	25 (10.6%)
III	26 (11.0%)
IV	77 (32.6%)
Grade (ccRCC only)	236
I	106 (44.9%)
II	73 (30.9%)
III	40 (16.9%)
IV	10 (4.2%)
Unknown	7 (3.0%)

Stage of the disease according to the 2009 TNM system, grading according to Fuhrman classification [10].

2.3. DNA isolation and genotyping

DNA for each individual was isolated from venous blood by using the QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. Genotyping was performed using the TaqManSNP Genotyping Assays as described previously [16].

2.4. Statistical analysis

The evaluation of Hardy-Weinberg equilibrium was performed by comparing the observed and expected frequencies of genotypes using χ^2 analysis. The χ^2 test was used to compare categorical data. Odds ratios (OR) and 95% CIs) were calculated using the binary logistic regression model. The haplotype frequencies were determined using the SHEsis program [17]. Haplotypes with frequencies <0.03 were not considered. Differences were considered statistically significant if $P < 0.05$. For the multiple comparisons, Bonferroni correction was employed to the level of significance.

3. Results

Each polymorphism in the *BTLA* gene was in Hardy-Weinberg equilibrium in both cases and controls. We found

that the polymorphism rs16859633, chosen on the basis of SNPinfo [15] does not occur in the Polish population, as genotyping of 293 individuals (100 controls/193 patients with RCC) revealed the presence of only the homozygotes AA.

3.1. BTLA gene polymorphisms and RCC risk

The distributions of the genotypes for all selected polymorphisms are presented in Table 2. Our results indicate that polymorphism rs1982809 is associated with susceptibility to RCC. The rs1982809G allele carriers (dominant model) were more frequent in patients RCC as compared with those of the control (0.49 vs. 0.41, $P = 0.03$) (Table 2). Furthermore, we observed trend for the overrepresentation of rs2705511C allele carriers (dominant model) in patients with RCC in comparison with the controls (0.51 vs. 0.44, $P = 0.08$).

Taking into account those 2 polymorphisms, we noticed that individuals possessing predisposing alleles for both polymorphisms (rs1982809G and rs2705511C carriers) have 40% increased risk of RCC than individuals possessing [AA] genotype for both SNPs (OR = 1.40; 95% CI: 1.02–1.93; $P = 0.04$). Haplotype estimation analysis showed that the haplotype of rs2705511C/rs1982809G/rs9288952A/rs9288953T/rs2705535C/rs1844089G (CGATCG)

Table 2
Distribution of alleles and genotypes of the *BTLA* SNPs in patients with RCC compared with the controls

