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 1235T/−1010G–containing diplo- types of *IL8RB* (odds ratio, 0.49; 95% confidence interval, 0.30-0.78; P = 0.003), whereas risk was increased with diplotypes 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 diplotypes 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 *IL6* (rs1800795; ref. 42) and *IL8* (rs4073; ref. 43) with altered risk of AIDS-associated Kaposi sarcoma among men. However, the extent to which this relationship is influenced by infection with HIV-1, if at all, is unknown.

Among HIV-1-seronegative cases and controls, we examined common variants in genes that encode proinflammatory (*IL1A, 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 invited to participate. HIV-1-seropositive cases and 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 chronicity, 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, Universitá degli studi di Palermo) and Central Italy (Laboratorio di Epidemiologia e Biostatistica, Istituto Superiore di Sanitá, 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 (Gentra 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 1. Characteristics of cases with CKS and KSHV LANA-seropositive controls genotyped for proinflammatory and anti-inflammatory cytokines, chemotactic molecules, and growth factors

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

Abbreviations: CKS, classic Kaposi sarcoma; KSHV, Kaposi sarcoma-associated herpesvirus; LANA, latent nuclear antigen.

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

Gene	Chromosome	Common variant (alias)*	dbSNP Identifier [†]	MAF [‡]	P_{HWE} [§]
Proinflammatory cytokines					
ILA	2q14	+114G>T (Ex5 +21)	rs17561	0.291	0.362
		IVS4 −96C>T	rs2856841	0.302	0.947
		IVS4 −109A>C	rs2071374	0.240	0.128
		Ex1 +12C>T (−889)	rs1800587	0.295	0.878
IL1B	2q14	Ex5 +14C>T (+3954)	rs1143634	0.240	0.128
		−580C>T (−31)	rs1143627	0.396	0.417
		−1060T>C (−511)	rs16944	0.401	0.859
IL2	Aq26	IVS1 −100G>T (−385)	rs2069762	0.349	0.265
IL6	7p21	−236C>G (−174)	rs1800795	0.246	0.244
IL12A	3p12-q13.2	IVS2 −798A>T	rs582054	0.440	0.559
		Ex7 +277A>G (+8685)	rs568408	0.120	0.665
TNF	6p21.3	−1036C>T (−857)	rs1799724	0.201	0.537
		−487A>G (−308)	rs1800629	0.082	0.882
IFNG	12q14	IVS3 +284G>A	rs1861494	0.203	0.660
Anti-inflammatory cytokines					
IL4	5q31.1	−1098G>T	rs2243248	0.076	0.990
		−588T>C (−524)	rs2243250	0.122	0.720
		Ex1 −168T>T(−33)	rs2070874	0.115	0.858
IL5	5q31.1	T-745C	rs2069812	0.364	0.034
IL10	1q31-q32	−853C>T(−819)	rs1800871	0.277	0.112
		C-1116A>G (−1082)	rs1800896	0.361	0.437
IL13	5q31	−1069C>T(−1112)	rs1800925	0.175	0.093
		Ex4 +98A>G (+130, +144)	rs20541	0.148	0.917
Chemotactic molecules and growth factors					
IL18	4q13-q21	−351A>T(−25I)	rs4073	0.409	0.658
		IVS1 +230G>T (+396)	rs2227307	0.401	0.394
		IVS1 −204C>T (+781)	rs2227306	0374	0.147
IL8RB	2q35	Ex3 +1235T>C	rs1126579	0.407	0.237
		Ex3 −1010G>A (+1440)	rs1126580	0.497	0.635
VEGF	6p12	236 bp 3′ of STP C>T	rs3025039	0.147	0.035

*Common genetic variant nomenclature adapted from Antonarakis SE and the Nomenclature Working Group (48).
[†]dbSNP database (http://www.ncbi.nlm.nih.gov/SNP).
[‡]Minor allele frequency among KSHV seropositive controls.
[§]Ps reported from Hardy-Weinberg equilibrium; Pearson's χ^2 (1 degree of freedom) among controls.

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; ref. 47). 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 recommendations set forth by the Nomenclature Working Group (48).

