Associations of Classic Kaposi Sarcoma with Common Variants in Genes That Modulate Host Immunity

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Abstract

Classic Kaposi sarcoma (CKS) is an inflammatory-mediated neoplasm primarily caused by Kaposi sarcoma–associated herpesvirus (KSHV). Kaposi sarcoma lesions are characterized, in part, by the presence of proinflammatory cytokines and growth factors thought to regulate KSHV replication and CKS pathogenesis. Using genomic DNA extracted from 133 CKS cases and 172 KSHV-latent nuclear antigen-positive, population-based controls in Italy without HIV infection, we examined the risk of CKS associated with 28 common genetic variants in 14 immune-modulating genes. Haplotypes were estimated for IL1A, IL1B, IL4, IL8, IL8RB, IL10, IL12A, IL13, and TNF. Compared with controls, CKS risk was decreased with 123ST/–1010G–containing diplotype of IL8RB (odds ratio, 0.49; 95% confidence interval, 0.30-0.78; P = 0.003), whereas risk was increased with diplotype of IL13 containing the promoter region variant 98A (rs20541, alias +130); odds ratio, 1.88; 95% confidence interval, 1.15-3.08; P = 0.01) when considered in multivariate analysis. Risk estimates did not substantially vary by age, sex, incident disease, or disease burden. Our data provide preliminary evidence for variants in immune-modulating genes that could influence the risk of CKS. Among KSHV-seropositive Italians, CKS risk was associated with diplotype of IL8RB and IL13, supporting laboratory evidence of immune-mediated pathogenesis. (Cancer Epidemiol Biomarkers Prev 2006;15(5):926–34)

Introduction

Kaposi sarcoma is an angioproliferative neoplasm caused primarily by infection with Kaposi sarcoma–associated herpesvirus (KSHV; refs. 1, 2). Four distinct clinical forms are described, including classic (3), endemic (4), iatrogenic (5), and AIDS-associated (6) forms, each following an incidence pattern that parallels KSHV seroprevalence (7). Histologically, Kaposi sarcoma lesions are similar for the four distinct clinical forms and are characterized by the presence of spindle-shaped tumor cells of vascular endothelial origin in addition to heterogeneous endothelial, fibroblast, and dermal dendritic cell populations and infiltrating inflammatory leukocytes (8-10).

The inflammatory infiltrate, composed of activated CD8+ T cells, macrophages, and monocytes (11-14), is recruited to early-stage Kaposi sarcoma lesions (15, 16) and is capable of producing a variety of proinflammatory cytokines [IFN-γ, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and IL-6; refs. 17-26], chemotactic molecules (IL-8 and IL-8Rβ), and growth factors [basic fibroblast growth factor and vascular endothelial growth factor (VEGF); refs. 27, 28]. These inflammatory molecules have been detected in Kaposi sarcoma tissue (29) and have been shown to support the growth of Kaposi sarcoma–derived cells in vitro (30-32). In addition, evidence suggests that high levels of IFN-γ induce endothelial cells to acquire the Kaposi sarcoma tumor cell phenotype (33) and that IL-6, IL-8, basic fibroblast growth factor, and VEGF enhance angiogenesis and spindle cell proliferation in culture (22, 34-38). Proinflammatory cytokines, chemotactic molecules, and angiogenic factors are tightly regulated and together promote the formation and progression of Kaposi sarcoma lesions by altering inflammation, angiogenesis, and growth of the Kaposi sarcoma spindle cell phenotype (39).
Clinical and experimental evidence suggests that proinflammatory cytokines, chemotactic agonists, and angiogenic factors contribute to both autocrine and paracrine effects in all stages of Kaposi sarcoma pathogenesis (22, 40). However, these observations were largely restricted to in vitro studies or AIDS-associated Kaposi sarcoma, where HIV infection disrupts cytokine expression and possibly influences the incidence of Kaposi sarcoma in the presence of KSHV. In addition, the interpretation of cytokine-mediated associations obtained from cross-sectional investigations is difficult given the predominantly paracrine and usually transient nature of cytokines (41). Because differences in cytokine concentrations between populations seem to be due, at least in part, to polymorphisms in cytokine genes, polymorphisms associated with protein regulation may provide an adequate time-independent measure of cytokine-mediated immune profiles associated with risk of classic Kaposi sarcoma (CKS). Findings from previously published genetic association studies suggest a possible role of promoter region common variants of IL1B, IL6, IL12A, TNFA, and IFNG and anti-inflammatory (IL2, IL4, IL5, IL10, and IL13) cytokines, chemotactic agonists (IL8 and IL8RB), and growth factors (VEGF) with shown function or clinical correlate, to identify common genetic variants associated with risk of CKS given KSHV infection.

