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-viremic 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 and 120 LANA-seronegative 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, 8.7; 95% confidence interval, 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 ≤ 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.


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 FcRIs. 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...
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-V158F 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 immunofluorescence without tetradecfluorescence assay using the BCBL-1 cell line (26). Specimens previously determined in plasma at a 1:120 dilution by immunofluorescence assay were considered KSHV+.

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 (Advanced Biotechnologies, Inc., Columbia, MD). 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 158F 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.
seropositivity, although not statistically significant. A modest gene-dose effect for the V-containing genotypes was observed for risk of KSHV infection among women $\left( P_{trend} = 0.06 \right)$.

**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 the 158V allele. In addition, in population I, gender substantially modified the association of $\text{FCGR3A}$ 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 KSHV-seronegative 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 KSHV-seronegative 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 $\text{FCGR3A}$ 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. $\text{FCGR3A}$ 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, $\text{FCGR3A}$ variants were not statistically significantly related to disease severity overall ($\leq 1$ lesion versus $>1$ lesion) or separately among men and women (data not shown; $\chi^2$, $P \geq 0.22$).

**Discussion**

We examined the relationship between a common genetic variant of $\text{FCGR3A}$–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

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Population I, KSHV infection</th>
<th>Population II, CKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KSHV+ cases</td>
<td>KSHV− controls</td>
</tr>
<tr>
<td>Sex, no. males/no. females (% female)</td>
<td>19/15 (44)</td>
<td>61/59 (50)</td>
</tr>
<tr>
<td>Median age at enrollment, y (range)</td>
<td>48 (31–60)</td>
<td>44 (31–60)</td>
</tr>
<tr>
<td>Detected PBMC KSHV DNA*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>No. CKS lesions†</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>1 lesion</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>&gt;1 lesion</td>
<td>82 (62)</td>
</tr>
</tbody>
</table>

**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%).

**Table 2. Risk of KSHV infection and CKS with common genetic variants of $\text{FCGR3A}$–V158F in two study populations in Italy**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Population I, KSHV infection</th>
<th>Population II, CKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KSHV+ n (%)</td>
<td>KSHV− n (%)</td>
</tr>
<tr>
<td>Overall</td>
<td>FF</td>
<td>4 (11)</td>
</tr>
<tr>
<td></td>
<td>VF</td>
<td>22 (64)</td>
</tr>
<tr>
<td></td>
<td>VV</td>
<td>8 (24)</td>
</tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td></td>
</tr>
</tbody>
</table>

**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 V158, 0.61 V158F and 0.26 V158, 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, V158 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 (KS, 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 (33,34).

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), the immune system to modify antigen presentation, lymphocyte activation, cytokine, and immune cell regulation (36–38), 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 (33,34).

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 (ORmale, 3.4 and ORfemale, 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 KHSV 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 KHSV 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 KHSV serostatus was misclassified irrespective of case or control status in both populations resulting in an overestimation or underestimation of the true association (50).

Cytokines 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
Additional members of the Kaposi Sarcoma Genetics Working Group are N. Romano (Dipartimento di Igiene e Microbiologia “Giuseppe D’Alessandro,” Universitàdegli studi di Palermo, Palermo, Italy); L. Gafa (Lega Italiana per la lotta contro i tumori-sez. Ragusa, Ragusa, Italy); D. Serraino (Dipartimento di Epidemiologia, Istituto Nazionale Malattie Infettive L. Spallanzani, IRCCS, Rome, Italy); M. Tamburini (Department of Epidemiology, National Cancer Institute, G. Pascale Foundation, Via M. Semmola, Naples, Italy); Stefania Stella (Department of Biosciences, Via Androne 83, Catania, Italy); and Maureen Kiley and Eunwha Choi (Core Genotyping Facility, National Cancer Institute, NIH, Department of Health and Human Services, Gaithersburg, Maryland).

References


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