Statistical Analysis

Single-Locus Markers. Common genetic variants in key candidate genes were selected a priori based on the following criteria: (*a*) a minor allele frequency of >5% in the SNP500Cancer Caucasian data set; (*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 χ^2 or the Fisher's exact test. Deviation from the Hardy-Weinberg equilibrium was examined for all loci separately among cases and controls using the Pearson's χ^2 test. Linkage disequilibrium (LD) between multiple loci was measured using the standardized disequilibrium coefficient (Lewotin's D') and correlation coefficient (r^2). For single loci, the relative risk of CKS was estimated by the odds ratio (OR) and corresponding 95% confidence interval (95% CI) calculated using logistic regression. The most frequent homozygous genotype occurring among KSHV-seropositive controls served as the referent. For genotypes occurring in 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 $P \leq 0.05$ 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 weighed 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 implemented in PHASE (version 2.0.2; Mathematical Genetics Group; refs. 51, 52) and then performing logistic regression to estimate diplotype effects weighed 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. Analyses 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; $P \leq 0.04$) 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 ($r^2 \geq 0.5$) 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 −109/IL1B +14; IL1A IVS4 −96/IL1B +14; IL5 −745/IL13 +98; and IL5 −745/IL13 −1069) yielded LD ($r^2 \geq 0.5$). However, LD and haplotype frequency estimates did not
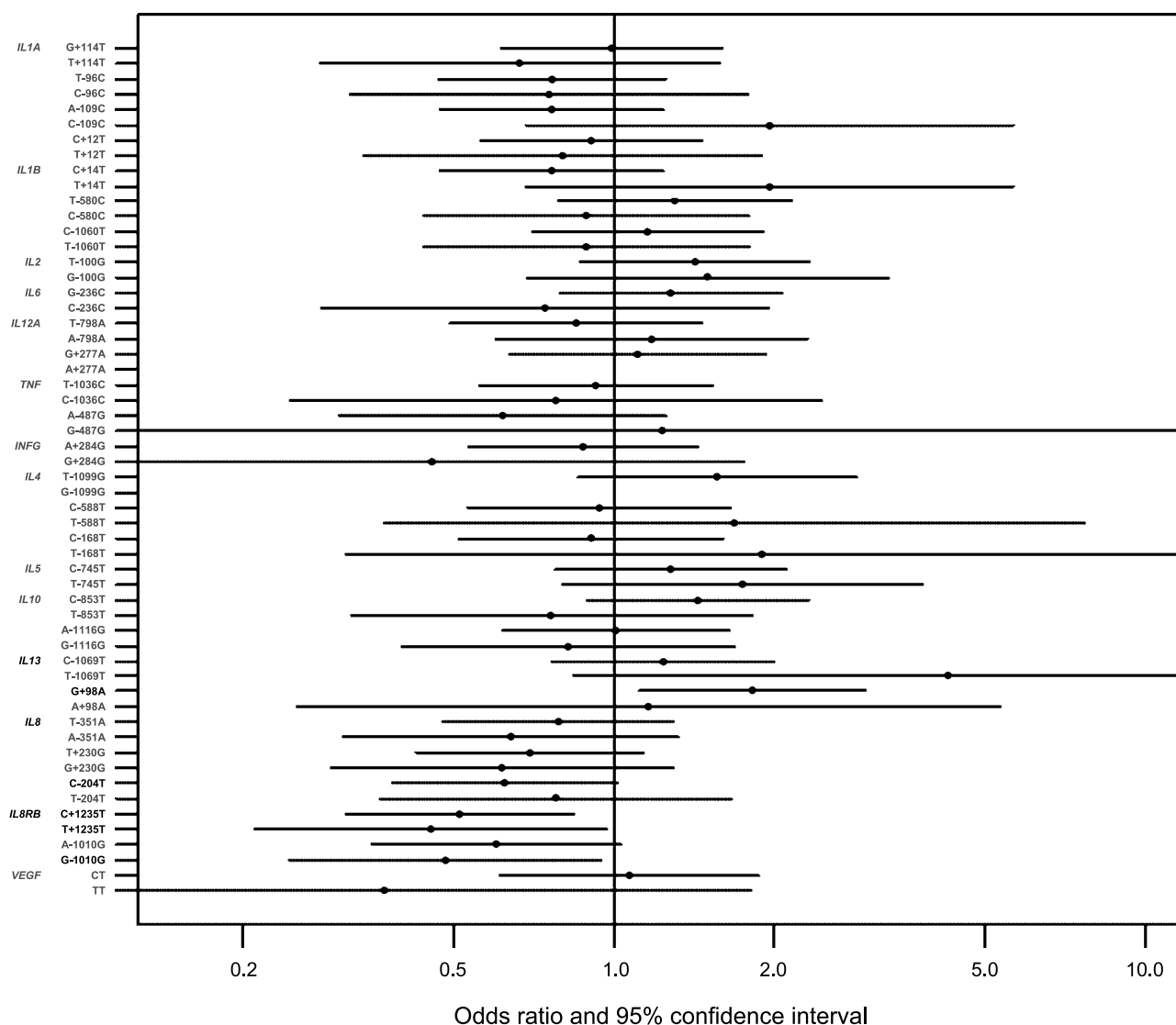
Figure 1. Proinflammatory and anti-inflammatory cytokine, chemotactic molecule, and growth factor genotypes and risk of CKS. •, OR; _, 95% CI. Bold font indicates significance level, $P \leq 0.05$. Genotype frequencies and ORs and corresponding 95% CIs are listed in Supplementary Table S1.

