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MDM2 and CDKN1A gene polymorphisms and risk of Kaposi's sarcoma in African and Caucasian patients

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Abstract

A single-nucleotide polymorphism in the *MDM2* promoter (SNP309; rs2279744) causes elevated transcription of this major negative regulator of p53 in several cancer types. We investigated *MDM2* SNP309 and *CDKN1A* (p21/Waf1/Cip1) codon 31 (rs1801270) polymorphisms in 86 cases of cutaneous Kaposi's sarcoma (KS) from African and Caucasian patients, and 210 healthy controls. A significant increase of the *MDM2* SNP309 T/G genotype was observed among classic KS cases (odds ratio 2.38, 95% confidence interval 1.0–5.5). Frequencies of *CDKN1A* codon 31 genotypes were not significantly different between cases and controls. The results suggest that the *MDM2* SNP309 G allele may act as a susceptibility gene for the development of classic KS in Caucasian patients.

Keywords: *Genetic polymorphisms; MDM2; CDKN1A; Kaposi's sarcoma.*

Introduction

Kaposi's sarcoma (KS) is a virus-associated tumour characterized by four different epidemiological and clinical manifestations (Buonaguro et al. 2003, Pyakurel et al. 2007, Schwartz et al. 2008): (1) classic or sporadic KS, mainly observed in elderly male subjects from Eastern and Mediterranean Europe (Kaposi 1872); (2) endemic KS, predominant in Eastern and Central sub-Saharan Africa (Hutt & Burkitt 1965); (3) epidemic or acquired immune deficiency syndrome (AIDS)-associated KS, the most frequent tumour in human immunodeficiency virus type 1 (HIV-l)-infected patients (Biggar et al. 1985); and (4) iatrogenic KS, seen in drug-related immune-suppressed patients, such as transplant patients (Penn 1983).

The human herpesvirus type 8 (HHV-8), first isolated in AIDS-associated KS, has been identified in virtually 100% of tumour biopsies and defined as the aetiological agent of all forms of KS (Chang et al. 1994, Buonaguro et al. 1996). Seroepidemiological surveys indicated that HHV-8 prevalence is generally high in the areas of high endemicity for classic or endemic KS (Boshoff & Weiss 2001, Dedicoat & Newton 2003). A recent study evaluating the prevalence of HHV-8 in the general female population from nine countries showed important geographical variations with higher prevalence in Nigeria (46%), Vietnam (11.3–15.5%), Colombia (13%) and Costa Rica (11.1%) (de Sanjose et al. 2009). Moreover, cancer registries of sub-Saharan Africa and Colombia reported incidence of >20 cases/100 000 women and of 0.2 cases/100 000 women, respectively, which are much higher compared with incidence of <0.1 cases/100 000 women observed in other areas (Parkin et al. 2010). These lines of evidence indicate that HHV-8 is necessary for the development of KS, but

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given the heterogeneous distribution of the virus, the incidence of KS in different populations and that only a small percentage of HHV-8-seropositive subjects develop KS, other genetic or environmental cofactors are clearly necessary for the development of this tumour (Brown et al. 2005, Mbulaiteye & Engels 2006, Whitby et al. 2007, Ruocco et al. 2010).

HHV-8, similarly to other DNA tumour viruses, expresses viral genes that directly or indirectly perturb p53 functions and thereby mediate viral oncogenesis (Si & Robertson 2006). The p53 protein plays a central role in cell cycle control for its ability to induce cell cycle arrest and DNA repair, or senescence and apoptosis in response to a variety of stimuli such as stress signals, genotoxic agents, hypoxia and oncogene activation (Jin & Levine 2001). The key function of p53 in oncogenesis as tumour suppressor protein is supported by the fact that *TP53* is the most frequently mutated gene in a variety of human cancers of diverse histological type (Petitjean et al. 2007). The HHV-8 virus interferes with the p53 pathway at several levels: (1) the latency-associated nuclear antigen (LANA), encoded by open reading frame (ORF) 73 of the HHV-8 genome, suppresses p53 transcription and transactivation activity, and interacts directly with the p53 protein inhibiting the ability of p53 to induce cell death (Friborg et al. 1999, Si & Robertson 2006); (2) the viral interferon regulatory factor 4 (vIRF4), encoded by ORFK10/K10.1 of HHV-8, specifically interacts with and stabilizes the MDM2 human homologue, a well-known negative regulator of p53 via proteasome-mediated degradation, leading to the consequent reduction of p53 levels and thereby contributing to the suppression of p53-mediated apoptosis (Rayburn et al. 2005, Lee et al. 2009); (3) the microRNA (miRNA)-K1, one of the 12 viral miRNAs expressed by HHV-8, inhibits the p53-induced growth arrest, targeting the mRNA of the cyclin-dependent kinase inhibitor *CDKN1A* gene (also known as p21/Waf1/CIP1), a downstream p53 effector (el-Deiry et al. 1993, Gottwein & Cullen 2010).

