Neighbourhood watch

BY CAROLYN BROWN

Emerging evidence points to a viral infection, low levels of vitamin D and genetics as culprits in multiple sclerosis, but how they combine to cause the disease is unclear.

When Jacques Dutrisac arrived at the Ottawa General Hospital in Canada for his first appointment after being diagnosed with multiple sclerosis (MS) in 1991, he met a former high-school classmate in the waiting room. She told him that a third schoolmate had also been diagnosed with the disease. They have since identified 15 people with MS from the neighbourhood where Dutrisac grew up in the late 1960s and 1970s, 14 of whom lived within a kilometre of each other. Four are from the same street, and "there were not even a hundred people on our little street", explains Dutrisac. Five attended the same elementary school, and seven the same high school. Their ages span just eight years. In an area that is predominantly English-speaking, ten are French Canadian.

This Ottawa cluster of MS cases has not been analysed statistically to determine whether it is the result of chance alone, but similar MS clusters in the Canadian province of Manitoba have. A study of new cases diagnosed over a 16 year period found several hotspots, mainly in the city of Winnipeg[1]. Rates of MS were more than twice as high in the city as they were in the rest of the province — more than three times as high in some neighbourhoods. Clearly something is causing these clusters.

Canada has the highest rate of MS in the world, with 291 cases per 100,000 people[2]. But rates vary widely from one region of Canada to another[3–5]. Several studies have concluded that something in the environment in Canada is contributing to MS — indeed, rates of MS in immigrants to Canada quickly rise to approach Canadian levels[6,7].

Differences between countries can suggest possible causes. Clusters in particular neighbourhoods, such as those in Ottawa and Winnipeg, raise suspicion about local environmental factors, including outbreaks of infectious disease or genetic differences in multicultural cities, says Mahmoud Torabi, a statistician at the University of Manitoba and an author of the Winnipeg study.

Until recently, researchers could not

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Jacques Dutrisac is one of a cluster of 15 people with multiple sclerosis who grew up in the same neighbourhood in Ottawa, Canada.

pinpoint why Canada in general, and specific places in particular, are so rife with this neurodegenerative disease[6–8]. But an international research effort — including database analyses, epidemiology, microbiology, genetics and immunology — is now yielding a growing body of evidence on the causes of MS. "It's difficult to get a grip on, it's so huge," says Egil Røsjø, a neurologist at Akershus University Hospital near Oslo, who is also completing a doctorate on MS aetiology. "There are lines of evidence that are pointing more or less in the same direction from all these different angles."

And they are pointing to three main culprits: Epstein–Barr virus (EBV), which is a herpesvirus that causes mononucleosis (glandular fever); low levels of vitamin D; and genetic variants that increase susceptibility to MS.

But each of these potential causes is common, and MS is quite rare, so none of these alone is likely to hold the key. "The development of MS is based on combinations of these," argues Amit Bar-Or, a neurologist at McGill University's Montreal Neurological Institute and Hospital. But how they interact to result in MS is not yet clear.

A LIFELONG INFECTION

Inspired by the geographical variation in MS, hundreds of epidemiological studies have been done to identify environmental factors. A wide range of potential causes have been the subject of meta-analysis studies, which pool data from several studies and can include thousands of patients, explains Ioanna Tzoulaki, an epidemiologist at Imperial College London. These meta-analyses have investigated infections, vaccinations, other diseases, surgery, accidents and exposure to toxins. Tzoulaki's team has conducted a review of these meta-analyses[9]. "They looked at every trend or player in these environmental factors," says Røsjø, who was not involved in the review, which he calls the "pinnacle" study of these factors.

The strongest evidence came from studies of links between MS and two signs of EBV infection: a history of mononucleosis, and a biological marker of EBV infection called EBNA1.

This chimes with Dutrisac's experiences. In his teens, he often felt tired, despite being an active adolescent who loved to play ice hockey in the winter. "I would just get tired for no reason sometimes," he says. Fatigue is common in MS, but it is also a symptom of mononucleosis, which strikes mainly adolescents and young adults, with symptoms that also include fever, sore throat, swollen lymph nodes and inflammation of the spleen. Dutrisac was not diagnosed with mononucleosis, but he may have had an EBV infection; many people who are exposed to the virus, especially as children, have no symptoms, and many mild cases go undiagnosed. So although only a minority of people have been diagnosed with mononucleosis, an estimated 90% of people globally have been exposed to EBV by the time they are 40 years old. And the virus is persistent. As Røsjø says, "You don't get rid of it. Once you've got it, you have it for life."