Materials and Methods

Study Population. We used participants from the Italian Kaposi Sarcoma Case-Control (KCC) study to examine the relationship of common regulatory polymorphisms to CKS susceptibility given KSHV infection. Cases with histologically confirmed CKS (International Classification of Disease-Oncology, Third Edition code M9140/3) were recruited from population-based cancer registries and major referral centers in Sicily, Rome and Naples between April 1998 and October 2001 (44). CKS cases were Italians ages ≥18 years, with evidence for KSHV latent antibody by immunofluorescence assay (IFA) and living in the designated geographic regions at the time of study enrollment. Controls were frequency matched to CKS cases on sex, age, and geographic region. Potential controls were screened for antibodies against KSHV latent nuclear antigen-1 (LANA-1) using IFA. KSHV LANA-seropositive controls were excluded. As previously described (44), 141 cases and 192 controls provided informed consent and were enrolled in the KCC study. Following enrollment, blood and answers to a questionnaire were collected. Institutional Review Boards at the participating centers in the United States and Italy approved the protocol, informed consent, and related study materials before initiation.

Genomic DNA was available from 133 (94.3%) CKS cases (71% males; median age, 72 years; range, 29-91) and 172 (89.6%) population-based KSHV LANA-seropositive controls (69% males; median age, 75 years; range, 37-92; Table 1). Consistent with the parent study population, participants were predominantly from Sicily, reflecting an increasing gradient in KSHV seroprevalence from northern to southern Italy (45). At the time of the interview, 82 (62%) cases with available DNA had Kaposi sarcoma lesions at multiple dermal sites, 20 (15%) had lesions at one dermal site, 29 (22%) had no current lesions, and 2 (2%) had missing information. DNA from 27 incident cases (diagnosed ≤1 year from enrollment), 102 prevalent cases (diagnosed >1 year from enrollment), 4 cases with unknown disease chronology, and 172 controls were available for analysis. Hematologic variables, including lymphocyte subpopulations, were available for 90% of the cases and controls.

Laboratory Methods

KSHV Serology. Controls were selected based on positive reaction to a previously described IFA using latently infected BCBL-1 cells (44). We note, however, that all participants were KSHV seropositive by IFA in addition to both lytic and latent ELISA (Viral Epidemiology Section, AIDS Vaccine Program, Science Applications International Corporation-Frederick, National Cancer Institute, Frederick, MD). Antibodies against lytic (replicative) KSHV antigens were tested at a 1:20 serum dilution using a K8.1 ELISA, as previously described (46). Antibodies against latent KSHV antigens were determined by ELISA using open reading frame 73 recombinant protein and a 1:100 serum dilution (7).

Hematologic Variables. We determined blood counts in fresh whole blood using conventional automated methods. Proportions of peripheral blood T-lymphocyte subsets (helper/inducer and suppressor/cytotoxic) were estimated using flow cytometry on fresh whole blood and monoclonal antibodies for CD4 and CD8, respectively. Absolute numbers of T-lymphocyte subpopulations were estimated as the product of total lymphocytes, from the complete blood count, and the respective T-lymphocyte proportions. All blood studies were analyzed at the time of study enrollment by centralized laboratories in Sicily (Dipartimento di Igiene e Microbiologia Giuseppe D’Alessandro, Università degli studi di Palermo) and Central Italy (Laboratorio di Epidemiologia e Biostatistica, Istituto Superiore di Sanità, Rome).

Genotype Analysis. Twenty-eight single nucleotide polymorphisms in 14 genes (IL1A, IL1B, IL2, IL4, IL5, IL6, IL8, IL8RB, IL10, IL12A, IL13, TNF, IFNG, and VEGF; Table 2) were genotyped by the National Cancer Institute Core Genotyping Facility (National Cancer Institute, Gaithersburg, MD). Genomic DNA was extracted from cryopreserved lymphocyte pellets by use of a modified salt precipitation extraction method (Centra Systems, Minneapolis, MN). Genotype analysis was done using validated and optimized Taqman assays and analyzed on the ABI 7900HT platform (ABI, Foster City, CA) that included negative and positive