The relevance of the vIRF4 and miRNA-K1 interference with MDM2 and CDKN1A proteins is strongly supported by their relevance in cell cycle control.

A number of studies have shown that MDM2 is overexpressed in several human cancers (Leach et al. 1993, Freedman & Levine 1999). The higher expression levels of MDM2 are mutually exclusive in respect to p53 mutations suggesting that they may substitute for mutational inactivation of p53 (Bond et al. 2004, Arva et al. 2005). A naturally occurring G to T sequence variation (single nucleotide polymorphism – SNP309) in the second promoter–enhancer region of the *MDM2* gene has been shown to increase the binding affinity of the transcriptional activator Sp1 resulting in high levels of MDM2 protein, formation of transcriptionally inactive p53–MDM2 complexes and alteration of the p53 pathway (Bond et al. 2004, Bougeard et al. 2006, Ruijs et al. 2007). These observations are consistent with an oncogenic function for the variant SNP309. The *MDM2* SNP309 polymorphism has been reported to be associated with earlier onset of breast cancer in Li-Fraumeni patients (Bond et al. 2006, Lind et al. 2006, Menin et al. 2006) and with earlier onset of soft tissue sarcoma, diffuse large B-cell lymphoma, colorectal cancer and non-small-cell lung cancer, particularly in women (Hu et al. 2010).

The *CDKN1A* gene exhibits two polymorphisms putatively associated with cancer risk: one is a common SNP at nucleotide position 98 in exon 2 (C to A change), which causes a non-synonymous serine-to-arginine substitution at codon 31; another polymorphism is located at nucleotide position 70 (C to T change) within the 3′ untranslated region, downstream of exon 3 stop codon. Several epidemiological studies found that *CDKN1A* codon 31 polymorphism was associated with increased risk of cervical cancer (Roh et al 2001), breast cancer(Keshava et al. 2002, Powell et al. 2002, Knappskog et al. 2007), and oesophageal cancer (Wu et al., 2003).

To our knowledge no studies have been performed on the role of *MDM2* and/or *CDKN1A* polymorphisms in the risk of KS development. In the present study, we evaluated the distribution of the *MDM2* SNP309 and *CDKN1A* codon 31 genotypes in a series of 86 KS, comprising classic, iatrogenic, endemic and epidemic KS, obtained from African patients (Uganda, Kenya and Cameroon) and Caucasian patients (Greece, Italy and USA), and compared them with healthy controls from the respective populations in order to possibly identify host genetic factors associated with different clinical types of KS.

Materials and methods

Study population and samples

This study included 76 liquid nitrogen frozen tissue specimens and ten paraffin-embedded biopsies of KS skin lesions. African samples were from the University Centre for Health Sciences, Yaounde, Cameroon ($n=3$ endemic), from the Pathology Department of Nairobi University in Kenya ($n=6$ epidemic) and from Mulago Hospital and Makerere University in Kampala, Uganda ($n=17$ endemic and $n=4$ epidemic). European cases were from the Pathology Department of Athens University Medical School, Greece ($n=5$ classic and $n=2$ iatrogenic), from the Institutes of Dermatological Sciences, University and IRCCS Ospedale Maggiore of Milan in North Italy ($n=7$ classic and $n=3$ epidemic), from the Department of Science for Health Promotion, Hygiene Section, University of Palermo ($n=10$ classic) and from the Dermatology Department of the Second University of

