A Common Genetic Variant in *FCGR3A*-V158F and Risk of Kaposi Sarcoma Herpesvirus Infection and Classic Kaposi Sarcoma

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Abstract

Associations of *FCGR3A* among men with HIV/acquired immunodeficiency syndrome suggest that host responses affect the pathogenesis of Kaposi sarcoma herpesvirus (KSHV) infection and risk of acquired immunodeficiency syndrome–associated Kaposi sarcoma. Using DNA from two HIV seronegative case-control populations in Italy, we examined whether the functional *FCGR3A*-V158F variant was associated with risk of KSHV infection or classic Kaposi sarcoma (CKS). In population I, we examined *FCGR3A* variants and risk of KSHV infection in 34 KSHV latent nuclear antigen (LANA)-seropositive and 120 LANA-seronegative adults from Sardinia (52% male; median age, 45 years; range, 31-60), whereas in population II, we examined risk of CKS from 133 CKS cases and 172 KSHV LANA-seropositive controls from Sicily, Rome, and Naples (70% males; median age, 74 years; range, 29-91). *FCGR3A* variants were determined by direct sequence analysis of a nested PCR of genomic DNA assay using allele-specific primers. KSHV LANA was determined by immunofluorescence assay. Overall, compared with the 158F allele, 158V was overrepresented among controls from both Mediterranean populations (frequency = 0.52 and 0.51, respectively). After controlling for age, 158V homozygous women were at increased risk of KSHV infection and CKS compared with 158F homozygous women [odds ratio (OR), 8.7; 95% confidence interval (95% CI), 0.8-98 and OR, 3.8; 95% CI, 1.0-14, respectively], whereas homozygous men were at decreased risk (OR, 0.4; 95% CI, 0.1-2.3 and OR, 0.4; 95% CI, 0.2-0.8, respectively). Significant gene-dose effects were observed among men and women at risk for CKS ($P_{trend} \leq 0.05$). Our findings suggest that gender differences could possibly modify the effect of *FCGR3A* on risk of KSHV infection and CKS. Additional studies are required to confirm these relationships and determine their etiologic significance. (Cancer Epidemiol Biomarkers Prev 2005;14(3):633–7)

Introduction

Classic Kaposi sarcoma (CKS) is an angioproliferative neoplasm induced by Kaposi sarcoma–associated herpesvirus (KSHV; ref. 1) that predominantly occurs among elderly men of Eastern Mediterranean descent (2). Its etiology is complex and includes alterations in immune function (3) that may be related to an imbalance of lytic and latent KSHV infection.

Natural killer (NK) cell–mediated, antibody-dependent cellular cytotoxicity contributes to host defense against viral infections (4) as well as immunosurveillance of transformed cells (5, 6). In immune-competent individuals, NK cells target and lyse latent KSHV-infected cells (7). In contrast, insufficient NK activity is associated with severe, chronic, and recurrent disseminating herpesvirus infections (8, 9), including herpes-simplex, Epstein-Barr, varicella zoster, and cytomegalovirus, and is observed in several solid tumor malignancies (10, 11), including CKS (12, 13).

The ability for NK cells to mediate cytotoxicity is regulated by a structurally and functionally diverse family of low-affinity transmembrane binding receptors for IgG (14), known as FcγRs. *FCGR3A*, the gene that encodes FcRIIIa (CD16), is predominantly expressed on NK cells (15). *FCGR3A*, located on the long arm of chromosome 1, carries a functional single nucleotide polymorphism that substitutes phenylalanine for valine at residue 158 (16). Despite identical levels of FcRIIIa expression, 158V homozygotes have higher binding affinity for IgG1 and IgG3 compared with 158F homozygotes (17-19); this is thought to result in greater susceptibility to KSHV infection and CKS.

Susceptibility to a number of infections and autoimmune diseases are linked to polymorphic forms of *FCGR3A* (20-23). In particular, the low-affinity binding *FCGR3A* –F158F variant was previously shown to be underrepresented among HIV-infected men with KSHV infection or acquired immunodeficiency syndrome–Kaposi sarcoma AIDS–KS, suggesting that the high-affinity variant (V158) may contribute to increased risk

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of infection and disease (24). It is possible, however, that HIV infection influences the reported association. Thus, we examined two HIV-seronegative study populations from KSHV and CKS endemic areas to determine whether the common genetic variant *FCGR3A*-V158V is associated with risk of KSHV infection and, among those with KSHV infection, risk of CKS.