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CKS cases (n = 133)</th>
<th>KSHV LANA° controls (n = 172)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, no. males/no. females (% male)</td>
<td>94/39 (71)</td>
<td>118/54 (69)</td>
<td>0.697</td>
</tr>
<tr>
<td>Median age at enrollment, y (range)</td>
<td>72 (29-91)</td>
<td>75 (37-92)</td>
<td>0.487</td>
</tr>
<tr>
<td>No. enrolled by region (%)</td>
<td>46 (35)</td>
<td>34 (20)</td>
<td></td>
</tr>
<tr>
<td>Central Italy</td>
<td>87 (65)</td>
<td>138 (80)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Abbreviations: CKS, classic Kaposi sarcoma; KSHV, Kaposi sarcoma-associated herpesvirus; LANA, latent nuclear antigen.
controls and 5% duplicate samples. All assays are publicly available (including primers, probes, and conditions) on the SNP500Cancer web site (http://snp500cancer.nci.nih.gov; available (including primers, probes, and conditions) on the SNP500Cancer web site (http://snp500cancer.nci.nih.gov; available on the SNP500 Cancer database; http://www.ncbi.nlm.nih.gov/snp)). Technicians conducting genotype assays were blinded to unique identifiers and participant characteristics. Internal laboratory quality control showed >99% concordance. All common variants are referred to according to the common genetic variant nomenclature adapted from Antonarakis SE and the Nomenclature Working Group (48).

### Table 2. Common genetic variants in proinflammatory and anti-inflammatory cytokines, chemotactic molecules, and growth factors evaluated in relation to risk of CKS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Common variant (alias)*</th>
<th>dbSNP Identifier †</th>
<th>MAF ‡</th>
<th>P_{HWE} ³</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proinflammatory cytokines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL6</td>
<td>5q31.1</td>
<td>−1098G&gt;T</td>
<td>rs2243248</td>
<td>0.076</td>
<td>0.990</td>
</tr>
<tr>
<td>IL5</td>
<td>5q31.1</td>
<td>−588T&gt;C</td>
<td>rs2243250</td>
<td>0.122</td>
<td>0.720</td>
</tr>
<tr>
<td>IL10</td>
<td>1q31-q32</td>
<td>−853C&gt;T</td>
<td>rs2069812</td>
<td>0.364</td>
<td>0.034</td>
</tr>
<tr>
<td>IL13</td>
<td>5q31</td>
<td>−847A&gt;G</td>
<td>rs1800925</td>
<td>0.175</td>
<td>0.093</td>
</tr>
<tr>
<td><strong>Chemotactic molecules and growth factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL18</td>
<td>4q13-q21</td>
<td>−351A&gt;T</td>
<td>rs4073</td>
<td>0.409</td>
<td>0.658</td>
</tr>
<tr>
<td>IL8RB</td>
<td>2q35</td>
<td>−1010G&gt;A</td>
<td>rs1126579</td>
<td>0.407</td>
<td>0.265</td>
</tr>
<tr>
<td>VEGF</td>
<td>6p12</td>
<td>236 bp 3’ of STP C&gt;T</td>
<td>rs3025039</td>
<td>0.147</td>
<td>0.035</td>
</tr>
</tbody>
</table>

*Common genetic variant nomenclature adapted from Antonarakis SE and the Nomenclature Working Group (48).
‡ Minor allele frequency among KSHV seropositive controls.
§ P values reported from Hardy-Weinberg equilibrium; Pearson’s χ² (1 degree of freedom) among controls.

Statistical Analysis

**Single-Locus Markers.** Common genetic variants in key candidate genes were selected on a priori basis on the following criteria: (a) a minor allele frequency of >5% in the SNP500Cancer database; (b) shown functional data; (c) previous associations of autoimmune, infectious, or cancer outcomes; or (d) plausible contribution to Kaposi sarcoma pathogenesis. Differences in genotype frequencies in participants with CKS were determined using the χ² test or the Fisher’s exact test. Deviation from the Hardy-Weinberg equilibrium was examined for all loci separately among the population <5%, rare allele-containing genotypes were combined. Statistical significance, based on multivariate logistic models, was calculated using the likelihood ratio test from logistic regression. A two-tailed P ≤ 0.05 was considered statistically significant.