Naples in South Italy ($n = 16$ classic and $n = 2$ epidemic). North American cases ($n = 6$ classic and $n = 5$ epidemic) were from New York's Sloan-Kettering/Memorial Hospital New York City, USA. DNA from frozen biopsies was extracted by proteinase K treatment (150 μg ml^{-1} at 60°C for 30 min) in lysis buffer (10 mM Tris–HCl, pH 7.6, 5 mM EDTA, 150 mM NaCl, 1% SDS), followed by DNA purification by phenol and phenol–chloroform–isoamyl alcohol (25:24:1) extraction and ethanol precipitation in 0.3 M sodium acetate (pH 4.6). Genomic DNA from 68 cases was previously extracted and subjected to polymerase chain reaction (PCR) amplification of HHV-8 ORF 26, T0.7, K1 and K14.1/15 (Tornesello et al. 2010), in parallel with exon 4 of the *TP53* gene for codon 72 polymorphism analysis (Buonaguro et al. 1996). Eighteen previously uncharacterized tumours from patients undergoing surgical resection from 1991 to 2008 were extracted, analysed for HHV-8 ORF26 sequences (100% positives) and included in this study. A DNA quality test was carried out on all DNA samples by amplification with specific oligoprimers targeting a fragment within exon 7 of the *TP53* gene (Tornesello et al. 2005), and DNA quantity analysis was evaluated by spectrophotometric measurements. All samples were suitable for the gene polymorphism analysis. Population-matched controls were selected among cancer-free Black Ugandan males ($n = 88$), previously analysed for the *TP53* polymorphism (Tornesello et al. 2008), and age-matched Caucasian Italian male blood donors ($n = 122$) who gave their consent to participate in the study during the period 2000–2004. Peripheral blood mononuclear cells (PBMCs) were purified from fresh blood samples by Leucoprep density gradient centrifugation (Higuchi 1989), and cellular lysates (approximately 1×10^6 cells) were prepared by proteinase K digestion at 56°C (Albert and Fenyo 1990).

The study protocol was approved by the local ethical review board of the involved institutions.

MDM2 SNP309 and CDKN1A codon 31 polymorphisms analysis

The *MDM2* SNP309 and *CDKN1A* codon 31 genotypes were determined by PCR and restriction fragment length polymorphism (RFLP) analysis of amplified products. A 174 base pairs (bp) amplimer of the *MDM2* intron 1 region, containing the *Msp*A1 polymorphic site at nucleotide 309, was amplified with the B-MDM2-309F (5′- GGGAGTTCAGGGTAAAGG-3′) and the B-MDM2-309R (5′- GACCAGCTCAAGAGGAAA-3′) oligoprimers, designed using the Beacon Designer 2.0 software (Premier Biosoft International, Palo Alto, CA, USA). PCR reactions were performed in a 50-μl reaction mixture containing 100–300 ng of target DNA, 20 pmol of each primer, 50 mM KCl, 2.5 mM $MgCl_2$, 100 mM Tris–HCl pH 8.3, 0.1% Triton X-100, 50 mM of each dNTP and 1.8 U of thermostable AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA, USA). DNA was amplified in a Perkin-Elmer GeneAmp PCR System 9700 thermal cycler with the following steps: an initial 1-min denaturation at 94°C, followed by 40 cycles of 55°C for 1 min, 72°C for 2 min, 94°C for 30 s and a final annealing at 55°C for 1 min with 5 min elongation at 72°C.

A 225-bp amplimer of the *CDKN1A* gene containing the *Blp*UI polymorphic site at codon 31 was amplified using primer pairs; reaction conditions have been described previously (Hirata et al. 2007). A reaction mixture without genomic DNA was used as a negative control and was included in every set of five clinical specimens (Tornesello et al. 2009). Ten-microlitre aliquots of *MDM2* and *CDKN1A* PCR products were digested with *Msp*A1I and *Blp*UI restriction enzymes, respectively, fractionated by electrophoresis on a 7% polyacrylamide gel in Tris–borate–EDTA running buffer and stained with ethidium bromide for DNA band visualization by UV transillumination. *MDM2* and *CDKN1A* PCR amplified products from ten independent samples were further subjected to direct nucleotide sequencing on both strands by Primm Srl Laboratories (Milan, Italy) using the fluorescent dye terminator technology and ABI 3730 DNA sequencers (Applied BioSystems). Nucleotide sequences were edited with Chromas Lite 2.01 (http://www.technelysium.com.au/chromas.html) and resulting genotypes were 100% concordant to that obtained with RFLP analysis.

