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Supplementary Materials: Complete Genome Sequence of Germline Chromosomally Integrated Human Herpesvirus 6A and Analyses Integration Sites Define a New Human Endogenous Virus with Potential to Reactivate as an Emerging Infection

Joshua Tweedy, Maria Alexandra Spyrou, Max Pearson, Dirk Lassner, Uwe Kuhl and Ursula A. Gompels

Table S1. Geographic prevalence studies separated into CiHHV-6A and CiHHV-6B.

Study	Country	n	CiHHV-6A (%)	CiHHV-6B (%)	References
Donors					
Cord blood	USA (NY)	5638	[19 *] 12 (0.2)	[38 *] 25 (0.4)	(Hall <i>et al.</i> , 2008, 2004)
Blood donor-adult	USA (Texas)	100	0 (<1.0)	1 (1.0)	(Hudnall <i>et al.</i> , 2008)
Blood donor	Canada (Ontario)	288	0 (<0.3)	0 (<0.3)	(Gravel <i>et al.</i> , 2013)
Blood donors-adult	France	200	0 (<0.5)	1 (0.5)	(Geraudie <i>et al.</i> , 2012)
Blood donor-adult	UK (London)	500	0 (<0.2)	4 (0.8)	(Leong <i>et al.</i> , 2007)
Serum bank-child	UK	610	4 + (0.7)	6 + (1.0)	(Ward <i>et al.</i> , 2005)
Blood controls	UK (Northern)	563	1 (0.2)	10 (1.7)	(Bell <i>et al.</i> , 2014) ^
Nails adult	Czech Republic	421	1 (0.2)	3 (0.7)	(Hubacek <i>et al.</i> , 2013)
Normal birth/infant-saliva, sera, nails	Southern Africa (Zambia)	495	0 (<0.2)	0 (<0.2)	#
Region Totals	North America N	6026	12 (0.2)	26 (0.4)	
	Europe E	2582	6 (0.2)	24 (0.9)	
Donor Total	NA + E	8608	18 (0.2)	50 (0.6)	
Patients					
SOT-Liver	USA	548	1 (0.2)	6 (1.1)	(Lee <i>et al.</i> , 2012)
SOT-Kidney	USA	46	0 (<2.0)	1 (2.2)	(Lee <i>et al.</i> , 2011)
Leukemic children	Canada (Ontario)	287	1 (0.3)	0 (<0.3)	(Gravel <i>et al.</i> , 2013)
SOT-mixed. Blood, tissue, hair samples	Italy	135	1 (0.7)	0 (<0.8)	(Potenza <i>et al.</i> , 2009)
SCT-Blood, hair samples	Italy	70	0 (<1.4)	1 (1.4)	(Potenza <i>et al.</i> , 2009)
CSF-encephalitis referral child/adult	UK	522	1 (0.2)	5 (1.0)	(Ward <i>et al.</i> , 2007)
Hodgkins lymphoma	UK (Northern)	936	1 (0.1)	15 (1.6)	(Bell <i>et al.</i> , 2014) ^
Cardiac referrals	Germany	3610	7 (0.2)	13 (0.4)	(Tweedy <i>et al.</i> , 2015)
Malignant disease-blood	Czech Republic	812	7 (0.9)	2 (0.2)	(Hubacek <i>et al.</i> , 2013)
Leukemia-child-blood	Czech Republic	339	4 (1.2)	1 (0.3)	(Hubacek <i>et al.</i> , 2009)
Transplant donors/recipients-blood, herpesvirus referrals	Japan (Osaka)	2332	1 (0.04)	4 (0.2)	(Tanaka-Taya <i>et al.</i> , 2004)
Neonatal intensive care unit, sera	Southern Africa (Zambia)	303	0 (<0.3)	0 (<0.3)	(Tembo <i>et al.</i> , 2014) ^
Leukemia-blood	North Africa (Tunisia)	73	0 (<1%)	1 (1.2%)	(Faten <i>et al.</i> , 2012)
	North America	881	2 (0.2)	7 (0.8)	
Region Totals	Europe	6424	21 (0.3)	37 (0.6)	
	Japan, J	2332	1 (0.04)	4 (0.2)	
Patient Total	NA + E + J + A	9719	23 (0.3)	34 (0.4)	

Table S1. Cont.

Study	Country	n	CiHHV-6A (%)	CiHHV-6B (%)	References
Donor + Patients					
Region Totals	North America	6907	14 (0.2)	33 (0.5)	
	Europe	9006	27 (0.3)	61 (0.7)	
	Japan	2332	1 (0.04)	4 (0.2)	
	Africa, A	868	0 (<0.1)	1 (0.1)	
ALL	NA + E + J + A	19,113	41 (0.2)	74 (0.4)	

* Original numbers screened, then those positively identified listed next; +4 and 6 positively identified; SOT—solid organ transplantation recipients; CSF—cerebral spinal fluid; NA North America; # Musonda, K. and Gompels, U.A., Analyses congenital infections with betaherpesviruses in Zambia, unpublished; manuscript in preparation; ^ References [1,15] type by polymerase gene, which HHV-6A variation may confound [4,16].