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

Gene	Haplotype			Haplotype frequency estimates				CKS, overall	Disease burden	
									CKS, no lesions*	CKS, current lesions*
				Overall	Case	Control	P	OR (95% CI)	OR (95% CI)	OR (95% CI)
IL8	−351	230	−204							
	T	T	C	0.611	0.643	0.587	0.189	Reference	Reference	Reference
	A	G	T	0.347	0.323	0.365	0.324	0.78 (0.55-1.12)	0.70 (0.37-1.32)	0.83 (0.56-1.23)
	A	G	C	0.025	0.015	0.033	0.157	0.39 (0.12-1.28)	0.91 (0.19-4.49)	0.27 (0.06-1.25)
	A	T	C	0.015	0.019	0.012	0.478	1.38 (0.36-5.30)	NA	1.86 (0.48-7.19)
Global score test							*0.292*		*0.617*	*0.232*
IL8RB	1235	−1010								
	C	A		0.543	0.595	0.502	0.017	Reference	Reference	Reference
	T	G		0.356	0.301	0.399	0.008	0.59 (0.40-0.85)	0.35 (0.17-0.71)	0.68 (0.46-1.02)
	C	G		0.099	0.104	0.095	0.697	0.89 (0.50-1.58)	0.96 (0.39-2.37)	0.91 (0.48-1.71)
Global score test							*0.031*		*0.048*	*0.288*
IL13	−1069	98								
	C	G		0.740	0.695	0.774	0.031	Reference	Reference	Reference
	T	A		0.122	0.142	0.105	0.132	1.52 (0.88-2.62)	1.57 (0.64-3.84)	1.46 (0.80-2.64)
	T	G		0.075	0.083	0.069	0.545	1.13 (0.56-2.29)	1.23 (0.41-3.69)	1.13 (0.52-2.46)
	C	A		0.064	0.079	0.052	0.179	1.73 (0.83-3.60)	0.34 (0.04-2.77)	2.30 (1.09-4.87)
Global score test							*0.167*		*0.438*	*0.069*

NOTE: Relative haplotype frequencies adjusted for sex and age. Diplotypes (haplotype pairs) based on log-additive models. Rare haplotypes with frequencies <1 percent were combined (data not shown). Empirical *P*s based on a minimum of 10,000 and maximum of 50,000 permutations. Frequencies add to 100% excluding the combined rare occuring haplotypes.
Abbreviation: NA, not applicable.
*Haplotype frequency estimates are not shown for strata of disease burden.

substantially differ between CKS cases and KSHV LANA-seropositive 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 *IL8RB* (P = 0.03).

As shown in Table 3, compared with the most common haplotype observed among KSHV-seropositive controls, a significant 2-fold reduction in CKS risk was observed for *IL8RB*, +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$_{-351A/230G/-204T}$, 0.78; 95% CI, 0.55-1.12 and OR$_{-351A/230G/-204C}$, 0.39; 95% CI, 0.12-1.28). Conversely, risk of CKS was nonsignificantly increased with 98A-containing haplotypes of *IL13* (OR$_{-1069T/98A}$, 1.52; 95% CI, 0.88-2.62 and OR$_{-1069C/98A}$, 1.73; 95% CI, 0.83-3.60). The magnitude of these associations was confirmed using the PHASE software and logistic regression approach (OR$_{IL8RB\ -1235T/-1010G}$, 0.65; 95% CI, 0.45-0.92; OR$_{IL8\ 351A/230G/-204T}$, 0.80; 95% CI, 0.57-1.14; OR$_{IL8\ -351A/230G/-204C}$, 0.42; 95% CI, 0.13-1.34; OR$_{IL13\ -1069T/98A}$, 1.53; 95% CI, 0.94-2.49; and OR$_{IL13\ -1069C/98A}$, 1.69; 95% CI, 0.84-3.37; data not shown).

