Familial Clustering of Classic Kaposi Sarcoma

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It is widely accepted that there is a causal association between Kaposi sarcoma–associated herpesvirus (KSHV) and Kaposi sarcoma (KS). However, the majority of individuals infected with KSHV never develop KS. Here, we present a unique familial case of classic KS, in which the disease occurs in 4 siblings who have no recognized underlying immunodeficiency. We examine risk factors that could play a role in this condition, including KSHV infection, KSHV DNA load, genetic variants of KSHV, infection with additional viruses, interleukin-6–promoter polymorphism, and HLA genotype. We hypothesize that a genetic susceptibility to KS, in combination with KSHV infection, may play an important role in the presented familial case.

Kaposi sarcoma–associated herpesvirus (KSHV), also known as "human herpesvirus–8," is an essential etiological agent of Kaposi sarcoma (KS) [1], yet the majority of individuals infected with KSHV never develop KS. The classic form of KS (CKS) is an indolent vascular neoplasm involving skin and subcutaneous tissue and is mostly observed in immunocompetent elderly men of Mediterranean or Jewish descent. A finely tuned balance between the regulation of virus propagation and the immune system likely controls the development and clinical course of KS. Thus, immunocompromised people—in particular, untreated individuals infected with both HIV-1 and KSHV—are prone to develop KS, with a 10-year probability of 49.6% [2], whereas, in the Mediterranean basin, CKS develops annually in only 0.03%–0.05% of individuals infected with KSHV who are >50 years of age [3].

Familial clustering of KSHV infection has been reported [4]. Still, the vast majority of CKS cases are sporadically distributed. Rare cases of familial clustering of the disease (i.e., families in which ⩾2 members are affected) have been documented [5]. The rarity of familial clustering of KS may be because individuals infected with KSHV have a low life-long risk of developing the disease. Familial clustering of KS suggests that virological, environmental, and genetically determined susceptibility factors, independently or in combination, may determine the pathogenesis of KS.

Patients, materials, and methods. CKS was clinically and histologically diagnosed in 4 siblings from a large consanguineous family (the father being the mother's uncle) that was of Jewish origin and that had immigrated from Morocco to Israel (figure 1). All 4 patients with CKS had been diagnosed at a relatively young age (38, 41, 45, and 53 years). The mother had died, and her family described undiagnosed lesions that she had had on her legs. When the present study was conducted, the father was a healthy 95-year-old. Clinical features of the patients are summarized in figure 1. None of the patients had either visceral lesions or lesions on mucous membranes, and no underlying immunocompromise was recognized. The family's medical history was unremarkable, except that several family members had a history of allergic rhinitis, asthma, and atopic dermatitis. Blood samples were provided by 11 of the 12 living family members (written, informed consent was obtained from each study participant, and ethics approval was obtained from the Helsinki Committee of the Israeli Ministry of Health), and peripheral-blood mononuclear cells (PBMCs) were separated.

KSHV seropositivity was determined by 3 serological assays: (1) latent indirect-immunofluorescence assay (IFA), at a 1:100 dilution, to detect antibodies against the KSHV latency–associated nuclear antigen–1 (LANA), using uninduced KSHV-infected BCP-1 cells and Ramos cells as controls [1]; (2) lytic indirect IFA, at a 1:40 dilution, to detect antibodies against KSHV lytic antigens (Biotrin International); and (3) a Western-blot assay, at a dilution of 1:100, to detect antibodies against the open-reading frame (ORF) 65 antigen (provided by Thomas F. Schulz, Hannover Medical School, Hannover, Germany) [1].

Antibodies against HIV-1 and HIV-2 were detected by the Abbott HIV-1/HIV-2 third-generation EIA (Abbott Laboratories); antibodies against the glycoprotein G encoded by herpes