BTLA			Controls[b]	RCC	OR [95% CI]	*P*
			N = 480	*N* = 282		
rs2705511	Genotype	A A	265 (55.9)	139 (49.3)	Reference	0.21
		A C	177 (37.3)	122 (43.3)	1.31 [0.97–1.79]	
		C C	32 (6.8)	21 (7.4)	1.25 [0.70–2.25]	
	Dominant model	A A	265 (55.9)	139 (49.3)	0.77 [0.57–1.03]	0.08
		A C + C C	209 (44.1)	143 (50.7)	1.30 [0.97–1.75]	
	Recessive model	A A + A C	442 (93.2)	261 (92.6)	0.90 [0.51–1.59]	0.72
		C C	32 (6.8)	21 (7.4)	1.11 [0.63–1.97]	
rs1982809	Genotype	A A	279 (59.4)	145 (51.4)	Reference	0.10
		A G	163 (34.7)	116 (41.1)	1.37 [1.00–1.87]	
		G G	28 (6.0)	21 (7.4)	1.44 [0.79–2.63]	
	Dominant model	A A	279 (59.4)	145 (51.4)	0.72 [0.54–0.98]	**0.03**
		A G + G G	191 (40.6)	137 (48.6)	1.38 [1.03–1.86]	
	Recessive model	A A + A G	442 (94.0)	261 (92.6)	0.79 [0.44–1.41]	0.42
		G G	28 (6.0)	21 (7.4)	1.27 [0.71–2.28]	
rs9288952[a]	Genotype	A A	425 (88.5)	246 (87.2)	Reference	0.78
		A G	53 (11.0)	34 (12.1)	1.11 [0.70–1.75]	
		G G	2 (0.4)	2 (0.7)	1.73 [0.24–12.34]	
	Dominant model	A A	425 (88.5)	246 (87.2)	0.88 [0.56–1.39]	0.59
		A G + G G	55 (11.5)	36 (12.8)	1.13 [0.72–1.77]	
rs9288953	Genotype	C C	186 (39.6)	108 (38.3)	Reference	0.42
		C T	219 (46.6)	125 (44.3)	0.98 [0.71–1.36]	
		T T	65 (13.8)	49 (17.4)	1.30 [0.84–2.02]	
	Dominant model	C C	186 (39.6)	108 (38.3)	1.06 [0.78–1.43]	0.73
		C T + T T	284 (60.4)	174 (61.7)	0.95 [0.70–1.28]	
	Recessive model	C C + C T	405 (86.2)	233 (82.6)	0.76 [0.51–1.14]	0.19
		T T	65 (13.8)	49 (17.4)	1.31 [0.87–1.96]	
rs2705535[a]	Genotype	C C	464 (97.1)	271 (96.1)	Reference	0.38
		C T	14 (2.9)	10 (3.5)	1.22 [0.54–2.79]	
		T T	0	1 (0.4)	–	
	Dominant model	C C	464 (97.1)	271 (96.1)	0.74 [0.33–1.66]	0.47
		C T + T T	14 (2.9)	11 (3.9)	1.35 [0.60–3.01]	
rs1844089[a]	Genotype	G G	397 (82.9)	238 (84.4)	Reference	0.43
		A G	80 (16.7)	41 (14.5)	0.85 [0.57–1.29]	
		A A	2 (0.4)	3 (1.1)	2.50 [0.42–15.08]	
	Dominant model	G G	397 (82.9)	238 (84.4)	1.12 [0.75–1.67]	0.59
		A G + A A	82 (17.1)	44 (15.6)	0.90 [0.60–1.34]	

The significant results were bolded.

[a]Owing to low number of mutated homozygotes, the analysis for recessive model was omitted.

[b]For the control, actual number of individuals for particular SNP genotyping was as follow: rs2705511, $n = 474$; rs1982809, $n = 470$; rs9288952, $n = 480$; rs9288953, $n = 470$; rs2705535, $n = 478$; and rs1844089, $n = 479$.

Table 3
Haplotypes frequency of SNPs in *BTLA* gene in the different group of patients with RCC and ccRCC compared with the controls

Haplotype[a]	Case (freq)	Control (freq)	*P*	OR	[95% CI]
	RCC				
	N = 282	*N* = 470[b]			
C G G C C A	9.7 (0.02)	28.6 (0.03)	0.11	0.55	[0.27–1.16]
A A A C C G	249.6 (0.44)	440.5 (0.47)	0.27	0.88	[0.71–1.10]
C A A C C G	23.4 (0.04)	38.8 (0.04)	1.00	1.00	[0.60–1.70]
C G A C C G	20.8 (0.04)	26.1 (0.03)	0.33	1.34	[0.75–2.41]
A A A T C G	110.2 (0.20)	195.7 (0.21)	0.52	0.92	[0.70–1.19]
C G A T C G[*]	95.9 (0.17)	116.0 (0.12)	**0.01**	1.46	[1.08–1.96]
	ccRCC				
	N = 236	*N* = 470[b]			
C G G C C A	9.9 (0.02)	28.6 (0.03)	0.31	0.68	[0.33–1.42]
A A A C C G	214.5 (0.45)	440.5 (0.47)	0.62	0.94	[0.75–1.19]
C A A C C G	19.2 (0.04)	38.8 (0.04)	0.96	0.98	[0.56–1.72]
C G A C C G	17.3 (0.04)	26.1 (0.03)	0.35	1.34	[0.72–2.49]
A A A T C G	86.6 (0.18)	195.7 (0.21)	0.28	0.85	[0.64–1.14]
C G A T C G	76.4 (0.16)	116.0 (0.12)	**0.04**	1.38	[1.01–1.90]

