Published in final edited form as:
J Infect Dis. 2010 November 15; 202(10): 1478–1481. doi:10.1086/657168.

XMRV Prevalence in Patients with Chronic Fatigue Syndrome or Chronic Immunomodulatory Conditions

Timothy J. Henrich[1,2,3,*], **Jonathan Z. Li**[1,2,3,*], **Donna Felsenstein**[2,3], **Camille N. Kotton**[2,3], **Robert M. Plenge**[3,4], **Florencia Pereyra**[1,3,5], **Francisco M. Marty**[1,3,6], **Nina H. Lin**[2,3], **Paul Grazioso**[2], **Danielle M. Crochiere**[2], **Daniel Eggers**[2], **Daniel R. Kuritzkes**[1,2,3], and **Athe M. N. Tsibris**[1,2,3,†]

[1] Division of Infectious Diseases, Brigham and Women's Hospital, Boston, MA, USA

[2] Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, USA

[3] Harvard Medical School, Boston, MA, USA

[4] Division of Allergy, Immunology and Rheumatology, Brigham and Women's Hospital, Boston, MA, USA

[5] Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University, Cambridge, MA, USA

[6] Dana-Farber Cancer Institute, Boston, MA, USA

Abstract

We investigated the prevalence of xenotropic murine leukemia virus-related virus (XMRV) in 293 participants seen at academic hospitals in Boston, Massachusetts. Participants were recruited from five groups of patients: chronic fatigue syndrome (CFS, *n* = 32), HIV infection (*n* = 43), rheumatoid arthritis (RA, *n* = 97), hematopoietic stem-cell or solid organ transplant (*n* = 26), or a general cohort of patients presenting for medical care (*n* = 95). XMRV DNA was not detected in any participant samples. We found no association between XMRV and patients with CFS or chronic immunomodulatory conditions.

Keywords

XMRV; chronic fatigue syndrome; HIV infection; rheumatoid arthritis; hematopoietic stem-cell transplantation; solid organ transplantation

Introduction

Murine and human xenotropic viruses were first described over 30 years ago [1]. The xenotropic murine leukemia virus-related virus (XMRV) was discovered in prostate tissue from prostate cancer patients homozygous for a reduced-activity variant of RNase L [2–4]. RNase L gene abnormalities have been described in patients with CFS [5], although these findings have not been verified in a more recent investigation [6].

†Corresponding author: Athe M.N. Tsibris, Division of Infectious Diseases, Massachusetts General Hospital, 65 Landsdowne St, Rm 422, Cambridge, MA 02139, Fax: (617) 768-8738, atsibris@partners.org.
*Authors contributed equally to this work

Financial disclosures: The authors have no relevant potential conflicts of interest to disclose.

A recent study in the United States found evidence of integrated XMRV DNA in 67% of subjects with CFS, compared to 3.7% of healthy controls [7]. Three subsequent CFS studies in European cohorts have not demonstrated the presence of XMRV DNA in CFS subjects or healthy controls [8–10]. The explanation for these contrasting results is unknown.

We designed a cross-sectional cohort study to determine XMRV prevalence in a variety of North American clinic populations, including healthy subjects, participants with CFS, and participants with states of chronic immune activation or suppression. We incorporated three different PCR primer sets from published XMRV studies into our testing strategy to determine if differences existed in their ability to amplify XMRV DNA.

Methods

Participant enrollment and study design

Samples were obtained from adult patients presenting to outpatient clinics, or from pre-existing repositories and cohorts at Brigham and Women's Hospital, Massachusetts General Hospital, and Dana-Farber Cancer Institute. Participants prospectively enrolled had a prior diagnosis of HIV infection, hematopoietic stem-cell or solid organ transplant, or CFS. This study was powered to detect XMRV prevalence with an upper confidence limit of 1.2% for the combined cohorts.