**Haplotype Identification.** Haplotype frequencies were estimated from unphased genotype data for nine genes (IL1A, IL1B, IL4, IL8, IL8RB, IL10, IL12A, IL13, and TNF) using the expectation-maximization progressive insertion algorithm in HaploStats v.1.1.0 (http://www.mayo.edu/hsr/people/schaid.html). Differences in the overall haplotype profile among cases and controls were evaluated using the global score test based on empirical distributions of a minimum of 10,000 and a maximum of 50,000 permutations and adjusted for covariates (sex and age). Associations of CKS and particular haplotypes were estimated using generalized linear models based on iterative weighted regression substitution, in which posterior probabilities of individual haplotype pairs are used as weights to update regression coefficients, and regression coefficients were used to update posterior probabilities (49, 50). Individual haplotypes with frequencies of <1% were not included. We examined additive, codominant diplotype (haplotype pairs containing 0 copy versus 1 copy versus 2 copies of a risk locus) and dominant diplotype (haplotype pairs containing 0 copy versus 1 or 2 copies of a risk locus) models. For internal validity, associations of CKS and haplotypes were confirmed using an alternative approach of first assigning individual posterior probabilities of phase to each person using the coalescent approach of phase 2 or 3 (version 2.0.2; Mathematical Genetic Group; refs. 51, 52) and then performing logistic regression to estimate diplotype effects weighted by these posterior probabilities.

Although no confounding by sex or category of age was observed, all risk estimates were adjusted for these variables to control for residual confounding. Additional potential
confounders were examined but excluded from final adjustment because they were not associated with CKS or immune-modulating genes. These factors included smoking status, comorbidities (allergy, asthma, cardiovascular, and renal disease), personal hygiene, sexual history, factors associated with childhood crowding, and low absolute platelet, RBC, and lymphocyte counts. Analysis are presented without adjustments for multiple comparisons. Genotypic distributions and LD were examined using Stata v.7.0 (College Station, TX).

Gene-gene and gene-environment interactions were assessed in two ways. First, for statistically significant single-locus markers and haplotypes, we compared full and nested recessive and dominant models with interaction terms by use of maximum likelihood methods. Interaction terms that did not contribute to the model \((P \geq 0.10)\) were excluded. Finally, for all single-locus markers, logic regression (an adaptive regression approach based on Boolean combinations of binary variables; refs. 53, 54) was used to identify potential interactions among three or more covariates. A two-tailed \(P \leq 0.05\) was considered statistically significant. Logic regression analyses were conducted using R version 2.1.0 (55).

Results

Among KSHV LANA-seropositive controls, genotypic distributions were consistent with the Hardy-Weinberg equilibrium (Table 2). Relative risk estimates for the 28 polymorphisms observed among CKS cases and controls are shown in Fig. 1 (genotype frequencies are shown in Supplementary Table S1). A reduction in CKS risk was significantly associated with genotypes of four single-locus markers, including \(IL8\) C-204T (rs2227306; \(P = 0.05\)), T-containing variants of \(IL8RB\) +1235 (rs1126579; \(P = 0.04\)) and \(IL8RB\) G-1010G (rs1126580; \(P = 0.04\)), whereas excess CKS risk was observed with the promoter variant of \(IL13\) A+98G (rs20541; \(P = 0.02\)).

Intralocus and interlocus pairwise LD was examined separately for cases and controls. Variants within \(IL1A\), \(IL1B\), \(IL4\), \(IL8\), \(IL8RB\), \(IL10\), \(IL12A\), \(IL13\), and \(TNF\) genes were correlated \((r^2 > 0.5)\) among controls (data not shown). Of the 28 polymorphisms on eight chromosomes, there were 13 pairs of unlinked loci, including seven between \(IL1A\) and \(IL1B\) (2q14) and six between \(IL4\), \(IL5\), and \(IL13\) (5q31). Four of the 13 pairs (\(IL1A\) IVS4/C0109/IL1B +14; \(IL1A\) IVS4/C096/IL1B +14; \(IL5\)/C0745/IL13 +98; and \(IL5\)/C0745/IL13/C01069) yielded LD \((r^2 \geq 0.5)\). However, LD and haplotype frequency estimates did not

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Proinflammatory and anti-inflammatory cytokine, chemotactic molecule, and growth factor genotypes and risk of CKS. **•**, OR; _, 95% CI. Bold font indicates significance level, \(P \leq 0.05\). Genotype frequencies and ORs and corresponding 95% CIs are listed in Supplementary Table S1.
substantially differ between CKS cases and KSHV LANARsopositive controls (data not shown).

Haplotypes were estimated for genes in which LD was observed between multiple loci with selected results shown in Table 3 and null findings shown in Supplementary Table S2. The difference in the overall haplotype profile among CKS cases and controls was significant for *IL8* (P = 0.03).