The significant results were bolded.
[a]The order of SNPs in estimated analysis of haplotypes frequency: rs2705511, rs1982809, rs9288952, rs9288953, rs2705535, and rs1844089.
[b]Only individuals for whom typing data for all SNPs were included to the analysis.
[*]After Bonferoni correction, $P = 0.05$.

increased risk of RCC of 46% (OR = 1.46; 95% CI: 1.08–1.96, $P_{corrected} = 0.05$) (Table 3).

3.2. *BTLA* gene polymorphisms and ccRCC risk

We observed overrepresentation of the rs1982809G alleles in patients with ccRCC in comparison with the controls (0.48 vs. 0.41; OR = 1.36; 95% CI: 1.00–1.87; $P = 0.05$). For other evaluated SNPs, no differences in genotype frequencies were found (data not shown). Moreover, there were also no differences in haplotypes' distribution (after Bonferroni correction) between patients with ccRCC and the controls (Table 3).

3.2.1. *BTLA* gene polymorphisms in patients with ccRCC in relation to the grade of tumor: Patients with high-grade tumors (3 and 4) (ccRCC-HG) vs. patients with low-grade tumors (1 and 2) and ccRCC-HG vs. the controls

The rs1982809[GG] genotype (recessive model) was observed more frequently in the ccRCC-HG than in the controls (0.14 vs. 0.06, $P = 0.04$) (Table 4). Moreover, the distribution of genotypes for rs2705535 in patients with ccRCC-HG was significantly different in comparison with the controls ($P_{corrected} = 0.02$), but due to low numbers of tested individuals in ccRCC-HG group the results must be treated with caution. In addition, rs9288953[TT] genotype (recessive model) tented to be more frequent in the ccRCC-HG than in the controls (0.24 vs. 0.14, $P = 0.06$). Distributions of genotypes for the remaining SNPs and haplotype frequencies did not differ between patients with ccRCC-HG and the controls.

The frequency of rs9288953[TT] and rs1982809[GG] genotypes were significantly higher in ccRCC-HG as compared with ccRCC-LG (0.24 vs. 0.11, $P = 0.02$ and 0.14 vs. 0.06, $P = 0.05$, respectively) (Table 4). We observed also the differences in genotypes' distribution for rs2705511 between those groups, but these differences did not reach statistical significance ($P = 0.06$). The distribution of haplotypes did not differ between patients with ccRCC-HG and ccRCC-LG and the controls (data not shown).

4. Discussion

RCC is an immunogenic tumor characterized by T cells, NK cells, dendritic cells, and macrophages' infiltration [7,18]. The tumor microenvironment impaired the function of protective immune cells and antitumor response, but on the contrary induced suppressive cells like myeloid-derived suppressor cells and cytokines such as MCP-1, IL-1β, and IL-5 [19]. It was shown that in patients with RCC, the levels of myeloid-derived suppressor cells are significantly higher [19]. In the tumor microenvironment, exhaustion of the effector T cells that mediate antitumor response is demonstrated by cell surface markers. The important role of coinhibitory molecules BTLA and PD-1 in this process was shown [8]. Moreover, in patients with ccRCC, the increased level of exhausted $BTLA^{+}CD8^{+}$ T cells was observed [19].

Taking into consideration the important role of coinhibitory molecule in antitumor immunity, we hypothesized that variations in *BTLA* gene might influence cancer susceptibility in particular susceptibility to RCC.

In the present case-control study, we investigated the association between 7 selected tagging *BTLA* SNPs and the risk of RCC in the Polish population.

