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Purple Paper – Issue No. 15

XMRV: A Virus in Search of a Disease or A Novel Virus that Causes Prostate Cancer and/or Chronic Fatigue Syndrome?

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The primary purpose of the *Purple Paper* is to provide public health practitioners with **timely and accurate** information about new infectious disease agents/diseases; new variations on "old foes"; or new approaches to diagnosis, treatment and control. Although we strive to bring our readers the most up-to-date peer-reviewed evidence, we realized, with the first *Papers* devoted to pH1N1, that synthesizing evidence in a rapidly evolving field meant that information quickly changes or becomes obsolete. The *Purple Paper* is not intended to be a systematic review. To ensure scientific rigor, the *Purple Paper* is reviewed by Canadian experts or is written by Canadian experts themselves, as with all other NCCID knowledge products. We do not yet know if inherent issues related to rapid reporting will be insurmountable for readers and welcome your feedback and suggestions.

We appreciated the feedback from several readers who pointed out to us that information presented in the recent *Purple Paper* issue on XMRV has become outdated and, in one instance, presented information subsequently determined to be inaccurate. The literature summarized in the XMRV issue is current as of May 10, 2010. Since the completion of the writing of the article, however, several new papers have been published.

In our original article with reference to the Lombardi study [2010] examining a possible link between XMRV and chronic fatigue syndrome (CFS), we had written that "Previously unreported information has recently surfaced in an editorial from the *British Medical Journal* that the patients in the Lombardi study came from a suspected outbreak of CFS at a village near Lake Tahoe in the mid-1980's [McClure, 2010]." The concern that these CFS patients were predominately from "outbreak" regions was also shared by other leading experts in the field [Sudlow et al, 2010; van der Meer et al., 2010] and was precipitated by a paucity of published information regarding the patients' origins. In the rebuttal recently published in *Science*, the senior authors of the Lombardi study indicated that not all their patients were from documented outbreaks of CFS. "In fact, only 25 samples in [the study] came from patients identified during the 1984 to 1988 CFS outbreak in Incline Village, Nevada. The remaining 76 samples included patients with sporadic cases from 12 U.S. states and Canada, including California, New York, North Carolina, Wisconsin, Michigan, Oregon, New Mexico, New Jersey, North Dakota, Texas, and Florida." [Mikovits and Ruscetti, 2010] Despite the authors' clarification, the fact remains that a definitive causal link between XMRV and chronic fatigue syndrome is not yet established – a point made in the prior *Purple Paper* and also agreed to by the authors of the Lombardi study [Mikovits and Ruscetti, 2010].

Given the tremendous interest generated by the topic, we will continue to monitor new research on XMRV. We will revisit and comprehensively update our original article once a considerable volume of new XMRV findings has accumulated.

If you have further inquiries or comments about this issue or the *Purple Paper* in general, please contact us at nccid@icid.com.

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Purple Paper

XMRV: A Virus in Search of a Disease or A Novel Virus that Causes Prostate Cancer and/or Chronic Fatigue Syndrome?

Key Points

- Xenotropic Murine leukemia virus-Related Virus (XMRV) is a gammaretrovirus that was first described in 2006. It has been isolated from human biological samples.
- Several reports have associated the virus with familial and sporadic prostate cancer but other reports do not find a link.
- Similarly, a possible association with chronic fatigue syndrome has been reported but other studies find no evidence of an association.
- XMRV has not been established as a cause of either prostate cancer or chronic fatigue syndrome.
- XMRV may be transmitted sexually.
- Because XMRV may be a blood-borne pathogen, Canadian Blood Services and Australian Red Cross Blood Service indefinitely defer individuals with a history of chronic fatigue syndrome from donating blood.
- The real population prevalence remains unclear.

What is XMRV? How was it Discovered?

Prostate cancer is the most common cancer among men in Canada and the USA, other than skin cancer. [Canadian Cancer Society, 2009; American Cancer Society, 2010]. According to the Canadian Cancer Society [2009], 1 in 7 men will develop prostate cancer in his lifetime and 1 in 27 will die of the disease. In 2009, an estimated 25,500 men were diagnosed with prostate cancer; among them, 4,400 will not survive.

