

Ugandan Kaposi's Sarcoma-Associated Herpesvirus Phylogeny: Evidence for Cross-Ethnic Transmission of Viral Subtypes

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Key Words

Kaposi's sarcoma-associated herpesvirus ·
Ugandan diversity · Viral phylogeny · Ethnicity

Abstract

Objective: The aim of this study was to test the relationship between Kaposi's sarcoma-associated herpesvirus (KSHV) phylogeny and host ethnicity at the within-country scale. **Methods:** KSHV genomic DNA samples were isolated from 31 patients across eleven Ugandan ethnic groups. Amino acid sequences of the ORF-K1 gene were used to construct a neighbor-joining phylogenetic tree. **Results:** A5 and B1 variants predominated with no evidence of distinct ethnic or geographic distribution. A new K1 subtype (F) was identified in a member of the Bantu Gisu tribe and a new subtype B variant (B3) among members of the Bantu Ganda tribe. **Conclusions:** The phylogeny may yet be structured by host ethnicity if members of Ugandan groups have convoluted biological origins, even as they identify with single tribes. An alternative possibility is that KSHV subtype evolution may have preceded major diversification of sub-Saharan Africans into ethnicities as we know them today, with

ethnic groups beginning their histories already hosting multiple subtypes. A third alternative is that horizontal transmission of multiple KSHV subtypes may have broken up vertical lineages of the virus passed down within Ugandan populations.

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Introduction

Kaposi's sarcoma (KS) is a cutaneous tumor, in some cases also found in visceral tissue [1]. The etiologic agent of KS is Kaposi's sarcoma-associated herpesvirus (KSHV) or human herpesvirus 8, a gamma-2 herpesvirus most closely related to chimpanzee rhadinovirus PanRHV1a [2–4]. The virus has been associated with four KS pathologies, including classic, endemic, HIV-associated and post-transplant phenotypes [5]. KSHV is also implicated in lymphoproliferative diseases such as primary effusion lymphoma and multicentric Castleman's disease.

Subtypes A through E of the KSHV K1 protein are distributed along broad geographic and ethnic lines [6, 7]. Subtype A appears predominant among patients of European descent in Europe and the United States, subtype B

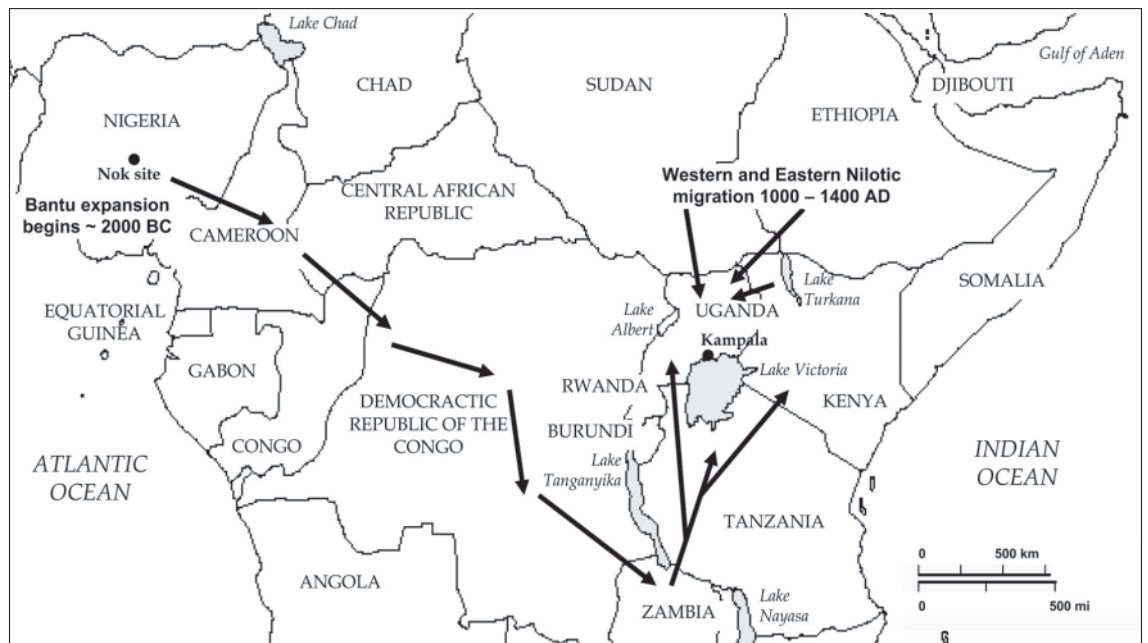


Fig. 1. Bantu and Nilotic migrations into Uganda overlaid on a map of the present-day geography of Africa. The map is an oversimplification. The migrations are schematic, and in the case of the Bantu, leave out myriad bifurcations down the west and east coasts of Africa. In addition, waves of Bantu and Nilotes moved in and out of Uganda, marking the ebb and flow of the groups' fortunes. Convergence zones are also marked at the east side of Lake Victoria (now Kenya and Tanzania), intermixing Bantu, Southern Nilotic and Eastern Nilotic populations.

in sub-Saharan Africa and among Haitians and African-Americans, subtype C in Eurasia and the United States, subtype D in South Asia, Australia and New Zealand, and subtype E among Brazilian Amerindians. Zong et al. [8] and Hayward [6] hypothesized that KSHV is a relatively old human virus that evolutionarily radiated along with early human populations migrating out of Africa. Ethnic distributions of KSHV are apparent within countries as well. Eight of 9 Taiwanese patients of Chinese descent were shown to host a novel C3' K1 variant, while a Taiwanese of Hualian descent was diagnosed with a virus of the Pacific-specific D1 clade [6]. In Israel, Ashkenazi Jewish patients from Russia typically expressed the A1' variant, while North African Sephardi Jews expressed C2, C3 or C6 [9].