As shown in Table 3, compared with the most common haplotype observed among KSHV-LANARsopositive controls, a significant 2-fold reduction in CKS risk was observed for *IL8*, +1235T/−1010G (OR, 0.59; 95% CI, 0.40-0.85). Similarly, reduced risk was observed with 230G-containing haplotypes of *IL8*, albeit not significantly (OR, 0.74; 0.55-1.12 and OR, 0.35; 0.17-0.71, respectively). The magnitude of these associations was confirmed using the PHASE software and logistic regression approach (OR, 0.74; 0.55-1.12 and OR, 0.35; 0.17-0.71, respectively). Conversely, risk of CKS was nonsignificantly increased with 98A-containing haplotypes of *IL13* (OR, 1.08; 0.23-5.05). In the dominant model, carriers of A-containing diplotypes were associated with a significant increased risk of CKS (P = 0.02). Findings for *IL8* and *IL13* were consistent based on both haplotype estimation methods.

Haplotypes frequencies estimated separately for incident and prevalent CKS cases did not reveal significant differences (P > 0.38). We examined haplotype frequencies by disease burden (no current lesions versus one or more current lesions). Twenty-nine prevalent CKS cases (22%) did not have lesions at enrollment. As shown in Table 3, haplotypes of *IL8* were slightly more heterogeneous among CKS cases with current lesions than among CKS cases with no lesions. Compared with the most common haplotype among KSHV-LANARsopositive controls, the haplotype of *IL8* 1235T/−1010G was associated with a significant reduction in CKS risk among patients without current lesions (OR, 0.35; 95% CI, 0.17-0.71), although the magnitude of the association was not substantially different than those with current lesions (OR, 0.68; 95% CI, 0.46-1.02; P = 0.28). Although the haplotypes among CKS cases without current lesions were rare, the presence of the IL13 −1069C/98A haplotype seemed to be a risk factor for increasing disease burden (OR, 2.30; 95% CI, 1.09-4.87; P = 0.03).

To assess genetic models, we did analyses considering pairs of haplotypes carried by each individual, termed diplotypes. For *IL8*, codominant diplotypes were less clear. Compared with all other diplotypes with a frequency of ≥1%, combined TG-containing homozygote and heterozygote diplotypes were associated with a significant decreased CKS risk (P = 0.006).

For *IL13*, codominant diplotypes were less clear. Compared with all other diplotypes with a frequency of ≥1%, CKS cases with one *IL13* A-containing haplotype were at increased risk, whereas no effect was observed among carriers with 2 copies (OR, 1.08; 95% CI, 1.12-2.99 and OR, 1.08; 95% CI, 1.08; 0.23-5.05). In the dominant model, carriers of A-containing diplotypes were associated with a significant increase in risk of CKS (P = 0.02). Findings for *IL8* and *IL13* were consistent based on both haplotype estimation methods.

### Table 3. Overall haplotype profile, individual haplotype frequency estimates, and risk of CKS overall and by disease burden

<table>
<thead>
<tr>
<th>Gene/Haplotype</th>
<th>Haplotype frequency estimates</th>
<th>CKS, overall</th>
<th>CKS, no lesions*</th>
<th>CKS, current lesions*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td><em>IL8</em> −351</td>
<td>230 −204</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T T C</td>
<td>0.611 0.643 0.587</td>
<td>0.189</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>A G T</td>
<td>0.345 0.323 0.365</td>
<td>0.324</td>
<td>0.78 (0.55-1.12)</td>
<td>Reference</td>
</tr>
<tr>
<td>A G A</td>
<td>0.013 0.015 0.033</td>
<td>0.157</td>
<td>0.79 (0.12-8.28)</td>
<td>Reference</td>
</tr>
<tr>
<td>A T C</td>
<td>0.015 0.019 0.012</td>
<td>0.478</td>
<td>1.36 (0.36-5.30)</td>
<td>Reference</td>
</tr>
<tr>
<td>Global score test</td>
<td></td>
<td>0.292</td>
<td>0.617</td>
<td>0.232</td>
</tr>
<tr>
<td><em>IL8RB</em> 1235</td>
<td>−1010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C A</td>
<td>0.543 0.595 0.502</td>
<td>0.017</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>T G</td>
<td>0.356 0.301 0.399</td>
<td>0.008</td>
<td>0.59 (0.40-0.85)</td>
<td>Reference</td>
</tr>
<tr>
<td>C G</td>
<td>0.099 0.104 0.095</td>
<td>0.697</td>
<td>0.80 (0.50-1.58)</td>
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<tr>
<td>Global score test</td>
<td></td>
<td>0.031</td>
<td>0.048</td>
<td>0.288</td>
</tr>
<tr>
<td><em>IL13</em> −1069</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C G A</td>
<td>0.740 0.695 0.774</td>
<td>0.031</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>T T A</td>
<td>0.122 0.142 0.105</td>
<td>0.132</td>
<td>1.52 (0.88-2.62)</td>
<td>Reference</td>
</tr>
<tr>
<td>T G A</td>
<td>0.075 0.083 0.069</td>
<td>0.545</td>
<td>1.13 (0.56-2.29)</td>
<td>Reference</td>
</tr>
<tr>
<td>C A</td>
<td>0.060 0.091 0.052</td>
<td>0.179</td>
<td>1.73 (0.83-3.60)</td>
<td>Reference</td>
</tr>
<tr>
<td>Global score test</td>
<td></td>
<td>0.167</td>
<td>0.438</td>
<td>0.069</td>
</tr>
</tbody>
</table>