Statistical analyses

The observed and expected genotype frequencies among the study groups were analysed using the Hardy–Weinberg equilibrium theory. A Fisher's exact test or χ^2 test was used, as appropriate, to compare the proportions of *MDM2* and *CDKN1A* genotypes between ethnic-matched cases and controls, and between HIV-positive and HIV-negative population-matched KS cases, as well as between HIV-positive KS cases and all respective controls (with unknown HIV status). An unpaired Student's *t*-test was used to evaluate differences between the mean age of cases and control groups. All analyses were performed with Epi Info 6 Statistical Analysis System Software (6.04d, 2001, Centers for Disease Control and Prevention, USA). Differences were considered to be statistically significant when *p*-values were less than 0.05.

Results

This study included a total of 86 cases of cutaneous KS lesions of which 20 cases (all in males) were endemic and ten cases (three in females and seven in males) were epidemic tumour types diagnosed in Black patients from

sub-Saharan African countries; the median age at the diagnosis was 41.5 years (mean 44.8 ± 14.5) for endemic and 29.5 years (mean 29.5 ± 7.8) for epidemic KS patients (p <0.001). The median age in the African control group was 30 years (mean 32.3 ± 11.4). Forty-four cases (10 in females and 34 in males) were classic, two cases (all in males) were iatrogenic tumours and 10 cases (all in males) were epidemic KS diagnosed in Caucasian patients from Europe and North America; the median age was 74 years (mean 71.73 ± 11.3) for classic, 50 years (mean 50 ± 4.2) for iatrogenic and 40.5 years (mean 443 ± 12.7) for AIDS-associated KS (p <0.0001). The median age in the Caucasian control group was 45.3 years (mean 48.4 ± 10.1). All KS samples were positive for HHV-8 DNA sequences.

The *MDM2* SNP309 and *CDKN1A* codon 31 genotypes were analysed using a PCR-RFLP based assay and ten samples confirmed through nucleotide sequencing analysis (Figure 1). The distribution of variant alleles in cases and controls are summarized in Table 1. The genotype frequencies of the *MDM2* SNP309 polymorphisms were found in Hardy–Weinberg equilibrium both in African and Caucasian healthy controls ($\chi^2 = 0.84$; df = 1; $p = 0.36$; and $\chi^2 = 2.33$; df = 1; $p = 0.13$, respectively) and in African cases ($\chi^2 = 3.58$; df = 1; $p = 0.06$) but not among Caucasian cases ($\chi^2 = 4.07$; df = 1; $p = 0.04$). The genotype frequencies of *CDKN1A*

