

CHRONIC FATIGUE SYNDROME

Studies Point to Possible Contamination in XMRV Findings

The stormy debate over a potential cause of chronic fatigue syndrome (CFS) is nearing hurricane force. Last month, it prompted headlines suggesting that researchers have reached a dead end, scores of blog posts from disappointed patients, and accusations that scientists had gone beyond their data. The 14-month-old row intensified when four papers appeared in *Retrovirology* suggesting that reports linking the virus XMRV to CFS were based on false positives.

The debate began in 2009 with a report in *Science* that XMRV, a retrovirus recently reported to have been found in prostate tumors, had been detected in 67% of a set of CFS patients but in only 4% of controls (*Science*, 9 October 2009, p. 215). Since then, one other group has found XMRV-like viruses in CFS patients' blood. But several teams have failed to detect the virus in CFS or cancer patients or in healthy people. Researchers have struggled to explain the discrepancies (*Science*, 17 September 2010, p. 1454).

The potential link to CFS has had important consequences: Some CFS patients have begun taking antiviral drugs, which can have side effects. Last month, after being briefed on the original XMRV studies, advisers to the U.S. Food and Drug Administration recommended that CFS patients be barred from donating blood.

The *Retrovirology* papers point to contamination as a possible source of positive results in previous studies. The polymerase chain reaction (PCR) test used to detect XMRV (a mouse retrovirus adapted to infect humans) could actually be picking up minute amounts of mouse DNA or similar mouse viruses.

Two of the four studies in *Retrovirology* used highly sensitive assays for mouse DNA and found that samples positive for XMRV-like viruses also tested positive for mouse DNA. Another study found mouse viral RNA in a commercial PCR kit. And the fourth study argues that XMRV sequences previously reported in patient samples don't show the diversity expected if the virus were spreading through the human population. Instead, these authors report, the sequences are similar to those found in a popular prostate cancer cell line, 22Rv1. This cell line, used in lab experiments, was already known to contain an XMRV-like sequence.

Greg Towers of University College London (UCL), who led the study of XMRV

diversity, says the evidence linking this virus and human disease "is really looking pretty shaky now." The Wellcome Trust, which cosponsored the research, and UCL issued a press release last week declaring flatly that the Towers study showed that "chronic fatigue syndrome is not caused by XMRV," a message some newspapers repeated. Towers says he was "comfortable" with the release. But John Coffin of the U.S. National Cancer Institute (NCI) and Tufts University Sackler School of Graduate Biomedical Sciences in Boston, who co-authored two of the contamination papers, is wary. He says these studies "just point out how careful one must be."

Virologists who have found a virus-disease link disagree with coverage of the Towers paper. "The data shown ... do not justify some of the sweeping statements made," says Ila Singh of the University of Utah, Salt Lake City, who has reported XMRV in prostate cancer samples. Moreover, the lead author of the *Science* paper on CFS and XMRV, Judy Mikovits of the Whittemore Peterson Institute (WPI) in Reno, Nevada, points

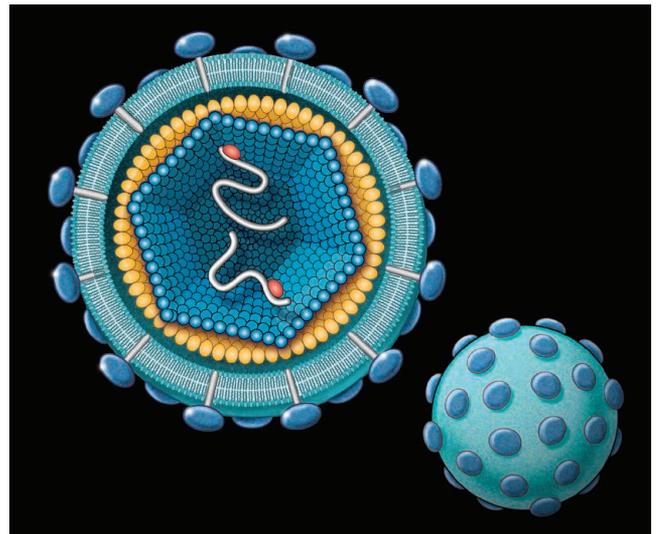
out that PCR wasn't the only test her studies used: For example, Mikovits's team also showed that XMRV-positive patients make antibodies to the virus and that XMRV isolated from their blood can infect cultured human cells. Mikovits said in a statement, "Nothing that has been published to date refutes our data."