Uganda would appear a good candidate for a similarly vicariant phylogeography, i.e. for a distribution brought about by coevolutionary divergences of host and virus. A multitude of ethnic groups of divergent historical origins live in Uganda. The progenitors of the Bantu tribes in Uganda originated in the Nigeria-Cameroon area before expanding into the Great Lakes region

of Uganda and Kenya by the first century AD (fig. 1) [10–12]. Nilotes appear to have an amalgam of origins, with waves of populations from the Nile River Valley in present-day Sudan, the Lake Turkana region and the Horn of Africa, an area historically characterized by a Middle Eastern, Indian and Levantine admixture [13, 14]. Nilotes entered Uganda from these regions between 1000 and 1400 AD. Groups of Sudanic and Nilo-Hamitic (Eastern Nilotic) descent are present in Uganda in lesser minorities.

Second, Uganda hosts some of the world's highest seroprevalences for KSHV across its population [15, 16]. Kakoola et al. [17] reported a seroprevalence of 74% among Ugandan blood donors tested. Third, Ugandan groups may differ in presenting KS. Two decades before the discovery of KSHV, Taylor et al. [18] showed that KS incidence differed in Uganda by tribe and geographic area. The Sudanic tribes tended to have greater incidences than the Bantu, which in turn had greater incidences than the Nilotes. With the caveats that districts may have differed in reporting rates and patients may have moved to urban centers for treatment, Taylor et al.

[18] showed greater standardized KSHV incidences in the western districts of Uganda.

Given the worldwide distribution of KSHV, the divergent origins of the host groups present and the history of KS in Uganda, we hypothesized that ethnic grouping largely structures KSHV phylogeny in Uganda. We expected that Bantu patients, of sub-Saharan origins, would host KSHV of subtype B, and Nilotic patients, of Northern African origins, would host subtypes A and C, save A5, a variant found throughout the African continent [8, 9, 17, 19, 20]. To test the hypothesis, we compared the ORF-K1 gene sequences of KSHV sampled from members of an array of Ugandan groups, representing both Bantu and Nilotic origins. K1, unique to KSHV, encodes for a transmembrane tyrosine kinase signaling glycoprotein with transforming properties and exhibits extensive amino acid variation (up to 30%) across populations [4, 21, 22, 23]. A phylogeny of the K1 samples was constructed and matched with patients' self-reported ethnicities.

Materials and Methods

Samples

Viral genomic DNA was isolated from white blood cells collected from 56 patients presenting with KS at the Uganda Cancer Institute in Kampala between June and August 2001. The blood samples were initially collected for routine laboratory evaluation of patients undergoing chemotherapy. Written consent was obtained from the patients to use a portion of their leftover blood, and ethical approval was obtained from the Uganda AIDS Research Committee and the National Council of Science and Technology.

Extraction, Amplification and Sequencing of the K1 Gene

Genomic DNA was extracted using a modification of Miller's salting-out procedure described by Welsh and Bunce [24]. A 650-bp fragment of the K1 gene including variable regions 1 (aa 54–93) and 2 (aa 191–228) was amplified by nested PCR using primers described by Zong et al. [9]: LGH No. 2521 and LGH No. 2522 (first round), LGH No. 2522/LGH No. 2090 (second round) and LGH No. 2090/LGH No. 2508 (third round). The PCR products were extracted from the agarose gel using a qiaex-2 gel extraction kit (Qiagen, Inc.) and sequenced using primers LGH No. 2090 and LGH No. 2508 by Big Dye cycle on an AB310 automated sequencer.

Phylogenetic Analysis

With MEGA 2.1 [25] we created a phylogenetic tree of the partial K1 amino acid sequences. Fifteen reference sequences from GenBank and published reports were included in the analysis to aid subtype identification. Three additional sequences that did not cluster with any known KSHV subtypes in previous studies – two from Lacoste et al. [26], one from Whitby et al. [27] – were included to verify the identity of a possible new subtype. All sequences were first aligned in ClustalW [28]. Pairwise distances were generated in MEGA with indels removed as needed in the course of dis-

tance computation. The distances were gamma-corrected for multiple substitutions at the same site, and substitution rates among sites were assumed unequal. The gamma shape parameter (α) was calculated in GZ-GAMMA [29] with a subset of sequences from the present study. The neighbor-joining method [30] was used to create a mid-rooted tree and a bootstrap test of 1,000 replications implemented to test branch consistency [31].

