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ORIGINAL ARTICLE

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Interleukin-10 and interleukin-18 promoter polymorphisms in an Italian cohort of patients with undifferentiated carcinoma of nasopharyngeal type

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Abstract *Purpose*: Cytokines such as IL-10 and IL-18 seem to be involved in the inflammatory response of undifferentiated carcinoma of nasopharyngeal type (UCNT). The aim of this study was to evaluate the correlation between functional single nucleotide polymorphisms (SNPs) in the promoter region of IL-10 and IL-18 genes and the virological and clinical characteristics in a large case series of Caucasian patients suffering from UCNT, a tumor regularly associated with the Epstein Barr Virus (EBV). *Methods*: Eighty-nine patients with histologically confirmed UCNT and 130 healthy donors were included in our study. DNA was examined for the polymorphisms of IL-10 gene at positions –1082, −819, −592 by direct sequencing and IL-18 gene at position −607 and −137 by allele –specific PCR. EBV DNA serum viremia was evaluated by QC-PCR. *Results*: The distributions of the IL-10 and IL-18 genetic variants were not different between UCNT patients and healthy controls. The frequency of IL-10 –1082G allele, which is associated with high IL-10 expression, showed a nearly statistically significant increase in UCNT patients EBV DNA-negative as compared to healthy controls (OR = 3.3 95% CI: 1.2–9.8). Subjects with C/C or C/G combined IL-18 genotypes showed an increased risk of being with Stages III-IV (OR = 2.1 95% CI: 1.2–6.6). *Conclusion*: This study was performed to improve the definition of the pathogenetic factors implicated in UCNT by addressing the correlation between cytokine polymorphisms and clinical parameters. This is the first study investigating the possible role of the IL-18 and IL-10 polymorphisms in the development and outcome of UCNT. In our genetic analysis there is no evidence for involvement of IL-10 promoter polymorphisms alone in the genetic predisposition to this tumor. On the other hand, IL18 genetic variants may represent a genetic risk factor for tumor aggressiveness.

Keywords Interleukin-10 · Interleukin-18 · Single nucleotide polymorphisms (SNPs) · Undifferentiated carcinoma of nasopharyngeal type (UCNT) · Epstein Barr virus (EBV)

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Introduction

Serological and histological evidences indicate that Epstein Barr virus (EBV) is pathogenetically linked with undifferentiated carcinoma of the nasopharyngeal type

(UCNT) [2, 19, 38], a tumor with high incidence in specific geographic areas such as China and Southeast Asia, but rare in Western Countries [2, 38]. There is a correlation reported between plasma/serum EBV DNA and disease-stage in Asian patients [27, 29], whereas clinical, virological and immunological characteristics of UCNT patients from non-endemic areas are still poorly investigated. We recently reported a detailed immunological and virological characterization of one of the largest series of UCNT patients in Western countries [31, 41]. EBV viremia was quantified in 58% of patients, with increased DNA levels among those with considerable extension of the primary tumor [31]. Then, we showed a specific immunological defect consisting in the impaired expression of IL-2 by circulating CD4+ T lymphocytes and of IFN-γ and of perforin by the CD8+ subpopulation [41].

The pathogenesis and the clinical progression of EBV-associated tumors have been widely studied [27, 38]. Many of the clinical manifestation of these diseases are based on the biological characteristics of the target cells for EBV infection and the expression and function of EBV genes which also perturb host immune functions.

It was recently proposed that the variability of cytokine production may influence the clinical and biological outcomes of viral infections [4, 10, 20]. In particular, evidence was provided suggesting that differences in the interleukin-10 (IL-10) levels may modulate the susceptibility to EBV infection and to the development of EBV-related malignancies [18, 28]. These observations were also supported by in vivo studies where high levels of IL–10 expression in UCNT tumor cells correlated with the presence of a low number of infiltrating cytotoxic T cells and a poor prognosis [6, 15]. Interestingly, the gene coding for human IL-10 (hIL-10) shows extensive sequence homology with the BCRF1 open reading frame of EBV and both the human and viral proteins share many biological activities.