Haplotype 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-seropositive controls, the haplotype of *IL8RB* 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 = 0.288). 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 using codominant models (haplotype pairs containing 0 risk haplotype versus 1 risk haplotype versus 2 risk haplotypes) and dominant models (haplotype pairs containing 0 risk haplotype versus 1 or 2 risk haplotypes). As shown in Table 4, in the codominant diplotype model, TG-containing haplotypes of *IL8RB* were associated with an ~2-fold reduction in CKS risk irrespective of the number of haplotype copies (TG/**, P = 0.006 and TG/TG, P = 0.14). Similarly, 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 diplotype models 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$_{*A/**}$, 1.83; 95% CI, 1.12-2.99 and OR$_{*A/*A}$, 1.08; 95% CI, 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 *IL8RB* and *IL13* were consistent based on both haplotype estimation methods.

Table 4. Combinations of haplotype pairs and risk of CKS

Gene/haplotype	OR (95% CI)	P
IL8RB		
/	Reference	
TG/**	0.50 (0.31-0.83)	0.006
TG/TG	0.57 (0.26-1.21)	0.14
TG/** and TG/TG	0.52 (0.32-0.82)	0.006
IL13		
/	Reference	
*A/**	1.83 (1.12-2.99)	0.02
*A/*A	1.08 (0.23-5.05)	0.92
*A/** and *A/*A	1.77 (1.09-2.86)	0.02

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 T_H1/T_H2 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), T_H2 cells promote the differentiation of B cells into antibody-secreting plasma cells, whereas T_H1 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 T_H1 and T_H2 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 T_H1 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 T_H1 mediated. Foster et al. (42) reported that the high-expressing proinflammatory *IL6* −236G allele (rs1800795, alias −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, alias −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 ($OR_{G\ allele}$, 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 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-8R* 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).

IL-8 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 (38), immortalize endothelial cells (84), 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 (89-91). 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 T_H1/T_H2 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 T_H2-mediated conditions, including bronchial hyper-responsiveness (AHR), atopic and nonatopic asthma, and allergic inflammation (92-94). *IL13*, *IL4*, and *IL5*, key T_H2-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 diplotypes 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 T_H2 cytokine capable of inhibiting production of proinflammatory cytokines (IL-1β, 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 T_H1-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 T_H1 and T_H2 pathways could influence the risk of CKS. Among KSHV-seropositive Italians, risk of CKS was associated with diplotypes 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 T_H1 and T_H2 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 Villafrate, and Maria Antonella Di Benedetto (Dipartimento di Igiene e Microbiologia Giuseppe D'Alessandro, Universitá degli studi di Palermo, Palermo, Italy); Mario Tamburini, Mauizio Montella, Anna Crispo, Sonia De Sicato, Maria Rosaria De Marco, and Paolo Ascierto (Unità operativa di Epidemiologia, Istituto dei Tumori di Napoli, Fondazione G. Pascale, Napoli, Italy); Pierluca Piselli (INMI Lazzaro Spallanzani, Istituti di Ricovero e Cura a Carattere Scientifico, Roma, Italy); Giovanni Rezza, Catia Valdarchi, and Francesca Farchi (Reparto di Epidemiologia, Dipartimento di Malattie Infettive, Parassitarie e Immunomediate, Istituto Superiore di Sanità, Rome, Italy); Rosa Maria Corona (Istituto Dermatopatico dell'Immacolata, Roma, Italy); Aldo Di Carlo and Concetta Castilletti (San Gallicano Hospital, Roma, Italy); Lorenzo Gafa (Lega Italiana per la lotta contro i tumori- sez. Ragusa, Ragusa, Italy); Stefania Stella and Michele Massimino (Departimento di Scienze Biomediche, Universitá degli studi di Catania, Catania, Italy); Barbara Kroner (Research Triangle Institute, Rockville, MD);