All CFS participants enrolled met the Centers for Disease Control and Prevention (CDC) revised CFS case definition (http://www.cdc.gov/cfs/cfsdiagnosis.htm). CFS participants had 10 mL of whole blood collected for peripheral blood mononuclear cell (PBMC) isolation and XMRV PCR testing, and completed a questionnaire. Demographic and clinical information were obtained from chart review. Cryopreserved PBMC and clinical information were obtained from HIV-infected patients from the International HIV Controllers Study [11–12]. Cryopreserved PBMC DNA and demographic data for rheumatoid arthritis participants, and an age- and gender-matched cohort of participants who had presented for either inpatient or outpatient clinical care, were obtained from the Crimson Biospecimen Core [13]. This study was approved by the relevant Institutional Review Boards.

XMRV PCR amplification

Human DNA was extracted from at least 5×10^6 PBMC (Qiagen). PCR reaction conditions and primer sets are described in Table 1. First round PCR amplifications were performed with 1 unit of Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen), 80–200ng of PBMC DNA, and 200nM of the outer primers of Lombardi, et al. (outer primer set) [7]. Nested PCRs were performed with first round product and 200nM of the inner primers of either Erlwein et al. (inner primer set #1) [8] or Urisman et al. (inner primer set #2) [4]. PBMC DNA from participant S6 was retested using an additional cryopreserved DNA aliquot, the original Platinum Taq HF Polymerase reagents, and USB *Taq* Polymerase (Affymetrix).

Each PCR amplification run included a negative control and positive controls of 10, 100, and 1000 copies of full-length XMRV (isolate VP62), generously provided by Robert H. Silverman, Cleveland Clinic. To test the efficiency of DNA extraction and PCR amplification, increasing amounts of XMRV plasmid, starting at 1 copy, were added to 200ng of PBMC DNA and to PBMCs prior to DNA extraction. Nested PCR amplification for each experiment was performed in triplicate for each primer set. To verify DNA integrity, human β-globin (hBG) was amplified from each participant sample with a single-round PCR reaction that used the outer hBG primer set of Erlwein, et al. [8]. All but one sample, from a general clinic participant, demonstrated β-globin amplification. This sample

was excluded from our analysis. Laboratory personnel who performed PCR amplification were blinded to the identity of each sample.

XMRV cloning and sequence analysis

DNA bands near the length of interest were purified with the QIAquick Gel Extraction kit (Qiagen), cloned into a pCR4-TOPO vector (Invitrogen) and bi-directionally sequenced (ABI 3730 automated DNA sequencer, Applied Biosystems).

Data analysis

Chi-squared (χ^2) testing with continuity correction was used to compare inter-group variation between non-parametric variables. Fisher's exact tests were used if expected counts were less than five. One-way analysis of variance testing was performed to define inter-group comparisons of continuous variables; a Bonferonni correction was used in pair-wise tests of individual hypotheses (SPSS version 10). To estimate the probability of detecting XMRV in CFS participants based on sample size, the Blyth-Still-Casella 95% exact confidence intervals (CIs) for disease prevalence were calculated.

Results

Participant cohorts

PBMC samples from 293 participants were tested for the presence of XMRV DNA (Table 2). Thirty-two subjects with a previous CFS diagnosis and 26 patients that had undergone either hematopoietic stem-cell or solid-organ transplant from participating outpatient hospital clinics were prospectively enrolled. Samples from 43 HIV-infected subjects were included either from an existing sample cohort or enrolled prospectively from the Brigham and Women's Hospital infectious disease clinic. Twenty-two of the HIV-infected patients (51%) were virologically suppressed on ART; the remainder were chronically infected persons not on treatment. We tested 97 samples from patients with rheumatoid arthritis and 96 samples from age-and gender-matched controls obtained from the Crimson Biospecimen Core.

