

SHORT REPORT

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# Characterization of herpes simplex virus clinical isolate Y3369 as a glycoprotein G variant and its bearing on virus typing

Daniel N Clark<sup>1</sup>, Brian D Poole<sup>1\*</sup>, Daniel V Hammond<sup>1</sup>, Tyler J Hedman<sup>1</sup>, Danny S Catts<sup>1</sup>, Amanda Stewart<sup>1</sup> and F Brent Johnson<sup>1,2</sup>

## Abstract

**Background:** Herpes simplex viruses exist as two major serotypes, type 1 (HSV-1) and type 2 (HSV-2). Determination of type, either HSV-1 or HSV-2, is important in accurate diagnosis and clinical control of transmission. Several tests are available for typing HSV, including a monoclonal antibody specific for glycoprotein G and several PCR assays.

**Findings:** A clinical isolate was identified as herpes simplex virus, but tested negative for both HSV-1 and HSV-2 antigens using type-specific monoclonal antibody assays. The isolate was determined to be HSV-1 by PCR analysis. A mutation which likely caused the monoclonal antibody non-reactivity was found in glycoprotein G. Phylogenetic analysis revealed two groups of HSV, one with the mutation and one without. Three population studies examining mutations in HSV-1 glycoprotein G were analyzed by chi-squared test. To this point, the epitope which the monoclonal antibody recognizes was only found in HSV-1 isolates from human European populations ( $p < 0.0001$ ).

**Conclusions:** These findings suggest that the PCR-based methods for HSV typing may be more useful than the standard monoclonal antibody test in areas of the world where the variant in glycoprotein G is more prevalent.

**Keywords:** Herpes Simplex Virus serotyping, glycoprotein G

## Findings

Herpes simplex viruses exist as two major serotypes, type 1 (HSV-1) and type 2 (HSV-2). Determination of type, either HSV-1 or HSV-2, is important in accurate diagnosis and clinical control of transmission. Tests which can determine HSV type include viral antigen tests, serological tests of human antibodies and PCR [1,2]. The importance of glycoprotein G as the test analyte is emphasized by the 2002 STD Treatment Guidelines from the CDC: "Accurate type-specific assays for HSV antibodies must be based on the HSV-specific glycoprotein G2 for the diagnosis of infection with HSV-2 and glycoprotein G1 for diagnosis of infection with HSV-1." [3].

A clinical sample of a herpes simplex virus, designated Y3369 was isolated and proved refractory to typing. The

isolate was obtained from an infected genital tract of a 48-year-old female patient. It was submitted to Richards Laboratories, Inc., Pleasant Grove, Utah, USA for diagnostic workup. The sample was incubated overnight, and then stained for virus-infected cells using a type-common polyclonal primary antibody and visualized by the immunoperoxidase technique using a rapid culture method [4,5]. The culture showed an abundance of cells positive for antibody labeling and had HSV-typical cytopathic effects, confirming the presence of HSV in the specimen (results not shown).

The Y3369 isolate was then tested using the Wampole type-specific viral antigen test for HSV glycoprotein G. A viral stock culture was generated by inoculation of a portion of the rapid culture isolate into a culture of MV1Lu cells (mink lung, ATCC CCL-64). The specimen was also incubated in C1008 cells (Vero subline, ATCC CRL-1586) and subjected to similar serotypic analysis by staining with virus-specific monoclonal antibodies (mAbs)

