

# Molecular characterization of human parvovirus B19 associated with neuromyelitis

Caracterização molecular do parvovirus humano B19 associado com neuromielite

Caracterización molecular del parvovirus B19 humano asociado a la neuromielitis

Maria Isabel de Oliveira  
Centro de Virologia, Instituto Adolfo Lutz, São Paulo, São Paulo, Brasil

Giselle Burlamaqui Klautau  
Departamento de Medicina da Santa Casa de Misericórdia de São Paulo, São Paulo, Brasil

Ana Maria Sardinha Afonso  
Centro de Virologia, Instituto Adolfo Lutz, São Paulo, São Paulo, Brasil

Maria Anice Mureb Sallum  
Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, Brasil

Cristina Adelaide Figueiredo  
Centro de Virologia, Instituto Adolfo Lutz, São Paulo, São Paulo, Brasil

Suely Pires Curti  
Centro de Virologia, Instituto Adolfo Lutz, São Paulo, São Paulo, Brasil

## ABSTRACT

**INTRODUCTION:** Human parvovirus B19 (B19V) is associated with a wide range of clinical symptoms. An acute infection can lead to anemia, erythema infectiosum, or hydrops fetalis, as well as arthritis and other clinical manifestations. Among other diseases associated with B19V, several demyelinating disorders may occur as a result of B19V infection. Phylogenetic analysis of partial erythrovirus sequences resulted in three possible B19V genotype groups. Our study involved analysis of samples from a patient with neuromyelitis that enabled the molecular characterization of human parvovirus B19. **OBJECTIVE:** To study B19V infection associated with a neurological manifestation and to perform phylogenetic analysis. **METHODS:** Serum and cerebrospinal fluid samples were tested for B19V infection. The serological assay for B19V was a commercial IgG and IgM enzyme immunoassay kit. Nucleic acid detection was performed using a PCR assay. The phylogenetic analyses were performed using PAUP and other software programs. **RESULTS:** Our study reports that the topology of viral isolate sequences from a patient with neuromyelitis fell within a clade consisting of B19V genotype 1. **CONCLUSION:** This result indicates that this virus genotype group has been circulating in Sao Paulo, Brazil. This report represents a rare case in which B19V might be the responsible agent for central nervous system infection.

**Keywords:** Human parvovirus B19; Neurologic manifestations; Parvoviridae infections; Sequence analysis, DNA.

## INTRODUCTION

Human parvovirus B19 (B19V) is classified in the Erythrovirus genus of the Parvoviridae family<sup>1</sup>. It may be associated with various pathological conditions, such as anemia, erythema infectiosum, hydrops fetalis and arthritis. The immunological response to the virus will vary greatly as a result of an individual's hematological status. The following are examples of conditions that may affect the immune response to infection: aplastic crisis, thrombocytopenia, hepatitis pancytopenia, renal disease, myocarditis and myositis<sup>2,3</sup>.

Generally, viral transmission takes place via an aerosol route through respiratory secretions. Viremia occurs one

week after exposure and usually lasts approximately five days, with virus titers peaking within the first two days. The course of infection is generally the same amongst individuals, which is likely a contributing factor in the lack of genetic diversity among B19V strains<sup>3</sup>.

A phylogenetic analysis has been conducted using partial sequences of B19V available in GenBank, and three genotype groups were proposed. Genotype 1 corresponds to B19V, and genotypes 2 and 3 are associated with V9 viruses<sup>4,5,6,7</sup>.

In addition to the disorders mentioned above, a number of demyelinating infections have been reported in association with B19V. Identification of the particular infectious agent involved is difficult. For this reason, B19V involvement in many of these disorders has not been clinically validated to date<sup>5</sup>.

Our study reports the case of a patient with neuromyelitis in which it was possible to characterize the genotype of the human parvovirus B19 that caused the infection.