Participants with rheumatoid arthritis and those from the age- and gender-matched general clinic/hospital cohort had the highest proportion of women, greater than 80%, within each group. The majority of CFS participants were women (66%), whereas the HIV-infected and hematopoietic stem-cell transplantation or organ transplantation cohorts contained fewer women (19% and 46%, respectively; significant inter-group difference compared to CFS cohort, $p < 0.001$). Mean CD4+ T lymphocyte counts for HIV-infected participants on or off ART were similar (566 and 554 cells/μl respectively, $P = 0.89$); 95.5% of participants receiving ART has plasma HIV-1 RNA levels <50 copies/mL. A majority of transplant patients had undergone solid organ transplantation, including eleven kidney, seven liver, two lung, two heart, and one liver/kidney transplants. One liver transplant recipient had underlying treated HIV infection. The remaining three transplant participants had undergone allogeneic hematopoietic stem-cell transplantation.

A majority of CFS participants had daily symptoms (75.9%), stopped work as a direct result of CFS symptoms (69%), or experienced fever, lymphadenopathy or swollen glands at the start of their illness (75%); 20.7% of participants had household contacts with similar symptoms or a diagnosis of CFS and 7% noted a tick-bite just prior to onset of their symptoms. The mean reported duration of symptoms for the CFS participants was 11.6 years.

PCR analysis

We could reliably detect 10 copies of XMRV per 200ng of PBMC DNA when control XMRV plasmid was added to PBMC aliquots either before or after DNA extraction. We were able to detect 1 copy of XMRV DNA in 2 of 3 assays when the VP62 plasmid was added to extracted PBMC DNA aliquots, a result consistent with the Poisson distribution. Using the PCR strategy described, we did not detect XMRV *gag* DNA in any of the 293 participant samples. In seven nested PCR reactions, DNA bands of a size similar to the expected XMRV *gag* PCR product were observed (data not shown). Cloning and sequence analysis of the PCR amplicon from participant S6, a subject with rheumatoid arthritis, demonstrated >99% sequence identity to a mouse endogenous retrovirus that was not XMRV [14]. Multiple repeat PCR amplifications with participant S6 PBMC DNA that used the original reagents, an additional aliquot of DNA, or another commercially available Taq polymerase failed to generate a PCR amplicon; this finding suggests that the original amplicon most likely resulted from contamination. Amplicons from the other six nested PCR reactions had sequences matching a portion of the human genome and shared no homology with XMRV.

Discussion

We assessed the prevalence of XMRV in a cohort of 293 American patients with CFS or chronic conditions associated with immune activation and/or immune deficiency and did not detect XMRV in any participant sample. A previous report showed that XMRV DNA could be detected in patient samples after a single round of 45-cycle PCR [7]. Our XMRV amplification strategy used similar amounts of input PBMC DNA and identical primer sets as were used in previous reports, and we increased the sensitivity of our methods by adding a nested PCR amplification that used two additional previously published XMRV primer sets [4,7–8]. These negative findings demonstrate that XMRV was not associated with any specific group that we investigated; the choice of PCR primers did not affect XMRV prevalence estimates. XMRV DNA could be present at levels below our detection threshold. However, we used PCR methodology that was comparable to previously published methods that detected XMRV DNA in CFS and healthy control subjects [7,15].

The upper limit of the 95% confidence interval around our CFS participant XMRV prevalence estimate (0%) was 9.5%. This result is similar to reports from Europe and suggests a far lower rate of XMRV infection, if any, in patients with CFS compared to the initial report [7]. Regional differences in XMRV prevalence among CFS patients could reflect geographical clustering of XMRV infection, and weakens the epidemiological link between XMRV infection and CFS.

To further characterize our CFS cohort and provide a basis of comparison to other CFS groups described in the literature, we administered a 43-item CFS questionnaire. Although our questionnaire may be confounded by recall bias, a majority of our CFS participants reported ongoing symptoms at the time of study entry. These symptoms had been present for an average of 12 years and were debilitating enough to cause the majority of participants to stop working.