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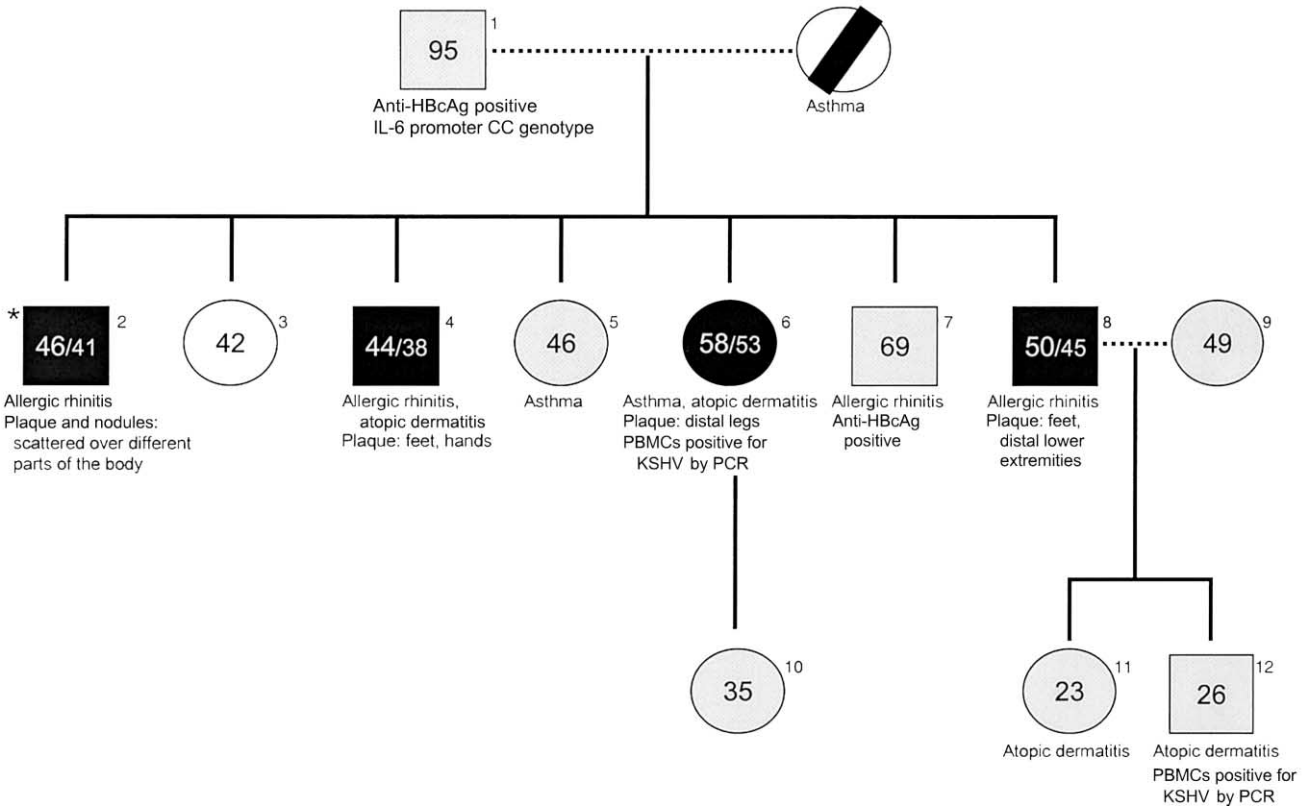

Figure 1. Family pedigree of presented family. Identification numbers for family members are at the upper right of each symbol; age (in years) at the time the present study was conducted is shown within each symbol, as is the age (in years) at diagnosis of classic Kaposi sarcoma (CKS), to the right of the virgules. Males are indicated by squares and females by circles; family members with CKS are indicated by black shading; family members infected with Kaposi sarcoma–associated herpesvirus (KSHV) are indicated by gray shading; the family member who is no longer living is indicated by a black diagonal bar. HBcAg, hepatitis B virus core antigen; IL-6, interleukin-6; PBMCs, peripheral-blood mononuclear cells; PCR, polymerase chain reaction. *Blood sample not available.

simplex virus type 2 (HSV-2) also were detected by EIA (MRL Diagnostics). Antibodies against hepatitis B virus (HBV) core antigen (HBcAg) and against hepatitis C virus (HCV) were detected by EIA, and the prevalence of HBV surface antigen (HBsAg) was determined by EIA; both EIAs were performed by the Abbott AxSYM system (CORE B7A410 67-6272/RS, HCV version 3.0 B3B440, and HBsAg V2 B7P400, respectively).

Polymorphism analysis for interleukin-6 (IL-6) promoter (−174G/C) was performed as described elsewhere [6], with genomic DNA that had been extracted from PBMCs. Polymerase chain-reaction (PCR) products were digested by use of *Nla*III, allowing the discrimination of the polymorphic alleles; DNA sequencing was used to confirm the restriction-digest results.

HLA class I typing was performed by use of the HLA Low-Resolution SSP UniTray (Pel-Freez Biologicals). HLA class II typing was performed by use of the One Lambda Micro SSP Generic HLA Class II DNA Typing Tray (Pel-Freez Biologicals).