Materials and Methods

Study Populations

Population I, KSHV. To examine genotypic associations of *FCGR3A* and risk of KSHV infection, we included 34 latent nuclear antigen (LANA)-seropositive and 120 LANA-seronegative HIV-seronegative adults from Sardinia enrolled in a cross-sectional KSHV seroprevalence survey. KSHV seropositive and seronegative participants were ≤60 years of age and had no evidence of CKS. As previously described (25), participants were enrolled from an outpatient clinic between 1996 and 1998.

All 34 KSHV seropositive and 120 seronegative individuals from Sardinia (52% male; median age, 45 years; range, 31-60) were genotyped to examine the risk for KSHV infection.

Population II, CKS. We assessed the distribution of *FCGR3A* variants on risk of CKS using DNA from individuals of central and southern Italy. This population included 141 CKS cases and 192 KSHV LANA-seropositive controls enrolled between April 13, 1998, and October 8, 2001, from Sicily, Rome, and Naples. As described elsewhere (26), CKS cases (International Classification of Disease-Oncology, third edition, M9140/3) were enrolled from population-based cancer registries and major referral centers. Population-based potential controls were screened for KSHV LANA antibodies. Of those testing KSHV LANA-seropositive, up to two controls, frequency matched to CKS cases on gender, age (±5 or ≥80 years of age), and physician clinic were enrolled during the same time frame. HIV-1 seropositive cases and controls were excluded.

Genomic DNA from a total of 138 (98%) CKS cases and 173 (90%) KSHV LANA-seropositive controls from central and southern Italy (70% males; median age, 74 years; range, 29-91) were genotyped for the *FCGR3A*-V158F variant to examine risk for CKS. Five cases and one control were excluded because *FCGR3A* genotypes were indeterminate. Of the 133 cases included, the majority had lesions at multiple dermal sites at the time DNA was obtained (62%); 20 (15%) had lesions at one dermal site, 29 (22%) had no current lesions, and 2 (2%) had missing information. Peripheral blood mononuclear cell (PBMC) KSHV DNA was assessed in 158 (92%) KSHV LANA-seropositive controls for which DNA was available and, of these, 26 (16%) had detectable PBMC KSHV DNA. Studies from populations I and II were reviewed and approved by the appropriate Institutional Review Boards.

KSHV Laboratory Assays

Population I, KSHV. We used two antibody assays to increase assay specificity. Antibodies against lytic KSHV antigens were tested at a 1:40 dilution using a commercially available enzyme immunosorbent assay according to the manufacturer's instructions (Advanced Biotechnologies, Inc., Columbia, MD). KSHV LANA seropositivity was determined by use of immunofluorescence assay at a 1:100 dilution using uninduced BCP-1 cells (25). Sardinians seropositive by enzyme immunosorbent assay and immunofluorescence assay were considered KSHV+. Controls were negative by both assays.

Population II, CKS. KSHV antibodies against LANA were previously determined in plasma at a 1:120 dilution by immunofluorescence assay using the BCBL-1 cell line (26). Specimens with punctate nuclear immunofluorescence without tetradecanoyl phorbol-ester acetate induction were considered positive.

In the absence of disease, detection of KSHV DNA was evaluated among KSHV-LANA seropositive controls from population II to examine *FCGR3A* variants and risk of active KSHV replication. As previously described (27-30), KSHV load among controls from population II was determined using DNA extracted from frozen PBMCs by TaqMan real-time PCR (SAIC–NCI, Frederick, Maryland). DNA copies of the *KSHV-K6* gene were detected in triplicate, averaged, and normalized to the number of PBMCs (copies/10^6 cells) as determined by parallel quantification of the human *ERV-3* gene (31). The lower limit of detection was three copies per 10^6 PBMCs.

FCGR3A-V158F Detection. Genomic DNA was extracted from stored frozen sera (KSHV infection, population I) and cryopreserved lymphocyte pellets (CKS, population II) by use of the QIAmp DNA Blood Mini kit (Qiagen, Valencia, CA) and Purgene DNA Extraction kit (Gentra Systems, Minneapolis, MN), respectively. A direct sequence analysis of a nested PCR assay was done using allele-specific primers (24, 32). Each genotype was run in triplicate and confirmed by direct sequence analysis (24). For all KSHV and genetic assays, laboratory personnel were masked to case-control status.