IL-10 secretion can be inhibited by IL-18, a member of IL-1 superfamily, which has a key role in driving the Th1 pro-inflammatory response [34]. Notably, a recent study by Hu et al. [22] demonstrated a constitutive secretion of this cytokine in UCNT tumor cells, suggesting a role for IL-18 in promoting the massive leukocyte infiltration process often characterizing the histopathologic picture of this tumor.

In general, genetic loci encoding cytokines were shown to be polymorphic, and some of these mutations were associated with variable levels of cytokine production.

In particular, several polymorphisms were identified within the IL-10 gene, but not all of them have a known clinical relevance. The best documented polymorphisms are two microsatellites, IL10.R and IL10.G, and three single nucleotide polymorphisms (SNPs) at position −1082(G/A), −819(C/T) and −592(A/C), which influence the transcription of IL-10 mRNA and the expression of IL-10 in vitro [12, 36]. Since a tight linkage disequilibrium (LD) exists between these SNPs, in the general population only three out of the eight possible haplotypes are present: GCC, ACC and ATA, defined as high, medium and low expression haplotypes, respectively. Interestingly, the involvement of these SNPs was reported with a more severe clinical course in several inflammatory diseases [13, 23], as well as with a different susceptibility to EBV infection and to the outcome of EBV-associated gastric carcinoma [18, 39].

With regard to the IL-18 gene, two SNPs have been recently described in its promoter region: one at position −607 (A/C) and the other at position −137 (G/C). These two SNPs seem to modulate gene expression at transcriptional level: alterations of the H4TF-1 nuclear factor binding site or of the cAMP-responsible element binding site were described when a change at position −137 from G to C or a substitution C to A at position −607, respectively, occurs. A tight LD exists between these SNPs, thus only three (GC, GA and CA) out of the four possible combinations were observed in healthy individuals. Studies on the effects of these polymorphisms on promoter activity discovered that, after stimulation in vitro, GC and GA haplotypes are associated with increased transcription of the IL-18 gene [16].

The assessment of possible correlations between these genotypic data and the clinicopathologic parameters of UCNT patients may allow the identification of new prognostic factors for these patients. On these grounds, we have investigated the promoter polymorphisms of IL-10 and IL-18 genes and searched for possible correlations with the EBV DNA load and clinical characteristics in an Italian cohort of UCNT patients.

Materials and methods

Cases and controls

Eighty-nine consecutive patients (60 native of and resident in northern Italy and 29 native of and resident in southern Italy) with histologically confirmed undifferentiated carcinoma of nasopharyngeal type (UCNT) were enrolled in this study. UCNT classification and staging evaluation was performed on the basis of the simplified World Health Organization (WHO) classification for nasopharyngeal carcinoma [3, 33]. One hundred and thirty controls (80 native of and resident in northern Italy and 50 native of and resident in southern Italy) randomly selected from the general healthy population were recruited.

All patients agreed to participate in the study by signing an informed consent approved by the Ethics Committee of our Institute.

Analysis of IL-10 gene promoter polymorphisms

Blood samples from healthy donors and UCNT patients at the time of diagnosis, were collected. Peripheral blood

mononuclear cells (PBMCs) were isolated by Ficoll/ Hypaque density gradient centrifugation and cryopreserved as dry pellets at −80°C together with serum samples.

Genomic DNA was extracted by standard techniques [9] and its concentration was determined by spectrophotometry.

For the determination of single allelic polymorphisms (SNPs) in the IL-10 promoter gene at positions −1082, −819 and −592 from the transcription start site, the 5′ flanking region containing all three SNPs, spanning from 8176 to 8920 nucleotide of the IL-10 gene (GenBank accession number X78437), was amplified by PCR and analyzed by sequencing. PCR reactions were carried out in 50 μl volumes using AmpliTaq Polymerase buffer with 1 mM $MgCl_2$ final concentration (PE Applied Biosystems, Foster City, CA, USA), 200 μM dNTPs, 0.5 μM each forward and reverse amplification primer and 1.5 units of AmpliTaq Polymerase (PE Applied Biosystems). Conditions used were: a denaturing step of 94°C for 3 min, 35 cycles at 94°C for 20 s, 55°C for 20 s, 72°C for 30 s and a final incubation at 72°C for 7 min.