Endogenous or latent viruses can become activated in patients with altered immune function. We explored the effect that immune activation or suppression could have on XMRV prevalence by including participants with rheumatoid arthritis, HIV infection (both treatment naïve and virologically suppressed), and hematopoietic stem-cell and solid organ transplant. We did not identify an association between XMRV prevalence and immune status. Healthy individuals were not included *per se* in this study and preclude us from drawing conclusions about the prevalence of XMRV in the general population. Our rheumatoid arthritis age- and

gender- matched hospital cohort may contain healthy patients that presented for routine clinical care, but we would reasonably expect greater morbidity in this control group, relative to the population-at-large in Boston. We did detect a mouse endogenous retroviral sequence in PBMC DNA from one participant, but could not replicate this finding. Mouse endogenous retroviral sequences are not present in the human genome; reagent testing did not identify the source of this contaminating sequence.

In summary, we found no evidence of XMRV infection in a cohort of patients cared for at Boston-area hospitals and no association of XMRV with either chronic fatigue syndrome or chronic conditions with altered immune function. Further research should be performed to define the demographic and geographic distribution of XMRV and to clarify its relationship with chronic fatigue syndrome.

Acknowledgments

We wish to thank the study participants for their participation. We thank Robert Silverman for his kind gift of the XMRV VP62 plasmid. We also wish to thank the clinic staff of the Dana Farber Cancer Institute Brigham and Women's Hospital and Massachusetts General Hospital Infectious Diseases clinics and the Massachusetts General Hospital transplant clinic for their efforts in support of this study. We are grateful to Hongying Wang for her statistical analysis advice.

Financial support: Supported in part by NIH grants T32 AI07387 (JL), KL2 Medical Research Investigator Training (MeRIT) program of Harvard Catalyst | The Harvard Clinical and Translational Science Center UL1 RR025758 (TJH), K24 RR016482 (DRK), and K08 AI081547 (AMNT).

References

1. Levy JA. Xenotropic type C viruses. Curr Top Microbiol Immunol. 1978; 79:111–213. [PubMed: 77206]
2. Fischer N, Hellwinkel O, Schulz C, et al. Prevalence of human gammaretrovirus XMRV in sporadic prostate cancer. J Clin Virol. 2008; 43:277–83. [PubMed: 18823818]
3. Schlaberg R, Choe DJ, Brown KR, Thaker HM, Singh IR. XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. Proc Natl Acad Sci U S A. 2009; 106:16351–6. [PubMed: 19805305]
4. Urisman A, Molinaro RJ, Fischer N, et al. Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog. 2006; 2:e25. [PubMed: 16609730]
5. Suhadolnik RJ, Reichenbach NL, Hitzges P, et al. Upregulation of the 2-5A synthetase/RNase L antiviral pathway associated with chronic fatigue syndrome. Clin Infect Dis. 1994; 18 (Suppl 1):S96–104. [PubMed: 8148461]
6. Gow JW, Simpson K, Behan PO, Chaudhuri A, McKay IC, Behan WM. Antiviral pathway activation in patients with chronic fatigue syndrome and acute infection. Clin Infect Dis. 2001; 33:2080–1. [PubMed: 11698994]
7. Lombardi VC, Ruscetti FW, Das Gupta J, et al. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science. 2009; 326:585–9. [PubMed: 19815723]
8. Erlwein O, Kaye S, McClure MO, et al. Failure to detect the novel retrovirus XMRV in chronic fatigue syndrome. PLoS One. 2010; 5:e8519. [PubMed: 20066031]
9. Groom HC, Boucherit VC, Makinson K, et al. Absence of xenotropic murine leukaemia virus-related virus in UK patients with chronic fatigue syndrome. Retrovirology. 2010; 7:10. [PubMed: 20156349]
10. van Kuppeveld FJ, de Jong AS, Lanke KH, et al. Prevalence of xenotropic murine leukaemia virus-related virus in patients with chronic fatigue syndrome in the Netherlands: retrospective analysis of samples from an established cohort. BMJ. 2010; 340:c1018. [PubMed: 20185493]