One outspoken scientist wavered on the significance of the *Retrovirology* papers. Columbia University virologist Vincent Racaniello, who runs a popular virology blog and podcast, initially e-mailed a *Chicago Tribune* reporter to say that they were "probably the beginning of the end of XMRV and CFS." But he retracted that statement (and a similar comment to *Science*) after reviewing the studies more closely. "It's pretty complicated," Racaniello concludes.

Some had hoped that a project in which several U.S. labs are testing for XMRV in the same samples would clear up the pic-

ture. But so far this effort has been inconclusive. Four CFS patients' blood initially tested positive for XMRV at WPI and the U.S. Centers for Disease Control and Prevention but not at an NCI lab. When all three labs tested new samples from the same patients, none found XMRV—for reasons that aren't yet clear, says Coffin. The group now plans to test blood from several dozen CFS patients and controls.

A bigger study is now under way. Funded by the U.S. National Institute of Allergy and Infectious Diseases, virologist W. Ian



Elusive. Scientists are still debating whether the XMRV virus has any connection to human disease.

Lipkin of Columbia University is leading a project that will collect blood from 150 CFS patients and 150 controls from six U.S. clinical sites. The samples will be tested blindly by several labs. Because all the clinicians have agreed on standard methods, the study should help resolve concerns that differences in how CFS patients are selected or how samples are handled could explain clashing conclusions, Lipkin says: "Results will be definitive."

As the new study gets started, some wonder whether it's worth the \$1.3 million it will cost. Jonathan Stoye of the MRC National Institute for Medical Research in London concedes that the Towers study was "overhyped." But he says "it's pointing people in a certain direction," away from chasing an elusive link to XMRV. Still, he says, a larger study may be the only way to satisfy patients.

—JOCELYN KAISER

With reporting by Martin Enserink.

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ME, or chronic fatigue syndrome, 'not caused by the XMRV virus', say researchers

By Helen Briggs
Health reporter, BBC News

A new study has cast further doubt on the idea that a virus called XMRV causes chronic fatigue syndrome.

US scientists linked the condition, also known as ME, to a mouse-like virus in 2009 after finding it in blood samples.

Now, UK experts say the discovery was a "false positive", caused by cross contamination in the lab.

The illness may still be caused by a virus, they say, but not the one at the centre of recent controversy.

"Our conclusion is quite simple: XMRV is not the cause of chronic fatigue syndrome," said Professor Greg Towers, a Wellcome Trust senior research fellow at University College, London, who led the research.

"It is vital to understand that we are not saying chronic fatigue syndrome does not have a virus cause - we cannot answer that yet - but we know it is not this virus causing it."

Mouse DNA

XMRV (xenotropic murine leukemia virus-related virus) is a virus found in mouse DNA.

It was discovered in 2006, and was later found in samples from some patients with prostate cancer and chronic fatigue syndrome.

This led to suggestions that the virus might be the cause of these conditions.

A paper providing some evidence in support of a link between chronic fatigue syndrome and the virus was published in the leading journal *Science* last year.

In the latest work, the team, from London and the University of Oxford, used DNA sequencing methods to study XMRV.

They say their evidence, published in the journal [Retrovirology](#), shows the virus found in patient samples arose from laboratory contamination.

What is more, they think it is unlikely that the virus could actually infect people.

Professor Tim Peto, consultant in infectious diseases at the University of Oxford, said the original paper in *Science* came as a great surprise to experts.

"There have now been a number of attempts which have failed to find the retrovirus in other samples, and this research suggests that in fact XMRV is probably a contamination from mouse DNA," he said.

"These latest findings add to the evidence and it now seems really very, very unlikely that XMRV is linked to chronic fatigue syndrome."

But the authors of the original research say they stand by their conclusions.

"Nothing that has been published to date refutes our data," Dr Judy Mikovits, of the Whittemore Peterson Institute for Neuro-Immune Disease, said in a statement.

Dr Charles Shepherd, medical advisor for the ME Association, said patients should keep an open mind on the issue.

"The jury is still out," he said.



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ME 'virus' was actually a lab mistake, study says

By Steve Connor, Science Editor

Tuesday, 21 December 2010

A virus that was believed to be the cause of chronic fatigue syndrome, also known as ME, has turned out to be a laboratory contamination that could not have caused an infection in humans, scientists said yesterday.

The discovery will embarrass the American scientists who said in 2009 that they had found convincing evidence to link the virus, called XMRV, with chronic fatigue syndrome, the debilitating condition that affects about three in every 1,000 people.