Here, we have found that rs1982809 SNP was associated with RCC risk as well with higher grade of tumor in ccRCC subgroups of patients. The functional role of this polymorphism is not established yet. In 2 databases SNPinfo and FastSNP [15,20], there are no data about the potential functional role of these polymorphisms. The rs1982809 SNP is situated in 3′ nearby gene region of *BTLA* between genes encoding CD200 and BTLA (-101081||-73 bp). Our results also indicated that rs2705511 that is in moderate linkage disequilibrium with rs1982809 ($r^2 = 0.596$ [16]) might be associated with RCC risk. This polymorphism is located in intragenic region between genes encoding *CD200* and *BTLA* (-97820bp||-3334 bp), but closer to *CD200* gene.

What is interesting is CD200 is also a type-1 membrane glycoprotein belonging to the immunoglobulin superfamily. CD200 has been shown to play an important role in the regulation of antitumor immunity, and overexpression of CD200 has been reported in a number of solid tumors [21–23] and in hematological malignancies [24], as well as on cancer stem cells [25].

Table 4
Distribution of genotypes of the *BTLA* SNPs in patients with ccRCC with high-grade tumors (3 and 4) compared with the patients with low-grade tumors (1 and 2) or with the controls

BTLA polymorphism			ccRCC high grade $N = 50$ (%)	ccRCC low grade $N = 179$ (%)	OR [95% CI]	*P*	ccRCC high grade $N = 50$ (%)	Controls $N = 480$ (%)	OR (95% CI)	*P*
rs2705511	Genotype	A A	29 (58.0)	86 (48.0)	Reference	0.06	29 (58.0)	265 (55.9)	Reference	0.30
		A C	15 (30.0)	83 (46.4)	0.54 [0.27–1.07]		15 (30.0)	177 (37.3)	0.77 [0.40–1.49]	
		C C	6 (12.0)	10 (5.6)	1.78 [0.59–5.32]		6 (12.0)	32 (6.8)	1.71 [0.66–4.44]	
	Dominant model	A A	29 (58.0)	86 (48.0)	1.49 [0.79–2.81]	0.21	29 (58.0)	265 (55.9)	1.09 [0.60–1.97]	0.78
		A C + C C	21 (42.0)	93 (52.0)	0.67 [0.36–1.26]		21 (42.0)	209 (44.1)	0.92 [0.51–1.66]	
	Recesive model	A A + A C	44 (88.0)	169 (94.4)	0.43 [0.15–1.26]	0.12	44 (88.0)	442 (93.2)	0.53 [0.21–1.34]	0.18
		C C	6 (12.0)	10 (5.6)	2.30 [0.79–6.69]		6 (12.0)	32 (6.8)	1.88 [0.75–4.75]	
rs1982809	Genotype	A A	27 (54.0)	91 (50.8)	Reference	0.08	27 (54.0)	279 (59.4)	Reference	0.10
		A G	16 (32.0)	78 (43.6)	0.69 [0.35–1.38]		16 (32.0)	163 (34.7)	1.01 [0.53–1.94]	
		G G	7 (14.0)	10 (5.6)	2.36 [0.82–6.79]		7 (14.0)	28 (6.0)	2.58 [1.03–6.47]	
	Dominant model	A A	27 (54.0)	91 (50.8)	1.14 [0.61–2.13]	0.69	27 (54.0)	279 (59.4)	0.80 [0.45 –1.44]	0.46
		A G + G G	23 (46.0)	88 (49.2)	0.88 [0.47–1.65]		23 (46.0)	191 (40.6)	1.24 [0.69–2.24]	
	Recesive Model	A A + A G	43 (86.0)	169 (94.4)	0.36 [0.13–1.01]	**0.05**	43 (86.0)	442 (94.0)	0.39 [0.16–0.94]	**0.04**