### Correspondence / Correspondência / Correspondencia:

Maria Isabel de Oliveira  
Instituto Adolfo Lutz, Centro de Virologia, Av. Dr. Arnaldo, n° 355  
CEP: 01246-902 São Paulo - São Paulo - Brasil  
E-mail: olive40@uol.com.br

## MATERIALS AND METHODS

A previously healthy 27-year-old female was admitted to the Hospital Irmandade da Santa Casa de Misericórdia (ISCMSP) in Sao Paulo State, Brazil, with dizziness for 2 days, cough, expectoration, nausea, vomiting and desquamative lesions in the left cervical region, but the patient had no signs of secondary infection. Two days after admission, her symptoms had evolved to mental confusion, unresponsiveness to verbal commands or painful stimuli, tachypnea and urinary retention. An orotracheal intubation was performed. During the neurological examination, signs of meningeal irritation were not detected. The results of magnetic resonance imaging (MRI) of the brain were normal. Laboratory investigation showed normal results for blood counts (Hb 14.00 g/dL; Hct 44%; 5,000 leukocytes/mm<sup>3</sup>; platelets 183,000/mm<sup>3</sup>), serum biochemistry and prothrombin time. The cerebrospinal fluid (CSF) was clear, glucose was 60 mg/dL, protein was 98 mg/100 mL, white blood cells were 2 leukocytes/mm<sup>3</sup>; and direct staining for bacteria and fungi produced negative results. Several image tests were performed and the following hypotheses were suggested following clinical evaluation: demyelinating disease, myelopathy, neuromyelitis, auto-immune disorder, aseptic meningoencephalitis or infection.

A screening for virus infection was initiated with the collection of blood and CSF and the utilization of serological assays and nucleic acid detection by PCR. The samples were analyzed at Instituto Adolfo Lutz, Sao Paulo, Brazil, to detect measles, rubella, cytomegalovirus (CMV), human immunodeficiency virus (HIV), syphilis, hepatitis, human T lymphotropic virus (HTLV), Epstein-Barr virus (EBV), Herpes simplex 1 and 2 and varicella zoster infection. Results of these serological and PCR assays showed no sign of recent infection with these viruses. Sera and CSF samples were then tested for B19V infection. Antibodies were detected using a commercial IgG and IgM enzyme immunoassay kit (Biotrin®, Dublin, Ireland) that was specific for identifying VP2. DNA was amplified from sera and CSF with QIAmp® DNA minikit (Courtabouf, France) Quiagen. The kits were used according to the manufacturers' recommendations. Sequences were amplified using oligonucleotide primers specific for the conserved region of the NS1 gene, which encodes the nonstructural protein of B19V (positions 1267 to 1691) described by Sanabani et al. For phylogenetic studies, amplified DNA fragments were purified with the PuriLink PCR Purification Kit (Invitrogen TM Life Technologies, Carlsbad, USA) and sequenced using ABI 3100 Prism Big Dye (Foster City, USA) Applied Biosystems, Inc.

The sequence generated from the erythrovirus HPV B19 sample was subjected to Blast searches using the GenBank database. The viral isolate sequences used for phylogenetic analysis, along with isolation locations, isolation years and genotype, are presented in table 1. Alignment was performed using the prototype sequences of genotype 1 (strain Pvbua; accession number: M13178), genotype 2 (strain Lali; accession number: AY044266), and genotype 3 (strain V9; accession number: AY647977) and the CLUSTAL X program (<http://www-igbmc.u-strasbg.fr/BioInfo/ClustalX/>). Analysis was performed using the MEGALIGN (DNASTAR, Inc.) software. Phylogenetic analysis was performed using the PAUP, version 4.0 beta 10 program for Windows, which was written by D. L. Swofford (Sinauer Associates, Sunderland, USA). The

Modeltest software, version 3.6 (<http://darwin.uvigo.es/11>), was used for statistical analysis<sup>8</sup>.