Sequence of forward and reverse primers used in PCR amplification and in sequencing analysis of the 745 bp target are given in Table 1. Primer pIL10_8920R was designed using Primer Express Version 2.0 software (Applied Biosystem) and all the other primers were selected from previously published studies, with minor modifications [1, 21, 25].

Amplified products were monitored by electrophoresis on 8% non–denaturing polyacrylamide mini-gels and staining with ethidium bromide (10 mg/l).

Two to three nanograms of PCR product, purified by "MICROCON 100" column (Amicon), were used for cycle sequencing with Big Dye Terminator chemistry (PE Biosystems, Foster City, CA, USA). Sequencing reactions were carried out in 10 μl final volume using either the forward or reverse primer, as used for the amplification, or internal primers as described in Table 1. In this way, all three SNPs were detected and confirmed in overlapping sequencing reactions. Cycle sequencing was performed as follows: 40 cycles at: 95°C for 30 s, 56°C for 30 s, 60°C for 4 min, 1 step at 72°C for 1 min and a final incubation at 4°C for 10 min. DNA sequence analysis was run on the ABI PRISM 310 Genetic Analyzer automated sequencer (PE Applied Biosystems) and collected data were automatically analyzed by the Sequencing analysis version 2.0 software.

Table 1 Nucleotide sequences of primers used to characterize IL-10 and IL-18 SNPs

Primer	Sequence (5′-3′)
pIL10_8176F[a]	AATCCAAGACAACACTACTAAGGCT
pIL10_8920R[a,b]	CGTGGACAAATTGCCCATT
pIL10_revp1[b]	TCTAAAGTTTAAAAGATGGGGTGGA
pIL10_819F[b]	TCTAAGGCCAATTTAATCCAAG
pIL10_819R[b]	TGTCCCCCACCCCAACTGTG
pIL10_592R[b]	TAACTTAGGCAGTCACCTTAGG
pIL18_607CNT	CTTTGCTATCATTCCAGGAA
pIL18_607A	GTTGCAGAAAGTGTAAAAATTATTA A
pIL18_607C	GTTGCAGAAAGTGTAAAAATTATTA C
pIL18_607R	TAACCTCATTCAGGACTTCC
pIL18_137CNT	CCAATAGGACTGATTATTCCGCA
pIL18_137C	CCCCAACTTTTACGGAAGAAAA C
pIL18_137G	CCCCAACTTTTACGGAAGAAAA G
pIL18_137R	AGGAGGGCAAAATGCACTGG

[a] Primers used to amplify the 745 bp fragment containing the three SNPs of IL-10 promoter region
[b] Primers used to sequence the 745 bp fragment containing the three SNPs of IL-10 promoter region

Analysis of IL-18 gene promoter polymorphisms

The SNPs at position −607(C/A) and −137 (G/C), from the transcription start site, in the IL-18 promoter gene, were determined by allelic- specific PCR (AS-PCR) as previously described [16].

Two reactions were performed to detect each of the two polymorphisms, carrying a total of four PCR amplifications for every sample. For the characterization of position −607, each PCR reaction was performed using a common antisense primer (pIL18_607R, Table 1) in combination with a sequence specific sense primer (pIL18_607A or pIL18_607C, Table 1). An amplification product of 196 bp was detected. As internal positive amplification control, a control sense primer (pIL18_607CNT, Table 1) was included to amplify a 301 bp fragment covering the polymorphic site.

PCR reactions were carried out in 50 μl volumes using AmpliTaq Polymerase buffer with 1.1 mM $MgCl_2$ final concentration (PE Applied Biosystems), 0.4 μM of one sequence-specific primer, common antisense primer and control sense primer, 200 μM each dNTP, 1.75 units of AmpliTaq Polymerase (PE Applied Biosystems) and 100 ng genomic DNA. The following cycle conditions were used: 94°C 2 min; 7 cycles of denaturation at 94°C for 20 s, annealing at 64°C for 40 s and extension at 72°C for 40 s, 25 cycles of denaturation at 94°C for 20 s, annealing at 57°C for 40 s and extension at 72°C for 40 s; finally 72°C 7 for min.

