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Myra McClure & Steve Kaye

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Can detection of xenotropic murine leukemia virus-related virus be linked to chronic fatigue syndrome?

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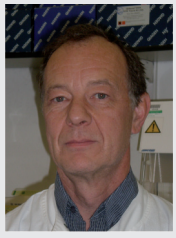
Myra McClure

Author for correspondence
Section of Infectious Diseases, Imperial College London, Jefferiss Research Trust Laboratories, St Mary's Campus, Norfolk Place, London W2 1PG, UK
Tel.: +44 7594 3902
Fax: +44 7594 3906
m.mcclure@imperial.ac.uk



Steve Kaye

Section of Infectious Diseases, Imperial College London, Jefferiss Research Trust Laboratories, St Mary's Campus, Norfolk Place, London W2 1PG, UK

"Chronic fatigue syndrome has long been considered to be a multifactorial condition, in which virus infection is likely to play a role."

Last year, a new retrovirus, xenotropic murine leukemia virus-related virus (XMRV), was reported to be present in the peripheral blood cells of patients with chronic fatigue syndrome (CFS) [1]. This finding has not, as yet, been independently confirmed by a second laboratory but, nevertheless, has attracted a great deal of attention.

As recently as 2006, Urisman *et al.*, in the USA, first found integrated proviral XMRV DNA in cases of familial prostate cancer (PC) [2]. Although the discovery of a new retrovirus was potentially exciting, no causal link to PC could be demonstrated, since the virus appeared to be restricted to stromal cells and not found within the tumor tissue. The authors built their breakthrough on the link between a genetic mutation in RNAseL (an enzyme key to the cellular antiviral response), implicated in familial PC cases [3,4]. Having identified the virus using a microarray capable of detecting all known virus families, they used a specific PCR to detect XMRV sequences in 40% (eight out of 20) of PC patients with the mutation.

A subsequent study in 2009, also from the USA, tried to find XMRV in 334 consecutive prostate resection samples [5]. Proviral DNA sequences were detected in the prostate tumor epithelium of 6% of patients, particularly those with high-grade cancers. This opened up the possibility that the virus might play a role in tumorigenesis. In contrast to the original report, infection was independent of the *RNAseL* gene mutation, implicating the virus in sporadic forms of PC, which are far more common in male populations.

The designation, XMRV, reveals the group of viruses to which this newcomer is most closely related, namely the xenotropic murine (endogenous) viruses. Genomic-sequence comparisons highlight its independence from its murine ancestor, as well as from any human endogenous retroviruses. This, together with the fact that the first XMRV sequences cloned from infected humans were not identical to one another (suggestive of separate episodes of human infection) underpins its discovery as the third human retrovirus [2], the others being the human T-cell leukemia virus type 1 (HTLV-1) and HIV-1.

In contrast to the two US studies, three European groups generated quite different results from their search for XMRV in PC by proviral DNA amplification. One German group detected a lower prevalence (one out of 105 patients) [6], while an Irish study failed to find the virus in a sample size of 139 patients [7]. A larger study from Germany also failed to detect the virus in any of 500 PC patients [8]. It is too early to speculate whether XMRV has a selective geographical distribution, as is the case for HTLV-1 [9].

So, how did a murine virus linked to PC become implicated in CFS? CFS is a condition characterized by unexplained long-term fatigue, chronic inflammation and immune dysfunction, which often appears following an episode of severe virus infection. Upregulation of the RNAseL pathway is a feature of CNS, which is consistent with an activated immunity, and with the idea of virus persistence in the pathogenesis of the condition [10,11]. Indeed,

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CFS has long been considered to be a multifactorial condition, in which virus infection is likely to play a role. Many viruses, including retroviruses, have been sought in CFS patients; some have been found, but none have been convincingly linked to the syndrome following prolonged scrutiny [12–19].

> "...of 101 chronic fatigue syndrome patients recruited to investigate the virus etiology of the disease, 68 (67%) were xenotropic murine leukemia virus-related virus positive..."

The new connection of XMRV with CFS reported from the Whittemore Peterson Institute (NV, USA), was published in *Science* last year [1], and claimed that, of 101 CFS patients recruited to investigate the virus etiology of the disease, 68 (67%) were XMRV positive by single-round PCR amplification of the proviral DNA from peripheral blood mononuclear cells. When the envelope of a virus related to XMRV (spleen focus-forming virus) was used as the antigen to which to measure an antibody response against XMRV, nine out of 18 patients tested had an antiviral response. Interestingly, eight out of 218 of healthy volunteers (3.7%) were also found to be infected with XMRV. There was no association with the RNAseL mutation in either group.