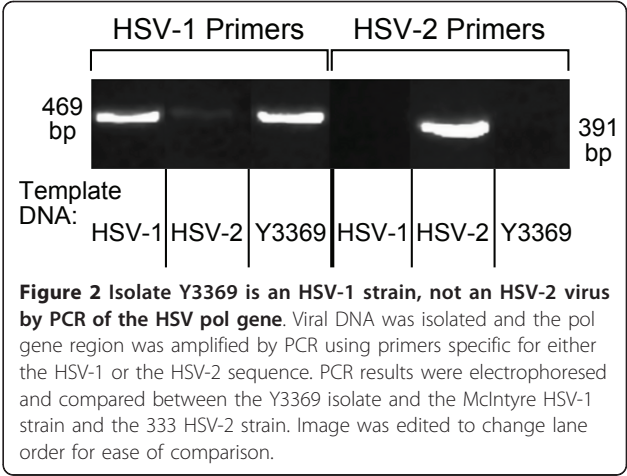
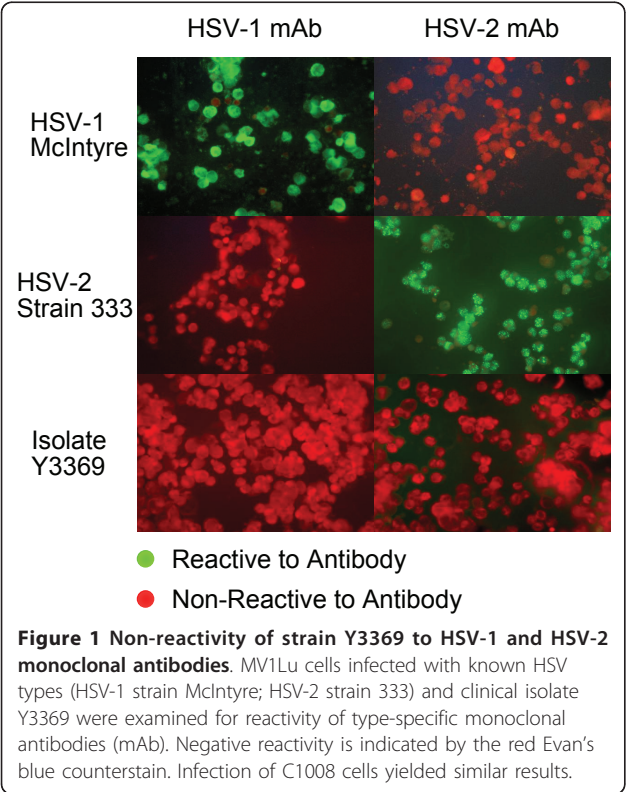
\* Correspondence: brian\_poole@byu.edu

<sup>1</sup>Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT, 84602, USA

Full list of author information is available at the end of the article

against HSV type 1 and type 2. These tests failed to yield a positive identification of the isolate as either HSV-1 or HSV-2 using type-specific mAb assays (Wampole Laboratories). The immunofluorescence result was negative against both reagent antisera in MV1Lu cells (Figure 1). The virus was also untypable in C1008 cells (not shown). The laboratory strains HSV-1 McIntyre and HSV-2 strain 333 were tested with mAb reagents and expected monotypic results were observed in these controls.

Determination of HSV type was done by PCR specific for the HSV pol gene using a common forward primer and type-specific reverse primers as performed by Abraham, et. al [6] and Kimura, et al. [7]. DNA was extracted (Invitrogen PureLink viral DNA/RNA mini kit) from purified virus of HSV-1 (McIntyre strain), HSV-2 (Strain 333), and from the Y3369 isolate. PCR products were then analyzed on a 1% agarose gel (Figure 2), which revealed that clinical isolate Y3369 contains the pol gene of an HSV-1 virus. To confirm the analysis, DNA was then extracted from the gel (QIAquick gel extraction kit, Qiagen) and sequenced (Parallab 350, ABI 3730xl). DNA sequencing confirmed Y3369 specimen was a strain of HSV-1 with the sequenced amplicon having 100% identity when compared to the published HSV-1 pol gene sequence (GenBank accession #X04771) and only 85% homology with the HSV-2 sequence.



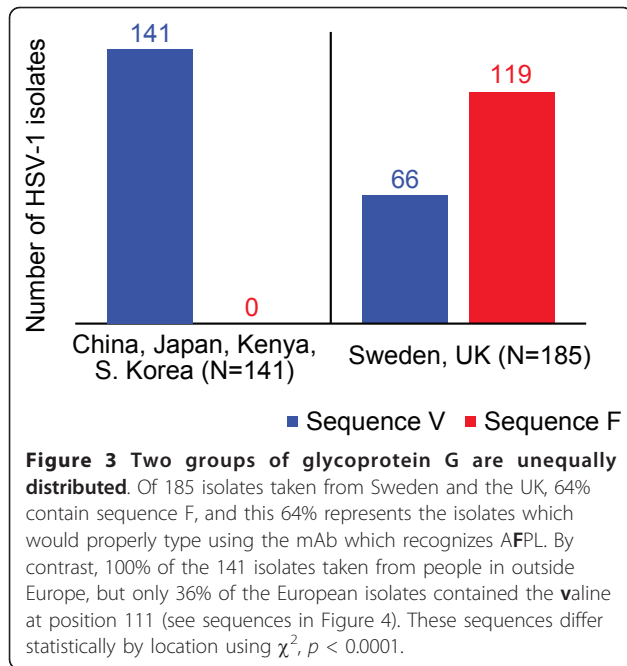
Confirmation of the isolate as an HSV-1 strain was done by successful PCR amplification of HSV-1 genes UL1, UL10, UL22, glycoprotein D, and glycoprotein G (data not shown, see Table 1 for PCR conditions and primers).