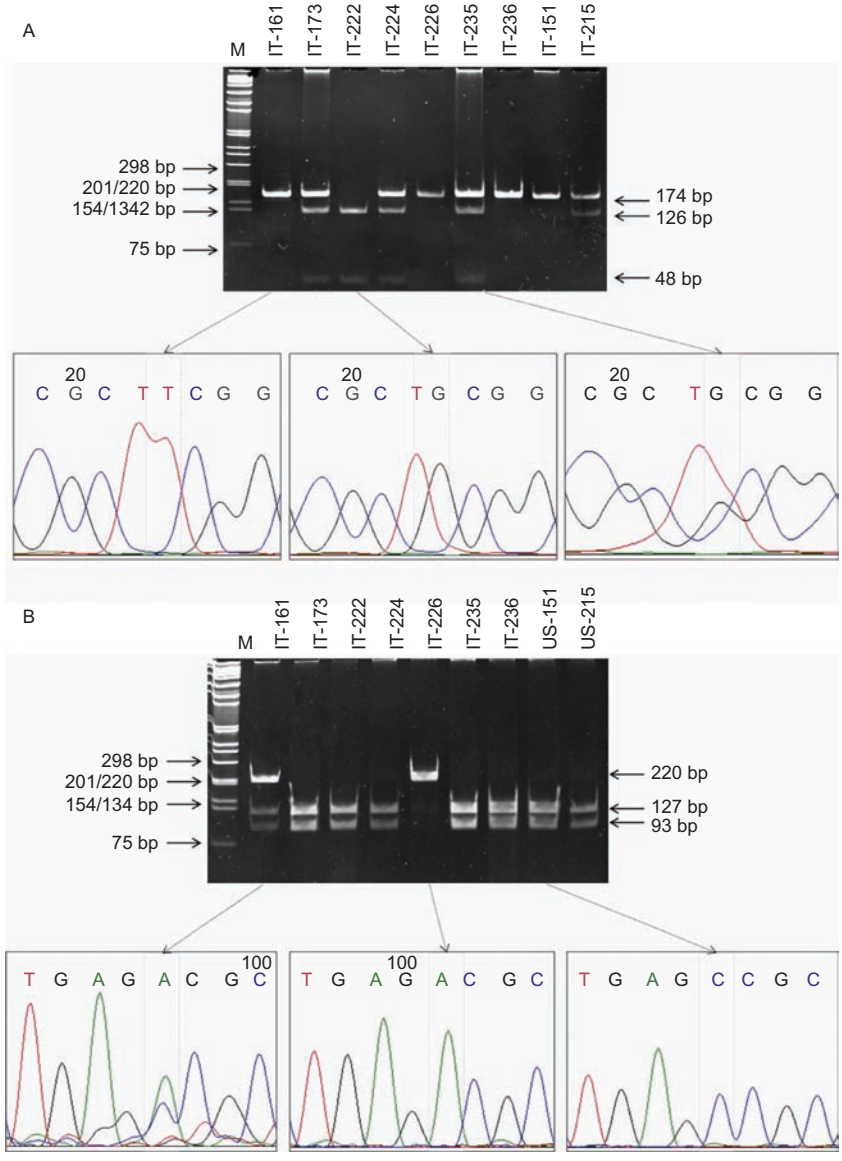

Figure 1. The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analyses and direct-sequence result of *MDM2* SNP309 (A) and *CDKN1A* codon 31 polymorphisms (B). (A) *MDM2* SNP309 T allele was not cleaved by *Msp*AI endonuclease and had a single band of 174 bp. The *MDM2* SNP309 G allele was cleaved by *Msp*AI and had two small fragments of 126 and 48 bp. The *MDM2* SNP309 heterozygote had three bands of 174, 126 and 48 bp. (B) *CDKN1A* with C at codon 31 is digested with *Blp*I endonuclease yielding 183 and 89 bp bands. The *CDKN1A* with A at codon 31 is not digested by *Blp*I and had a single band of 220 bp. RFLP products of heterozygotes had three bands of 220, 127 and 93 bp.

Table 1. Distribution of *MDM2* SNP309 and *CDKN1A* codon 31 genotypes in African and Caucasian Kaposi's sarcoma (KS) patients (cases) and respective controls.

	African			Caucasian		
	KS cases ($n=30$)n(%)	Controls ($n=88$)n(%)	OR[a] (95% CI)	KS cases ($n=56$)n(%)	Controls ($n=122$)n(%)	OR[a] (95% CI)
MDM2 SNP309						
T Allele	55 (91.7)	156 (88.6)	1	71 (63.4)	154 (63.1)	1
G Allele	5 (8.3)	20 (11.4)	0.71 (0.2–2.1)	41 (36.6)	90 (36.9)	0.99 (0.6–1.6) [a]
T/T	26 (86.7)	70 (79.5)	1	19 (33.9)	55 (45.1)	1
T/G	3 (10.0)	16 (18.2)	0.50 (0.1–2.0)	33 (58.9)	48 (39.3)	1.99 (0.95–4.2)[b]
G/G	1 (3.3)	2 (2.3)	1.35 (0.0–26.8)	4 (7.1)	19 (15.6)	0.61 (0.1–2.2)
G/G + T/G	4 (13.3)	18 (20.4)	0. 06 (0.1–2.1)	37 (66.1)	67 (54.9)	1.01 (0.7–1.6)
CDKN1A codon 31						
C Allele	44 (73.3)	128 (72.7)	1	96 (87.3)	217 (88.9)	1
A Allele	16 (26.7)	48 (27.3)	0.97 (0.5–2.0)	14 (12.7)	27 (11.1)	1.17 (0.6–2.4)
C/C	19 (63.3)	47 (53.4)	1	45 (80.3)	98 (80.3)	1
C/A	6 (20.0)	34 (38.6)	0.44 (0.1–1.3)	8 (14.3)	21 (17.2)	0.83 (0.3–2.2)
A/A	5 (16.7)	7 (7.9)	0.73 (0.2–2.5)	3 (5.3)	3 (2.5)	2.18 (0.3–16.8)
A/A + C/A	11 (36.7)	41 (46.6)	0.66 (0.3–1.7)	11 (19.6)	24 (19.7)	1.02 (0.4–2.4)

[a]T allele or T/T *MDM2* SNP309 and C allele or C/C *CDKN1A* codon 31 genotypes were considered as the baseline when calculating the relative crude odds ratios (ORs). [b]$p=0.0479$. CI, confidence interval.

codon 31 were consistent with the Hardy–Weinberg equilibrium in African and Caucasian controls ($\chi^2 = 0.06$; df = 1; $p = 0.81$; and $\chi^2 = 1.92$; df = 1; $p = 0.17$, respectively), and slightly deviated from the equilibrium in African and Caucasian cases ($\chi^2 = 7.16$; df = 1; $p = 0.007$; and $\chi^2 = 6.74$; df = 1; $p = 0.009$, respectively).