		G G	7 (14.0)	10 (5.6)	2.75 [0.99–7.65]		7 (14.0)	28 (6.0)	2.57 [1.06–6.23]	
rs9288952	Genotype	A A	43 (86.0)	152 (84.9)	Reference	0.57	43 (86.0)	425 (88.5)	Reference	0.35
		A G	6 (12.0)	26 (14.5)	0.82 [0.32–2.11]		6 (12.0)	53 (11.0)	1.12 [0.45–2.75]	
		G G	1 (2.0)	1 (0.6)	3.53 [0.22–57.69]		1 (2.0)	2 (0.4)	4.94 [0.44–55.63]	
	Dominant model	A A	43 (86.0)	152 (84.9)	1.09 [0.44–2.68]	0.85	43 (86.0)	425 (88.5)	0.80 [0.34–1.85]	0.60
		A G + G G	7 (14.0)	27 (25.1)	0.92 [0.37–2.25]		7 (14.0)	55 (11.5)	1.26 [0.54–2.93]	
rs9288953	Genotype	C C	19 (38.0)	71 (39.7)	Reference	0.06	19 (38.0)	186 (39.6)	Reference	0.14
		C T	19 (38.0)	88 (49.2)	0.81 [0.40–1.64]		19 (38.0)	219 (46.6)	0.85 [0.44–1.65]	
		T T	12 (24.0)	20 (11.2)	2.24 [0.93–5.39]		12 (24.0)	65 (13.8)	1.81 [0.83–3.93]	
	Dominant model	C C	19 (38.0)	71 (39.7)	0.93 [0.49–1.78]	0.83	19 (38.0)	186 (39.6)	1.07 [0.59–1.95]	0.83
		C T + T T	31 (62.0)	108 (60.3)	1.07 [0.56–2.04]		31 (62.0)	284 (60.4)	0.94 [0.51–1.71]	
	Recesive Model	C C + C T	38 (76.0)	159 (88.8)	0.40 [0.18–0.89]	**0.02**	38 (76.0)	405 (86.2)	0.51 [0.25–1.02]	**0.06**
		T T	12 (24.0)	20 (11.2)	2.51 [1.13–5.58]		12 (24.0)	65 (13.8)	1.97 [0.98–3.96]	
rs2705535	Genotype	C C	48 (96.0)	171 (95.5)	Reference	0.12	48 (96.0)	464 (97.1)	Reference	0.008
		C T	1 (2.0)	8 (4.5)	0.45 [0.05–3.65]		1 (2.0)	14 (2.9)	0.69 [0.09–5.37]	
		T T	1 (2.0)	0	–		1 (2.0)	0	–	
	Dominant model	C C	48 (96.0)	171 (95.5)	1.12 [0.23–5.46]	0.89	48 (96.0)	464 (97.1)	0.72 [0.16–3.28]	0.68
		C T + T T	2 (4.0)	8 (4.5)	0.89 [0.18–4.33]		2 (4.0)	14 (2.9)	1.38 [0.30–6.26]	
rs1844089	Genotype	A A	42 (84.0)	148 (82.7)	Reference	0.83	42 (84.0)	397 (82.9)	Reference	0.33
		A G	7 (14.0)	29 (16.2)	0.85 [0.35–2.08]		7 (14.0)	80 (16.7)	0.83 [0.36–1.91]	
		G G	1 (2.0)	2 (1.1)	1.76 [0.16–19.91]		1 (2.0)	2 (0.4)	4.73 [0.42–53.23]	
	Dominant model	A A + A G	42 (84.0)	148 (82.7)	1.06 [0.45–250]	0.89	42 (84.0)	397 (82.9)	1.08 [0.49–2.40]	0.84
		G G	8 (16.0)	31 (17.3)	0.91 [0.39–2.13]		8 (16.0)	82 (17.1)	0.92 [0.42–2.04]	

The significant results were bolded.

At present, it is hard to distinguish if the polymorphisms rs1982809 and rs2705511 influence or not influence the expression of *BTLA* or *CD200* genes or both the genes. We believe that rs1982809 is associated rather with *BTLA* expression, as it is located definitely closer to *BTLA* than *CD200* gene. Moreover, our unpublished yet study on patients with CLL, indicates that this polymorphism is associated with mRNA expression level of *BTLA*. Weaker association of rs2705511 polymorphism with RCC risk indirectly confirmed this hypothesis.