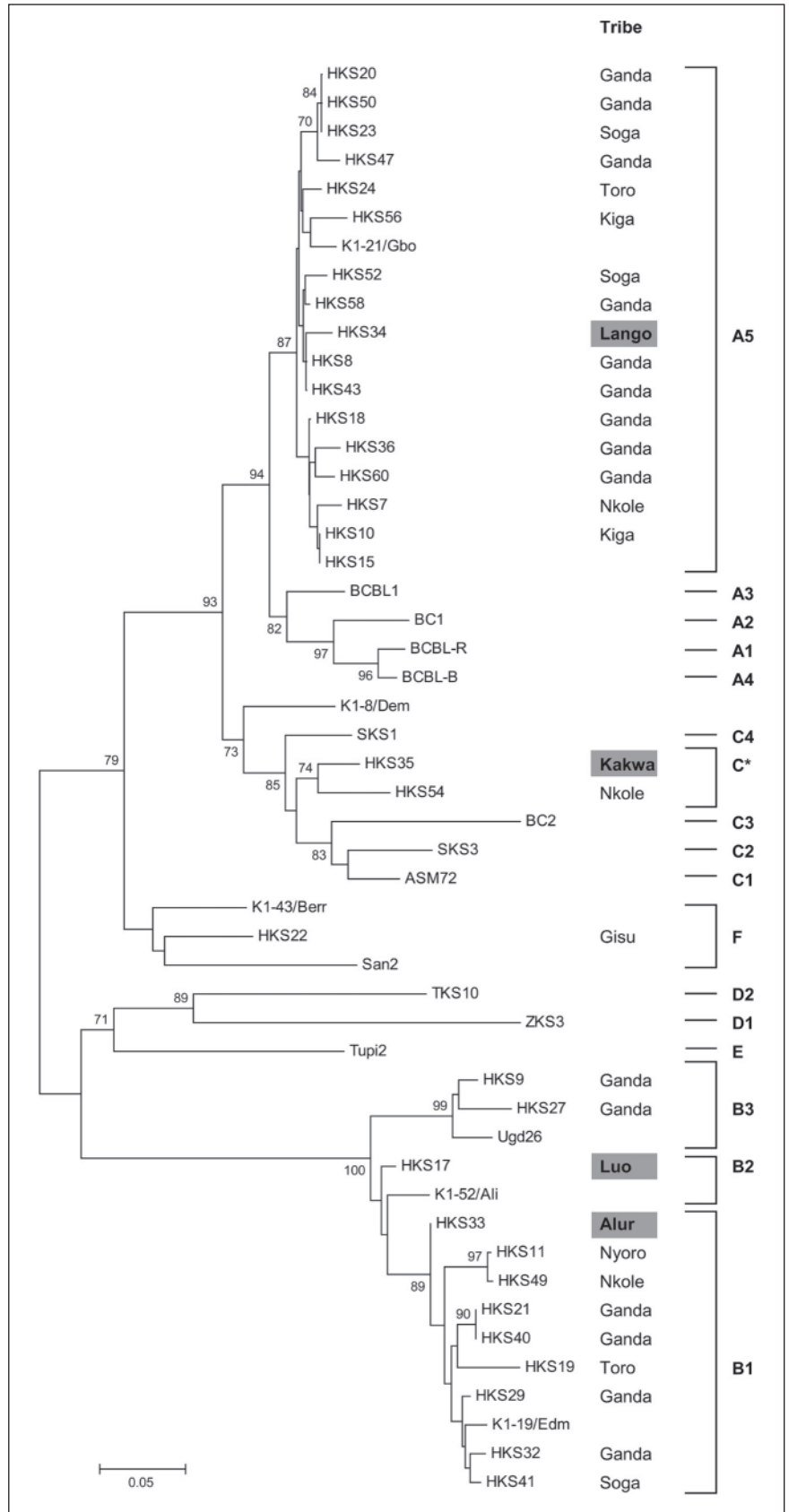
Results

PCR products were obtained for 32 of the 56 white blood cell samples (GenBank Accession Numbers AY953871–AY953903). Samples HKS10 and HKS15 were obtained from the same patient at different times and resulted in identical K1 sequences.

Figure 2 shows the neighbor-joining tree relating the 32 samples and bootstrap values ≥ 70 . The tribes of the donating participants are listed along with the subtypes of reference sequences. Seventeen sequences were identified as A5 variants, nine as B1 and one (HKS17) as B2. Despite its relatively low bootstrap value, the latter unambiguously clusters with other B2 clones when included in a separate phylogenetic analysis of 47 subtype B clones (fig. 3), including sequences from Kakoola et al. [17], Cook et al. [19], Lacoste et al. [26] and Treurnicht et al. [35]. Two sequences (HKS9 and HKS27) appear to constitute a new B variant (B3) and cluster with six other Ugandan samples reported by Kakoola et al. [17], indicating a local cladogenetic event. The B variants appear distinguishable by a motif at the beginning of VR2 (residues 194–198). Two additional sequences (HKS35 and HKS54) appear to comprise a new C variant and appear distinct from the majority of C variants described in Europe, the United States, Asia and the Middle East.