In a search for the genetic root of prostate cancer predisposition, several mutations in the *RNASEL* gene have been linked to increased susceptibility to the disease [Carpten et al., 2002; Casey et al., 2002; Rennert et al., 2002; Rokmon et al., 2002]. The *RNASEL* gene encodes an enzyme (RNase L) with anti-viral properties that is ubiquitously found in a wide range of human cell types and tissues. Once activated, Rnase L limits viral spread by directly destroying the viral genetic material and mediating the programmed death of virus-infected cells [Silverman, 2007]. One particular mutation resulting in a change from arginine (R) to glutamine (Q) in amino acid position 462 (R462Q) of Rnase L has received much attention. It has been reported that, compared to men with two copies of the normal gene, men with one copy of the R462Q gene and one copy of the normal gene had a 50% increased risk of prostate cancer; and carrying two copies of the mutated gene doubles a man's risk of developing the disease [Casey et al., 2002]. This, combined with the fact that the Rnase L R462Q variant showed reduced anti-viral activity compared to Rnase L without the mutation [Casey et al., 2002], has prompted scientists to question the possible involvement of a virus in the etiology of prostate cancer.

To answer this question, a group of American scientists used DNA microarray technology[a] to screen for viruses from all known viral families [Urisman et al., 2006]. The presence of a gammaretrovirus was initially detected in 7 of 11

[a] All cells in the body have the identical genetic makeup – it is the array of genes that are active (i.e. expressed) which determines the functions of the cell. As such, what distinguishes one cell type from another, or a healthy cell from a diseased cell, is the pattern of active genes. DNA microarray is a powerful nucleic acid-based technology platform that is originally used by scientists to examine how active thousands of genes are at a point in time. The application of DNA microarray is broad. In cancer studies, scientists can use DNA microarrays to classify different types of cancers based on the pattern of gene activity in the tumour cells. DNA microarray can also be adapted to the study of infectious diseases. Because each pathogen has a preferred tissue location for replication and that replication requires activation and/or heightened activity of the pathogen's own genes, DNA microarrays can be used to identify the type and strain of the pathogen that is actively proliferating in the host.

prostate cancer patients with two copies of the R462Q gene, in 0 of 3 patients with one copy and in 1 of 5 patients with no mutated gene. Expanding the survey to an additional 86 prostate cancer patients found that 8 of 20 (40%) cases with two copies of the R462Q gene harboured the gammaretrovirus, compared to 1 of 66 (1.5%) patients with one copy or none of the mutated gene. Comparison of the viral genetic sequences from different tumour isolates indicated that cases who tested positive for the gammaretrovirus were infected with the same virus [Urisman et al., 2006].

The identified gammaretrovirus was named Xenotropic Murine leukemia virus-Related Virus (XMRV). It is a member of the family Retroviridae and the genus Gammaretrovirus. The genome of XMRV is approximately 8,100 nucleotides in length. Although it is closely related to other murine leukemia viruses (MuLV), XMRV is evolutionarily distinct from other MuLV isolates. However, like some MuLV members in the group, XMRV had lost the ability to infect its original rodent host – thus the word "xenotropic" in its name [Urisman et al., 2006].

What is the Evidence for the Causal Relationship between XMRV and Prostate Cancer?

Evidence supporting a causal link between XMRV and prostate cancer has been largely circumstantial, and in some instances, lacking or conflicting.

The mechanism for host cell transformation by XMRV is still unknown. To replicate, a retrovirus is obligated to insert its genome into that of the host. Through this intermediary integration step, a retrovirus can transform the host cell (i.e. turn the host cell into a cancer cell) by: 1) introducing an oncogene[b] into the host genome; 2) activating host proto-oncogenes[c]; or, 3) inactivating host tumour suppressor genes. Given that no oncogenes have been found in the XMRV genome [Urisman et al., 2006], XMRV likely depends on the latter two mechanisms for transforming prostate cells if a causal link between XMRV and prostate cancer exists. Although integration of XMRV genetic sequences does occur in infected host cells, integration sites appear to be unique for each retrovirus [Kim et al., 2010]. To date, no integration sites have been detected within or near proto-oncogenes or tumour suppressor genes [Dong et al., 2007; Kim et al., 2008]. Furthermore, XMRV showed little or no transformation capacity as morphological changes of cultured cells – a hallmark of tumour virus infection – were not observed when the cells were exposed to XMRV [Metzger et al., 2010].

Perhaps a more contentious issue is that XMRV has not been consistently found in prostate cancer patients in different studies. In the original study by Urisman and colleagues [2006], XMRV was reported in 40% of American prostate cancer patients who have two copies of the *RNASEL* R462Q gene. While this finding was reproduced in another cohort of American prostate cancer patients [Arnold et al., 2010], European researchers detected a much lower prevalence or even complete absence of XMRV in prostate cancer patients from two German cohorts [Fischer et al., 2008; Hohn et al., 2009] and one Irish cohort [D'Arcy et al., 2008].