The frequency of *MDM2* SNP309 T/T, T/G and G/G genotypes among the 30 African cases were 86.7% ($n=26$), 10% ($n=3$) and 3.3% ($n=1$), and the corresponding figures among the 88 Ugandan controls were 79.5% ($n=70$), 18.2% ($n=16$) and 2.3% ($n=2$), respectively. The distribution of the genotypes among the 56 Caucasian cases was 33.9% ($n=19$), 58.9% ($n=33$) and 7.1% ($n=4$) and among controls it was 45.1 % ($n=55$), 39.3% ($n=48$) and 15.6% ($n=19$), respectively. A significant increase in the *MDM2* SNP309 T/G genotype was observed only in Caucasian KS patients (58.9%) compared with respective controls (39.3%), $p=0.048$.

The frequencies of *CDKN1A* codon 31 genotypes (C/C, C/A and A/A) among the African cases were 63.3% ($n=19$), 20.0% ($n=6$) and 16.7% ($n=5$), and among the 88 Ugandan controls were 53.4% ($n=47$), 38.6% ($n=34$) and 7.9% ($n=7$), respectively. The corresponding genotypes among the 56 Caucasian cases were 80.3% ($n=45$), 14.3% ($n=8$) and 5.3% ($n=3$), and among controls they were 80.3% ($n=98$), 17.2% ($n=21$) and 2.5% ($n=3$), respectively.

Crude odds ratios (OR) and 95% confidence intervals (CI) were used to evaluate the association between the *MDM2* SNP309 and *CDKN1A* codon 31 genotypes and the risk for KS (Table 1). Overall, there was no significant increase in the risk of KS associated with the *MDM2* SNP309 G/G compared with T/T genotype (OR 1.35, 95% CI 0.0–26.8, Africans; OR 0.61, 95% CI 0.1–2.2, Caucasians). A higher frequency of *MDM2* SNP309 T/G genotype was observed among Caucasian KS cases compared with controls (OR 1.99, 95% CI 0.95–4.2). The *MDM2* SNP309 T/G genotype was over-represented particularly in the group of classic KS patients (OR 2.38, 95% CI 1.0–5.5) (Table 2). *CDKN1A* codon 31 allele and genotype frequencies did not differ between African and Caucasian cases and respective controls, and ORs did not differ according to KS type (Table 1). ORs remained unchanged following age adjustment. Furthermore, there was no difference in the distribution of *MDM2* and *CDKN1A* genotypes neither between HIV-positive (epidemic KS) and HIV-negative (classic and endemic KS) case subjects nor between HIV-positive Caucasian cases and respective controls.

Sixty-seven out of the 86 KS included in the present study, along with relative controls, were previously analysed for codon 72 polymorphism of the *TP53* gene and no association was found between cancer risk and specific *TP53* genotypes (Tornesello et al. 2009). The combined effect of the *TP53* codon72 polymorphism and the *MDM2* SNP309 or *CDKN1A* codon 31 polymorphism was also analysed. However, no additional cancer risk effect associated with the *TP53* codon 72 polymorphism was identified when the risk of combined genotypes was analysed.

Discussion

The HHV-8 is the aetiological agent of KS, as well as of primary effusion lymphoma (PEL) and of multicentric Castleman's disease (MCD) (Boshoff & Weiss 2001). Several viral oncogenic proteins encoded by HHV-8,

Table 2. Distribution of *MDM2* SNP309 and *CDKN1A* codon 31 genotypes in endemic and classic Kaposi's sarcoma (KS) patients (cases) and respective controls.

