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Lack of evidence for human infection with *Xenotropic murine leukemia virus-related virus* in the Brazilian Amazon basin

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ABSTRACT

Introduction: This study confirmed the absence of natural infection with Xenotropic murine leukemia virus-related virus (XMRV) or XMRV-related disease in human populations of the Brazilian Amazon basin. We demonstrated that 803 individuals of both sexes, who were residents of Belem in the Brazilian State of Pará, were not infected with XMRV. **Methods:** Individuals were divided into 4 subgroups: healthy individuals, individuals infected with human immunodeficiency virus, type 1 (HIV-1), individuals infected with human T-lymphotrophic virus, types 1 or 2 (HTLV-1/2), and individuals with prostate cancer. XMRV infection was investigated by nested PCR to detect the viral *gag* gene and by quantitative PCR to detect *pol*. **Results:** There was no amplification of either *gag* or *pol* segments from XRMV in any of the samples examined. **Conclusions:** This study supports the conclusions of the studies that eventually led to the retraction of the original study reporting the association between XMRV and human diseases.

Keywords: Xenotropic murine leukemia virus-related virus. Retrovirus. Amazon region. Brazil.

INTRODUCTION

Xenotropic murine leukemia virus-related virus (XMRV) was first identified by Urisman et al.[1], who studied the correlation between XMRV infection and the R462Q mutation of the ribonuclease L (RNase L) antiviral protein in familial-linked cases of prostate cancer (PC). Schlaberg et al.[2] detected XMRV proteins in malignant epithelial cells, although no correlation was observed between XMRV infection and the R462Q mutation. A similar lack of association was found in other studies performed in Mexico[3] and the United States[4].

The study of XMRV in blood samples from chronic fatigue syndrome (CFS) patients by Lombardi et al.[5] revealed that 67% of patients tested positive for the virus, compared to 3.7% in the control population. XMRV infection has also been investigated in patients with respiratory tract infections, and approximately 10% of immunodeficient patients were infected with XMRV, compared to 3.2% of immunocompetent patients. In addition, salivary viral ribonucleic acid (RNA) concentrations in infected individuals, although relatively low, were suggestive of a potential transmission route[6].

Xenotropic murine leukemia virus-related virus proteins were detected in 4.2% of Japanese people infected with human T-lymphotrophic virus, type 1 (HTLV-1), although this result may have been an artifact of the high degree of similarity between gp21 in HTLV-1 and p15E in XMRV. Positive serological findings from previous studies may have also been the result of cross reactions[7]; however, antibodies specific to the virus were apparently detected[8,9]. Other studies showed a prevalence of between 1-3% in the general (i.e., healthy) population, which indicated a possible role of blood transfusions in the dissemination of the virus[10,11].

New information about XMRV has raised controversy regarding the prevalence of the virus. Potential confounding factors include: a) the difficulty of reproducing results obtained for both the general population and patients with PC or CFS; b) the origin of the virus in relation to endogenous murine leukemia virus; c) the contamination of human blood and tissue samples with murine deoxyribonucleic acid (DNA); and d) the contamination of commercial polymerase chain reaction (PCR) reagents with potential sources of false-positive results[12-14].

The results of subsequent studies involving PC and CFS patients failed to show detectable XMRV or found extremely

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low levels of proviral DNA in the samples, indicating that PCR detection of the virus is not always feasible or reliable[13,15-17]. In addition, the lack of an association between viral infection, new cases of PC, and the RNase L R462Q mutation further questioned the potential of XMRV as a human oncovirus[14,18].

The present available data led to the retraction of the original data reporting an association between XMRV and human diseases[19-21]. A wide variety of infectious agents, many of which are capable of infecting humans, are endemic to the Brazilian Amazon. Thus, the present study aimed to determine the presence or absence of natural XMRV infection in human populations in the Amazon and its potential associations with diseases.

METHODS

Sample collection

DNA samples acquired in previous studies conducted at the Virus Laboratory of the ICB/UFPA were obtained from 803 individuals of both sexes who were residents of the City of Belém, State of Pará, Brazil. The subjects were divided into 4 groups as described in **Table 1**: healthy individuals (n = 290), individuals infected with human immunodeficiency virus, type 1 (HIV-1) (n = 292), individuals infected with HTLV-1/2 (n = 107), and individuals with prostate cancer (n = 114).

DNA extraction

DNA samples were extracted using the Pure Link Total DNA Kit (Life Technologies, USA), according to the manufacturer's instructions. Samples from the healthy individuals and HIV-1 and HTLV-1/2-infected individuals were obtained from leukocytes. DNA from patient PC samples was extracted from paraffin blocks and frozen tumor tissues.