For the characterization of position −137, each PCR reaction was performed using a common antisense primer (pIL18_137R, Table 1) in combination with a sequence specific sense primer (pIL18_137C or pIL18_137G, Table 1). An amplification product of 261 bp was detected. As internal positive amplification control, a control sense primer (pIL18_137CNT, Table 1) was included to amplify a 446 bp fragment covering the polymorphic site.

PCR reactions were carried out in 50 μl volumes using AmpliTaq Polymerase buffer with 1.5 mM $MgCl_2$ final concentration (PE Applied Biosystems, Foster City, CA), 0.5 μM of one sequence-specific primer and common antisense primer, 0.46 μM of control sense primer, 260 μM each dNTP, 1.75 units of AmpliTaq Polymerase (PE Applied Biosystems) and 100 ng genomic DNA

The following cycle conditions were used: 94°C 2 min; 5 cycles of denaturation at 94°C for 20 s,

annealing and extension at 68°C for 60 s, 25 cycles of denaturation at 94°C for 20 s, annealing at 62°C for 20 s and extension at 72°C for 40 s; finally 72°C 7 for min.

All PCR products were separated on 8% non-denaturing polyacrylamide mini-gels stained with ethidium bromide.

To confirm our results, PCR products of A and C homozygotes in locus −607 and G and C homozygotes in locus −137 were sequenced on an ABI PRISM 310 Genetic Analyzer automated sequencer (PE Applied Biosystems)

Detection of EBV DNA viremia

Serum samples available from 19 patients from northern Italy at the time of diagnosis (before they started any therapy) were assessed for EBV DNA viral load by QC-PCR as described elsewhere [31]. As controls, serum samples from healthy blood donors were also evaluated and no detectable EBV DNA was found [31].

The samples were first screened by qualitative EBV DNA PCR. Amplifiability was evaluated by co-amplifying DNA sample with an EBV DNA competitor, containing a fragment of the LMP2A unique region of the EBV genome with a 28 bp internal deletion. Quantitative competitive PCR was then performed on samples by adding increasing amounts of the EBV competitive template. The PCR products were resolved on 8% non-denaturing polyacrylamide mini-gels and quantified by densitometric analysis.

Statistical analysis

Statistical analysis for group comparisons was performed using Chi-Square Analysis or Fisher's exact test when appropriate. Odds ratios (ORs) and their corresponding 95% confidence intervals (CI) were estimated using unconditional multiple logistic regression models [5], after including terms for age (in the age group of 10-years-olds), sex and, given the differences of: allele and genotype frequencies between northern and southern Italy [26, 30, 32, 37], for place of birth and place of living.

Haplotype frequencies and Linkage Disequilibrium estimates between the threes SNPs were evaluated by a maximum likelihood method using EH program (35; web Resources for Genetic Linkage analysis [Rockefeller University]).

Statistical analyses were performed using SAS language program (reference version 8.2; SAS Institute Inc. Cary, NC USA)

Results

The frequencies of −1082 G/A −819 C/T and −592 A/C SNPs within the IL-10 gene promoter and −607 C/A and −137 G/C SNPs within the IL–18 gene promoter were determined in 89 patients with UCNT and 130 matched controls. The features of all enrolled individuals are described in Table 2.

The frequency distributions of the SNPs and the combined expression genotypes among UCNT patients and healthy controls are shown in Tables 3 and 4. Within each study group, all the genotypes studied were consistent with those predicted by the Hardy–Weinberg equilibrium, showing a random distribution.

IL-10 promoter polymorphisms in UCNT patients

The prevalence of each IL-10 genotype found in healthy controls was in agreement with data previously reported [32]. Furthermore, the differences in the distribution of IL-10 genetic variants among patients and local controls were not statistically significant (Table 3).

In agreement with previous findings [13, 36], our samples also showed complete LD of the SNP at position −819 with the −592 SNP, indicating that they are completely dependent from each other. Therefore, the frequency of each allele was identical between these two genetic sites (Table 3).