PBMCs were tested, in triplicate, for KSHV DNA by a quantitative real-time PCR assay that is specific for the K6 gene region, as described elsewhere [7]. To determine the viral genotype, nested PCR was used to amplify KSHV DNA from formalin-fixed, paraffin-embedded tissues, as described elsewhere [8]; PCR products were directly sequenced.

Results. Of the 4 patients with CKS, 3 provided blood samples (the fourth patient refused); and all 3 of them, as well as 7 (87.5%) of the 8 family members without CKS, were found, by all serological assays, to be positive for KSHV (figure 1). This rate of KSHV infection is ~9-fold greater than the 9.9% rate of KSHV seroprevalence in the Israeli Jewish population [4]. To quantify KSHV DNA in PBMCs, we used a real-time quantitative PCR assay that enables the detection, on 100,000-cell equivalents, of as few as 3 copies of KSHV per PCR [7]. Amplification was detected in only 2 members of the family, but at a level below the quantitative sensitivity of the assay.

All family members were found to be negative for anti-HIV, HSV-2, and HCV antibodies. Two family members without CKS were positive for HBcAg antibody, but all family members were negative for HBsAg antibody.

Extensive evidence indicates that IL-6 may play a role in the pathogenesis of KS [6]. It has recently been demonstrated that

the −174 IL-6 GG and GC promoter genotypes, which are associated with increased IL-6 production, are overexpressed in HIV-positive patients with KS [6]. Except for the healthy father, who was found to harbor a CC genotype at the −174 IL-6 locus, all 10 other family members whom we tested had the GC genotype.

Because different HLA loci could affect individuals' susceptibility to a given disease, we defined the HLA alleles in the presented family. Among the 8 family members whom we analyzed, there were no differences between the frequencies of HLA classes I and II genes in the family members with CKS and those in the family members without CKS (table 1). Of note, 2 of the 3 family members with CKS who provided blood samples were homozygous for the HLA A*24/B*18/Cw*12/DRB1*11/DQB1*03 haplotype, whereas none of the 4 family members without CKS who were positive for KSHV were homozygous for that haplotype. Of the 8 family members whom we analyzed, 7, including the 3 with CKS, presented DRB1*11, an HLA subtype previously categorized in the DR5 allele. Conflicting reports regarding the association of the DR5 allele with KS have been published [5].

A unique, highly pathogenic, and transforming variant of KSHV could account for the familial occurrence of CKS. Nucleotide-sequence analysis of the *ORF26* gene has shown that KSHV falls into 4 subgroups, A–D [9]. We isolated DNA from paraffin-embedded biopsies of KS legions from all 4 patients with CKS and performed nested PCR to amplify the *ORF26* gene. The amplicons were directly sequenced to determine the viral subgroup. In the presented family, 3 of the 4 patients were infected with a virus belonging to subgroup C, whereas the fourth patient was infected with a novel variant belonging to subgroup A.

Discussion. We have presented a family in which 4 siblings were diagnosed with CKS, and we have investigated an interaction that could possibly cause this unique cluster—the interaction between a malignancy-linked virus, KSHV, and several host factors. On the basis of (1) a 9.9% seroprevalence rate (equally distributed among men and women) for KSHV in the Israeli population [4] and (2) data from Israel on the incidence of CKS in the Jewish population [10], we would expect that, annually, 2.1/10,000 men infected with KSHV would develop CKS. Thus, the occurrence of 3 males with CKS among 4 brothers infected with KSHV is 3587-fold (95% confidence interval [CI], 740–10,482-fold) greater than expected ($P<.001$); similarly, the number of women infected with KSHV who would be expected to eventually develop CKS is 0.76/10,000 annually. In the 2 sisters positive for KSHV, the occurrence of CKS is 6600-fold (95% CI, 167–36,776-fold) greater than expected ($P<.001$). Furthermore, given the possibility that additional siblings could develop the disease in the future, these ratios may become even higher. Therefore, we assume that, in addition to infection with KSHV, other underlying factors may be involved in the development of this cluster of familial CKS.

In the presented family, the average age at diagnosis of CKS was 44.25 years, which is significantly younger than the average ± SD age at diagnosis of sporadic CKS cases in Israel (men, 69.5 ± 13.9 years; women, 73.5 ± 12.7 years) [10]. An early age at onset of cancer is a recognized characteristic of familial cancers, such as ovarian cancer and nasopharyngeal carcinoma (NPC).