In the present study, we have also noticed the overrepresentation of rs9288953[TT] genotype in patients with ccRCC with high-grade tumors as compared to the controls and to patients with ccRCC with low-grade tumors. This SNP was previously investigated by 2 researchers' groups in Asian population. The first work was performed by Inuo et al. [13] who found no associations between this SNP and the risk of type 1 diabetes mellitus and systemic lupus erythematosus. The second study performed by Ge at al. [26] showed that rs9288953 SNP was associated with the risk of colon cancer. In our previous study, we have found the association between presence of rs9288953T allele with CLL risk [27]. The rs9288953 SNP is situated in the first intron of *BTLA* gene. It is well established that first intron is important for splicing process and may regulate gene expression more efficiently than other introns [28], therefore SNPs situated in this region may have potential functional role. Ge et al. postulate that according to human splicing finder software, rs9288953 SNP could active 6 new splice sites in splicing enhancer motifs and break 1 splicing site in silencer motif and in this way may enhance the splicing signal and strengthen the expression of BTLA.

To date, only 2 publications present data on an association between *BTLA* gene polymorphisms and cancer. In the first study (mentioned above), Ge et al. [26] investigated the association between 3 SNPs in *BTLA* gene: rs1844089, rs2705535, and rs9288953 and the risk of colon cancer in Chinese population. The authors found the association between rs9288953 and rs2705535 SNPs with the colon cancer risk, whereas for rs1844089 SNP this association was modified by pork food intake.

The second study by Fu et al. [12] investigated the association between rs1844089, rs2705535, rs9288952, rs2633562, and rs2931761 SNPs and the risk of malignant breast cancer in Chinese women. The authors observed that rs1844089, rs2705535, and rs9288952 were associated with disease risk, tumor size, and estrogen and progesterone receptor expression, as well as C-erbB and P53 status. Here, we also noticed associations of rs2705535 polymorphism with tumor grade, but because of very limited number of cases in compared group of patients these results must be treated with caution.

The rs2705535 and rs1844089 SNPs are both situated in first intron of *BTLA* gene and similarly to rs9288953, these SNPs might induce the aberrant splicing owing to disruption of the splice site such as the splicing enhancers, silencers, or alterations of the mRNA secondary structure [29].

The rs9288952 (C+800T) was to date the most commonly studied SNP, because this polymorphism is a missense mutation. The nucleotide transition causes amino acid residue change Pro-Leu in position 219. It was shown that this SNP is associated with susceptibility to the rheumatoid arthritis in the Japanese population [14], rheumatoid arthritis in the Taiwan population [11], and as mentioned above with breast cancer risk in Chinese women [12]. The functional role of this polymorphism is not well established. It is postulated that this amino acid substitution located near the proximal immunoreceptor tyrosine-based inhibitory motif might deregulate inhibitory function of BTLA [11].

Our trial has several limitations. Firstly, population accrued for this trial is based on a tertiary urological cancer center with overrepresentation of stage III and IV patients, which might influence the results of the study. But this fact gives unique opportunity to study a subpopulation of patients with aggressive and advanced disease.

Prospective nature of this trial, trying to overcome selection bias of retrospective studies, causes significant heterogeneity in kidney tumor types. To overcome this problem, we have selectively analyzed the group of patients with RCC and additionally separate analysis for the most common (clear cell) variant of kidney cancer.

5. Conclusion

To our knowledge, this study is the first prospective, largest, and most comprehensive evaluation to date of the association between polymorphisms in the *BTLA* gene and RCC. The results of our investigation indicate that polymorphisms in *BTLA* gene, especially rs1982809 SNP might be considered as potentially low-penetrating risk factor for RCC, but our results are required to be confirmed in further studies.

Conflict of interests

K.T.: consultant for Pfizer, clinical trials: Pfizer, Roche, Bristol-Myers Squibb, GlaxoSmithKline, immatics. R.Z.: clinical trials: Pfizer, Roche, Bristol-Myers Squibb, GlaxoSmithKline, immatics. Other authors declare that they have no competing interests.

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