**NOTE:** Relative haplotype frequencies adjusted for sex and age. Diplotypes (haplotype pairs) based on log-additive models. Rare haplotypes with frequencies <1% were combined (data not shown). Empirical P values based on a minimum of 10,000 and maximum of 50,000 permutations. Frequencies add to 100% excluding the combined rare occurring haplotypes.

**Abbreviation:** NA, not applicable.

*Haplotype frequency estimates are not shown for strata of disease burden.

### Table 4. Combinations of haplotype pairs and risk of CKS

<table>
<thead>
<tr>
<th>Gene/Haplotype</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>IL8</em> <strong>/</strong></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>TG/**</td>
<td>0.50 (0.31-0.83)</td>
<td>0.006</td>
</tr>
<tr>
<td>TG/TG</td>
<td>0.57 (0.26-1.21)</td>
<td>0.14</td>
</tr>
<tr>
<td>TG/** and TG/TG</td>
<td>0.52 (0.32-0.82)</td>
<td>0.006</td>
</tr>
<tr>
<td><em>IL13</em>*/**</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>A*/A**</td>
<td>1.83 (1.12-2.99)</td>
<td>0.02</td>
</tr>
<tr>
<td>A*/A and A*A**</td>
<td>1.08 (0.23-5.05)</td>
<td>0.92</td>
</tr>
<tr>
<td>A<em>A** and A</em>A**</td>
<td>1.77 (1.09-2.86)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**NOTE:** Asterisks refers to other alleles. Relative risk estimates adjusted for sex and age.
No significant gene-gene interactions on CKS risk were observed among these single-locus markers or between statistically significant genotypes or haplotypes (IL8, IL8RB, and IL13; data not shown). Because it is possible for combinations of statistically insignificant markers to contribute to a complex disease outcome, we examined multiple gene-gene interactions (≥3 genes) for all single-locus markers by use of logic regression. New possible predictors for the outcome were considered by creating Boolean statements of the common genetic variant data (encoded as two binary predictors for each variant, using dominant and recessive notation), embedded in a logistic regression framework. Model selection, carried out using cross-validation and permutation tests, did not reveal any association between the variants (and their interactions) and the outcome.

The univariate associations of IL8RB and IL13 did not substantially differ when considered together in a multivariate logistic model that included sex and age. A reduction in CKS risk was observed with combined heterozygous and homozygous diplotypes of IL8RB (TG/** and TG/TG: OR, 0.49; 95% CI, 0.30-0.78; \( P = 0.003 \)), and increased risk was observed with combined diplotypes of IL13 (A/* and A/A: OR, 1.88; 95% CI, 1.15-3.08; \( P = 0.01 \); data not shown).

Discussion

Our analysis of haplotypes of select cytokine (IL1A, IL1B, IL2, IL4, IL5, IL6, IL10, IL12A, TNFA, and IFNG), chemotactic (IL8 and IL8RB), and growth factor (VEGF) genes revealed that compared with KSHV-seropositive controls, CKS cases were significantly less likely to have diplotypes of IL8RB containing TG allelic combinations and significantly more likely to have 98A allele-containing diplotypes of IL13, each accounting for ∼14% of the population attributable risk. To our knowledge, this is the first report to identify genetic variants that could influence susceptibility to CKS in individuals with latent KSHV infection.