Table S2. HHV-6A SNPs detected in CiHHV-6A by deep sequencing.

No.	SNPs U54 HHV-6A > CiHHV-6A	SNPs U54 Amino Acid	HHV-6A U1102	% HHV-6A Minor Variant SNPs in CiHHV-6A		
	HHV-6A:U1102, GS & AJ	Changes	Position	2284	5055	5814
1	A > G	-	86,106	<	<	<
2	A > G	-	86,142	17	<	<
3	G > A (AJ only)	-	86,195	17	<	<
4	A > G (AJ only)	I > T	86,329	16	<	<
5	G > T (AJ only)	L > I	86,372	16	<	<
6	T > C	[S, A, T > M]	86,379	17	<	<
7	G > A	[S, A, T > M]	86,380	18	<	<
8	C (U1102) A (GS) > T	S, A, T > M	86,381	<	<	<
9	T > C	T > A	86,387	16	<	<
10	G > C	-	86,400	17	<	<
11	T > C (GS only)	-	86,514	14	<	<
12	T > C	T > A	86,537	30	<	4
13	G > T	T > N	86,608	31	<	<
14	A > G	-	86,613	31	<	<
15	A > G	-	86,619	31	<	<
16	C > A	V > F	86,627	30	<	<
17	G > A	-	86,638	28	<	<
18	G > A	P > S	86,645	25	<	<
19	A > G	-	86,720	17	<	<
20	T > A (GS only)	-	86,724	<	<	<
21	G > T	A > D	86,761	16	<	<
22	A > C	[I, L > R]	86,791	17	<	<
23	T > G (GS only)	I, L > R	86,792	<	<	<
24	T > C (AJ only)	N > D	86,801	17	<	<
25	G > T (U1102)	K, N > I	86,811	<	<	<
26	T > A	[K, N > I]	86,812	16	<	<
27	A > G	-	86,859	14	<	<
28	T > C	N > D	86,891	19	<	<
29	C > G (U1102 only)	G > A	86,893	<	<	<
30	G > T	T > N	86,896	19	<	<
31	C > T (U1102 only)	-	86,943	<	<	<
32	A > C (GS only)	N > K	86,949	<	<	<
33	G > T (U1102 only)	Q > K	87,011	<	<	<
34	T > C	K > R	87,016	22	<	<
35	A > G	-	87,036	21	<	<
36	C > T	A > T	87,092	16	<	<
37	C > T (U1102 and AJ)	R > H	87,100	16	<	<
38	A > G (GS only)	S > P	87,110	<	<	<

Table S1. Cont.

No.	SNPs U54 HHV-6A > CiHHV-6A	SNPs U54 Amino Acid	HHV-6A U1102	% HHV-6A Minor Variant SNPs in CiHHV-6A		
	HHV-6A:U1102, GS & AJ	Changes	Position	2284	5055	5814
39	C > A (GS only)	A > S	87,119	<	<	<
40	T > C (GS only)	M > V	87,128	<	<	<
41	G > C	N > K	87,129	20	<	<
42	G > A	H > Y	87,137	19	<	<
43	T > G	R > S	87,171	18	<	<
44	A > T	I > N	87,199	18	<	2
45	C > A	Q > R	87,204	18	<	<
46	T > C	Q > R	87,205	18	<	3
47	T > C	T > A	87,266	15	<	<
48	A > G	I > T	87,289	15	<	<
49	T > C (GS)	-	87,299	<	<	<
50	A > G (U1102 and AJ)	S > P	87,308	15	<	<
51	A > G (GS)	S > P	87,314	<	<	<

Mean read depths from new sequences from this study (methods 2.3) were for endogenous CiHHV-6A genomes from patients 2284, 5055 and 5814 it was 314, 9943 and 8941 respectively. Comparisons were made to all available reference genomes, from exogenous HHV-6A strains U1102, GS and AJ (methods 2.2). SNP% cutoffs were <0.5% reads indicated by <. Coding from the opposite strand. Brackets [] indicate same codon giving the coding change.

a. telomere - DR-L-----UL-----DR-R-sub-telomere-----//----centromere
CiHHV-6A/B

b. DR: pac1-T1-DR1-DR6-T2-pac2

Figure S1. (a) Structure of CiHHV-6A/B underlined as integrated into the sub-telomeric region of human chromosomes. DR-L is the left direct repeat and DR-R is the right direct repeat in the prototype orientation of the virus genome, and bound the unique region, U, encoding most coding sequences; (b) The structure of the DR region from HHV-6A/B which includes the pac 1 and pac 2 DNA packaging signals, imperfect telomeric repeat region T1, perfect telomeric repeat region T2, and spliced coding sequences for genes DR1 and DR6. In the CiHHV-6A/B genomes the DR regions do not have the terminal pac sites.