Sample HKS22, from a Bantu Gisu patient, clusters with clones K1–43/Berr (from France) and San2 (from Botswana), reported by Lacoste et al. [26] and Whitby et al. [27]; together, they are designated as a proposed new KSHV subtype F. HKS22 is characterized by a unique combination of minority residues in the putative VR* loop (residues 52–76), including singularly unique residues H-69 and S-71 (fig. 4). HKS22 also exhibits a unique combination of residues, including singular residues K-79 and S-83, in a region identified as homologous to the variable region of the immunoglobulin light chain and where variation differentiates KSHV subtypes [8, 36, 37]. Additionally, HKS22 exhibits unique residues L-127, F-158, K-161 and P-163 in the CR2 domain, which, with the immunoglobulin region, appears to comprise the outer surface of the K1 extracellular domain [37].

Fig. 2. Neighbor-joining phylogeny of KSHV K1 amino acid sequences (HKS series) collected from 31 Ugandan patients of different ethnic groups (GenBank Accession Numbers AY953871–AY953903). Samples from patients of Nilotic origins are annotated in bold. The other samples indicate Bantu groups. HKS10 and HKS15 are from the same patient sampled at different times during infection. Sequences of a known subtype were included to aid subtype identification: BCBL1 [32], BC1 [33], BCBL-R, BCBL-B, BC2, ASM72, TKS10, ZKS3 [34], Tupi2 [7], Ugd26 [17], K1-21/Gbo, K1-52/Ali, K1-19/Edm [26], SKS1 and SKS3 [8]. Three additional sequences that did not cluster with known subtypes in previous studies were also included to verify a possible new subtype: San2 [27], K1-43/Berr and K1-8/Dem [26]. The mid-rooted tree was built by pairwise deletion: the alignment gaps were removed as needed in the course of pairwise distance computations. The distances were gamma-corrected for multiple substitutions at the same site and unequal substitution rates across sites, using a gamma shape parameter (α) of 0.64. A bootstrap test of 1,000 replications was implemented to test branch consistency. Bootstrap values ≥ 70 are shown for support.



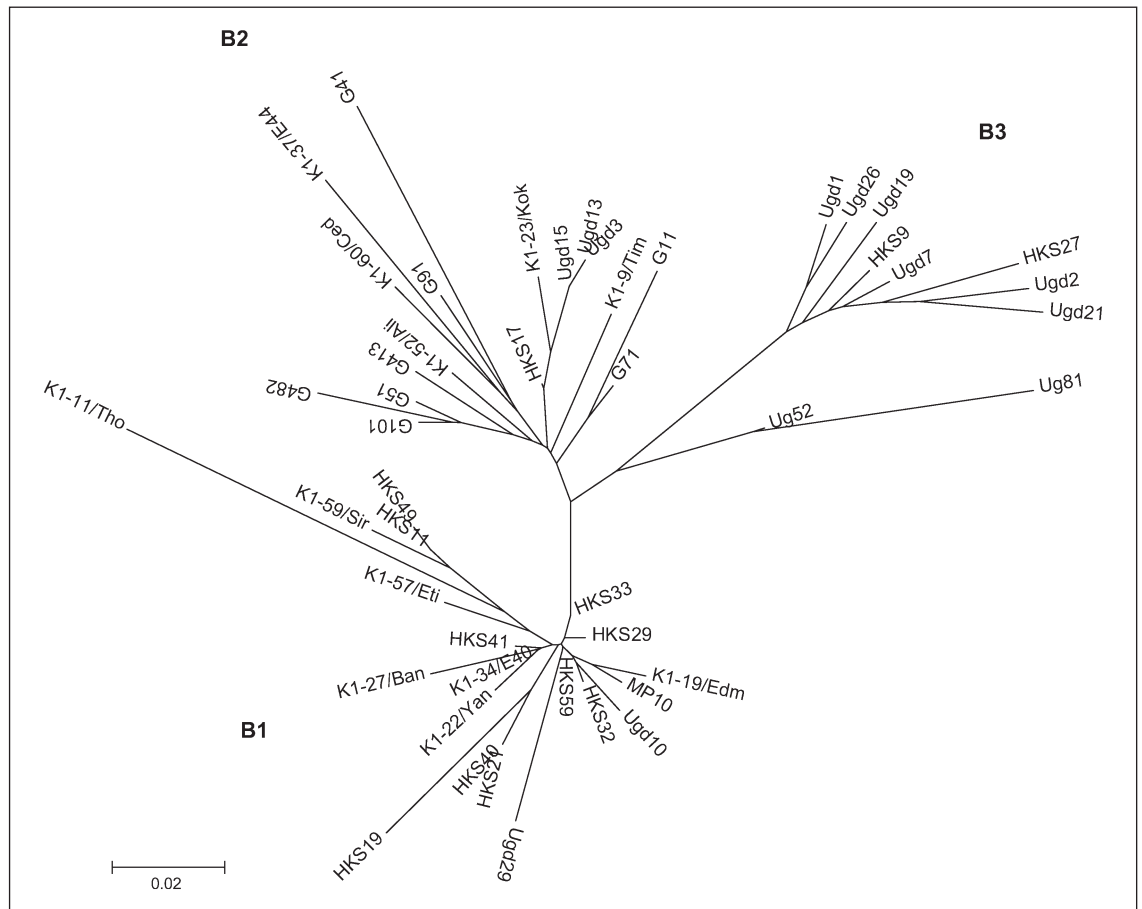


Fig. 3. Radial neighbor-joining tree of KSHV ORF-K1 subtype B phylogeny defines three variants. The tree was constructed as described in figure 2, using clones from Kakoola et al. [17], Cook et al. [19], Lacoste et al. [26] and Treurnicht et al. [35], in addition to the HKS series introduced here. Two clones previously defined as ‘B3’ – MP10 [35] and K1–60/Ced [26] – appear well-situated within B1 and B2, respectively. In a consensus tree, the B variants are supported by bootstrap values greater than 70. The variants appear diagnosable by a motif at the beginning of VR2. B1 expresses amino acids VKVL at residue positions 194–198, while B2 expresses FKTL and B3 F/IKMS.