**Table 1** – Characteristics of B19V virus isolates subjected to phylogenetic analyses

Accession number	Sample from	Locality	Isolation year	Genotype
AB126271	Liver	Japan	1987	1
M13178	Serum	United States	1986	1
AF161226	Synovial tissue	Finland	1999	1
HQ224945*	Cerebrospinal fluid	Brazil	2009	1
Z70560	No information	Germany	1996	1
AY386330	Serum	Canada	2004	1
AY064476	Plasma /serum	Denmark	2001	2
AY044266	Skin	Finland	2001	2
AJ717293	Serum	Berlin	2004	2
AY647977	Serum	Brazil	2004	3
AY083234	Serum	France	2002	3

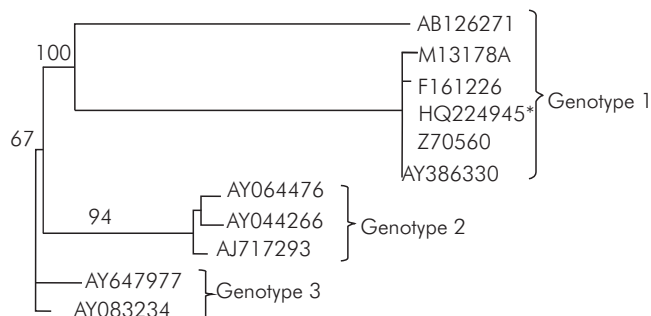
\*This work

## RESULTS

Serum samples were positive for B19V-specific IgG and negative for B19V-specific IgM. CSF samples were negative for both B19V-specific IgG and IgM. B19V DNA was detected only in CSF by PCR. The sequence was deposited in GenBank (accession number: HQ224945) and was aligned to a 423 bp fragment at position 1,282 to 1,704 in the NS1 gene sequence with accession number M13178.

Comparisons between the NS1 nucleotide and amino acid sequences from the isolate in this study and those from representative groups of B19V showed a high level of identity with genotype 1. Pairwise nucleotide identity among strains ranged from 99.1% to 98.6% in genotype 1, from 87.2% to 87.7% in genotype 2 and from 88.2% to 86.8% in genotype 3 (MEGALIGN, DNASTAR, Inc.).

Sequence HQ224945 generated from the SP Brazil isolate was recovered in the clade formed by sequences representatives of the genotype 1 (Figure 1). Aligned sequences with the reference genotype showed that the amino acid sequence had a mutation at the A619T position (alanine changed to threonine) through the analysis of the partial NS1 region from the erythrovirus variant studied in this work.



**Figure 1** – Phylogenetic relationships among B19 viruses based on the NS1-VP1 sequence analysis performed using the neighbor-joining algorithm and the distance matrix obtained in the PAUP package. A graphical representation of the distribution into three distinct groups: 1, 2 and 3. The strains are listed in table 1, and "\*" indicates the proposed genotype in this study

## DISCUSSION

Molecular test results for the patient described in this study suggested a B19V infection. The viral DNA detected in CSF indicated an acute viral infection that was likely the cause of the neuromyelitis. However, the precise mechanism whereby B19V affects the central nervous system (CNS) is unclear. P-antigen is the cellular receptor for B19V and it plays an important role in viral infection and replication. This antigen is present in endothelial, macrophage and microglial cells<sup>9</sup>.

The patient described in this report had a history of undetermined disease with acute neurological symptoms. Of note in the clinical history of this patient is the presence of an infectious condition preceding the neurological symptoms and diagnosis of neuromyelitis. Discovery of the etiologic agent could only be performed after a PCR assay analysis of CSF. It is unclear why patients exhibit different neurologic manifestations following infection despite having similar severe demyelinating lesions. Extensive investigation of possible causative infectious agents is required in cases without a definite etiology.

Our study of B19V infection in a patient with neuromyelitis showed that the isolate belonged to genotype 1. The study by Zakrzewska et al<sup>9</sup> showed B19V infection in systemic sclerosis (SSc) patients, indicating that infection with genotype 1 viruses was more frequent in the skin and that there was simultaneous persistence of genotype 2, which was detected in SSc skin and bone marrow samples. Piotr<sup>2</sup> reported genotypes 1 and 2 in immunocompromised patients. However, both genotypes were associated with high viremia in renal transplant patients treated with chemotherapy.

There is evidence that the large nonstructural protein NS1 transactivates both regulatory and structural gene

expression and that the synthesis of the NS1 transcripts occurs early in the course of infection<sup>10</sup>.

In our study, we did not make an association of mutation at position 619 with a probable infection. This mutation in the NS1 gene has not been reported in any previously described virus isolate. Further studies of the clinical significance of other erythrovirus variants should be performed.