Start of U41 Start ori-lyt (mori) 67610

HHV-6A.U1102 AACGGGAGCAGAAACTACCGTTTCGTTTTTCATCAGCCATCTTTGTGGATTTGATCACAGA 67641
GS AACGGGAGCAGAAACTACCGTTTCGTTTTTCATCAGCCATCTTTGTGGATTTGATCACAAA
AJ AACGGGAGCAGAAACTACCGTTTCGTTTTTCATCAGCCATCTTTGTGGATTTGATCACAAA
CiHHV-6A.5055 AACGGGAGCAGAAACTACCGTTTCGTTTTTCATCAGCCATCTTTGTGGATTTGATCACAAA
2284 AACGGGAGCAGAAACTACCGTTTCGTTTTTCATCAGCCATCTTTGTGGATTTGATCACAAA
5814 AACGGGAGCAGAAACTACCGTTTCGTTTTTCATCAGCCATCTTTGTGGATTTGATCACAAA
HHV-6B.Z29 AACAGGAGCAGAAACCACCGTTTCGTTTTTCATCAGCCATCTTTGTGGATTCGATCACAAA
HST AACAGGAGCAGAAACCACCGTTTCGTTTTTCATCAGCCATCTTTGTGGATTCGATCACAAA
*** *****

HHV-6A.U1102 AACAGAGATAATGGGGTTTTGTGGTGAATCCTTATATATATGTTTGACGTAACATAAC
GS AACAAAGATAATGGGGTTTTGTGGTGAATCCTTATATATATGTTTGACGTAACATAAC
AJ AACAAAGATAATGGGGTTTTGTGGTGAATCCTTATATATATGTTTGACGTAACATAAC
CiHHV-6A.5055 AACAAAGATAATGGGGTTTTGTGGTGAATCCTTATATATATGTTTGACGTAACATAAC
2284 AACAAAGATAATGGGGTTTTGTGGTGAATCCTTATATATATGTTTGACGTAACATAAC
5814 AACAAAGATAATGGGGTTTTGTGGTGAATCCTTATATATATGTTTGACGTAACATAAC
HHV-6B.Z29 AACAAAGATAATGGGGTTTTGTGGTGAATCCTTATATATATGTTTGACGTAACATAAC
HST AACAAAGATAATGGGGTTTTGTGGTGAATCCTTATATATATGTTTGACGTAACATAAC

HHV-6A.U1102 ACGCGTCATCAAAACATAAGAGTAAACCACAAGTTGAATTATACCGTTTTCTATATGAGGT
AJ ACGCGTCATCAAAACATAAGAGTAAACCACAAGTTGGATTATACCGTTTTCTATATGAGGT
GS ACGCGTCATCAAAACATAAGAGTAAACCACAAGTTGGATTATACCGTTTTCTATATGAGGT
CiHHV-6A.5055 ACGCGTCATCAAAACATAAGAGTAAACCACAAGTTGGATTATACCGTTTTCTATATGAGGT
2284 ACGCGTCATCAAAACATAAGAGTAAACCACAAGTTGGATTATACCGTTTTCTATATGAGGT
5814 ACGCGTCATCAAAACATAAGAGTAAACCACAAGTTGAATTATACCGTTTTCTATATGAGGT
HHV-6B.Z29 ACGCGTCATCAAAACATAAGAGTAAACCACAAGTTGAATTATACCGTTTTCTATATGAGGT
HST ACGCGTCATCAAAACATAAGAGTAAACCACAAGTTGAATTATACCGTTTTCTATATGAGGT

HHV-6A.U1102 TTACGGTCAAAGAAAAACGATTTTTTATGCAAATATTTCCACGCAGATGATATGACA
GS TTACGGACAAAAGAAAAACGATTTTTTATGCAAATATTTCCACGCAGATGATATGACA
AJ TTACGGACAAAAGAAAAACGATTTTTTATGCAAATATTTCCACGCAGATGATATGACA
CiHHV-6A.5055 TTACGGACAAAAGAAAAACGATTTTTTATGCAAATATTTCCACGCAGATGATATGACA
2284 TTACGGACAAAAGAAAAACGATTTTTTATGCAAAGATTTCCACGCAGATGATATGACA
5814 TTACGGACAAAAGAAAAACGATTTTTTATGCAAAGATTTCCACGCAGATGATATGACA
HHV-6B.Z29 TTACGGACAAAAGAAAAACGATTTTTCTTATGCAAATATTTCCACGCAGATGATATGACA
HST TTACGGACAAAAGAAAAACGATTTTCTTATGCAAATATTTCCACGCAGATGATATGACA