Overall, the Ugandan K1 sequences did not group by tribal identity, as self-reported by study participants. Ganda samples were repeatedly represented in both the A5 and B1 clades. The only four Nilotic samples included in the analysis were represented in the A5, C*, B1 and B2 clades. Samples HKS29 and HKS33, from a Bantu and a Nilotic patient, respectively, are identical B1 sequences, save for a 10-aa deletion event in the variable region 2 (fig. 4). According to the sequences included here, there appears to be little geographic structure to the phylogeny (fig. 5). All of the samples were of distinctly sub-Saharan African variants but were widely distributed throughout the Ugandan groups’ traditionally defined geography.

One of two C* samples was found in a member of the Kakwa (Nilote), traditionally located in northwest Uganda, and the other in a member of the Nkole (Bantu), traditionally of southern Uganda.

Discussion

Much of the work on the phylogeny of KSHV has been directed towards developing a ‘natural history’ of the virus: demonstrating where variants are located and showing the genomic mutations and recombination events that have occurred. The works of Hayward [6] and Zong

	← VR* Loop →										Ig
	←		VR1		→						
	37	57	77	97							
HKS54	TLTcpsdasL	PTswycNDTR	LFRLKLQIVT	VVNLTCNFSC	VGQSGHRHSV	WITWYEQPVL	QTLCGQPSNT	VTCCGHVTTY			C*
HKS35T.....L.QR.KL.T.A.....F.....			C*
HKS36L....	.I.....	.L.TKPSF.	.ATIH...T.	.E....Q.INA...A.....			A5
HKS60L....	.I.....	.L.SDPFS.	.ATI...T.	.E....Q.INA...A.....			A5
HKS15L....	.I.....	.L.TD.SF.	.ATI...T.	.E....Q.INA...A.....			A5
HKS10L....	.I.....	.L.TD.SF.	.ATI...T.	.E....Q.INA...A.....			A5
HKS7L....	.I.....	.L.TDRSF.	.DTI...T.	.E....Q.INA...A.....			A5
HKS18L....	.I.....	.L.TD.SF.	.ATI...T.	.E....Q.INA...A.....			A5
HKS8L....	.I.....	.W.TN.SF.	.ATI...T.	.E....Q.INA...A.....			A5
HKS43L....	.I.....	.W.TN.SF.	.ATI...T.	.E....Q.INA...A.....			A5
HKS34L.G..	.I.....	.W.TN.SF.	.ATI...T.	.E....Q.INA...A.....			A5
HKS58L....	.I.....	.R.TN.SF.	.ATI...T.	.E....Q.INA...A.....			A5
HKS50L....	.I.....	.W.TD.SF.	.ATI...T.	.E....Q.INA...A.....	.A...Q...			A5
HKS23L....	.I.....	.W.TD.SF.	.ATI...T.	.E....Q.INA...A.....	.A...Q...			A5
HKS20L....	.I.....	.W.TD.SF.	.ATI...T.	.E....Q.INA...A.....	.A...Q...			A5
HKS47L....	.I.....	.P.TD.SF.	.ATI...T.	.E....Q.INA...A.....	.A...Q...			A5
HKS24L....	.IH.....	.W.TD.SF.	.ATI...T.	.E....Q.INA...A.....			A5
HKS52L....	.I.....	.L.TN.SF.	.ATF...T.	.E....Q.INA...A.A.			A5
HKS56L....	.I.....	.IW...D.SF.	.ATI...T.	.E....Q.INA...T.....			A5
HKS22I.....	.G...H..	.TQRTLP	.SH.S...T.	.K...PS..	.I...E..A...			F
HKS49NR..	.I...G..	.L.ITASNL	.FS...T.	MTT..PT..	.I...E..FT...A.....	.S...P...			B1
HKS11NR..	.I...G..	.L.ITASNL	.FS...T.	MTT..PT..	.I...E..FT...A.....	.S...P...			B1
HKS40NR..	.I...E..	.H.ITASNL	.SS...T.	MTT..PT..	.I...E..T...A.....	.S...P...			B1
HKS21NR..	.I...E..	.H.ITASNL	.SS...T.	MTT..PT..	.I...E..T...A.....	.S...P...			B1
HKS32NR..	.I...G.Q	.Y.ITASNL	.SS...T.	MTT..PT..	.I...E..T...A.....	.S...P...			B1
HKS41NR..	.I...G..	.L.ITASNL	.SS...T.	MTT..PT..	.I...E..T...A.....	.S...P...			B1
HKS29NR..	.I...G..	.H.ITASNL	.SS...T.	MTT..PT..	.I...E..T...A.....	.S...P...			B1
HKS33NR..	.I...G..	.H.ITASNL	.SS...T.	MTT..PT..	.I...E..T...A.....	.S...P...			B1
HKS19NR..	.I...E..	.Y.ITASNL	.SS...T.	MTT..PT..	.I...E..T...A.....	.S...P...			B1
HKS17NR..	.I...G..	.L.ITASNL	.SS...T.	MTT..PT..	.I...E..S...A.....R...			B2
HKS9NR..	.I...G.Q	.Y.ITESTL	.SS..G..T.	MTA..PT..	.I...E..TT...A.....R...			B3
HKS27NR..	.I...G.Q	.W.IRQFSL	.SS..G..T.	MTA..PT..	.I...E..TT...A.....R...			B3
	Ig	→								← VR2	
	117	137	157	177							
HKS54	CSTSGNNVTV	WHLpNGQNET	VSQTKYYNFT	LMNQTEGCYA	CSNGLSSRLS	NRLCFWARCA	NITPETHTVS	VSSTTGFRTF			C*
HKS35V			C*
HKS36R.....TI.....V			A5
HKS60R.....TI.....V			A5
HKS15R.....TI.....V			A5
HKS10R.....TI.....V			A5
HKS7R.....	...Q.....TI.....V			A5
HKS18R.....TI.....V			A5
HKS8R.....TI.....			A5
HKS43R.....TI.....			A5
HKS34R.....TI.....			A5
HKS58R.....TI.....			A5
HKS50R.....TI.....			A5
HKS23R.....TI.....			A5
HKS20R.....TI.....			A5
HKS47R.....TI.....			A5
HKS24R.....TI.....			A5
HKS52R.....TI.....			A5
HKS56R.....TI.....			A5
HKS22I	.L.Q.R.Q.	...N.....F.K.P...	...PI.....			F
HKS49	.H.S...I	.R.Q.H.Q.M...Q..TI...S.IP...	A...MPVKVL				B1
HKS11	.H.S...I	.R.Q.H.Q.M...Q..TI...S.IP...	A...MPVKVL				B1
HKS40	.D.S...I	.R.Q.H.Q.M...Q..T	...D.....I...S.IP...	A...MAVKVL				B1
HKS21	.D.S...I	.R.Q.H.Q.M...Q..T	...D.....I...S.IP...	A...MAVKVL				B1
HKS32	.D.S...I	.R.Q.H.Q.M...Q..TI...S.IP...	A...MAVKVL				B1
HKS41	.D.S...I	.R.Q.H.Q.M...Q..TI...S.IP...	A...SMAVKVL				B1
HKS29	.D.S...I	.R.Q.H.Q.M...Q..TI...S.IP...	A...VAVKVL				B1
HKS33	.D.S...I	.R.Q.H.Q.M...Q..TI...S.IP...	-----L				B1
HKS19	.D.S...I	.R.Q.H.QAM...Q..TI...S.I	D.S...P...	A.T.MAVTVL				B1
HKS17	.H.SD...I	.R.Q.H.Q.M...Q..TI...S.IP...	A...A.K.L				B2
HKS9	.H.S.G.I	.R.Q.H.Q.M...R..I	...D.....I...S.IP...	A...A.KMS				B3
HKS27	.H.S.G.I	.R.Q.H...M...R..I	...AD.....I...S.IP...	A...A.KMS				B3