	Endemic African			Classic Caucasian		
	KS cases ($n=20$) n (%)	Controls ($n=88$) n (%)	OR[a] (95% CI)	KS cases ($n=44$) [b] n (%)	Controls ($n=122$) n (%)	OR[a] (95% CI)
MDM2 SNP309						
T Allele	36 (90.0)	156 (88.6)	1	53 (60.2)	154 (63.1)	1
G Allele	4 (10.0)	20 (11.4)	0.87 (0.2–2.8)	33 (37.5)	90 (36.9)	1.07 (0.6–1.8)
T/T	17 (85.0)	70 (79.5)	1	13 (29.5)	55 (45.1)	1
T/G	2 (10.0)	16 (18.2)	0.51 (0.05–2.56)	27 (61.4)	48 (39.3)	2.38 (1.0–5.5)[c]
G/G	1 (5.0)	2 (2.3)	2.06 (0.03–41.3)	4 (9.1)	19 (15.6)	0.89 (0.2–3.4)
G/G + T/G	3 (15.0)	18 (20.4)	0.69 (0.12–2.8)	30 (70.4)	67 (54.9)	1.89 (0.8–4.2)
CDKN1A codon 31						
C Allele	28 (70.0)	128 (72.7)	1	76 (88.4)	217 (88.9)	1
A Allele	12 (30.0)	48 (27.3)	1.14 (0.5–2.6)	10 (11.6)	27 (11.1)	1.06 (0.5–2.4)
C/C	12 (60.0)	47 (53.4)	1	36 (81.8)	98 (80.3)	1
C/A	4 (20.0)	34 (38.6)	0.46 (0.1–1.7)	6 (13.6)	21 (17.2)	0.78 (0.2–2.2)
A/A	4 (20.0)	7 (7.9)	2.24 (0.4–10.5)	2 (4.5)	3 (2.5)	1.81 (0.2–16.4)
A/A + C/A	8 (40.0)	41 (46.6)	0.76 (0.–2.3)	8 (18.2)	24 (19.7)	0.91 (0.3–2.4)

[a]T allele or T/T *MDM2* SNP309 and C allele or C/C *CDKN1A* codon 31 genotypes were considered as the baseline when calculating the crude odds ratios (ORs). [b]The two iatrogenic KS are combined with the classic KS. [c]$p=0.0252$ (Mantel–Haenszel). CI, confidence interval.

homologues of cellular proteins, are able to deregulate cell proliferation, induce cell transformation, and prevent cell apoptosis interfering directly with p53 or p53-related modulators, particularly with MDM2 and CDKN1A proteins (Lee et al. 2009, Gottwein & Cullen 2010). These complex mechanisms of interaction can substitute for p53 mutations given that the *TP*53 gene is rarely mutated in the HHV8-associated malignancies (Nador et al. 1996, Katano et al. 2001, Petre et al. 2007, Tornesello et al. 2009, Boulanger et al. 2009). Several epidemiological studies have evaluated the association of *MDM2* SNP309 or *CDKN1A* polymorphisms and risk of or survival from several types of cancer (Hu et al. 2007), but none evaluated the association of these polymorphisms in KS.

In the current study the *MDM2* SNP309 and polymorphism of *CDKN1A* at codon 31 were investigated in a series of 86 African and Caucasian patients with classic, iatrogenic, endemic and AIDS-associated KS, all positive for HHV-8 DNA (Tornesello et al. 2010), and 210 population-matched controls, in order to verify the impact of these gene variants on the risk of tumour development. The *MDM2* SNP309 and *CDKN1A* codon 31 genotypes were distributed in accordance with the Hardy–Weinberg equilibrium in controls and their frequencies were in broad agreement with those in previously published studies of Caucasian and African subjects (Birgander et al. 1996, Menin et al. 2006, Hu et al. 2007, Capasso et al. 2010). The results showed no significant differences in *MDM2* SNP309 or *CDKN1A* codon 31 allele distribution between cases and controls, and no increase in the frequency of the *MDM2* SNP309 G/G and *CDKN1A* codon 31 A/A or C/A genotypes among KS cases. However, the heterozygous *MDM2* SNP309 T/G genotype was significantly increased among Caucasian classic KS cases ($p=0.0252$). The homozygous *MDM2* SNP309 G/G genotype in classic KS, on the other hand, was lower (9.1%) than that observed in controls (15.6%). The decreased frequency of *MDM2* SNP309 G/G genotype in cutaneous KS patients could have several explanations including that G/G carriers with HHV-8 infection could be at increased risk of developing visceral KS, or highly aggressive HHV-8-related lymphoproliferative disorders such as PEL or MCD (Gaidano et al. 1999). Boulanger et al. have recently reported the analysis of seven PEL cell lines for mutations and SNPs in ten genes involved in apoptosis and cell cycle regulation including SNP309 in *MDM2* and codon 31 in *CDKN1A* genes (Boulanger et al. 2009). Interestingly, three (42.8%) Epstein–Barr virus (EBV)-negative cell lines, namely BC3, BCBL-1 and BCP, were homozygous for SNP309 G suggesting a major role of this polymorphic allele in cell transformation particularly in the absence of EBV co-infection. Future research, however, is needed in order to address this hypothesis accurately.