11. Walker BD. Elite control of HIV Infection: implications for vaccines and treatment. Top HIV Med. 2007; 15:134–6. [PubMed: 17720999]
12. Pereyra F, Addo MM, Kaufmann DE, et al. Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. J Infect Dis. 2008; 197:563–71. [PubMed: 18275276]
13. Murphy S, Churchill S, Bry L, et al. Instrumenting the health care enterprise for discovery research in the genomic era. Genome Res. 2009; 19:1675–81. [PubMed: 19602638]
14. Policastro PF, Fredholm M, Wilson MC. Truncated gag products encoded by Gv-1-responsive endogenous retrovirus loci. J Virol. 1989; 63:4136–47. [PubMed: 2789292]
15. Fischer N, Schulz C, Stieler K, et al. Xenotropic murine leukemia virus-related gammaretrovirus in respiratory tract. Emerg Infect Dis. 2010; 16:1000–2. [PubMed: 20507757]

Table 1

XMRV and Human Beta-Globin (hBG) Oligonucleotide Primers and PCR Reaction

Primer	Sequence	Location[a]	PCR Condition
XMRV Forward outer [7]	ATCAGTTAACCTACCCGAGTCGGAC	424–448	94°C × 2m
XMRV Reverse outer [7]	GCCGCCTCTTCTTCATTGTTCTC	1154–1132	40 cycles: 94°C × 30s, 57°C 30s, 68°C × 60s 68°C × 5m
XMRV Forward inner #1 [8]	GACTTTTTGGAGTGGCTTTGT	446–466	94°C × 2m
XMRV Reverse inner #1 [8]	ACAGAAGAACAACAAAACAAATC	571–549	30 cycles: 94°C × 30s, 55°C 30s, 68°C × 30s 68°C × 5m
XMRV Forward inner #2 [4]	TCTCGAGATCATGGGACAGA	603–622	94°C × 2m
XMRV Reverse inner #2 [4]	AGAGGGTAAGGGCAGGGTAA	1015–996	30 cycles: 94°C × 30s, 60°C 30s, 68°C × 30s 68°C × 5m
hBG Forward	TGGTGGTCTACCCTTGGACC	148–162	94°C × 2m
hBG Reverse	GAGGTTGTCCAGGTGAGCCA	296–277	40 cycles: 94°C × 30s, 55°C 30s, 68°C × 30s 68°C × 2m

[a] Locations in XMRV isolate VP62 (GenBank accession DQ399707) and human beta-globin (GenBank accession NM000158.4) genes. XMRV gag-pro-pol gene starts at nucleotide location 613.

Table 2

Demographics of Screened Participant Cohorts

	Cohort					
	CFS	**HIV**	**SOT/HCT**	**Rheumatoid Arthritis (RA)**	**RA-Matched Hospital Control[a]**	**All Subjects**
N	32	43[b]	26[c]	97	95	293
Median Age (Years)	46	46	56	66	62	58
Female	21	8	12	83	84	208
Gender	(65.6%)[d]	(18.6%)	(46.2%)	(85.6%)	(88.4%)	(71.0%)

Note: CFS = chronic fatigue syndrome; SOT = solid organ transplant; HCT = hematopoietic stem-cell transplant

[a] DNA obtained from discarded in- or outpatient phlebotomy samples, age and gender matched with the rheumatoid arthritis cohort

[b] 22 subjects currently receiving and 21 subjects not receiving antiretroviral therapy

[c] 3 HCT, 11 kidney, 2 lung, 2 heart, 7 liver, and 1 liver and kidney transplants. 1 liver transplant patient also had concurrent HIV infection

[d] N (percent female gender within specific cohort)

J Infect Dis. Author manuscript; available in PMC 2011 November 15.