	VR2		→		
	197		217		237
HKS54	STNS-----H ATTHDVVVVQ	EAKSTNPHIE	VPFLAFITLV	ALI	C*
HKS35KM	C*
HKS36	...LLNII.KV.M	A5
HKS60	..H.LVNII.KV.M	A5
HKS15	...LVNII.	..N.....KV.M	A5
HKS10	...LVNII.	..N.....KV.M	A5
HKS7	...LVNII.	..N.....KV.M	A5
HKS18	...LVNII.KV.M	A5
HKS8	...LVNII.KV.M	A5
HKS43	...LVNII.KV.M	A5
HKS34	...FVNII.KV.M	A5
HKS58	...LVNII.KV.M	A5
HKS50	...LVNII.KV.M	A5
HKS23	...LVNII.KV.M	A5
HKS20	...LVNII.KV.M	A5
HKS47	...FVNII.KV.M	A5
HKS24	...LVNII.KV.M	A5
HKS52	...LVNII.KV.M	A5
HKS56	...LVNMI.KV.M	A5
HKS22KV.M	F
HKS49	T..GLLKIIP	...AA.A.E	.V...T..Q	...V.M...V	B1
HKS11	R..GLLKIIP	...AA.A.E	.V...T..Q	...V.M...V	B1
HKS40	T..GLLKIIP	...AA.A.E	.V...T..Q	...V.M...V	B1
HKS21	T..GLLKIIP	...AA.A.E	.V...T..Q	...V.M...V	B1
HKS32	R..GLLKIIP	...AA.A.E	.V...T..Q	...V.M...V	B1
HKS41	R..GLLKIIP	...AA.A.E	.V...T..Q	...V.M...V	B1
HKS29	R..GLLKIIP	...AA.A.E	.V...T..Q	...V.M...V	B1
HKS33	R..GLLKIIP	...AA.A.E	.V...T..Q	...V.M...V	B1
HKS19	R..GLLKIIP	...AA.A.E	.V...T..Q	...V.M...V	B1
HKS17	R..GLLKIIP	...AA.A.E	.V.....Q	...V.M...V	B2
HKS9	R..GLLKIIP	...AA.A.E	.V.....Q	...V.M...V	B3
HKS27	R..GLLKIIP	...AA.A.E	.V.....Q	...V.M...V	B3