The proper regulation of MDM2 levels has been shown to be critical for p53 tumour suppression, and even a modest change in levels could affect the p53 pathway and, subsequently, increase the risk of cancer development in mouse models (Mendrysa et al. 2003). MDM2 binds directly to and inhibits p53 by regulating its location, stability and ability to activate transcription (Bond et al. 2004). HHV-8 vIRF4, on the other hand, interacting directly with MDM2, leads to specific suppression of MDM2 ubiquitination and to the consequent further

enhancement of p53 degradation (Lee et al. 2009). This implies that *MDM2* polymorphism together with HHV-8 vIRF4 action may result in a potentiated mechanism of p53 inactivation.

The study by Bond et al. (2004) showed that MDM2 protein levels in homozygous and heterozygous SNP309 G cell lines were on average 4-fold and 1.9 fold higher, respectively, higher than TT cell lines. Furthermore, Hirata et al. reported that the renal cell carcinoma tissue from G/G and G/T carriers was more frequently positively stained for MDM2 than that with the TT genotype (50% and 26%, respectively, versus 13%) (Hu et al. 2007). A meta-analysis of 25 published case–control studies found that *MDM2* 309 G/G associates with a significantly increased risk of all types of cancers (OR 1.17; 95% CI 1.04–1.33) (Hirata et al. 2007). In both patients with hereditary Li-Fraumeni syndrome and with adult sporadic soft tissue sarcoma, the presence of one SNP309 G-allele accelerated tumour formation as a rate-limiting event, and in tumour-prone Li-Fraumeni syndrome individuals, SNP309 can cause the occurrence of multiple primary tumours over the course of a lifetime (Bond et al. 2004).

Few studies have investigated the role of *MDM2* SNP309 polymorphism in virus-associated cancers. Yoon et al. (2008) found the *MDM2* SNP309 and p53 Arg72Pro associated with the early development of hepatocellular carcinoma in Korean patients with chronic hepatitis B virus infection (Yoon et al. 2008). Conversely, Hu et al. (2010) found no association between *MDM2* SNP309 and risk of human papillomavirus-related cervical cancer.

The p21, one of major effectors of p53, negatively controls cell proliferation by inhibiting several cyclin-dependent kinase complexes. In response to DNA damage, p53 upregulates p21, resulting in G1 phase cell cycle arrest. The loss of p53 function leads to the attenuation of p21 expression, although the p53-independent pathway also regulates p21 expression (Xi et al. 2004). Although p21 mutation is rare in tumour cell lines, SNPs of p21 are thought to change its function and the p21 codon 31 SNP has been reported to be associated with several cancers. However, in this study, the p21 codon 31 polymorphism was not significantly associated with risk for any form of KS. Gottwein & Cullen (2010) have recently shown that HHV-8 miR-K1 specifically targets cellular mRNAs encoded by the *CDKN1A* gene inhibiting the expression of the endogenous p21 in HHV-8-negative cells and resulting in strong attenuation of the cell cycle arrest. The interaction of HHV-8 elements at RNA level more than at protein level is in line with the absence of a polymorphic changes at protein level.

Studies in other cancers have reported a combined effect of both *MDM2* and p53 polymorphisms on cancer risk and survival (Hong et al. 2005, Zhang et al. 2006, Hirata et al. 2007). In this study, we found no association with risk of KS when the genotypes of the two SNPs were combined. The effect of *TP53* codon 72 polymorphism in human cancer remains controversial; only one study has been done reporting no association between p53 arginine homozygous genotype at codon 72 and risk of KS (Tornesello et al. 2009).

In conclusion, these results provide the first evidence that the *MDM2* SNP309 polymorphism may be a low penetrance gene risk factor for classic KS but not for endemic and epidemic KS. Conversely, the *CDKN1A* polymorphism at codon 31 is not a potential risk factor for development of KS in subjects of either African or Caucasian ethnicity.

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