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References

1. Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 1994;266:1865−9.
2. Schalling M, Ekman M, Kaaya EE, Linde A, Biberfeld P. A role for a new herpes virus (KSHV) in different forms of Kaposi's sarcoma. Nat Med 1995;1:707−8.
3. Kaposi M. Idiopathisches multiples pigmentsarkom der haut. Arch f Dermat u Syph 1872;4:265−73.
4. Oettle A. Geographical and racial differences in the frequency of Kaposi's sarcoma as evidence of environmental or genetic causes. Acta Un Int Cancer 1962;18:330−63.
5. Myers BD, Kessler E, Levi J, Pick A, Rosenfeld JB, Tikvah P. Kaposi sarcoma in kidney transplant recipients. Arch Intern Med 1974;133:307−11.
6. Pneumocystis pneumonia among homosexual men: New York City and California. MMWR MOrb Mortal Wkly Rep 1981;30:305−8.
7. Whitby D, Luppi M, Barozzi P, Boshoff C, Weiss RA, Torelli G. Human herpesvirus 8 seroprevalence in blood donors and lymphoma patients from different regions of Italy. J Natl Cancer Inst 1998;90:395−7.
8. Friedman-Kien AE. Disseminated Kaposi's sarcoma syndrome in young homosexual men. J Am Acad Dermatol 1981;5:468−71.
9. Gottlieb GJ, Ackerman AB. Kaposi's sarcoma: an extensively disseminated form in young homosexual men. Hum Pathol 1982;13:882−92.
10. McNutt NS, Fletcher V, Conant MA. Early lesions of Kaposi's sarcoma in homosexual men. An ultrastructural comparison with other vascular proliferations in skin. Am J Pathol 1983;111:62−77.
11. Tabata M, Langford A, Becker J, Reichart PA. Distribution of immunocompetent cells in oral Kaposi's sarcoma (AIDS). Eur J Cancer B Oral Oncol 1993;29B:209−13.
12. Uccini S, Ruco LP, Monardo F, et al. Co-expression of endothelial cell and macrophage antigens in Kaposi's sarcoma cells. J Pathol 1994;173:23−31.
13. Regezi JA, MacPhail LA, Daniels TE, DeSouza YG, Greenspan JS, Greenspan D. Human immunodeficiency virus-associated oral Kaposi's sarcoma. A heterogeneous cell population dominated by spindle-shaped endothelial cells. Am J Pathol 1993;143:240−9.
14. MacPhail LA, Dekker NP, Regezi JA. Macrophages and vascular adhesion molecules in oral Kaposi's sarcoma. J Cutan Pathol 1996;23:464−72.
15. Galea P, Frances V, Dou-Dameche L, Sampol J, Chermann JC. Role of Kaposi's sarcoma cells in recruitment of circulating leukocytes: implications in pathogenesis. J Hum Virol 1998;1:273−81.
16. Ganem D. KSHV and Kaposi's sarcoma: the end of the beginning? Cell 1997;91:157−60.
17. Moore PS, Boshoff C, Weiss RA, Chang Y. Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. Science 1996;274:1739−44.

18. Cesarman E, Nador RG, Bai F, et al. Kaposi's sarcoma-associated herpesvirus contains G protein-coupled receptor and cyclin D homologs which are expressed in Kaposi's sarcoma and malignant lymphoma. J Virol 1996;70:8218–23.

19. Arvanitakis L, Geras-Raaka E, Varma A, Gershengorn MC, Cesarman E. Human herpesvirus KSHV encodes a constitutively active G-protein-coupled receptor linked to cell proliferation. Nature 1997;385:347–50.

20. Neipel F, Albrecht JC, Ensser A, et al. Human herpesvirus 8 encodes a homolog of interleukin-6. J Virol 1997;71:839–42.

21. Guo HG, Browning P, Nicholas J, et al. Characterization of a chemokine receptor-related gene in human herpesvirus 8 and its expression in Kaposi's sarcoma. Virology 1997;228:371–8.

22. Ensoli B, Nakamura S, Salahuddin SZ, et al. AIDS-Kaposi's sarcoma-derived cells express cytokines with autocrine and paracrine growth effects. Science 1989;243:223–6.

23. Barillari G, Buonaguro L, Fiorelli V, et al. Effects of cytokines from activated immune cells on vascular cell growth and HIV-1 gene expression. Implications for AIDS-Kaposi's sarcoma pathogenesis. J Immunol 1992;149:3727–34.

24. Miles SA, Rezai AR, Salazar-Gonzalez JF, et al. AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6. Proc Natl Acad Sci U S A 1990;87:4068–72.

25. Yang J, Hagan MK, Offermann MK. Induction of IL-6 gene expression in Kaposi's sarcoma cells. J Immunol 1994;152:943–55.

26. Nair BC, DeVico AL, Nakamura S, et al. Identification of a major growth factor for AIDS-Kaposi's sarcoma cells as oncostatin M. Science 1992;255:1430–2.

27. Samaniego F, Markham PD, Gallo RC, Ensoli B. Inflammatory cytokines induce AIDS-Kaposi's sarcoma-derived spindle cells to produce and release basic fibroblast growth factor and enhance Kaposi's sarcoma-like lesion formation in nude mice. J Immunol 1995;154:3582–92.

28. Samaniego F, Markham PD, Gendelman R, Gallo RC, Ensoli B. Inflammatory cytokines induce endothelial cells to produce and release basic fibroblast growth factor and to promote Kaposi's sarcoma-like lesions in nude mice. J Immunol 1997;158:1887–94.