Statistical Analysis. Among controls from populations I and II, genotype frequencies were consistent with Hardy-Weinberg equilibrium. Differences in genotype frequencies in patients with and without KSHV infection and CKS were determined using the χ^2 or the Fisher's Exact test when less than five participants were available for comparison. The relative risk of KSHV infection and CKS was estimated by the odds ratio (OR) and corresponding 95% confidence interval (CI) calculated by use of logistic regression (33). Homozygous genotypes for the low-functioning allele (158F) served as the reference. Gene-dose trends were determined by modeling ordered categorical variables as continuous (reference genotype: FF = 0; heterozygotes: VF = 1; and functional homozygotes: VV = 2). Risk estimates did not differ by using age defined as a categorical (median or 10-year intervals) or continuous variable. Analyses were stratified by gender and adjusted for dichotomous category of age [>45 years for population I (KSHV infection) and >74 years for population II (CKS)] defined by the median in the two control groups. Significant differences between strata were determined by the Breslow-Day χ^2 test for homogeneity. Interactions between gender and *FCGR3A* genotypes were formally tested by comparing the likelihood ratio between separate models containing the joint and main effects. A two-tailed P value <0.05 was considered statistically significant. All analyses were conducted using STATA version 7.0 (College Station, TX, USA).

Results

Population I, KSHV. KSHV seropositive cases and seronegative controls did not differ significantly by gender or age (Table 1). In controls at risk for KSHV infection, the allele frequency for 158V was 51% (Table 2). The VV genotype was present in 8 (24%) LANA seropositives compared with 25 (21%) LANA seronegatives (P = 0.75) and was associated, albeit not significantly, with increased risk of KSHV infection (OR, 1.7; 95% CI, 0.4-6.4).

Because the previously reported association of *FCGR3A* and AIDS–KS was based in men only, we examined the combined effect of gender and *FCGR3A* genotypes on the risk of KSHV infection. In this analysis, gender modified the association of *FCGR3A* on risk of KSHV infection (P = 0.07). Stratified analyses by gender are shown in Table 2. Among males, the VV genotype was associated with a nonsignificant reduction in the risk of LANA seropositivity (OR, 0.4; 95% CI, 0.1-2.3). In contrast, among females, the VV genotype was associated with an approximate 9-fold increase in the risk of LANA

Table 1. Characteristics of KSHV seropositive and seronegative participants (population I) and cases with CKS and KSHV LANA+ controls (population II) genotyped for *FCGR3A*

Characteristic	Population I, KSHV infection			Population II, CKS		
	KSHV+ cases	KSHV− controls	*P*	CKS cases	KSHV LANA+ controls	*P*
Sex, no. males/no. females (% female)	19/15 (44)	61/59 (50)	0.91	94/39 (29)	118/54 (31)	0.78
Median age at enrollment, y (range)	48 (31-60)	44 (31-60)	0.49	72 (29-91)	75 (37-92)	0.52
Detected PBMC KSHV DNA*	NA	NA		42 (39.6)	26 (16.5)	
No. CKS lesions†						
None	NA	NA		29 (22)	NA	
1 lesion	NA	NA		20 (15)	NA	
>1 lesion	NA	NA		82 (62)	NA	

NOTE: Population I (KSHV infection) includes 34 KSHV LANA seropositives and 120 KSHV LANA seronegatives and population II (CKS) includes 133 CKS cases and 172 KSHV+ controls.
*KSHV DNA in peripheral blood mononuclear cells was assessed among 106 CKS cases and 158 KSHV LANA-seropositive controls from population II (CKS).
† The number of lesions at the time of enrollment was not available for two CKS cases (2%).

seropositivity, although not statistically significant. A modest gene-dose effect for the V-containing genotypes was observed for risk of KSHV infection among women (P_{trend} = 0.06).

Population II, CKS. No notable difference in the distribution of gender and age was observed between CKS cases and KSHV seropositive controls (Table 1). As shown in Table 2, the allele frequency observed for 158V among controls at risk for CKS given KSHV LANA seropositivity was similar to that observed among KSHV seronegative controls from population I (52%). Thirty (23%) CKS cases were homozygous for *FCGR3A*-158V compared with 45 (26%) LANA-seropositive controls without CKS (P = 0.47). Thus, the VV genotype did not significantly contribute to risk of CKS compared with KSHV LANA-seropositive controls (OR, 0.7; 95% CI, 0.4-1.3).