Interestingly, the association between XMRV infection and carriage of two copies of the *RNASEL* R462Q gene – the very rationale that led to the discovery of XMRV – has been recently questioned. Schlaberg and colleagues [2009] found that while XMRV genetic material and protein were detected respectively in 6% and 23% of prostate cancer patients, the presence of XMRV was not associated with the presence of the *RNASEL* R462Q variant in these patients. Instead, the higher prevalence of XMRV was correlated with higher-grade tumours (measured by the Gleason score) and more advanced stage prostate cancers. Further fuelling the controversy is the fact that several epidemiological studies do not support the involvement of the RNASEL R462Q mutation in prostate cancer etiology [Downing et al., 2003; Wiklund et al., 2004; Maier et al., 2005]. Therefore, whether XMRV is the causative agent of prostate cancer remains an open question. Further research is needed to delineate how XMRV infection and

[b] An oncogene is a gene that can turn a normal cell into a tumour cell.

[c] Proto-oncogenes expressed in the appropriate cell type under normal cellular control are not oncogenic. A proto-oncogene only becomes oncogenic when it is overexpressed or when its sequence is mutated.

RNASEL R462Q gene carriage are related to the etiology of prostate cancer.

What is the Evidence for the Causal Relationship between XMRV and Chronic Fatigue Syndrome?

Chronic fatigue syndrome (CFS), also named myalgic encephalitis, is a complex, debilitating illness that likely encompasses more than one entity. CFS is characterized by persistent disabling physical and mental fatigue – lasting for at least six months and without apparent physical cause – that is not improved by bed rest [Prins et al., 2006]. According to the CFS definition from the Centers for Disease Control and Prevention (CDC), in addition to unexplained chronic fatigue, the sufferer may also experience impaired memory or concentration, sore throat, tender cervical or axillary lymph nodes, muscle pain, pain in several joints, new headaches, unrefreshing sleep, or malaise after exertion [CDC, 2006]. A viral origin of the disorder has long been suspected as three-quarters of patients have reported having an infection, such as an acute influenza-like illness or infectious mononucleosis, before the onset of CFS [Prins et al., 2006]. Because RNase L dysfunction has also been implicated as a potential cause of CFS [Suhadolnik RJ et al., 1997; Nijs and De Meirleir K, 2005], shortly after the discovery of XMRV among prostate cancer patients, scientists began their search for the same gammaretrovirus in CFS patients [Lombardi et al., 2009].

In the American study by Lombardi and colleagues [2009], investigators detected XMRV genetic sequences in the blood samples of 68 of 101 (68%) CFS patients, compared to only 8 of 218 (3.7%) healthy controls. Using antibody reagents that recognize a broad spectrum of common elements shared among all xenotropic MuLV (of which XMRV is a member), investigators found indirect evidence for the presence of XMRV proteins within the blood cells of CFS patients. Cell culture experiments revealed that patient-derived XMRV was infectious. By directly exposing uninfected permissive cell lines (that are susceptible to infection by XMRV) to infected blood cells or cell-free plasma from CFS patients, it was shown that cell-associated and cell-free transmission of XMRV were possible. This study also provided indirect evidence for the development of an XMRV-specific antibody response in CFS patients, but not in healthy controls [Lombardi et al., 2009].

The study by Lombardi and colleagues [2009] has generated much interest, concern and criticism. For example, the report has been criticized for not providing adequate description of the CFS patients in question, despite the complexity of the disorder [van Kuppeveld et al., 2010]. Some experts also queried whether it is biologically plausible for a single infectious agent to trigger two-thirds of CFS cases [McClure, 2010]. Previously unreported information has recently surfaced in an editorial from the *British Medical Journal* that the patients in the Lombardi study came from a suspected outbreak of CFS at a village near Lake Tahoe in the mid-1980's [McClure, 2010]. Hence, even if the XRMV cause of CFS is real, the prevalence of XMRV among these CFS patients might have been overestimated when the finding is extrapolated to the general population. Following the initial publication of the Lombardi study, three independent studies from the UK and the Netherlands found no evidence of XMRV in their CFS patients [Erlwein et al., 2010; Groom et al., 2010; van Kuppeveld et al., 2010], thus injecting further doubt about the validity of the American findings.

At this juncture, there is no conclusive evidence supporting XMRV as the causative agent of CFS. The mechanism by which XMRV triggers CFS continues to be a mystery.

Can XMRV Infect Other Parts of the Body?