We detected antibodies against KSHV in all 3 patients with CKS whom we tested and in most of the other family members, although we did not specifically assess the titer of virus-neutralizing antibody (which could play a biological role). That the family members were not infected with sexually transmitted agents (HIV and HSV-2) suggests that the high rate of KSHV infection in the presented family does not reflect an increased exposure to these pathogens. Furthermore, this finding enabled us to rule out the possibility that a synergistic model of interaction between KSHV and these other viruses was at work in the presented setting. A heterozygous GC genotype of the IL-6 promoter was found in most family members, which may have increased IL-6 expression and, therefore, their risk for the

Table 1. HLA haplotypes, in patients with classic Kaposi sarcoma (CKS) and in their family members.

Family member identification no. (age, years [sex])	CKS diagnosis	KSHV serostatus	HLA				
			Class I			Class II	
			A*	B*	Cw*	DRB1*	DQB1*
6 (58 [F])	Positive	Positive	24, –	18, –	12, –	11, –	03, –
8 (50 [M])	Positive	Positive	24, 30	18, 41	12, 17	11, 07	03, 02
4 (44 [M])	Positive	Positive	24, –	18, –	12, –	11, –	03, –
1 (95 [M])	Negative	Positive	24, 30	18, 41	12, 17	11, 07	03, 02
7 (69 [M])	Negative	Positive	24, 30	18, 41	12, 17	11, 07	03, 02
5 (46 [F])	Negative	Positive	24, 30	18, 41	12, 17	11, 07	03, 02
3 (42 [F])	Negative	Negative	24, –	18, –	12, –	11, –	03, -
11 (23 [F])	Negative	Positive	3, 30	44, 41	16, 17	04, 07	03, 02

NOTE. F, female; KSHV, Kaposi sarcoma–associated herpesvirus; M, male; –, suggestive of homozygosity.

development of CKS; the homozygous CC genotype, found only in the elderly father, could protect against the development of CKS. However, these findings are probably not sufficient to account for the presented rare clustering of familial CKS. HLA typing revealed no differences between the allele distribution in family members with CKS and that in family members without CKS, except for a homozygosity in 2 family members with CKS; this finding is interesting because an association between homozygosity of HLA haplotypes and poor control of viral replication has been recognized [11]. We detected the HLA-DRB1*11 allele in most family members; yet, the importance of HLA-DRB1*11 as a risk factor in the development of KS is controversial [5]. In a consanguineous family, it is to be expected that family members would share similar genotypes (e.g., IL-6 promoter and HLA), and therefore we did not attempt to compare their genotype frequencies to those in a reference control population. Nevertheless, part of the HLA haplotype shared by many members of the presented family—namely, A*24/B*18/DRB1*11—comprises alleles that are among the 10 most frequent in Moroccan Jews [12].

We amplified 2 different KSHV genetic variants from KS lesions; 3 brothers were infected with the same KSHV variant, whereas the sister with CKS harbored a different KSHV variant. Therefore, it is possible that the development of CKS in the 3 brothers could have resulted from infection with a unique, virulent KSHV variant, whereas the CKS diagnosed in the sister could be regarded as a sporadic form; yet the possibility of an individual harboring several variants of KSHV has not been formally ruled out, and the sister with CKS could be carrying an additional viral variant, identical to that amplified from the brothers' lesions. Finally, a finding of infection with an identical KSHV variant could reflect intrafamilial transmission and, therefore, may not be the cause of this unique case.

An elevated risk of KS is associated with allergies and a history of asthma [13]. Both allergies and asthma were noted in the presented family and, thus, may be causally associated with the development of CKS.

KSHV belongs to a group of viruses that are etiologically linked to certain forms of human neoplasia that appear only in a small subset of infected individuals. In the Chinese population, familial clustering of NPC, an Epstein-Barr virus–associated disease, has been widely documented, and a major susceptibility locus on chromosome 4 has been identified in a subset of the affected families [14]. Similarly, it has been observed that the risk for cervical cancer, which is strongly associated with genital infection with oncogenic types of human papillomavirus, increases with advancing degrees of genetic relatedness, indicating that inherited genetic factors can contribute to the development of the disease [15]. The rarity of familial KS argues against simple Mendelian inheritance but does not exclude the possibility that complex predisposing factors are involved. We suggest that a genetic susceptibility to KS, possibly provided by the IL-6 promoter GC genotype and/or the HLA-DRB1*11 allele, in conjunction with KSHV infection, may have played a role in the development of the familial CKS cluster presented here. The data obtained from this family permit us to suggest possible causative factors that can be tested in larger studies in the future. Both pedigree-based gene mapping and familial case–based chromosome analyses will be necessary to locate unidentified genes that could confer susceptibility to KS. The identification of risk factors for the development of KS has important clinical implications for prevention and therapy and could increase our understanding of this multifaceted disease.

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