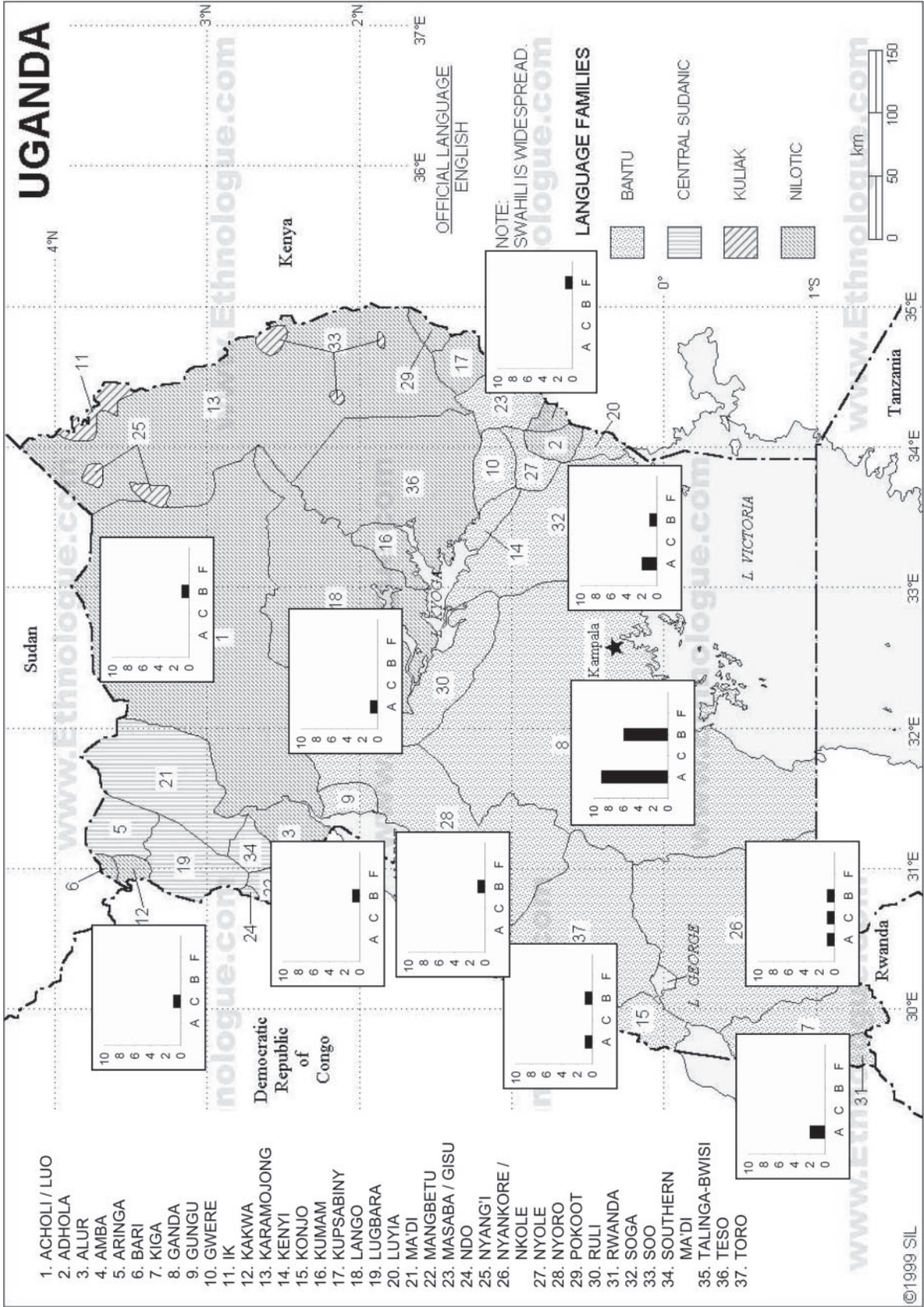
Fig. 4. Amino acid sequence alignment for 32 Ugandan KSHV K1 samples (aa residues 37–239). Top line (HKS54) shows entire sequence. Dots for the other sequences indicate residues that are the same as those for HKS54. Dashes mark deletion events. Variable regions 1 and 2, the putative VR* loop, the immunoglobulin region and the variant designations for each sample are indicated as well.

et al. [8] represented an important step forward in proposing the first explanatory theory of the evolutionary and molecular processes by which these distributions arose. As has been proposed for other viruses [38, 39], Hayward et al. [6] hypothesized that KSHV is an evolutionarily old human virus distributed worldwide along broad ethnic and geographic lines and acts as a marker for ancient human migration.