29. Brockmeyer NH, Willers CP, Anders S, Mertins L, Rockstroh JK, Sturzl M. Cytokine profile of HIV-positive Kaposi's sarcoma derived cells *in vitro*. Eur J Med Res 1999;4:95–100.

30. Sturzl M, Brandstetter H, Zietz C, et al. Identification of interleukin-1 and platelet-derived growth factor-B as major mitogens for the spindle cells of Kaposi's sarcoma: a combined *in vitro* and *in vivo* analysis. Oncogene 1995;10:2007–16.

31. Oxholm A, Oxholm P, Permin H, Bendtzen K. Epidermal tumour necrosis factor alpha and interleukin 6-like activities in AIDS-related Kaposi's sarcoma. An immunohistological study. APMIS 1989;97:533–8.

32. Cai J, Gill PS, Masood R, et al. Oncostatin-M is an autocrine growth factor in Kaposi's sarcoma. Am J Pathol 1994;145:74–9.

33. Fiorelli V, Gendelman R, Samaniego F, Markham PD, Ensoli B. Cytokines from activated T cells induce normal endothelial cells to acquire the phenotypic and functional features of AIDS-Kaposi's sarcoma spindle cells. J Clin Invest 1995;95:1723–34.

34. Ensoli B, Markham P, Kao V, et al. Block of AIDS-Kaposi's sarcoma (KS) cell growth, angiogenesis, and lesion formation in nude mice by antisense oligonucleotide targeting basic fibroblast growth factor. A novel strategy for the therapy of KS. J Clin Invest 1994;94:1736–46.

35. Ensoli B, Gendelman R, Markham P, et al. Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. Nature 1994;371:674–80.

36. Cornali E, Zietz C, Benelli R, et al. Vascular endothelial growth factor regulates angiogenesis and vascular permeability in Kaposi's sarcoma. Am J Pathol 1996;149:1851–69.

37. Xerri L, Hassoun J, Planche J, et al. Fibroblast growth factor gene expression in AIDS-Kaposi's sarcoma detected by *in situ* hybridization. Am J Pathol 1991;138:9–15.

38. Bais C, Santomasso B, Coso O, et al. G-protein-coupled receptor of Kaposi's sarcoma-associated herpesvirus is a viral oncogene and angiogenesis activator. Nature 1998;391:86–9.

39. Sturzl M, Zietz C, Monini P, Ensoli B. Human herpesvirus-8 and Kaposi's sarcoma: relationship with the multistep concept of tumorigenesis. Adv Cancer Res 2001;81:125–59.

40. Ensoli B, Sturzl M. Kaposi's sarcoma: a result of the interplay among inflammatory cytokines, angiogenic factors and viral agents. Cytokine Growth Factor Rev 1998;9:63–83.

41. Thomson A. The cytokine handbook. 3rd ed. San Diego: Academic Press; 1998.

42. Foster CB, Lehrnbecher T, Samuels S, et al. An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. Blood 2000;96:2562–7.

43. van der Kuyl AC, Polstra AM, Weverling GJ, Zorgdrager F, van den Burg R, Cornelissen M. An IL-8 gene promoter polymorphism is associated with the risk of the development of AIDS-related Kaposi's sarcoma: a case-control study. AIDS 2004;18:1206–10.

44. Goedert JJ, Vitale F, Lauria C, et al. Risk factors for classical Kaposi's sarcoma. J Natl Cancer Inst 2002;94:1712–8.

45. Whitby D, Boshoff C. Kaposi's sarcoma herpesvirus as a new paradigm for virus-induced oncogenesis. Curr Opin Oncol 1998;10:405–12.

46. de Sanjose S, Marshall V, Sola J, et al. Prevalence of Kaposi's sarcoma-associated herpesvirus infection in sex workers and women from the general population in Spain. Int J Cancer 2002;98:155–8.

47. Packer BR, Yeager M, Staats B, et al. SNP500Cancer: a public resource for sequence validation and assay development for genetic variation in candidate genes. Nucleic Acids Res 2004;32 Database issue:D528–32.

48. Antonarakis SE. Recommendations for a nomenclature system for human gene mutations. Nomenclature Working Group. Hum Mutat 1998;11:1–3.

49. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002;70:425–34.

50. Lake SL, Lyon H, Tantisira K, et al. Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. Hum Hered 2003;55:56–65.

51. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89.

52. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;73:1162–9.

53. Kooperberg C, Ruczinski I, LeBlanc ML, Hsu L. Sequence analysis using logic regression. Genet Epidemiol 2001;21 Suppl 1:S626–31.