OBP2

HHV-6A.U1102 CGCCCTTAATTTAAATTTATGCAAATCGTCGTCACCTCAGGTACAATAGTATATTTATA 67822
GS CGCCCTTAATTTAAATTTATGCAAATCGTCGTCACCCAGGTACAATAGTATATTTATA
AJ CGCCCTTAATTTAAATTTATGCAAATCGTCGTCACCTCAGGTACAATAGTATATTTATA
CiHHV-6A.5055 CGCCCTTAATTTAAATTTATGCAAATCGTCGTCACCTCAGGTACAATAGTATATTTATA
2284 CGCCCTTAATTTAAATTTATGCAAATCGTCGTCACCTCAGGTACAATAGTATATTTATA
5814 CGCCCTTAATTTAAATTTATGCAAATCGTCGTCACCTCAGGTACAATAGTATATTTATA
HHV-6B.Z29 CGCCCTTAATTTAAATTTATGCAAATCGTCGTCACCTCAGGTACAATAGTATATTTATA
HST CGCCCTTAATTTAAATTTATGCAAATCGTCGTCACCTCAGGTACAATAGTATATTTATA

OBP1

HHV-6A.U1102 TATATATTTATTTAATAAACTTATTGAGGACGGGAGAACGAGGGCGTGGCGTTTACGTCA 67882
GS TATATATTTATTTAATAAACTTATTGAGGACGGGAGAACGAGGGCGTGGCGTTTACGTCA
AJ TATATATTTATTTAATAAACTTATTGAGGACGGGAGAACGAGGGCGTGGCGTTTACGTCA
CiHHV-6A.5055 TATATATTTATTTAATAAACTTATTGAGGACGGGAGAACGAGGGCGTGGCGTTTACGTCA
2284 TATATATTTATTTAATAAACTTATTGAGGACGGGAGAACGAGGGCGTGGCGTTTACGTCA
5814 TATATATTTATTTAATAAACTTATTGAGGACGGGAGAACGAGGGCGTGGCGTTTACGTCA
HHV-6B.Z29 TATATAGTTTTTTAATAAACTTATTGAGGACGGGAGAACGAGGGCGTGGCGTTTACGTCA
HST TATATAGTTTTTTAATAAACTTATTGAGGACGGGAGAACGAGGGCGTGGCGTTTACGTCA

HHV-6A.U1102 TAGCCTAATTATGCATTCCTCAGAACAGGATTTAAAAGGCTGCGAGCGGCGGACTGTTCA
GS TAGCCTAATTATGCATTCCTCAGAACAGGATTTAAAAGGCTGCGAGCGGCGGACTGTTCA
AJ TAGCCTAATTATGCATTCCTCAGAACAGGATTTAAAAGGCTGCGAGCGGCGGACTGTTCA
CiHHV-6A.5055 TAGCCTAATTATGCATTCCTCAGAACAGGATTTAAAAGGCTGCGAGCGGCGGACTGTTCA
2284 TAGCCTAATTATGCATTCCTCAGAACAGGATTTAAAAGGCTGCGAGCGGCGGACTGTTCA
5814 TAGCCTAATTATGCATTCCTCAGAACAGGATTTAAAAGGCTGCGAGCGGCGGACTGTTCA
HHV-6B.Z29 TAGCCTAATTATGCATTCCTCAGAACAGGATTTAAAAGGCTGCGAGCGGCGGACTGTTCA
HST TAGCCTAATTATGCATTCCTCAGAACAGGATTTAAAAGGCTGCGAGCGGCGGACTGTTCA
*** *****

HHV-6A.U1102 GAGGGACGCTGGGGTACGGCTTGATACGTTTGATTGAAAATGATTCCTCGTGTGCTATTTT
GS GAGGGACGCTGGGGTACGGCTTGAGACGTTTGATTGAAAATGATTCCTCGTGTGCTATTTT
AJ GAGGGACGCTGGGGTACGGCTTGAGACGTTTGATTGAAAATGATTCCTCGTGTGCTATTTT
CiHHV-6A.5055 GAGGGACGCTGGGGTACGGCTTGAGACGTTTGATTGAAAATGATTCCTCGTGTGCTATTTT
2284 GAGGGACGCTGGGGTACGGCTTGAGACGTTTGATTGAAAATGATTCCTCGTGTGCTATTTT
5814 GAGGGACGCTGGGGTACGGCTTGAGACGTTTGATTGAAAATGATTCCTCGTGTGCTATTTT