British researchers, led by Professor Greg Towers of University College London, believe that the DNA techniques used in the US research were so sensitive that they inadvertently picked up laboratory contaminants that had been in contact with XMRV, which normally infects mice.

"Our conclusion is quite simple: XMRV is not the cause of chronic fatigue syndrome. Our evidence shows that the sequences from the virus genome in cell culture have contaminated human chronic fatigue syndrome samples," Professor Towers said.

"It is vital to understand that we are not saying chronic fatigue syndrome does not have a virus cause – we cannot answer that yet – but we know it is not this virus causing it."

The study, involving scientists from the Wellcome Trust Sanger Institute in Cambridge and the University of Oxford and published in the journal *Retrovirology*, showed that the "virus" detected by the American researchers, led by Judy Mikovits of the Whittemore Peterson Institute in Reno, Nevada, was actually a contamination probably arising from growing laboratory reagents in mouse cells infected with the XMRV virus.

The researchers also showed that as many as one in 50 "cell lines" used by scientists in laboratory experiments are infected with XMRV, which significantly increases the prospect of inadvertent contamination of samples taken from patients with chronic fatigue syndrome.

In addition, the scientists found that the DNA sequence of XMRV found within the samples indicated that it was not an actively replicating virus, but one that was being passed on passively, further supporting the idea that it is a laboratory contamination rather than a genuine infection.

Scientists conclude mouse virus does not cause ME

Hopes of breakthrough dashed as four papers conclude virus originating in mice is not the cause of chronic fatigue syndrome

Sarah Boseley

The Guardian, Monday 20 December 2010 17:50 GMT



It is likely that the evidence for mouse virus found in human samples was due to contamination by mouse DNA.

Photograph: Steve Chenn/Corbis

A virus that originates in mice, which last year was hailed as a possible cause of chronic fatigue syndrome, or ME, is not the cause of the disease, say scientists.

Four papers published by the journal Retrovirology all come to the conclusion that the finding of the mouse virus XMRV in human cell samples was not the breakthrough that researchers and doctors had hoped for. Further research suggests that the samples were contaminated with mouse DNA.

"Our conclusion is quite simple: XMRV is not the cause of chronic fatigue syndrome," says Professor Greg Towers, a Wellcome Trust Senior Research Fellow at University College London (UCL) and an author of one of the papers.

"All our evidence shows that the sequences from the virus genome in cell culture have contaminated human chronic fatigue syndrome and prostate cancer samples.

"It is vital to understand that we are not saying chronic fatigue syndrome does not have a virus cause – we cannot answer that yet – but we know it is not this virus causing it."

The discovery of xenotropic murine leukemia-related virus (XMRV) in patients with CFS by the Whittemore Peterson Institute in Reno, Nevada, published in the highly reputable journal Science, set the CFS/ME community alight. Many people who had been desperately ill for years without any idea of the cause, believed that this could be the answer. With a viral cause, CFS should be treatable and preventable.

But attempts to replicate the institute's findings have largely failed and the four papers published today leave little scope for further work on XMRV.

"Studies conducted by four completely independent research groups working around the globe have all reached the same conclusion: it is likely that the evidence for mouse virus found in human samples was due to contamination by mouse DNA," says Mark Wainberg, editor of the open-access journal *Retrovirology*.

The research includes evidence from a team led by Professor Myra McClure from Imperial College, London who looked at samples from prostate cancer patients, where XMRV had also been identified. They noted that XMRV was routinely found in around 5% of US citizens with prostate cancer, but was only very rarely found in European patients. Analysing British, Korean and Thai samples with a highly sensitive assay technique, however, revealed markers for mouse DNA other than XMRV. Professor Brigitte Huber and her team from Tufts University, near Boston, found similar results in samples from CFS patients.

In another paper, Dr Takayuki Miyazawa from Kyoto University offered evidence that the mouse DNA contamination may come from a particular manufacturer of testing kits commonly used to identify XMRV in tissue samples. When analysing the reagents in the kits without any human tissue present, the team found markers for mouse DNA.

The fourth paper came from Greg Towers and colleagues at UCL. They carried out retrospective analysis of previous research that seemed to support the claim and found that mouse DNA contamination was very likely in most of these studies. They concluded that researchers have been trying to identify XMRV using a DNA marker that may not be unique to XMRV after all. "Collectively, these results cast serious doubts on the PCR evidence used to support claims of MLV- related viruses in prostate cancer and CFS patients," writes Prof Robert Smith, Department of Pathology, University of Washington, Seattle in the USA in an editorial. "Future assessments of the prevalence of XMRV should include more rigorous PCR and phylogenetic tests to exclude the possibility of contamination."

latimes.com

Studies cloud chronic fatigue research

Syndrome's link to certain viruses is put in doubt

By Trine Tsouderos, Tribune newspapers

4:33 PM PST, December 20, 2010

Contamination is a likely explanation for scientific data that seemed to link a retrovirus and other mouse viruses to chronic fatigue syndrome and prostate cancer, according to four papers published Monday in the journal *Retrovirology*.

