

this mutation was inherited. Genomic DNA was isolated from blood samples obtained from both living, unaffected parents and from the healthy brother and sequenced (Fig. 1, A). None of the immediate family members carried the mutation found in the DNA from the patient's tumor. This finding argues against a new familial form of breast cancer. However, we cannot definitively exclude the occurrence of a mutation in the germline of one of the parents, since the lack of a normal DNA sample from the patient hampers verification of a possible somatic origin of the mutation.

Our finding supports the hypothesis that somatic or constitutive mutations affecting BRCA1 gene expression could identify a subset of "sporadic" breast cancers and that such mutations could potentially represent a useful prognostic marker. We are currently investigating the functional effect of this candidate BRCA1 gene mutation.

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## Notes

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## Human Herpesvirus 8 Seroprevalence in Sardinia

Italy has one the highest rates of classic Kaposi's sarcoma (KS) in the world, with a particularly high incidence occurring in Sicily, in the Po valley, and in Sardinia (1-3). In a recent issue of the *Journal*, Whitby et al. (4) reported a higher prevalence of antibodies against human herpesvirus 8 (HHV-8) among blood donors from southern Italy (i.e., Apulia, Calabria, and Sicily; 24.6%) compared with blood donors from northern and central Italy (i.e., Lombardia, Friuli-Venezia Giulia, and Emilia-Romagna; 7.3%). Although it is not clear from the data presented by the authors that these blood donors from dif-

ferent regions in Italy were comparable in age and sex, the geographic distribution of HHV-8 seropositive individuals seems to parallel the national rates of incidence for classic KS (1-3). Their findings suggest that an association exists between the prevalence of HHV-8 antibodies and the increased incidence of classic KS. These data also support a role for HHV-8 in determining the risk for KS development (4,5).

We report similar data derived from pregnant women in Sardinia, where a bank of sera has been collected since 1986, within the framework of the Sardinia Insulin Dependent Diabetes Mellitus Study (6). To determine the prevalence of HHV-8 infection in pregnant women living in Sardinia, 100 individual sera were prepared from umbilical cord blood taken at delivery in 1993 and 1994. These sera were assayed for antibodies directed against both latent nuclear antigens (antilatent antibodies) and cytoplasmic antigens (antilytic antibodies) of HHV-8, using the method described by Lennette et al. (7).

We determined that 25 of 100 sera were seropositive for HHV-8 antilytic antibodies (95% confidence interval [CI] = 16.5%-33.5%) (Table 1). HHV-8 seropositivity for the cytoplasmic antigens ranged from 29.1% in women under 30 years of age to 21.6% in women greater than or equal to 30 years of age (data not shown). A greater percentage of women living in northern Sardinia (i.e., the provinces of Sassari and Nuoro), an area with especially higher incidence rates of classic KS (2), were seropositive (31.0%) than those women living in the southern part of the region (i.e., Cagliari province (12.0%) (Table 1). This difference between the two groups persisted after adjustment by quinquennia of age ( $X^2_1 = 4.13$ ;  $P = .04$ ). The geometric mean titer was 183

**Table 1.** Prevalence of HHV-8 infection in pregnant women, at time of delivery, according to type of antibodies and area of residence in Sardinia 1993 and 1994

Residence*	No. of sera tested	Sera positive for antilytic antigens No. (%)	Sera positive for antilatent antigens No. (%)
Northern Sardinia	71	22 (30.1)	3 (4.2)
Southern Sardinia	25	3 (12.0)†	0 (0.0)
Total	100	25 (25.0)	3 (3.0)

\*The sum does not add up to the total because of missing values.

† $X^2_1$ , adjusted for age = 4.13,  $P = .04$ , two-sided.

for women who resided in northern Sardinia but 129 for those living in southern Sardinia. Antilient HHV-8 antibodies were found in only three women (95% CI = 0.0%–6.3%); all of these individuals were from northern Sardinia (Table 1).

Our results confirm the association between seroprevalence of HHV-8 and incidence rates of KS in different regions of Italy as reported by Whitby et al. (4). The results also highlight the possibility that substantial variations exist between neighboring areas. However, HHV-8 seroprevalence rates must be interpreted with caution on account of limitations of assay specificity for different HHV-8 antigens (7) and the predominance of selected population groups such as blood donors (4).

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## No Association Between Human Herpesvirus Type 8 Infection and Multiple Myeloma

Human herpesvirus type 8 (HHV-8) is a human herpesvirus that is associated with Kaposi's sarcoma (KS), Castemans disease, and a rare form of body cavity lymphoma (1,2). Recently, Retting et al. (3) have also demonstrated the presence of HHV-8 in cultured bone marrow stromal dendritic cells from 15 of 15 samples obtained from patients with multiple myeloma and in two of eight samples from patients with monoclonal gammopathy of undetermined significance (MGUS). However, the virus was not found not in malignant plasma cells. Therefore, the researchers believed that the virus might cause the bone marrow cancer by infecting nonmalignant cells, possibly through alteration in the bone marrow microenvironment and production of viral interleukin 6 (3). Since then, other investigators have evaluated the possible link between HHV-8 and multiple myeloma through either serologic assays or polymerase chain reaction (PCR)-based experimental designs. None of these investigators

could confirm this association, but one group (4) did detect HHV-8 in 18 of 20 acetone-fixed and paraffin-embedded bone marrow biopsy samples tested.

We have demonstrated (5) the presence of HHV-8 by PCR in biologic samples of human immunodeficiency virus-positive and -negative individuals in western Sicily, an area with one of the highest incidence rates of classical KS in developed countries, suggesting that HHV-8 is widespread in the general population (5). Therefore, to establish the possible association between HHV-8 and plasma cell dyscrasias, we used a nested PCR to amplify the HHV-8 KS330 sequence (a 233-base-pair herpesvirus-like DNA sequence). We extracted DNA from peripheral blood mononuclear cells (PBMCs), bone marrow aspirates, and bone marrow stromal cells isolated from 20 patients with multiple myeloma and two patients with MGUS as described (5). PBMCs and bone marrow mononuclear cells were separated by Ficoll-Hypaque density sedimentation from EDTA-treated blood samples and fresh bone marrow aspirates. Bone marrow mononuclear cells were used to establish long-term bone marrow stromal cell cultures as described (3). An immunofluorescence assay, using body-cavity-based lymphoma-1 cells, that detects antibodies against latent and lytic antigens of HHV-8 was also performed to investigate the presence of antibodies to HHV-8 in patients with multiple myeloma and MGUS.

Table 1 shows that none of the 22 patients had HHV-8 DNA sequences in PBMCs, in bone marrow aspirates, or in dendritic stromal cells. Antibodies to HHV-8 antigens were detected in four of 22 patients. This seroprevalence rate is similar to that found in our earlier stud-

**Table 1.** Detection of human herpesvirus type 8 (HHV-8) DNA by polymerase chain reaction (PCR) and serologic response (as measured by an immunofluorescence assay [IFA]) to HHV-8 antigen(s) of 20 patients with multiple myeloma and two patients with gammopathy of undetermined significance (eight females and 14 males; age range, 50–75 years)

Biologic sample	Test	
	PCR, No. positive/No. tested	IFA, No. positive/No. tested
Peripheral blood mononuclear cells	0/22	—
Bone marrow aspirates	0/22	—
Bone marrow stromal dendritic cells	0/22	—
Serum	—	4/22