At the finer geographic scale of analysis conducted in this study, limited to the ORF-K1 gene, the KSHV subtypes did not segregate by Ugandan ethnicity (fig. 2), replicating results reported by Kakoola et al. [17]. Bantu and Nilotic patients hosted all three subtypes previously shown to be present in Uganda. Ganda tribe samples were repeatedly represented in both the A5 and B1 clades. The Nilotic tribes included in the analysis were represented in the A5, C* and B1 clades and provided the only B2 sample. There was no geographic pattern to Uganda's KSHV phylogeny (fig. 5). The most prevalent variants (A5 and B1) appear widely distributed throughout the traditional geography of the sampled ethnic groups, a phylogeography similar to those shown elsewhere for Af-

rican samples [9, 17, 19, 25, 26], save for Kasolo et al. [20] who identified all 15 Zambian clones they sampled as A5. The two C* sequences sampled here (HKS35 and HKS54) are from the Kakwa and Nkole, tribes traditionally located at opposite ends of the country.

One explanation for the resultant phylogeny consistent with the ethnicity hypothesis of KSHV evolution invokes Uganda's history. The Bantu expansion out of the first African iron-smelting center at Nok in Nigeria (fig. 1) likely placed the Bantu at Urewe, one of several secondary iron centers near Lake Victoria, by the first century AD [10, 40, 41]. Nilotic groups arrived in the area not long after. In East Africa, the opportunity for intermixing appears to have been considerable [14, 42], convoluting biological origins even as contemporary subjects identify with single tribes alone. Therefore, the patients may not have identified their biological ancestry, the adjustment for which might yet result in an ethnically structured KSHV phylogeny. Analyses of Y-chromosomal and human leukocyte antigen haplotypes are in preparation to account for the 'biological' components of patient ethnicity.



Other explanations consistent with the data but not with the ethnicity hypothesis are possible. KSHV diversity may have arisen in Africa before the evolution of the major ethnicities that exist today, with one or more newly originated groups beginning their histories already hosting multiple subtypes. Zong et al. [8] proposed that the B subtype originated with the first migrations of modern humans throughout Africa 100,000 years ago. Zong et al. [8] and Cook et al. [19] placed the divergence of subtype A from subtype C at several thousand to 35,000 years ago, well before Ugandan populations locally radiated into their current ethnic groups. The novel subtype F may also have had an African genesis.

Horizontal transmission provides a second alternative for the observed phylogeny. The familial/ethnic descent model depends on a vertical mode of transmission through which older family members infect younger members. Non-sexual transmission of KSHV, by breast milk and/or saliva, appears to predominate in endemic countries [43] and may be consistent with both vertical and horizontal transmission. Horizontal transmission may occur more readily in KS-endemic regions than in other populations, because KSHV is shed more readily in saliva and at higher levels, particularly in children [D. Whitby, pers. commun.]. Some work suggests that sexual transmission occurs in endemic populations as well [44–48]. In Uganda, for example, KSHV seroprevalence continues to increase in adulthood (to 50–60% in healthy adults) [15, 49].

Successful horizontal transmission of multiple KSHV subtypes could break up vertical lineages of the virus passed down within ethnic groups, leading to a dispersed phylogeography for KSHV. Phylogenetic analysis of 8 additional polymorphic genes from the Ugandan isolates (along with K1) defined 26 different KSHV chimeric genotypes [9; Zong et al., in preparation]. These data suggest that a significant amount of intertypic recombination has occurred within the viral genomes now found in Uganda. For example, the bulk of the K1-A5 Ugandan KSHV genome appears to be that of a sub-Saharan B1

pattern [Zong et al., in preparation]. In fact, the genetic diversity of the Uganda virus is far more complex than any other KSHV ecosystem yet studied, including those of Eurasian, Pacific Rim, Southern African and other sub-Saharan regions [9]. The origins of such recombination may be quite ancient. Each of the 32 Ugandan white blood cell samples analyzed here appeared to be infected by only a single KSHV genotype, indicating that recombination is sporadic and generally occurs over long periods of time.

A third alternative to the ethnicity hypothesis is that ethnically defined biomolecular selection filters do not act as the sole means by which K1 subtypes are segregated. Linguistic differences may separate networks of infected people in such a way that cultural practices (1) define divergent modes of transmission and/or (2) isolate strains by cultural distance, differentiating the KSHV subtypes that host populations epidemiologically support. When cultural boundaries become porous and group-specific modes of transmission become geographically spread, KSHV subtypes may breach ethnic gaps. From a historical perspective, Ugandan society has become increasingly integrated, with kinship, ethnic and local governing structures interlaced with state institutions, albeit to varying degrees and in the face of civil wars and persistent social divisions [50–52]. English and Swahili are widespread in Uganda, and increased rural-urban migration over the past 3 decades has tied more of the country to the capital, Kampala. It is a reasonable possibility that KSHV subtype host ranges have increased in kind.

A fourth alternative explanation for the K1 phylogeny presented here arises from sampling limitations. Molecular approaches have the power to identify viral genotypes and, if run over time, infection kinetics. However, such tests involve relatively few individuals because of the cost, time and effort required and may not embody a representative sample that captures the full range of viral diversity present. In all likelihood, the more samples collected, the greater the range of viral clades any one ethnicity will harbor.

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Fig. 5. Frequency distributions of KSHV subtypes by self-reported ethnic group and overlaid on a map of the traditional geographic distribution of Ugandan languages/ethnicities. The map was adapted from SIL International's online *Ethnologue: Languages of the World* available at www.ethnologue.com/show_map.asp?name=Uganda. The frequency distributions mark the traditional geography of patients' ethnicity, not where samples were collected.

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