Glycoprotein G was PCR amplified (see supplementary table) and sequenced. Examination of the sequences showed that the probable cause for the non-reactivity of the mAb assay was the presence of a valine residue in glycoprotein G at amino acid (AA) 111. This valine is near the immunodominant region of antibody binding during normal immune response [8]. Sequencing results were deposited [GenBank:HQ833203], and compared to other isolates on GenBank. Sequencing revealed that the clinical isolate Y3369 contains an amino acid sequence consistent with a common HSV-1 sequence found in many parts of the world [9] (Figure 3).

A meta analysis of three population studies which have sequenced this region of the HSV-1 US4 gene was conducted to determine the prevalence of valine at position 111, as was identified in our sample [9-11]. Included were isolates from individuals from China,

Table 1 PCR condistions and Primers	
Primer	Sequence
UL1 for	5'-GAGACCCCTCGGCTATAAA-3'
UL1 rev	5'-CGTTTCTGTTCTGGGTGT-3'
UL10 for	5'-GAGCCTTGTTGGGCACTTATG-3'
UL10 rev	5'-GTGATCTGCAGCAACCAAGA-3'
UL 22 for	5'-AAACAAAAGCGCTCCTCGTA-3'
UL22 rev	5'-GACAGACCCATGGTTTTTGG-3'
glycoprotein G for	5'-GCTGTTTGCGGTTGGCACA-3'
glycoprotein G rev	5'-TCCCCGCCCCATACCCTAC-3'
glycoprotein D for	5'-TTTGTGTGGTGC GTTCCGGT-3'
glycoprotein D rev	5'-TCCCATCCCAACCCGCGAGA-3'

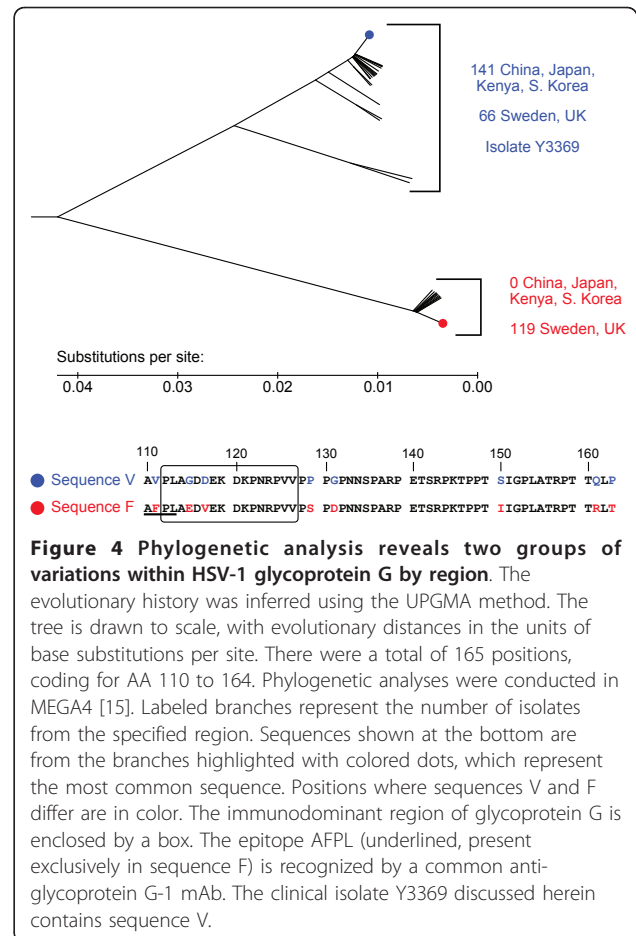
PCR conditions for all reactions:  
94°C 2 min, 40 cycles of [94°C 15 s, 57°C 15 s, 72°C 1:15], 72°C 2 min.