IL-18 promoter polymorphisms in UCNT patients

The allele frequencies for the two IL-18 promoter SNPs detected in our control group were similar to those reported from other Caucasian populations [16, 24]. Subjects carrying −607 AA genotype at position −607 showed a nearly statistically significant increased risk of UCNT (OR = 1.4 95% CI: 0.9–3.3). Allelic frequencies and genotype rates of the −137 SNPs did not show any

Table 2 Baseline characteristics of UCNT patients and healthy controls from northern and southern Italy according to selected demographic and clinical characteristics

	UCNT $n = 89$, n (%)	CONTROLS $n = 130$, n (%)
Gender		
Male	70 (78.7)	100 (76.9)
Female	19 (21.3)	30 (23.1)
Age (Years)[a]		
< 50	45 (51.7)	97 (74.6)
≥50	42 (48.3)	33 (25.4)
Residential diagnosis		
Northern	60 (67.4)	80 (61.5)
Southern	29 (32.6)	50 (38.5)
Lymph node metastasis[a]		
Positive	67 (80.7)	–
Negative	6 (19.3)	–
Tumor size[a]		
T1	14 (17.1)	–
T2	42 (51.2)	–
T3	13 (15.9)	–
T4	13 (15.9)	–

[a] The sum does not add up to the total because of a few missing values

Table 3 Odds Ratios (OR)[a] and corresponding 95% confidence intervals (95% CI) of UCNT according to frequency of IL-10 and IL-18 SNPs in the Italian population

Position	Genotype	UCNT $n=89$, n (%)	Controls $n=130$, n (%)	OR (95% CI)
IL-10				
−1082	A/A	29 (32.6)	46 (35.4)	1[b]
	A/G	41 (46.1)	58 (44.6)	1.1 (0.7–2.8)
	G/G	19 (21.3)	26 (20.0)	1.1 (0.8–2.8)
−819	C/C	48 (53.9)	70 (53.8)	1[b]
	C/T	36 (40.4)	54 (41.5)	1.0 (0.5–3.1)
	T/T	5 (5.6)	6 (4.6)	1.2 (0.5–3.4)
−592	C/C	48 (53.9)	70 (53.8)	1[b]
	C/A	36 (40.4)	54 (41.5)	1.0 (0.5–3.1)
	A/A	5 (5.6)	6 (4.6)	1.2 (0.5–3.4)
IL-18				
−607	C/C	26 (29.2)	43 (33.1)	1[b]
	A/C	42 (47.2)	64 (49.2)	1.0 (0.7–2.6)
	A/A	21 (23.6)	23 (17.7)	1.4 (0.9–3.3)
−137	G/G	43 (48.3)	72 (55.4)	1[b]
	G/C	39 (43.8)	53 (40.8)	1.2 (0.5–3.0)
	C/C	7 (7.9)	5 (3.8)	2.1 (0.4–4.3)

[a] Estimates from multiple logistic regression models including terms for age, gender and population's origin

[b] Reference category

difference between UCNT patients and local controls (Table 3).

Complete LD of allele −137 C with allele −607A and the presence of three haplotypes (CG, AC and AG) were characteristic of samples from our population, as also observed in other reports [7]. No statistically significant difference was observed between UCNT cases and controls with regard to all of the estimated haplotypes frequencies (Table 4).

IL-10 gene polymorphisms and EBV DNA viremia

To identify a possible association between the genetically determined capacity for IL-10 production and EBV viremia at diagnosis of UCNT, we assessed EBV DNA load by QC-PCR in the serum of 19 patients. The single IL-10 gene variants and the expression genotypes among these patients, stratified according to EBV DNA viremia, were then compared.