The T141/T142 cytokine network is complex and regulates the balance between humoral and cell-mediated immunity. Shifts in the expression or function of key cytokines can have pleiotropic effects, resulting in marked alterations in host response. Emerging evidence indicates that common genetic variants in cytokine genes can modify protein expression or biological function. Through the release of cytokines (IL-4, IL-5, IL-10, and IL-13), T142 cells promote the differentiation of B cells into antibody-secreting plasma cells, whereas T141 cytokines (TNF-α, IFN-γ, IL-1, IL-6, and IL-8) are capable of regulating antiviral activity (56), both thought to be important for Kaposi sarcoma and KSHV pathogenesis. Both T141 and T142 cells regulate each other’s functions through a series of tightly regulated checks and balances predominantly via the effects of IFN-γ and IL-4/IL-10 (41).

Kaposi sarcoma is an inflammatory-mediated disease characterized by localized pathogenesis involving the expression of proinflammatory molecules (IL-1α, IL-1β, IL-6, TNF-α, and IFN-γ; refs. 24, 31, 32), that favor a shift toward a T141 cytokine dominated profile (57, 58). Previously published studies of cytokine gene polymorphisms and risk of AIDS-associated Kaposi sarcoma support laboratory evidence that Kaposi sarcoma pathogenesis may be T141 mediated. Foster et al. (42) reported that the high-expressing proinflammatory IL6 –256C allele (rs1802379, allele –174) was associated with increased risk of AIDS-associated Kaposi sarcoma compared with KSHV-seropositive controls (calculated OR, 1.84; 95% CI, 1.49-2.27). In a different investigation, among dually HIV-1- and KSHV-infected men enrolled in the Amsterdam Cohort, van der Kuyl et al. (43) reported decreased risk of AIDS-associated Kaposi sarcoma with the presence of the low-expressing IL8 –351T allele (rs4073, allele –251) compared with KSHV-seropositive men with AIDS-defining conditions other than AIDS-Kaposi sarcoma (calculated OR, 0.67; 95% CI, 0.52-0.86).

In our study of classic, non–AIDS-associated Kaposi sarcoma, no difference in allele frequencies of IL6 –236 were observed between CKS cases and KSHV-LANA seropositive controls (ORc allele, 1.05; 95% CI, 0.81-1.36; \( P = 0.82 \); Supplementary Table S1), and contrary to van der Kuyl (43), we found that the IL8 –351T allele, associated with decreased IL-8 expression, was overrepresented among CKS cases compared with KSHV-LANA-seropositive controls (OR, 1.25; 95% CI, 1.05-1.49). Genetic susceptibility to both HIV-1 infection and progression to AIDS and AIDS-defining events has been established (59). Thus, it is possible that HIV infection could influence the relationship of common germ line variants in immune-modulating genes on risk of AIDS-Kaposi sarcoma, potentially accounting for the differences observed in our study and the previous ones.

To maximize population homogeneity and minimize phenotype misclassification, we compared histologically confirmed CKS cases and controls in Italy, all of whom were seropositive for both anti-lytic and anti-latent KSHV antibodies. We recognize that chance alone could account for the observed associations and additional loci in close proximity to IL13 and IL8RB may be involved in development of the CKS phenotype. We sought to minimize the likelihood of false positives due to imputation by reconstructing haplotypes using two approaches including expectation-maximization–based and Bayesian algorithms. Each of the methods are independently highly accurate (51, 60, 61), and together provide a higher degree of confidence that findings are internally valid.

In the present study, we found consistent associations of single marker loci of IL8RB (1235T and –1010G) and 1235T/–1010G-containing combinations with decreased risk of CKS. Although the exact function of these IL8RB variants is unknown, we selected this gene because of homology with the KSHV IL-8 gene, also known as the viral G protein-coupled receptor (vGPCR; refs. 19, 62, 63), emphasizing a possible relationship among viral gene homologues, host IL8RB, and Kaposi sarcoma pathogenesis. To date, variants of IL8RB have been associated with other immune-mediated diseases, including rheumatoid arthritis (64) and cryptogenic fibrosing alveolitis (65).