HHV-6B.Z29 GAGGGACGCTGGGGTACGACTTGAGACGTTTGACTGAAAAATGATCCTTCGTGTACTATTT
HST GAGGGACGCTGGGGTACGACTTGAGACGTTTGACTGAAAAATGATCCTTCGTGTACTATTT

HHV-6A.U1102 TCTGCAAAAAAATTAATTCGCCGGCGACAGTAAACTTTTCAGCGGAATTTCAAAAAATTA
GS TCTGCAAAAAAATTAATTCGCCGGCGACAGTAAACTTTTCAGCGGAATTTCAAAAAATTA
AJ TCTGCAAAAAAATTAATTCGCCGGCGACAGTAAACTTTTCAGCGGAATTTCAAAAAATTA
CiHHV-6A.5055 TCTGCAAAAAAATTAATTCGCCGGCGACAGTAAACTTTTCAGCGGAATTTCAAAAAATTA
2284 TCTGCAAAAAAATTAATTCGCCGGCGACAGTAAACTTTTCAGCGGAATTTCAAAAAATTA
5814 TCTGCAAAAAAATTAATTCGCCGGCGACAGTAAACTTTTCAGCGGAATTTCAAAAAATTA
HHV-6B.Z29 TCTACAAAAAATTAATTCGCCGGCGACAGTAAACTTTTCAGCGGAATTTCAAAAAATTA
HST TCTACAAAAAATTAATTCGCCGGCGACAGTAAACTTTTCAGCGGAATTTCAAAAAATTA

I DR1

HHV-6.U1102 TTCCATATGTAATTTAAGCATTTTAAAACGTATAACTCACACGCGTAAAAACATATTTACG 68112
GS TTCCACATGTAATTTAAGCATTTTAAAACGTATAACTCACACGCGTAAAAACATATTTACG
AJ TTCCACATGTAATTTAAGCATTTTAAAACGTATAACTCACACGCGTAAAAACATATTTACG
CiHHV-6A.5055 TTCCACATGTAATTTAAGCATTTTAAAACGTATAACTCACACGCGTAAAAACATATTTACG
2284 TTCCACATGTAATTTAAGCATTTTAAAACGTATAACTCACACGCGTAAAAACATATTTACG
5814 TTCCACATGTAATTTAAGCATTTTAAAACGTATAACTCACACGCGTAAAAACATATTTACG
HHV-6B.Z29 TTCCACATGTAATTTAAGCATTTTAAAACGTATAACTCACACGCGTAAAAACATATTTACG
HST TTCCACATGTAATTTAAGCATTTTAAAACGTATAACTCACACGCGTAAAAACATATTTACG

HHV-6A.U1102 AATACAGTAGTTTTCGTGATATTTT-CGAAATTAATAAATTTTAAATCGGGTAAATGAT
GS AATACAGTAGTTTTCGTGATATTTTTCGAAATTAATAAATTTTAAATCGGGTAAATGAT
AJ AATACAGTAGTTTTCGTGATATTTTTCGAAATTAATAAATTTTAAATCGGGTAAATGAT
CiHHV-6A.5055 AATACAGTAGTTTTCGTGATATTTTTCGAAATTAATAAATTTTAAATCGGGTAAATGAT
2284 AATACAGTAGTTTTCGTGATATTTTTCGAAATTAATAAATTTTAAATCGGGTAAATGAT
5814 AATACAGTAGTTTTCGTGATATTTTTCGAAATTAATAAATTTTAAATCGGGTAAATGAT
HHV-6B.Z29 AATACAGTAGTTTTCGTGATATTTTTCGAAATTAATAAATTTTAAATCGGGTAAATGAT
HST AATACAGTAGTTTTCGTGATATTTTTCGAAATTAATAAATTTTAAATCGGGTAAATGAT

U1102 indel-1

HHV-6.U1102 AAAGCATACTATTAGATTCCTCACGTTAACAAAGCAAGTTTTTTGAGGTTTTTCGGTATA 68242
GS AAAGCATACTA-----AGTTTTTT-GAGGTTTTTCGGTATA
AJ AAAGCATACTA-----AGTTTTTT-GAGGTTTTTCGGTATA
CiHHV-6A.5055 AAAGCATACTA-----AGTTTTTT-GAGGTTTTTCGGTATA
2284 AAAGCATACTATTAGATTCCTCACGTTAACAAAGCAAGTTTTTT GAGGTTTTTCGGTATA
5814 AAAGCATACTATTAGATTCCTCACGTTAACAAAGCAAGTTTTTT-GAGGTTTTTCGGTATA
HHV-6B.Z29 AAAGCATACTATTAGATTCCTCACGTTAACAGAGCAAGCTCTTC-GAGGTTTTTCAGTAGA
HST AAAGCATACTATTAGATTCCTCACGTTAACAGAGCAAGCTCTTC-GAGGTTTTTCAGTAGA