As was the case with PC, three European studies have failed to find XMRV in CFS patients. The first from Erlwein *et al*. used nested proviral DNA PCR amplification to amplify different parts of the genome from the Whittemore study [20], but included amplification of a control gene (*B-globin*) to testify to the integrity and sufficiency of the DNA being assayed. Despite a sensitivity of detection of a single copy of XMRV, they were unable to find the virus in any of the 186 CFS blood samples.

The second UK study, by Groome *et al*., used the PCR primers and protocol described in the *Science* paper [1], but failed to detect XMRV sequences in the 170 CFS patients tested [21]. In addition, they carried out a real-time PCR able to detect fewer than 16 copies per reaction and, again, failed to amplify the virus, either in their CFS samples or in the 395 controls. When this approach failed, they looked for a neutralizing antibody response, as evidence for virus infection. The fact they were able to detect neutralizing activity in one CFS patient, as well as in a number of control sera, gives some indication of the sensitivity of the assay. Further investigation of the specificity of the neutralizing response, by testing the sera against the envelope proteins of viruses other than XMRV, showed that the sera neutralized other viruses, suggesting that the response from the CFS patient was likely to be one of crossreactivity. Nevertheless, they found that, among their healthy control sera, there were four that specifically neutralized XMRV, which raises questions about the prevalence of this virus in the general UK population.

A third paper, by van Kuppeveld *et al*., further weakened the case for XMRV involvement in CFS [22]. Two amplification procedures were used. The first was a *gag*-nested XMRV PCR, adapted from the Urisman paper [2], and the second a quantitative PCR, identifying conserved XMRV *pol* sequences described previously in the 2009 USA PC paper. The assays, sufficiently sensitive to detect fewer than 10 copies of XMRV, failed to detect virus in either the previously described 298 Dutch CFS patient cohort [23,24], or any of the controls matched by age, sex and geographical location.

One technical difference stands out from these studies. PCR was employed to amplify XMRV sequences in all four studies. Only the study from the Whittemore Peterson Institute [1] was able to detect XMRV sequences following single-round PCR, indicating that copies of the XMRV genome were not in short supply. All the others found it necessary to employ a nested PCR, a modification of the standard reaction designed to enhance sensitivity and specificity.

Is there an explanation for such discrepant findings? It is true that no one study is a replicate of another in the sense that the cohort of patients investigated in each case is not the same. It is certainly not beyond the bounds of impossibility that the patients investigated by the Whittemore Peterson Institute may be quite different in their medical experience, immunological or genetic background, from patients studied in Europe and could, therefore, be more susceptible to this particular virus infection. This remains to be clarified.

One way to address this is for patient material to be sent to the three laboratories unable to detect virus in their respective cohorts and Mikovits from the Whittemore Peterson Institute has, indeed, offered to do this with at least one of the groups involved. However, retrovirologists know the pitfalls associated with studying retroviral association with disease [25]; therefore, a simple posting of DNA samples and reagents to laboratories capable of carrying out PCR is an insufficiently robust means of executing a definitive study. Despite the CFS patient community being, understandably, impatient to know whether or not XMRV is playing a role in CFS, any informative investigation requires thoughtful planning.

> "As was the case with prostate cancer, three European studies have failed to find xenotropic murine leukemia virus-related virus in chronic fatigue syndrome patients."

Who is best equipped to carry out the investigation? It is critical to engage those skilled in dealing with biological or public health controversy. National Monitoring Laboratories, such as the CDC and NIH in the USA, and the Health Protection Agency, National Institute of Biological Standards and Control or the National Institute for Medical Research in the UK are best placed to carry this out. Once an investigator from participating laboratories has been identified, a discussion on practical operational issues can start, the most important being which patients should be tested for the virus? If, as would seem logical, this is to be the same cohort of patients who appear to be carrying the virus, then each patient requires to be recalled to the clinic to provide fresh, multiple, blood samples, one of which should be provided to each participating laboratory for independent DNA analyses. The results of this experiment

will demonstrate whether XMRV is undeniably present in those patients, or whether its original detection resulted from a technical artefact.

Future sample exchange between interested parties is likely. Meanwhile, at least two leading US groups are carrying out similar investigations into the link between XMRV and CFS. Their results are awaited with interest.

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