IL8 is a potent neutrophil chemoattractant (66-68) particularly in response to inflammatory stimuli (69, 70). It plays an important role in angiogenesis (71-74), T-cell recruitment and monocyte adherence (75-77), and proliferation, migration, and invasion of endothelial cells (71), all thought to be important for Kaposi sarcoma pathogenesis. The effects of IL-8 are mediated by its binding receptor genes (CXCR1 and CXCR2; refs. 78, 79), which are clustered at chromosome 2q35 (80). Variants of IL8RB are located in the 3’ untranslated region of exon 3 and have the capacity to alter mRNA translation (81, 82). Functional studies suggest that IL8RB indirectly activates fibroblasts by mediating the recruitment of T cells (83), a process similar to that observed in Kaposi sarcoma spindle cell transformation. Similarly, vGPCR seems sufficient to transform cells (84), immortalize endothelial cells (85), promote cellular VEGF-mediated angiogenesis (19, 38, 62, 84-89), and up-regulate proinflammatory transcription, including cellular IL-8 in Kaposi sarcoma-derived cell lines (90-91). Thus, the plausibility of the association between haplotypes of IL8RB and CKS risk would be further strengthened if it could be established that IL8, IL8RB, and vGPCR interact to influence viral replication, the T141/T142 balance, and promotion of angiogenesis leading to Kaposi sarcoma lesion formation or maintenance.
Should an imbalance be precipitated by IL-8 or IL-8RB, IL-13 could possibly be one of several anti-inflammatory cytokines left unchecked. Compared with the IL13 98G allele, the A allele is associated with low serum IL-13 levels (92). Carriers of the 98G allele are at increased risk for several Th2-mediated conditions, including bronchial hyper-responsiveness (AHR), atopic and nonatopic asthma, and allergic inflammation (92-94). IL13, IL4, and IL5, key Th2-type response cytokines, lie in close proximity on chromosome 5q 23-31 (96, 97). Although we did not observe associations of CKS with single-locus markers or haplotypes of IL13, IL4, or IL5, the effect of IL13 98A-containing diplotype on CKS risk could possibly be influenced by loci or haplotypes in the region that includes IL4 and IL5.

The association of 98A-containing haplotypes of IL13 with CKS risk is particularly intriguing. In addition to immune-stimulatory activities (95), IL-13 is a potent Th2 cytokine capable of inhibiting production of proinflammatory cytokines (IL-1α), TNF-α, IL-6, IL-8, and GRO-α) by peripheral blood monocytes (98). Together with IL-2, IL-13 antagonizes the induction of IFN-γ (41, 95), previously reported to be important for KSHV reactivation and Kaposi sarcoma lesion formation. It is notable that IL-13 induces B-cell and monocyte differentiation (98), important for Kaposi sarcoma tumorigenesis (99, 100), and up-regulates class II expression and IgE class switching (98). In this regard, carriers of the low IL-13-producing 98A allele could shift the balance toward a Th1-dominated cellular profile, a local hallmark of Kaposi sarcoma lesions and Kaposi sarcoma-derived cells in culture (30-32).

Our findings are the first to provide preliminary evidence that genetic variants in cytokines from both Th1 and Th2 pathways could influence the risk of CKS. Among KSHV-seropositive Italians, risk of CKS was associated with diplotype of IL8RB and IL13 that may work directly or indirectly to promote CKS pathogenesis. The redundancy inherent in cellular immunity make it difficult to speculate how these genes from both Th1 and Th2 pathways may work together to promote CKS pathogenesis. However, these preliminary findings suggest aberrant cytokine expression may affect the risk of CKS. Further studies are required to confirm these findings, preferably using finer haplotype structure and to explore a biological basis for alterations in cytokine pathways in the pathogenesis from KSHV infection to CKS.

Appendix 1

Study group membership: Additional investigators and institutions belonging to the Classical Kaposi Sarcoma Working Group include Nino Romano, Francesca Ajello, Filippa Bonura, Anna Maria Perna, Enza Viviano, Fabio Tramuto, Maria Rosaria Villafrape, and Maria Antonella Di Benedetto (Dipartimento di Igiene e Microbiologia Giuseppe D’Alessandro, Università degli studi di Palermo, Palermo, Italy); Mario Tamburini, Mauizio Rezza, Catia Valdarchi, and Francesca Ajello, Filippa Bonura, Anna Maria Perna, Enza Viviano, Fabio Tramuto, Maria Rosaria Villafrape, and Maria Antonella Di Benedetto (Dipartimento di Igiene e Microbiologia Giuseppe D’Alessandro, Università degli studi di Palermo, Palermo, Italy); Pierluca Piselli (INMI Lazzaro Spallanzani, Istituti di Patologia del Sintomo 23–31. University and Messina University; in Washington, DC, Rockville, MD, and Buenos Aires, Argentina (Research Triangle Institute); Maryanne Ardini for study management; Daniel Ringer for specimen processing and shipping advice; and Susan Wilson and Liliana Preiss for computer programming; the research participants and the nearly 100 local primary care physicians for providing population control subjects, without whom this study would not have been possible.

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