Similar to population I, gender substantially modified the association of *FCGR3A* on risk of CKS (P = 0.007). As shown in Table 2, the VV genotype showed a statistically significant reduction in the risk of CKS among males compared with LANA-seropositive controls (OR, 0.4; 95% CI, 0.2-0.8). In contrast, among women, the VV genotype was associated with a borderline statistically significant 4-fold increase in CKS risk compared with LANA-seropositive controls. A significant gene-dose effect for the V-containing genotypes was observed for risk of CKS that was increased among men and decreased among women ($P_{trend} \leq 0.05$) in this population.

Among KSHV LANA-seropositive controls, we examined whether *FCGR3A* variants were associated with the presence of KSHV DNA in PBMCs and, among CKS cases in this population, with extent of disease. Of the 26 (16%) controls that had detectable KSHV DNA load, 17 were male and 9 were female. *FCGR3A* 158V homozygous women were more likely to have KSHV DNA detected in PBMCs compared with 158F homozygotes (OR, 2.5; 95% CI, 0.6-10), whereas among men, no difference was observed (OR, 1.0; 95% CI, 0.5–2.1). Among CKS cases, *FCGR3A* variants were not statistically significantly related to disease severity overall (≤1 lesion versus >1 lesion) or separately among men and women (data not shown; χ^2, $P \geq 0.22$).

Discussion

We examined the relationship between a common genetic variant of *FCGR3A*–V158F and risk of KSHV infection and CKS in two populations in Italy. Among controls, the 158V allele was overrepresented in both Mediterranean populations compared with the 158F allele. In addition, in populations I and II, respectively, frequencies of V-containing

Table 2. Risk of KSHV infection and CKS with common genetic variants of *FCGR3A*-V158F in two study populations in Italy

	Genotype	Population I, KSHV infection					Population II, CKS				
		KSHV+ *n* (%)	KSHV− *n* (%)	P_{locus}	$OR_{(AGE)}$*	95% CI	CKS case *n* (%)	Control *n* (%)	P_{locus}	$OR_{(AGE)}$*	95% CI
Overall	FF	4 (11)	22 (18)		1.0		39 (29)	39 (23)		1.0	
	VF	22 (64)	73 (61)	0.66	1.4	0.4-1.3	64 (48)	88 (51)	0.40	0.7	0.4-1.2
	VV	8 (24)	25 (21)		1.7	0.4-6.4	30 (23)	45 (26)		0.7	0.4-1.3
P_{trend}					0.47					0.22	
Males	FF	3 (16)	9 (15)		1.0		33 (35)	23 (20)		1.0	
	VF	12 (63)	34 (56)	0.77	0.6	0.1-3.0	42 (45)	58 (49)	0.02	0.5	0.3-1.0
	VV	4 (21)	18 (30)		0.4	0.1-2.3	19 (20)	37 (31)		0.4	0.2-0.8
P_{trend}					0.28					0.008	
Females	FF	1 (7)	13 (22)		1.0		6 (15)	16 (30)		1.0	
	VF	10 (67)	39 (66)	0.20	3.2	0.4-27	22 (56)	30 (56)	0.14	1.9	0.7-5.7
	VV	4 (27)	7 (12)		8.7	0.8-98	11 (28)	8 (15)		3.8	1.0-14.0
P_{trend}					0.06					0.05	

NOTE: Population I (KSHV infection) includes 34 KSHV LANA seropositives and 120 KSHV LANA seronegatives and population II (CKS) includes 133 CKS cases and 172 KSHV+ controls. Among males, there are 19 cases and 61 controls from population I (KSHV) and 94 cases and 118 controls from population II (CKS). Among females, there are 15 cases and 59 controls from population I (KSHV) and 39 cases and 54 controls from population II (CKS). In population I (KSHV), the gene-dose effect for men and women is P_{trend} = 0.28 and P_{trend} = 0.06, respectively. In population II (CKS), the gene-dose effect for men and women is P_{trend} = 0.008 and P_{trend} = 0.05, respectively.
*Adjusted for dichotomous category of age defined by the median in the respective control groups (population I, >45 years and population II, >74 years).

genotypes of *FCGR3A* (0.21 V158V, 0.61 V158F and 0.26 V158V, 0.51 V158F) and alleles (0.51, 158V and 0.52, 158V) were higher than previously published ranges for healthy populations of European ancestry (genotypes: 0.11 0.17, V158V and 0.39-0.51, V158F; allele frequencies: 0.30-0.43, 158V; refs. 17, 32). Because the distribution of common low-affinity Fc receptors (e.g., *FCGR2A*, *FCGR3A*, and *FCGR3B*) are independent of race in normal blood donors in the United States (32), it is unlikely that differences in ethnicity sufficiently account for the observed variation in allele frequencies. Instead, selective pressures specific to these two Mediterranean islands may contribute to the observed difference in allele distributions.

