Tweedy, J; Spyrou, MA; Pearson, M; Lassner, D; Kuhl, U; Gompels, UA (2016) 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. Viruses, 8 (1). ISSN 1999-4915 DOI: https://doi.org/10.3390/v8010019

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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

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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 et al., 2008, 2004) |
| Blood donor-adult            | USA (Texas)    | 100 | 0 (<1.0)     | 1 (1.0)      | (Hudnall et al., 2008) |
| Blood donor                  | Canada (Ontario) | 288 | 0 (<0.3)     | 0 (<0.3)     | (Gravel et al., 2013) |
| Blood donors-adult           | France         | 200 | 0 (<0.5)     | 1 (0.5)      | (Geraudie et al., 2012) |
| Blood donor-adult            | UK (London)    | 500 | 0 (<0.2)     | 4 (0.8)      | (Leong et al., 2007) |
| Serum bank-child             | UK             | 610 | 4 + (0.7)    | 6 + (1.0)    | (Ward et al., 2005) |
| Blood controls               | UK (Northern)  | 563 | 1 (0.2)      | 10 (1.7)     | (Bell et al., 2014) |
| Nails adult                  | Czech Republic | 421 | 1 (0.2)      | 3 (0.7)      | (Hubacek et al., 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 et al., 2012) |
| SOT-Kidney                   | USA            | 46  | 0 (<2.0) | 1 (2.2) | (Lee et al., 2011) |
| Leukemic children            | Canada (Ontario) | 287 | 1 (0.3) | 0 (<0.3) | (Gravel et al., 2013) |
| SOT-mixed. Blood, tissue, hair samples | Italy | 135 | 1 (0.7) | 0 (<0.8) | (Potenza et al., 2009) |
| SCT-Blood, hair samples      | Italy          | 70  | 0 (<1.4) | 1 (1.4) | (Potenza et al., 2009) |
| CSF-encephalitis referral child/adult | UK     | 522 | 1 (0.2) | 5 (1.0) | (Ward et al., 2007) |
| Hodgkins lymphoma            | UK (Northern)  | 936 | 1 (0.1) | 15 (1.6) | (Bell et al., 2014) |
| Cardiac referrals            | Germany        | 3610| 7 (0.2) | 13 (0.4) | (Tweedy et al., 2015) |
| Malignant disease-blood      | Czech Republic | 812 | 7 (0.9) | 2 (0.2) | (Hubacek et al., 2013) |
| Leukemia-child-blood         | Czech Republic | 339 | 4 (1.2) | 1 (0.3) | (Hubacek et al., 2009) |
| Transplant donors/recipients-blood, herpesvirus referrals | Japan (Osaka) | 2332 | 1 (0.04) | 4 (0.2) | (Tanaka-Taya et al., 2004) |
| Neonatal intensive care unit, sera | Southern Africa (Zambia) | 303 | 0 (<0.3) | 0 (<0.3) | (Tembo et al., 2014) |
| Leukemia-blood               | North Africa (Tunisia) | 73  | 0 (<1%) | 1 (1.2%) | (Faten et al., 2012) |
| Region Totals                | North America  | 881 | 2 (0.2) | 7 (0.8) |                  |
|                             | 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 HHV-6A > CiHHV-6A | HHV-6A % HHV-6A Minor Variant | HHV-6A % HHV-6A Minor Variant | HHV-6A % HHV-6A Minor Variant |
|-----|----------------------------|----------------------------|-------------------------------|-------------------------------|-------------------------------|
|     | 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.

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**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.
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