I DR2

HHV-6A.U1102 ATTTTAAACATTTACTTCCACATGTAATTTAAGCATTTTAAAACGTATAACTCACACGCGT 68302
GS ATGTTAAACATTTAGTTCCACATGTAATTTAAGCGTTTTAAAACGTATAGCTCACACGCGT
AJ ATGTTAAACATTTAGTTCCACATGTAATTTAAGCGTTTTAAAACGTATAGCTCACACGCGT
CiHHV-6A.5055 ATGTTAAACATTTAGTTCCACATGTAATTTAAGCGTTTTAAAACGTATAGCTCACACGCGT
2284 ATTTTAAACATTTAGTTCCACATGTAATTTAAGCATTTTAAAACGTATAACTCACACGCGT
5814 ATTTTAAACATTTAGTTCCACATGTAATTTAAGCATTTTAAAACGTATAACTCACACGCGT
HHV-6B.Z29 ATTTCAATATTTA-----
HST ATTTCAATATTTA-----

HHV-6A.U1102 AAAACATTTACGAATACAGTAGTTTTCGTGATATTTTTCGAAATTCAAAAAGTTTAAAT
GS AAAACATTTACGAATACAGTAGTTTTCGTGATATTTTTCGAAATTCAAAAATTTTAAAT
AJ AAAACATTTACGAATACAGTAGTTTTCGTGATATTTTTCGAAATTCAAAAATTTTAAAT
CiHHV-6A.5055 AAAACATTTACGAATACAGTAGTTTTCGTGATATTTTTCGAAATTCAAAAAGTTTAAAT
2284 AAAACATTTACGAATACAGTAGTTTTCGTGATATTTTTCGAAATTCAAAAAGTTTAAAT
5814 AAAACATTTACGAATACAGTAGTTTTCGTGATATTTTTCGAAATTCAAAAAGTTTAAAT
HHV-6B.Z29 -----
HST -----

I DR3

HHV-6A.U1102 CGGGTAATGATAAAGCATATTTAGATTACACATGTAATTTAAGCATTTTAAAACGTA 68422
GS CGGGTAATGATAAAGCATATTTAGATTCCACATGTAATTTAAGCATTTTAAAACGTA
AJ CGGGTAATGATAAAGCATATTTAGATTCCACATGTAATTTAAGCATTTTAAAACGTA
CiHHV-6A.5055 CGGGTAATGATAAAGCATATTTAGATTCCACATGTAATTTAAGCATTTTAAAACGTA
2284 CGGGTAATGATAAAGCATATTTAGATTCCACATGTAATTTAAGCATTTTAAAACGTA
5814 CGGGTAATGATAAAGCATATTTAGATTACACATGTAATTTAAGCATTTTAAAACGTA
HHV-6B.Z29 -----ATTCCACATGTAATTTAAGCATTTTAAAATGTA
HST -----ATTCCACATGTAATTTAAGCATTTTAAAATGTA

HHV-6A.U1102 GAATTCACAAAGTGACAAAACATT--AAATACAGTAGTTTTCACGGTATTTTTCGAAAT
GS GAATTCACAAAGTGACAAAACATTCACAAATACAGTAGTTTTCACGGTATTTTTCGGGAT
AJ GAATTCACAAAGTGACAAAACATTCACAAATACAGTAGTTTTCACGGTATTTTTCGGGAT
CiHHV-6A.5055 GAATTCACAAAGTGACAAAACATTCACAAATACAGTAGTTTTCACGGTATTTTTCGGGAT

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2284      GAATTCACAAAGTGACAAAACATTGACGAATACAGTAGTTTTTCACGGTATTTTTTGAAT
5814      GAATTCACAAAGTGACAAAACATTGACGAATACAGTAGTTTTTCACGGTATTTTTTGAAT
HHV-6B.Z29 GAATTCACAAAGTGACAAAACATTGACGAATACAGTAGTTTTTCACGGTATTTTTT-GAAAT
HST      GAATTCACAAAGTGACAAAACATTGACGAATACAGTAGTTTTTCACGGTATTTTTT-GAAAT
*****