Molecular analysis

Infection by XMRV was investigated using nested PCR to amplify a 413-bp fragment of the *gag* gene, as described previously[10]. In addition, the XMRV *pol* gene was detected by quantitative polymerase chain reaction (qPCR), using the StepOnePLUS™ Real-Time PCR System (Life Technologies, USA), as described by Schlaberg et al.[2]. Detection of the human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an endogenous control for qPCR reactions. The AO-H4 and AM2-9 plasmids, encoding XMRV cDNA derived from viral RNA isolated from a prostate cancer specimen (VP62), were used as positive controls[1]. TaqMan FAM-labeled probes were designed to target conserved regions of both the XMRV *gag* and *pol* genes and in the GAPDH gene. Plasmid samples were kindly provided by Dr. Nicole Fisher of the Institute for Medical Microbiology, Virology and Hygiene, at the Hamburg-Eppendorf University Medical Center in Hamburg, Germany.

Ethical considerations

The present study was submitted to and approved by the Ethics Committee of the Federal University of Pará (Number: 0024.0.324.324-11).

RESULTS

Analysis by qPCR indicated that the *pol* segments of XMRV were not amplified in any of the samples tested (**Table 1** and **Figure 1**), although positive control templates (i.e., XMRV cDNA plasmids) and endogenous controls (i.e., GAPDH) were detected. Nested PCR also failed to amplify the targeted *gag* segment in patient samples, but it was amplified in the controls.

DISCUSSION

Xenotropic murine leukemia virus-related virus was initially regarded as a virus that was capable of infecting humans and being transmitted through similar routes to those used by other human retroviruses. It was additionally thought to occur in co-infections, for example with HIV-1 or HTLV, as proposed by Groom et al.[16] and Qiu et al.[9] Although a previous study described a high rate of HIV-1/HTLV-1/2 co-infection[22], the present study did not find evidence for co-infection with XMRV among people infected with HIV-1 or HTLV-1/2, confirming previous results from HIV-1 carriers in Germany[23].

TABLE 1 - Characteristics of the population groups investigated in the present study for the presence of XMRV infection.

Population	Number	Sample	Mean age (± SD)	Gender		Nested PCR	qPCR
				male	female	*Gag*$^+$	*pol*$^+$
Healthy control	290	blood	29.98 ± 9.39	102	188	0	0
HIV-1	292	blood	39.87 ± 10.68	176	116	0	0
HTLV-1/2	107	blood	46.82 ± 15.76	33	74	0	0
Prostate cancer	114	tumor tissue	61.59 ± 7.33	114	0	0	0
Total	803	—	41.39 ± 14.83	425	378	0	0

XMRV: *xenotropic murine leukemia virus-related virus*; **SD:** standard deviation; **PCR**: polymerase chain reaction; **qPCR**: quantitative polymerase chain reaction; **HIV-1**: human immunodeficiency virus, type 1; **HTLV-1/2:** human T-lymphotrophic virus, types 1 and 2.