XMRV genetic material and proteins have been found in stromal and epithelial cells of the prostate tumour tissue in prostate cancer patients [Urisman et al., 2006; Schlaberg et al., 2009] and blood cells of CFS patients [Lombardi et al., 2009], suggesting that these tissues are the location for active XMRV replication in the respective disease. Although direct detection of XMRV and its viral products have not yet been performed in other tissues, scientists have attempted to determine the tissue tropism of XMRV by studying XMRV infectivity on cultured cell lines of different organ origins. Indeed, a wide range of cultured cells from different tissue types are

susceptible to XMRV infection, including smooth muscle cells, fibroblasts, B cells, peripheral blood promyeloblast, and cells derived from prostate stroma, prostate epithelium, cervix, brain, kidney, lung, and umbilical vein endothelium [Stieler et al., 2010; Bhosle et al., 2010]. This in turn indicates that all these cell types possess the appropriate surface receptors for XRMV infection. In spite of this finding, cell entry alone is not sufficient to promote productive replication of XMRV [Stieler et al., 2010]. Studies have shown that the male sex hormone, androgen, can enhance the replication and spread of XMRV [Dong and Silverman, 2010; Rodriguez and Goff, 2010], thus partially explaining why cells of prostate origin, which express androgen receptors, appeared to be superior to other cell types in supporting a high level of XMRV proliferation [Knouf et al., 2009; Stieler et al., 2010]. As such, the significance of the ability of XMRV to infect other cell types remains unclear.

How is XMRV Transmitted?

XMRV may be transmitted sexually. This is based on the finding that XMRV genetic material has been found in the semen of men with prostate cancer. Protein fragments originated from the prostate appeared to enhance XMRV infectivity in cell culture experiments [Hong et al., 2009]. In addition, given that XMRV was detected in the blood samples of CFS patients [Lombardi et al., 2009], XMRV may also be spread by contact with blood and blood products.

What are the Implications if XMRV is Proven to Cause Disease?

The discovery of XMRV has very important implications for medicine, health care and public health, especially if the prevalence of the new human retrovirus is as high as the American studies have suggested. The fact that XMRV may be a blood-borne pathogen has already alerted officials from Canadian Blood Services and Australian Red Cross Blood Service to indefinitely defer individuals with a history of CFS from donating blood [Canadian Blood Services, 2010; Australian Red Cross Blood Service; 2010]. In the USA, agencies within the Department of Health and Human Services (HHS) are working to find the most sensitive and reliable method for XMRV detection, to determine the prevalence of XMRV in the blood supply and to determine the transmissibility of XMRV by blood transfusion [CDC, 2010]. Contaminated organs donated for transplantation will also become an issue if XMRV is transmissible by blood.

If XMRV is confirmed to be the causative agent of prostate cancer and CFS, more options will become available for prevention, screening and diagnosis, and treatment of the disease. For instance, preventive measures may involve a vaccine targeting XMRV. Assuming that XMRV is sexually transmitted, barrier methods and preventive precautions used for other STIs could be applied to the prevention of XMRV. Screening and diagnosis of prostate cancer and CFS may rely on molecular identification of XMRV. Treatment options may include the use of antivirals for other human retrovirus infections. In fact, nucleoside reverse transcriptase inhibitors, zidovudine and tenofovir, and integrase inhibitor, raltegravir – medication used for HIV treatment – have already been shown to be effective against XMRV in cell culture experiments [Sakuma et al., 2010; Singh et al., 2010; Paprotka et al., 2010].

What are some Unresolved Issues?

Many questions are still unanswered.

- Why is XMRV seemingly geographically confined to the USA? Is it an artefact as a result of differences in experimental protocols or reagents? Or, is the disparity in the geographical distribution of XMRV between European countries and the USA a real phenomenon?
- What is preventing XMRV spread from North America to Europe?
- If a causal link between XMRV and prostate cancer does exist, what is the mechanism for disease initiation and progression?
- If a causal link between XMRV and CFS does exist, what is the mechanism for disease initiation and progression?
- Since prostate cancer affects only males and CFS affects mostly females, is it possible that the illnesses experienced by the two sexes are just different manifestation of the same disease caused by XMRV infection?

- What is the role of RNase L in the relationship between XMRV and prostate cancer, and between XMRV and CFS?
- How is the immune response of individuals who can control and eliminate XMRV different from the immune response of those who succumb to disease?
- How is XMRV transmitted? At what age does one become prone to infection by XMRV?
- Is it possible to transmit XMRV from mother to child?
- Does XMRV have the potential to become stably integrated into the human genome such that individuals can inherit the virus from his/her parents as a "genetic trait"?
- What is the prevalence of XMRV in Canada?
- Can a vaccine be developed against XMRV? Would it be useful in preventing XMRV given its prevalence in Canada?

At present, it is apparent that there are more questions than answers. As scientists continue to work through some of these outstanding issues, a clearer picture related to the burden of XMRV on the Canadian population, health care, and public health will gradually emerge. Until then, health care and public health officials must be vigilant and respond accordingly as more information becomes available.

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