Table 4 IL-10 and IL-18 expression genotypes in UCNT patients and healthy controls in the Italian population

Cytokine	Genotype	UCNT $n=89$, n(%)	Controls $n=130$, n(%)
IL-10[a]			
	GCC/GCC	19 (21.3)	26 (20.0)
	GCC/ACC	19 (21.3)	27 (20.8)
	GCC/ATA	22 (24.7)	31 (23.8)
	ACC/ACC	10 (11.2)	17 (13.1)
	ACC/ATA	14 (15.7)	23 (17.7)
	ATA/ATA	5 (5.6)	6 (4.6)
IL-18[b]			
	AA/CC	7 (7.9)	5 (3.8)
	AA/GC	12 (13.5)	11 (8.5)
	AA/GG	2 (2.2)	7 (5.4)
	AC/GG	15 (16.9)	22 (16.9
	AC/GC	27 (30.3)	42 (32.3)
	CC/GG	26 (29.2)	43 (33.1)

[a] $X_5^2 = 0.81$ p = 0.98

[b] $X_5^2 = 4.41$ p = 0.49

Interestingly, none of the UCNT patients with undetectable EBV DNA viremia carried the genotype A/A, when compared to patients EBV DNA-positive or healthy controls. Moreover, the frequency of IL-10 −1082G allele, which is associated with an high IL-10 expression, showed a nearly statistically significant increase in UCNT patients EBV DNA-negative as compared to healthy controls (Table 5).

Any correlation was found between the different IL-10 promoter polymorphisms evaluated and the TNM or WHO stage (data not shown).

IL-18 gene polymorphisms and overall TNM stage

An association between IL-18 promoter polymorphisms and different clinical stage of disease at diagnosis was found. Subjects with C/C or C/G combined genotypes at −137 site showed an increased risk of being with Stages III-IV (OR = 2.1 95% CI: 1.2–6.6) (Table 6). In addition, subjects with the A allele at −607 site had an increased risk, even not statistically significant, of being with Stages III-IV (OR = 1.4 95% CI: 0.7–3.7).

Discussion

UCNT patients characteristically show a marked leukocyte infiltration in tumor tissue. The mechanisms involved in this process are still poorly understood, but there is increasing evidence that the severity and persistence of the local inflammatory response may be related to an imbalance in the release of pro- and anti-inflammatory cytokines at the site of tissue injury. In particular, an increased production of IL-10 and IL-18, two cytokines with different immunological activity, was observed and their possible involvement in the tumor growth was also hypothesized [14, 15, 22].

Table 5 Odds Ratios (OR)[a] and corresponding 95% confidence intervals (95% CI) of UCNT according to distribution of –1082 genotypes and alleles among EBV DNA-positive and -negative patients with UCNT (northern Italian population)

SNP, −1082	Control, *n* = 80, *n* (%)	UCNT EBV-positive, *n* = 10, *n* (%)	OR (95% CI)	UCNT EBV-negative, *n* = 9, *n* (%)	OR (95% CI)
Genotype					
G/G	13 (16.3)	2 (20.0)	1[b]	4 (44.4)	1[b]
G/A	33 (41.2)	4 (40.0)	0.8 (0.1–4.9)	5 (55.6)	0.6 (0.1–2.2)
A/A	34 (42.5)	4 (40.0)	0.8 (0.1–4.8)	0 (–)	–
Allele					
A	101 (63.1)	12 (60.0)	1[b]	5 (27.8)	1[b]
G	59 (35.3)	8 (40.0)	1.1 (0.3–3.4)	13(72.2)	3.3 (1.2–9.8)

[a] Estimates from multiple logistic regression models including terms for age, gender
[b] Reference category

Although the complex mechanisms of cytokine production remain largely undetermined, host genetic factors are believed to play an important role.

We, therefore, investigated functionally relevant genetic variants in the IL-10 and IL-18 promoter region as possible markers of genetic susceptibility and clinical outcome of UCNT. In this study, we characterized, by genotypic analysis of three IL-10 SNPs and two IL-18 SNPs, 89 Italian UCNT patients and 130 healthy controls.

Our data disclosed findings of some interest regarding the role of immunogenetic factors in UCNT. First, similar genetic frequencies of the three IL-10 gene polymorphisms were found among UCNT patients and healthy donors in both geographic areas. Furthermore, in case-to-case comparison by stage of the disease, no-correlation between IL-10 genetic variants and tumor features was found (data not shown). These data seem to exclude a role for IL-10 gene polymorphisms alone as a predisposing factor for the development of disease and its severity.