54. Ruczinski I, Kooperberg Cl, LeBlanc M. Logic regression. J Computational and Graphical Statistics 2003;12:475–511.

55. Computing TRPfS. 2.1.0 ed. Available: http://www.r-project.org/; 2005.

56. Abbas AK LAaPJ. Cellular and molecular immunology. 3rd ed. Philadelphia: WB Saunders Co.; 1997.

57. Sirianni MC, Vincenzi L, Fiorelli V, et al. gamma-Interferon production in peripheral blood mononuclear cells and tumor infiltrating lymphocytes from Kaposi's sarcoma patients: correlation with the presence of human herpesvirus-8 in peripheral blood mononuclear cells and lesional macrophages. Blood 1998;91:968–76.

58. Fiorelli V, Gendelman R, Sirianni MC, et al. gamma-Interferon produced by CD8+ T cells infiltrating Kaposi's sarcoma induces spindle cells with angiogenic phenotype and synergy with human immunodeficiency virus-1 Tat protein: an immune response to human herpesvirus-8 infection? Blood 1998;91:956–67.

59. O'Brien SJ, Nelson GW. Human genes that limit AIDS. Nat Genet 2004;36:565–74.

60. Fallin D, Cohen A, Essioux L, et al. Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. Genome Res 2001;11:143–51.

61. Fallin D, Schork NJ. Accuracy of haplotype frequency estimation for biallelic loci, via the expectation-maximization algorithm for unphased diploid genotype data. Am J Hum Genet 2000;67:947–59.

62. Schwarz M, Murphy PM. Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor constitutively activates NF-kappa B and induces proinflammatory cytokine and chemokine production via a C-terminal signaling determinant. J Immunol 2001;167:505–13.

63. Arvanitakis L, Mesri EA, Nador RG, et al. Establishment and characterization of a primary effusion (body cavity-based) lymphoma cell line (BC-3) harboring kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in the absence of Epstein-Barr virus. Blood 1996;88:2648–54.

64. Kato H, Tsuchiya N, Tokunaga K. Single nucleotide polymorphisms in the coding regions of human CXC-chemokine receptors CXCR1, CXCR2 and CXCR3. Genes Immun 2000;1:330–7.

65. Renzoni E, Lympany P, Sestini P, et al. Distribution of novel polymorphisms of the interleukin-8 and CXC receptor 1 and 2 genes in systemic sclerosis and cryptogenic fibrosing alveolitis. Arthritis Rheum 2000;43:1633–40.

66. Hechtman DH, Cybulsky MI, Fuchs HJ, Baker JB, Gimbrone MA, Jr. Intravascular IL-8. Inhibitor of polymorphonuclear leukocyte accumulation at sites of acute inflammation. J Immunol 1991;147:883–92.

67. Colditz I, Zwahlen R, Dewald B, Baggiolini M. *In vivo* inflammatory activity of neutrophil-activating factor, a novel chemotactic peptide derived from human monocytes. Am J Pathol 1989;134:755–60.

68. Leonard EJ, Skeel A, Yoshimura T, Noer K, Kutvirt S, Van Epps D. Leukocyte specificity and binding of human neutrophil attractant/activation protein-1. J Immunol 1990;144:1323–30.

69. Matsushima K, Morishita K, Yoshimura T, et al. Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J Exp Med 1988;167:1883–93.

70. Schroder JM, Mrowietz U, Christophers E. Purification and partial biologic characterization of a human lymphocyte-derived peptide with potent neutrophil-stimulating activity. J Immunol 1988;140:3534–40.

71. Koch AE, Polverini PJ, Kunkel SL, et al. Interleukin-8 as a macrophage-derived mediator of angiogenesis. Science 1992;258:1798–801.

72. Murdoch C, Monk PN, Finn A. Cxc chemokine receptor expression on human endothelial cells. Cytokine 1999;11:704–12.

73. Salcedo R, Ponce ML, Young HA, et al. Human endothelial cells express CCR2 and respond to MCP-1: direct role of MCP-1 in angiogenesis and tumor progression. Blood 2000;96:34–40.

74. Li A, Dubey S, Varney ML, Dave BJ, Singh RK. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. J Immunol 2003;170:3369–76.

75. Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines-CXC and CC chemokines. Adv Immunol 1994;55:97–179.
76. Gerszten RE, Garcia-Zepeda EA, Lim YC, et al. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature 1999;398:718–23.
77. Yoshimura T, Matsushima K, Tanaka S, et al. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. Proc Natl Acad Sci U S A 1987;84:9233–7.
78. Murphy PM, Tiffany HL. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. Science 1991;253:1280–3.
79. Holmes WE, Lee J, Kuang WJ, Rice GC, Wood WI. Structure and functional expression of a human interleukin-8 receptor. Science 1991;253:1278–80.
80. Mollereau C, Muscatelli F, Mattei MG, Vassart G, Parmentier M. The high-affinity interleukin 8 receptor gene (IL8RA) maps to the 2q33–36 region of the human genome: cloning of a pseudogene (IL8RBP) for the low-affinity receptor. Genomics 1993;16:248–51.
81. Sprenger H, Lloyd AR, Lautens LL, Bonner TI, Kelvin DJ. Structure, genomic organization, and expression of the human interleukin-8 receptor B gene. J Biol Chem 1994;269:11065–72.
82. Ahuja SK, Shetty A, Tiffany HL, Murphy PM. Comparison of the genomic organization and promoter function for human interleukin-8 receptors A and B. J Biol Chem 1994;269:26381–9.
83. Santamaria Babi LF, Moser B, Perez Soler MT, et al. The interleukin-8 receptor B and CXC chemokines can mediate transendothelial migration of human skin homing T cells. Eur J Immunol 1996;26:2056–61.
84. Bais C, Van Geelen A, Eroles P, et al. Kaposi's sarcoma associated herpesvirus G protein-coupled receptor immortalizes human endothelial cells by activation of the VEGF receptor-2/KDR. Cancer Cell 2003;3:131–43.
85. Sodhi A, Montaner S, Patel V, et al. The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1alpha. Cancer Res 2000;60:4873–80.
86. Cesarman E, Mesri EA, Gershengorn MC. Viral G protein-coupled receptor and Kaposi's sarcoma: a model of paracrine neoplasia? J Exp Med 2000;191:417–22.
87. Montaner S, Sodhi A, Pece S, Mesri EA, Gutkind JS. The Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor promotes endothelial cell survival through the activation of Akt/protein kinase B. Cancer Res 2001;61:2641–8.
88. Yang TY, Chen SC, Leach MW, et al. Transgenic expression of the chemokine receptor encoded by human herpesvirus 8 induces an angioproliferative disease resembling Kaposi's sarcoma. J Exp Med 2000;191:445–54.
89. Pati S, Cavrois M, Guo HG, et al. Activation of NF-kappaB by the human herpesvirus 8 chemokine receptor ORF74: evidence for a paracrine model of Kaposi's sarcoma pathogenesis. J Virol 2001;75:8660–73.
90. Gershengorn MC, Geras-Raaka E, Varma A, Clark-Lewis I. Chemokines activate Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor in mammalian cells in culture. J Clin Invest 1998;102:1469–72.
91. Chiou CJ, Poole LJ, Kim PS, et al. Patterns of gene expression and a transactivation function exhibited by the vGCR (ORF74) chemokine receptor protein of Kaposi's sarcoma-associated herpesvirus. J Virol 2002;76:3421–39.
92. Heinzmann A, Mao XQ, Akaiwa M, et al. Genetic variants of IL-13 signalling and human asthma and atopy. Hum Mol Genet 2000;9:549–59.
93. Graves PE, Kabesch M, Halonen M, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. J Allergy Clin Immunol 2000;105:506–13.
94. Wang M, Xing ZM, Lu C, et al. A common IL-13 Arg130Gln single nucleotide polymorphism among Chinese atopy patients with allergic rhinitis. Hum Genet 2003;113:387–90.
95. Minty A, Chalon P, Derocq JM, et al. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. Nature 1993;362:248–50.
96. Morgan JG, Dolganov GM, Robbins SE, Hinton LM, Lovett M. The selective isolation of novel cDNAs encoded by the regions surrounding the human interleukin 4 and 5 genes. Nucleic Acids Res 1992;20:5173–9.
97. Smirnov DV, Smirnova MG, Korobko VG, Frolova EI. Tandem arrangement of human genes for interleukin-4 and interleukin-13: resemblance in their organization. Gene 1995;155:277–81.
98. McKenzie AN, Culpepper JA, de Waal Malefyt R, et al. Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function. Proc Natl Acad Sci U S A 1993;90:3735–9.
99. Monini P, Colombini S, Sturzl M, et al. Reactivation and persistence of human herpesvirus-8 infection in B cells and monocytes by Th-1 cytokines increased in Kaposi's sarcoma. Blood 1999;93:4044–58.
100. Blasig C, Zietz C, Haar B, et al. Monocytes in Kaposi's sarcoma lesions are productively infected by human herpesvirus 8. J Virol 1997;71:7963–8.