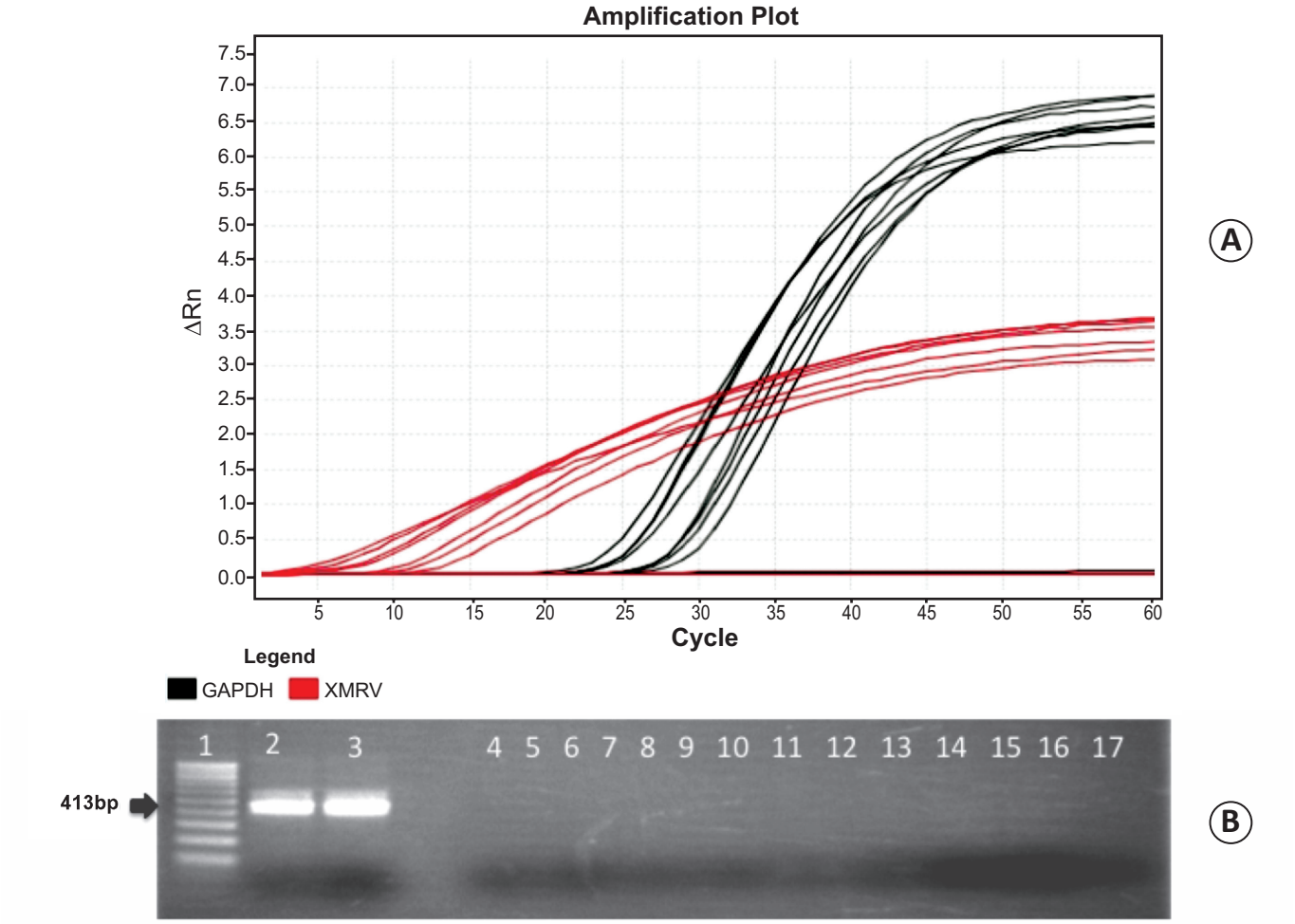

FIGURE 1 - A: Dose-dependent qPCR detection of the XMRV plasmid control (red) at concentrations of 333.6, 83.4, 20.8, 5.2, 1.3, 0.33, and 0.08ng/uL and qPCR detection of the *GADPH* gene (black). The *GAPDH* gene was used as an endogenous control for qPCR reactions, and XMRV cDNA encoded in the AO-H4 and AM2-9 plasmids was used as a positive control for *pol* detection. All patient samples were XMRV-negative as assessed by real time PCR and nested PCR. B: Ethidium bromide staining of a 1.5% agarose visualized under UV light, showing nested PCR results for the amplification of a 413-bp fragment of the *gag* gene. Lanes: 1: 100-bp DNA ladder; 2-3, plasmid controls; 4-17, representative negative XMRV patient samples. ΔRn (Delta Rn) is the normalization of Rn (fluorescence of the reporter dye divided by the fluorescence of a passive reference dye) obtained by subtracting the baseline (ΔRn = Rn – baseline).

qPCR: quantitative polymerase chain reaction; **XMRV:** *xenotropic murine leukemia virus-related virus*; **GAPDH:** glyceraldehyde 3-phosphate dehydrogenase; **cDNA:** complementary DNA; **UV**: ultraviolet.

We also found no evidence of XMRV infection in prostate tumor samples, contradicting the results of previous studies that found an apparent association between this retrovirus and prostate cancer[1-4]. Similar negative results were attributed to the failure of PCR procedures[13,15-17]. However, it is important to note that in the present study, nested and real time PCR were used, and both procedures were effective in detecting proviral DNA of HIV-1 and HTLV-1/2 in several different groups, even when viral copy numbers were extremely low[24-27].

Several factors can interfere with the diagnosis of XMRV infections in humans. The most likely explanation for false positive results is contamination of commercial PCR reagents[12-14]. Additionally, Tuke et al.[28] proposed that a specific Taq DNA polymerase may contain monoclonal mouse antibodies with varying quantities of murine DNA. This DNA is detectable in some batches and can thus generate false positive results. The results of the present study did not support this conclusion; the reagents used were provided by several different manufacturers, including the manufacturer used by Tuke et al.[28], and no false positives were observed. It thus seems more likely that contamination of reagents, human blood or tissue samples with murine genomic DNA was restricted to only a few research laboratories, which would account for the divergence of results.

Paprotka et al.[29] proposed that XRMV originated from a single recombination event between 1993 and 1996, involving 2 endogenous MLVs (i.e., PreXMRV-1 and PreXMRV-2) derived from a xenograft of the 22R*v1* cell line in nude mice. The possibility that this recombination event occurred in multiple locations is highly unlikely, given the marked similarity of sequences isolated in different studies. XMRV was therefore

considered a virus that originated from a single recombination event and did not have a natural human reservoir[13,30]. It thus seems likely that all the subsequent isolates of the virus were derived from this unique recombination event because of cross-contamination in various laboratories when handling 22R*v1* and other susceptible cell lines. From 1999 onwards, 22R*v1* cell lines were distributed worldwide and were largely used in biological studies of prostate cancer[29].

Clearly, there was no contamination in the present study. Furthermore, the absolute lack of XMRV in the various groups investigated herein challenges the results of previous studies that showed a high prevalence of XMRV in human populations and alleged an association with prostate cancer. This study supports the conclusions of studies that eventually led to the retraction of the original study reporting the association between XMRV and human diseases[19-21].

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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