The sequence generated in this study had the same reproducibility described in previous studies that used a small fragment of the NS1 gene to identify three distinct genotypic groups<sup>3</sup>. To date, only a limited number of studies have been conducted to detect virus in cerebrospinal fluid. There are reports showing the circulation of these genotypic groups in Brazil but not in association with infections of the CNS<sup>3,11,12,13</sup>.

## CONCLUSION

An accumulation of similar cases is required to elucidate the role the virus plays in the development of neuromyelitis. The patient described in this case report recovered without sequelae and represents a rare case of B19V as a possible cause of CNS infection.

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## Caracterização molecular do parvovírus B19 humano associado à neuromielite

### RESUMO

**INTRODUÇÃO:** O parvovírus B19 humano (B19V) é associado a vários sintomas clínicos. Uma infecção aguda pode levar à ocorrência de anemia, eritema infeccioso ou hidropsia fetal, bem como artrite e outras manifestações clínicas. Infecções por B19V podem também ocasionar várias doenças desmielinizantes. A análise filogenética das sequências parciais de eritrovírus resultou em três possíveis grupos de genótipos de B19V. Este estudo consistiu na análise de amostras de uma paciente com neuromielite, o que possibilitou a caracterização molecular do parvovírus B19 humano. **OBJETIVO:** Estudar a infecção por B19V associada a uma manifestação neurológica e realizar sua análise filogenética. **MÉTODOS:** Amostras de soro e de líquido cefalorraquidiano foram testadas para a observação de infecção por B19V. O teste sorológico utilizado para a pesquisa de B19V foi um kit comercial de ensaio imunoenzimático para IgG e IgM. A detecção de ácido nucleico foi realizada utilizando-se um teste de PCR. As análises filogenéticas foram realizadas utilizando PAUP e outros programas. **RESULTADOS:** Este artigo registra que a topologia de sequências de isolados virais de uma paciente com neuromielite foram inseridas em um clado constituído de grupos dentro do genótipo 1 de B19V. **CONCLUSÃO:** Esses achados indicam que este genótipo do vírus tem se mantido circulante em São Paulo, Brasil. Este relato representa um caso raro no qual o B19V pode ter sido o agente responsável pela infecção no sistema nervoso central.

**Palavras-chave:** Parvovírus B19 Humano; Manifestações Neurológicas; Infecções por Parvoviridae; Análise de Sequência de DNA.

## Caracterización molecular del parvovirus B19 humano asociado a la neuromielitis

### RESUMEN

**INTRODUCCIÓN:** El parvovirus B19 humano (B19V) está asociado a varios síntomas clínicos. Una infección aguda puede conducir a anemia, eritema infeccioso o hidropesía fetal, así como a artritis y a otras manifestaciones clínicas. Infecciones por B19V pueden también ocasionar varias enfermedades desmielinizantes. El análisis filogenético de las secuencias parciales de eritrovirus resultó en tres posibles grupos de genotipos de B19V. Este estudio consistió en el análisis de muestras de una paciente con neuromielitis, lo que posibilitó la caracterización molecular del parvovirus B19 humano. **OBJETIVO:** Estudiar la infección por B19V asociada a una manifestación neurológica y realizar su análisis filogenético. **MÉTODOS:** Se analizaron muestras de suero y de líquido cefalorraquídeo para la observación de infección por B19V. La prueba serológica utilizada para la investigación de B19V fue un kit comercial de ensayo inmunoenzimático para IgG e IgM. La detección de ácido nucleico se realizó utilizando una prueba de PCR. Los análisis filogenéticos se realizaron utilizando PAUP y otros programas. **RESULTADOS:** Este artículo registra que la topología de secuencias de aislados virales de una paciente con neuromielitis fueron insertas en un caldo constituido de grupos dentro del genotipo 1 de B19V. **CONCLUSIÓN:** Estos hallazgos indican que este genotipo del virus se ha mantenido en circulación en São Paulo, Brasil. Este relato representa un caso raro en el cual, el B19V puede haber sido el agente responsable por la infección en el sistema nervioso central.

**Palabras clave:** Parvovirus B19 Humano; Manifestaciones Neurológicas; Infecciones por Parvoviridae; Análisis de Secuencia de ADN.



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