UK Biobank

Inherited chromosomally integrated HHV-6 data

Version 1.0

http://www.ukbiobank.ac.uk/

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Contents

1. Background

1.1. This document provides information on the inherited chromosomally Integrated herpesvirus (iciHHV-6) data available in UK Biobank.

1.2. DNA samples from over 400k participants in the UK Biobank were screened using a triplex TaqMan qPCR assay to identify likely positive samples.

1.3. Analysis was performed between August 2021 and March 2022.

2. Research Overview

The genomes of human herpesvirus 6A and 6B (HHV-6A and 6B) can integrate into human DNA. Integration occurs specifically into telomeres, the repetitive elements found at the end of each chromosome. Most individuals are infected by exogenous HHV-6 but the viral genome can integrate into the germline and can be inherited by offspring as inherited chromosomally

integrated HHV-6 (iciHHV-6). It is estimated that tens of millions of people worldwide have iciHHV-6, but accurate prevalence estimates are lacking.

The study identified iciHHV-6 carriers by screening DNA samples from UKB participants using a TaqMan qPCR assay. In March 2022, the initial TaqMan screen of the samples was completed. Following the exclusion of missing samples, controls, replicates, and withdrawn samples, there were results on over 416,000 samples.

3. TaqMan Assay protocols

The HHV-6 genome comprises a long unique (U) region spanning U2 to U100, flanked by left and right Direct Repeat (DR) regions (DR1 and DR6). Additionally, telomere-like regions are present at the ends of the genome. Primers and probes targeting the U7 and DR1 regions were used for the molecular assays.

3.1 Methodology

Initial screening of DNA samples for iciHHV-6 was carried out using triplex qPCR incorporating three hydrolysis probe assays, DR1, U7 and β-globin (Table 1). The DR1 and U7 assays target the direct repeat (DR) and unique (U) regions of HHV-6, respectively. The DR1 and U7 assays are not species-specific; both assays amplify from iciHHV-6A and iciHHV-6B. The β-globin (BG) assay targets a human single-copy reference. 10 µL volume reactions consisted of 1× TaqPath ProAmp Multiplex Mastermix, 300 nM U7 and β-globin, 200 nM DR1 primers, 200 nM U7 and β-globin fluorescently-labelled probes, and 100 nM DR1 fluorescently-labelled probes. Thermocycling was performed on a QuantStudio™ 7 Flex Real-Time PCR System. Results were exported to a custom database for semi-automated analysis based on C_t and Δ C_t values.

Control DNAs from iciHHV-6A and 6B-positive cell lines and an iciHHV-6-negative cell line were included in every reaction plate, and accepted C_t thresholds for each control were used to quality-control each reaction plate: four iciHHV-6-positive control wells, two iciHHV-6 negative control wells, and two no-template control (NTC) wells. Daily checks for contamination were conducted using NTC reaction plates.

Table 1 lists the primers and probe sequences used in the TaqMan screen.

Table 1 qPCR triple iciHHV-6 screen primers and probes sequences.

Primer/Probe Name	Sequence	Label	Quencher
β-globin Forward	GGCAACCCTAAGGTGAAGGC		
β-globin Reverse	GGTGAGCCAGGCCATCACTA		
β-globin Probe	ATGGCAAGAAAGTGCTCGGTGCCT	JUN	QSY
HHV-6 DR1 Forward	GAAACTGTAACGGCCACGTT		
HHV-6 DR1 Reverse	GTGCTCCGCCACGACTAC		
HHV-6 DR1 Probe	CGCCGCCGCCGTTACTGTC	FAM	QSY

HHV-6 U7 Forward	AAAATTTCTCACGCCGGTATTC		
HHV-6 U7 Reverse	CCTGCAGACCGTTCGTCAA		
HHV-6 U7 Probe	TCGGTCGACTGCCCGCTACCA	VIC	QSY

3.2 Classification of results

iciHHV-6 positive individuals typically have one copy of the viral genome per cell (one copy of the unique region and two copies of the direct repeat region, U_1DR_2), but other genome compositions are also detected including genomes consisting of only a single direct repeat. The PCR assay C_t (Cycle thresholds) values for the U7, DR and β-globin genes were used to automatically derive the TaqMan assay screen result, based on the following cut-off values:

- If β-globin $C_t > 28$, sample scored as "Failed".
- $\Delta U7\ C_t$ = β-globin C_t - U7 C_t
- $\Delta DR1\ C_t$ = β-globin C_t – DR1 C_t
    - If $\Delta U7\ C_t < 6$ and $\Delta DR1\ C_t < 6$, sample scored as "Likely iciHHV-6".
    - If $\Delta U7\ C_t \geq 6$ and $\Delta DR1\ C_t < 6$, sample scored as "DR-only".
    - If $\Delta U7\ C_t < 6$ and $\Delta DR1\ C_t \geq 6$, sample scored as "U-only".
    - If $6 \leq \Delta U7\ C_t$ or $6 \leq \Delta DR1\ C_t < 8$, sample scored as "Unlikely iciHHV-6".
    - If $\Delta U7\ C_t \geq 8$ and $\Delta DR1\ C_t \geq 8$, sample scored as "iciHHV-6 negative".

In certain instances, manual assignment of the status was preferred when it appeared to be more appropriate.

4. Data preparation steps

4.1. The herpesvirus data have been pre-processed by removing all samples that had a failed QC, broken well or where well-to-well contamination was suspected. For the TaqMan assay, samples failed QC if the Ct value for beta-globin was greater than 28 and have been excluded.