Japan, Kenya, South Korea, Sweden, and the United Kingdom. We discovered the valine at position 111 to be present in all HSV-1 isolates (100%,  $N = 141$ ) taken from human populations from Asia and Africa. The other populations, from the UK and Sweden, contained the valine at position 111 in 36% ( $N = 185$ ) of isolates (Figure 3). This valine at position 111 is located within the binding site for a commonly used typing mAb, which recognizes the epitope AFPL [10]. The phenylalanine is replaced to form the sequence AVPL in this variant.

Sequences for the middle region encoding AA 110 to 164 of glycoprotein G were analyzed and a phylogenetic tree created (Figure 4). Phylogenetic analysis groups our isolate Y3369 as an HSV-1 with sequence V (representing valine at 111) which contains the sequence AVPL instead of AFPL, as well as other common nucleotides as shown in Figure 4. All isolates from populations from Africa and Asia, as well as 36% of the European population contained the sequence AVPL, which would not be recognized by the mAb which tests for the AFPL epitope. Another study found that all isolates with a valine residue at position 111 of glycoprotein G were untypable when assaying viral antigens [10]. This specific test would not be likely to function diagnostically in these African or Asian populations.

Our analysis of these studies provides evidence that glycoprotein G variation is likely significant in clinical typing discrepancies and also in isolate variations. Analysis of the amino acid sequences of Y3369 and other isolates indicates that there is a shared significant



variation among HSV-1 strains that alters viral antigen assay specificity. PCR analysis is likely to succeed in HSV typing where the isolate is not recognized by the monoclonal antibody. In addition to results presented here, PCR has been used to type HSV samples on other occasions. In one study, 75 HSV-positive isolates yielded two which were untypable using type-specific antibody tests, later confirmed HSV-1 by PCR [12]. Another study yielded 1 untypable isolate of 37 tested HSV-positive isolates, which was also confirmed as HSV-1 by PCR [13]. These represent about 2% of the HSV-positive isolates in these two studies.

We have determined the presence of two phylogenetic groups of glycoprotein G. One group was only found in Europe (Figure 4, sequence F), and all the isolates in this group contain the epitope AFPL, which a common assay uses to type HSV-1. The other group (Figure 4, sequence V) was found in all tested regions, which include Africa, Asia and Europe. This group was characterized by the AVPL sequence. Y3369 is a member of this group. The two sequences differ by location statistically ( $\chi^2 = 142.8$ ,  $p < 0.0001$ ).

The identification of these two groups, as well as their localization to different parts of the world, may aid in developing strategies for clinical viral antigen assays for HSV typing. Although the isolates included in the meta analysis which have the AVPL sequence were not tested by us, they would likely fail to type as HSV-1 using this same test. It should be considered that tests for the viral antigen epitope AFLP be used with caution in Africa or Asia.

This variation may also alter the interaction of virus with host. The presence of the variations in the immunodominant region of the protein suggests these mutations could be a result of viral immune evasion. These mutations may also affect the functioning of glycoprotein G, which involves attachment and entry [14]. Further tests are being performed to study what other effects this mutation has on the virus's efficiency of infection.

## Human Subjects

The specimen was submitted to a clinical laboratory for diagnostic workup by code number only and the work was performed under clinical laboratory licensure: Richards Laboratories of Utah, Inc., 55 East Center St., Pleasant Grove, UT 84062, Laboratory Director: Dr. F. Brent Johnson

## List of abbreviations used

AA: amino acid; HSV: herpes simplex virus; mAb: monoclonal antibody;

## Author details

<sup>1</sup>Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT, 84602, USA. <sup>2</sup>Richards Laboratories, Inc., Pleasant Grove, UT 84062, USA.

## Authors' contributions

DC was involved in experimental design and data acquisition, performed data analysis, conducted the meta analysis, and wrote the manuscript and figures. BP designed the study, participated in data analysis, and aided in writing and reviewing the manuscript. DH designed the study, performed PCR, gels, and sequencing; and was involved in writing the manuscript. TH, DK and AS all participated in data acquisition and writing the manuscript. FBJ performed antigen testing, conceived of the study, participated in study design, and participated in manuscript writing. All authors have read and do approve the final manuscript.

## Competing interests

The authors declare that they have no competing interests. This work was funded by Brigham Young University.

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