HHV-6A.U1102 TAA---ATTTAAATCGGGTACATGATAAAGCATGCTGTTAAATTCACGTTTTTAGGGCA
GS      TAA---ATTTAAATCGGGTAAATGACAAAGCATGCTGTTAAATTCACGTTTTTAGGGCA
AJ      TAA---ATTTAAATCGGGTAAATGACAAAGCATGCTGTTAAATTCACGTTTTTAGGGCA
CiHHV-6A.5055 TAA---ATTTAAATCGGGTAAATGACAAAGCATGCTGTTAAATTCACGTTTTTAGGGCA
2284      TAA---ATTTAAATCGGGTAAATGATAAAGCATGCTGTTAAATTCACATTTTTAGGGCA
5814      TAA---ATTTAAATCGGGTAAATGATAAAGCATGCTGTTAAATTCACATTTTTAGGGCA
HHV-6B.Z29 TAATAAATTTTAAATCGGGTAAATGGTAAAGCATGCTGTTAAATTCACGTTAACACAGCA
HST      TAATAAATTTTAAATCGGGTAAATGGTAAAGCATGCTGTTAAATTCACGTTAAAGAGCA
***      *****

HHV-6A.U1102 AGTTCGCGAGGTTTGCCTTGTGATTATCAGTGAATGTATTCAGCGTTTCAATTTTAGAT
GS      AGTTCCTTCGAGGTTTGCCTGTGATTATCAGTGAAT--ATTCAGCGTTTCAATTTTAGAT
AJ      AGTTCCTTCGAGGTTTGCCTGTGATTATCAGTGAAT--ATTCAGCGTTTCAATTTTAGAT
CiHHV-6A.5055 AGTTCCTTCGAGGTTTGCCTGTGATTATCAGTGAAT--ATTCAGCGTTTCAATTTTAGAT
2284      AGTTCGCGAGGTTTGCCTGTGATTATCAGTGAATGTATTCAGCGTTTCAATTTTAGAT
5814      AGTTCGCGAGGTTTGCCTGTGATTATCAGTGAATGTATTCAGCGTTTCAATTTTAGAT
HHV-6B.Z29 AGTTCCTTCGAGGTTTGCCTATGTGTTTATCAGTGAATGTATTTAGTATTTCAATTTTAGAT
HST      AGTTCCTTCGAGGTTTGCCTATGTGTTTATCAGTGAATGTATTTAGTATTTCAATTTTAGAT
*****

                                  U1102 indel-2      End ori-lyt (mori) 68712
HHV-6A.U1102 TATGGTTCGGTAAAAATATAGGTCTTTGTAATTCATTTGATTTAGCTTTTATATGTATT 68714
GS      TATGGTTCGGTAAAAATATAGGTCTTTG-----
AJ      TATGGTTCGGTAAAAATATAGGTCTTTG-----
CiHHV-6A.5055 TATGGTTCGGTAAAAATATAGGTCTTTG-----
2284      TATGGTTCGGTAAAAATATAGGTCTTTGTAAATCATTTGATTTAGCTTTTATATGTATT
5814      TATGGTTCGGTAAAAATATAGGTCTTTGTAAATCATTTGATTTAGCTTTTATATGTATT
HHV-6B.Z29 CATGGTTCGGTAAAGATATAAGTCCGTGTAATTTTTTGGTTTAGTTTTTCATATCTACT
HST      CATGGTTCGGTAAAGATATAAGTCCGTGTAATTTTTTGGTTTAGTTTTTCATATCTACT
*****

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Figure S2. The origin of lytic replication, *mori*, of CiHHV-6A 5055/1623, 5814, and 2284/4305 compared to virus reference HHV-6A U1102, AJ, GS and HHV-6B Z29, HST.

References

1. Bell, A.J.; Gallagher, A.; Mottram, T.; Lake, A.; Kane, E.V.; Lightfoot, T.; Roman, E.; Jarrett, R.F. Germ-line transmitted, chromosomally integrated HHV-6 and classical Hodgkin lymphoma. *PLoS ONE* **2014**, *9*, e112642.
2. Faten, N.; Agnes, G.D.; Nadia, B.F.; Nabil, A.B.; Monia, Z.; Abderrahim, K.; Henri, A.; Salma, F.; Mahjoub, A. Quantitative analysis of human herpesvirus-6 genome in blood and bone marrow samples from Tunisian patients with acute leukemia: a follow-up study. *Infect. Agent Cancer* **2014**, *7*, 31, doi:10.1186/1750-9378-7-31.
3. Geraudie, B.; Charrier, M.; Bonnafous, P.; Heurte, D.; Desmonet, M.; Bartoletti, M.A.; Penasse, C.; Agut, H.; Gautheret-Dejean, A. Quantitation of human herpesvirus-6A, -6B and -7 DNAs in whole blood, mononuclear and polymorphonuclear cell fractions from healthy blood donors. *J. Clin. Virol.* **2012**, *53*, 151–155.
4. Gravel, A.; Sinnett, D.; Flamand, L. Frequency of chromosomally-integrated human herpesvirus 6 in children with acute lymphoblastic leukemia. *PLoS ONE* **2013**, *8*, e84322.
5. Hall, C.B.; Caserta, M.T.; Schnabel, K.; Shelley, L.M.; Marino, A.S.; Carnahan, J.A.; Yoo, C.; Lofthus, G.K.; McDermott, M.P. Chromosomal integration of human herpesvirus 6 is the major mode of congenital human herpesvirus 6 infection. *Pediatrics* **2008**, *122*, 513–520.
6. Hall, C.B.; Caserta, M.T.; Schnabel, K.C.; Boetrich, C.; McDermott, M.P.; Lofthus, G.K.; Carnahan, J.A.; Dewhurst, S. Congenital infections with human herpesvirus 6 (HHV6) and human herpesvirus 7 (HHV7). *J. Pediatr.* **2004**, *145*, 472–477.
7. Hubacek, P.; Hrdlickova, A.; Spacek, M.; Zajac, M.; Muzikova, K.; Sedlacek, P.; Cetkovsky, P. Prevalence of chromosomally integrated HHV-6 in patients with malignant disease and healthy donors in the Czech Republic. *Folia Microbiol.* **2013**, *58*, 87–90.
8. Hubacek, P.; Muzikova, K.; Hrdlickova, A.; Cinek, O.; Hyncicova, K.; Hrstkova, H.; Sedlacek, P.; Stary, J. Prevalence of HHV-6 integrated chromosomally among children treated for acute lymphoblastic or myeloid leukemia in the Czech Republic. *J. Med. Virol.* **2009**, *81*, 258–263.