The *FCGR3A*-V158V variant was not statistically significantly associated with risk of KSHV infection in population I or risk of CKS in population II in our initial analyses. In contrast to the previously published report of American-Caucasian men with HIV/AIDS (24), our men had a higher frequency of the VV genotype with KSHV infection (KSHV, 30% versus KSHV and HIV/AIDS, 10%) and with KS (CKS, 31% versus AIDS-KS, 12%; ref. 24). However, it is possible that HIV infection, in addition to selective pressures, influences the relationship of variants in *FCGR3A* and risk of KSHV infection and KS, potentially contributing further to the difference in gene frequencies observed in our study and the previous one. This is a particularly attractive hypothesis in light of recent findings that suggest NK cells serve as a reservoir for HIV infection and, consequently, are important for HIV persistence (34).

In contrast to men, among women, the 158V homozygotes were associated with increased risk of KSHV infection and CKS. Differences in gender and immune function are well established (35, 36). For example, sex steroids act directly on the immune system to modify antigen presentation, lymphocyte activation, cytokine, and immune cell regulation (36-38), as well as the expression of disease resistance genes including FcγRs and the IgG superfamily (39, 40). According to one report, genetic variants of *IL13*, a gene that influences IgE expression, were associated with risk of asthma among men but not women (OR_{male}, 3.4 and OR_{female}, 1.1; ref. 41). Of note, both *FCGR3A* and *IL13* have specific effector functions that regulate immunoglobulin levels, which are typically higher among women (42).

CKS provides a unique model for evaluating immunologic differences by gender because men are ~3-fold more likely to develop CKS than women given equal KHSV infection by gender (43-45). Thus, we hypothesize that given KSHV infection, CKS risk must be related to differences in how men and women manage persistent virus over time. KSHV persists in the host and alternates between a lytic and latent life cycle. In an active replication state, the presence of KSHV-DNA load is highly correlated with presence of KS (46). Therefore, we would expect to see a relationship between V-containing variants and risk of viral replication that directly corresponds with the risk observed with CKS among men and women. Although not statistically significant, 158V homozygous women were more likely to have KSHV DNA detected in their PBMCs compared with 158F homozygotes, and among men no relationship between *FCGR3A* variants and active viral replication was observed. Similarly, because the high-affinity binding polymorphism is associated with greater antibody-dependent cellular cytotoxicity, we might expect to see a dose response with severity of disease given *FCGR3A* V-containing variants. However, this was not observed in our investigation overall or separately among men and women.

Although differential expression of gene products by gender is plausible, the observed difference in the distribution of *FCGR3A* genotypes by gender is unexpected. In both population I (KSHV) and II (CKS), the pattern of association by gender was in the same direction providing internal consistency. Nonetheless, we cannot rule out the possibility that differences observed in *FCGR3A* genotype frequencies by gender are spurious. We note that findings based on small sample size further diminished by stratification are subject to false-positive interpretation (47, 48). Thus, caution in interpreting these results must be exercised and replication is required in a larger population. In addition, our findings may be limited by the possibility that the observed *FCGR3A* associations might be due to linkage disequilibrium with other variants not studied (49). Finally, it is possible that *FCGR3A* genotyping or KSHV serostatus was misclassified irrespective of case or control status in both populations resulting in an overestimation or underestimation of the true association (50).

Overall, among HIV-seronegative Italians, we did not observe a statistically significant association of genetic variants in *FCGR3A* and risk of KSHV infection or CKS compared with KSHV LANA-seropositive controls. Stratified by gender, risk of both KSHV infection and CKS were significantly increased among women and decreased among men who had *FCGR3A*-158V containing variants. Additional studies are required to confirm possible relationships between *FCGR3A* variants and risk of KSHV infection as well as CKS and to determine their etiologic significance.

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Appendix A Kaposi Sarcoma Genetics Working Group

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