The papers provide a possible solution to a mystery that has dogged researchers for the past year: Why have a few labs been able to find evidence of the retrovirus XMRV or related mouse viruses in patients with chronic fatigue syndrome, or CFS, and prostate cancer while many others have not?

Approaching the question in different ways, four independent teams of scientists found that contamination of specimens, the lab or chemicals used in experiments could produce results that could be mistaken for XMRV and the other related mouse viruses.

"These four papers are probably the beginning of the end of XMRV and CFS," virologist Vincent Racaniello of Columbia University wrote in an email. "They don't prove that XMRV (and related viruses) don't cause CFS, but they go a long way to explaining many of the different findings in different labs."

Retrovirologist John Coffin of Tufts University, who co-authored two of the papers, said that although the papers do not settle the question, there is now reason to be concerned about the original findings on XMRV.

Slightly more than a year ago, a team of scientists led by Judy Mikovits at the Whittemore Peterson Institute for Neuro-Immune Disease reported in the prestigious journal *Science* they had found evidence of XMRV in the blood of more people diagnosed with chronic fatigue syndrome than their healthy peers.

The chronic fatigue syndrome community reacted with excitement to the idea that scientists could have finally figured out what was wrong with them and to the prospect of an effective therapy, a vaccine or even a cure. Patients began sending blood samples to commercial labs claiming they could detect XMRV, and some patients started taking potent antiretroviral drugs, as the *Chicago Tribune* reported in June.

But several other teams of researchers, including one led by the U.S. Centers for Disease Control and Prevention and another from the National Institutes of Health and the U.S. Food and Drug Administration, have published papers failing to find evidence linking XMRV to chronic fatigue syndrome.

One of the teams, from the NIH and FDA, found evidence of other mouse viruses in the patients, but it did not confirm the original study. The new papers suggest contamination could explain that team's findings, too.

The discordant results sparked talk of contamination among virologists. Many times in the history of their field, reported links between a virus and disease have later been shown to be caused by lab contamination,

a phenomenon known as a "rumor virus."

The researchers behind the new papers found various routes to contamination, confirming what virologists know well – that mouse DNA is everywhere in labs and can produce phantom results.

One team led by scientists at University College London presented genetic evidence suggesting that previously described XMRV sequences are derivatives of the ones found in a human cell line known as 22Rv1, which is often used in prostate cancer research.

"There really is no evidence for XMRV replicating in people," said Greg Towers, professor of molecular virology at University College London and a co-author of the paper. "We can explain the test results as contamination."

A Japanese team led by researchers from Kyoto University found that a reagent in one of the commercial kits used to detect genetic material is contaminated with mouse DNA. Another pair of papers showed human samples easily can be contaminated by mouse DNA, potentially leading to results that look like evidence of XMRV or related mouse viruses.

"I think this is: end of story," said Tufts professor Brigitte Huber, another author of one of the papers. "It is overwhelming."

On Monday, the Whittemore Peterson Institute released a statement forcefully denying the possibility that contamination had skewed its results, citing the multiple ways they have shown the link between the disease and the retrovirus. "The coauthors stand by the conclusions," Mikovits said in the statement. "Nothing that has been published to date refutes our data."

Researchers said a large study being led by Dr. Ian Lipkin at Columbia University should help settle the question.

Virologist Robin Weiss of University College London, has sounded the alarm about the reported link between XMRV and chronic fatigue syndrome for months. In 1997, his own team reported finding a retrovirus genome in patients with rheumatoid arthritis. Two teams — one in Sweden, one in the U.S. — "confirmed" his results. It seemed to be a breakthrough in rheumatoid arthritis.

After Weiss reported his findings, he discovered he was actually detecting contamination from a newly discovered rabbit retrovirus.

Asked earlier this month whether he think this is another case of a rumor virus, Weiss wrote, "yes."

On message boards, some chronic fatigue patients reacted to the quartet of new papers with dismay. "I've got to admit, it feels like a bit of a blow," wrote esther12 at Phoenix Rising. "We'll have to see how it all works out."

Wrote withhope on the same board: "This seems like really unsettling news at Xmas!"

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