9. Hudnall, S.D.; Chen, T.; Allison, P.; Tyring, S.K.; Heath, A. Herpesvirus prevalence and viral load in healthy blood donors by quantitative real-time polymerase chain reaction. *Transfusion* **2008**, *48*, 1180–1187.
10. Lee, S.O.; Brown, R.A.; Eid, A.J.; Razonable, R.R. Chromosomally integrated human herpesvirus-6 in kidney transplant recipients. *Nephrol. Dial. Transplant.* **2011**, *26*, 2391–3239.
11. Lee, S.O.; Brown, R.A.; Razonable, R.R. Chromosomally integrated human herpesvirus-6 in transplant recipients. *Transpl. Infect. Dis.* **2012**, *14*, 346–354.
12. Leong, H.N.; Tuke, P.W.; Tedder, R.S.; Khanom, A.B.; Eglin, R.P.; Atkinson, C.E.; Ward, K.N.; Griffiths, P.D.; Clark, D.A. The prevalence of chromosomally integrated human herpesvirus 6 genomes in the blood of UK blood donors. *J. Med. Virol.* **2007**, *79*, 45–51.
13. Potenza, L.; Barozzi, P.; Masetti, M.; Pecorari, M.; Bresciani, P.; Gautheret-Dejean, A.; Riva, G.; Vallerini, D.; Tagliazucchi, S.; Codeluppi, M.; *et al.* Prevalence of human herpesvirus-6 chromosomal integration (CIHHV-6) in Italian solid organ and allogeneic stem cell transplant patients. *Am. J. Transplant.* **2009**, *9*, 1690–1697.
14. Tanaka-Taya, K.; Sashihara, J.; Kurahashi, H.; Amo, K.; Miyagawa, H.; Kondo, K.; Okada, S.; Yamanishi, K. Human herpesvirus 6 (HHV-6) is transmitted from parent to child in an integrated form and characterization of cases with chromosomally integrated HHV-6 DNA. *J. Med. Virol.* **2004**, *73*, 465–473.
15. Tembo, J.; Kabwe, M.; Chilukutu, L.; Chilufya, M.; Mwaanza, N.; Chabala, C.; Zumla, A.; Bates, M. Prevalence and risk factors for betaherpesvirus DNAemia in infants aged between 3 weeks and 2 years of age, admitted to a large referral hospital in sub-Saharan Africa. *Clin Infect Dis.* **2014**, doi:10.1093/cid/ciu853.
16. Tweedy, J.; Spyrou, M.A.; Hubacek, P.; Kuhl, U.; Lassner, D.; Gompels, U.A. Analyses of germline, chromosomally integrated human herpesvirus 6A and B genomes indicate emergent infection and new inflammatory mediators. *J. Gen. Virol.* **2015**, *96*, 370–389.
17. Ward, K.N.; Andrews, N.J.; Verity, C.M.; Miller, E.; Ross, E.M. Human herpesviruses-6 and -7 each cause significant neurological morbidity in Britain and Ireland. *Arch. Dis. Child* **2005**, *90*, 619–623.
18. Ward, K.N.; Leong, H.N.; Thiruchelvam, A.D.; Atkinson, C.E.; Clark, D.A. Human herpesvirus 6 DNA levels in cerebrospinal fluid due to primary infection differ from those due to chromosomal viral integration and have implications for diagnosis of encephalitis. *J. Clin. Microbiol.* **2007**, *45*, 1298–1304.



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