Secondly, despite the likely association between IL-10 and severity of EBV infection, the UCNT patients (from northern Italy) whose serum was EBV DNA-negative, had a borderline statistically significant increase in frequency of IL-10 –1082G allele. This result could be confounded by the presence of an association between EBV viremia and the stage at diagnosis. However, within our data, such association was not confirmed. Moreover, the previous borderline association found between IL-10 polymorphism and EBV DNA viremia, although conducted in a small number of patients, is in keeping with the other recently published data on gastric cancer [39]. The pleiotropic effects mediated by IL-10 can only partially explain these findings. In fact, it has been established that this cytokine may promote important immunosuppressive effects, including down-regulation of MHC class I and class II expression, inhibiting cytotoxic and the production of pro-inflammatory cytokines, such as IL-1, TNF and IL-6. High IL-10 levels would allow lower production rates of these cytokines which may act as mediators of virus reactivation from latent state [17]. Among the patients carrying G allele, a higher IL-10 production may be hypothesized to down-regulate the EBV-specific CTL response with a related reduced apoptosis with no or limited EBV DNA release from tumor cells. In fact, it has been recently reported that, in UCNT, apoptosis seems to be one of the main mechanisms responsible for the presence of free circulating EBV DNA [8].

Although we could not demonstrate a direct positive association between IL-10 polymorphism and EBV DNA viremia, our results may stimulate further studies on the possible role of a differential production of IL-10 alone or in concert with other cytokines and growth factors, leading to a better understanding of the viral mechanisms involved into UCNT oncogenesis.

With regard to IL-18 polymorphisms, we observed that subjects with AA genotype at −607 position, showed a nearly statistically significant risk of UCNT. Furthermore, patients with C/C or C/G combined genotypes at −137 site were at increased risk of being with advanced stage of disease. This association could be a consequence of a lower IL-18 gene transcription and protein production due to disruption of the H4TF-1 nuclear factor binding site, as a result of the C allele. Because inflammatory cytokines like IL-18 are known to inhibit IL-10

Table 6 Odds Ratios (OR)[a] and corresponding 95% confidence interval (95% CI) of Stage III + IV vs Stage I+II UCNT according to IL-18 polymorphisms in the Italian population

[a] Estimates from multiple logistic regression models including terms for age, gender, and population's origin
[b] Reference category

UCNT				
Variable		STAGE (I-II) N = 30, N (%)	STAGE (III-IV) N = 53, N (%)	OR (95% CI)
	Genotype			
Position –137	G/G	19 (63.3)	23 (43.3)	1
	C-	11 (36.7)	30 (56.6)	2.1 (1.2–6.6)
	Allele			
Position –607	C	37 (61.7)	54 (50.9)	1
	A	23 (38.3)	52 (49.1)	1.4 (0.7–3.7)

synthesis in vivo, a reduced production of IL-18 could allow an enhanced IL-10 secretion that seems to represent one of the mechanisms allowing UCNT cells to escape local antitumor immune responses [6, 15, 40].

However, these borderline associations could be due to a linkage disequilibrium between these marker loci and a potential UCNT susceptibility site in the IL-18 gene or nearby gene. Furthermore, the relatively small size of the study indicates that the power to detect any association is low. Thus, the few statistically significant associations found are potential clues for further investigation.

In conclusion, this is the first study investigating the possible role of IL-18 and IL-10 polymorphisms in affecting the development and clinical outcome of UCNT. In our genetic analysis there is no evidence for an involvement of IL-10 promoter polymorphisms alone in the genetic predisposition to this tumor. On the other hand, IL18 genetic variants may represent a genetic risk factor for tumor aggressiveness.

However, it must be taken into account that our results refer to UCNT patients from a non-endemic area and consequently they are not representative of all UCNT. Consistent with epidemiological studies indicating that the susceptibility genes for UCNT might not be identical in different geographic areas [11], we believe that further and larger studies in different ethnic populations may help to elucidate whether IL-10 and IL-18 polymorphisms may constitute markers of predictive/prognostic value in this EBV- associated malignancies.

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