There are three broad theories to explain how EBV infection could result in MS, explains Bar-Or. One possibility is that the EBV infects the central nervous system (CNS), especially the brain. The virus may then kill oligodendrocytes, the cells that produce the myelin sheath — the insulating envelope that coats the nerve fibres (axons). Myelin speeds up the nerve impulses travelling through the axons and is essential for normal nerve function.

In the CNS, the infection may also trigger an immune response in which a type of immune cell known as CD8 tries to kill the virus but also damages oligodendrocytes and neurons. Bar-Or admits that this theory is controversial, because although some researchers have found the virus in the CNS, others have been unable to replicate the finding.

A second theory involves molecular mimicry, in which the immune system, Bar-Or says, may mistake myelin basic protein (MBP), which is important for the myelination process, for a similar piece of the invading EBV. The immune system's T cells have receptors that can recognize both the virus and MBP, and when the T cells are activated during the immune response to the virus, they could attack MBP as well as EBV in parts of the brain, resulting in a loss of myelin.

A third possible mechanism involves immune cells known as B cells. In normal immune responses, there are interactions between B cells, myeloid cells and T cells when responding to invading bacteria and viruses, and reversing the immune response when it is no longer needed. Any imbalance in this delicate dance can cause a strong or long-term response that goes beyond its original purpose and injures the body by interfering with basic cell functions, says Bar-Or. Perhaps EBV causes such an imbalance in MS by activating the B cells that they no longer perform their crucial regulatory functions, but instead start to promote inflammation. There is some support for this theory because MS treatments that remove B cells have succeeded in decreasing MS relapses, says Bar-Or.

> *"The body of evidence is difficult to get a grip on, it's so huge."*

VITAMIN D LINK COMES TO LIGHT

World maps of MS (see page S2) show that its prevalence increases as you move further from the Equator. This geographical distribution suggests that MS may be linked to the lower levels of vitamin D found in people living in regions with long, dark winters. But evidence supporting this theory has come mainly from observational studies, which cannot show cause and effect, says Brent Richards, an endocrinologist who studies vitamin D and genetics at McGill University in Montreal. For example, it is unclear which way the causality runs — rather than low levels of vitamin D causing MS, it is possible that the disease somehow lowers vitamin D levels, Richards says. Another complication is that "vitamin D is associated with a host of healthy lifestyle behaviours", he says. "If you go outside, you tend to be healthier and your vitamin D levels tend to be higher." In this

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case the healthy behaviours may prevent MS, and the geographical variation would need another explanation.

Demonstrating a causal link through a randomized controlled trial (RCT) of vitamin D would be complex, lengthy and costly, and such a trial may not be funded anyway because it would not test a drug. Richards' team tackled this problem by conducting a study that he calls "nature's RCT". They analysed large databases to find genetic variants that lowered vitamin D levels and then, by using another genetic database of people with MS, determined the MS risk in people with these variants. They found a strong risk of MS in people who have naturally low levels of vitamin D — and the risk increased as the vitamin D level decreased[10]. "The person had their genetic make-up before they had MS. That's important, because the disease cannot influence the genes," he says.

The mounting evidence of the link with vitamin D has sparked discussion about how low levels could lead to MS. Bar-Or points out that vitamin D has been shown to affect the immune response in many of the cell types involved in MS, including myeloid, B and T cells. "There's an enormous amount of literature that associates vitamin D deficiency with risk of disease," adds Richards.

Røsjø thinks that this points to the connection with EBV. His research follows up a theory[11] that vitamin D may boost immune responses to EBV and, conversely, that a lack of vitamin D may lead to poor responses to EBV. He thought that a weak immune response would increase levels of the biomarker EBNA1. "This in some way reflects increased risk of MS disease development," says Røsjø, although the molecular basis is still unknown. But *in vitro* studies have shown that the form of vitamin D that is active in the body, 1,25-dihydroxyvitamin D3, inhibits the production of antibodies from B cells, which are the cells infected by EBV.

Røsjø's study has borne out this theory. In a long-term placebo-controlled study of high-dose vitamin D supplementation in people with MS, Røsjø found[12] that levels of antibodies against EBNA1 fell significantly over the first 48 weeks of the study, but then rebounded, although never to their original levels, over the following 48 weeks. Røsjø says that this study was the first indication of an interaction between vitamin D and EBV. "There might be a short-term effect" of vitamin D on MS, he adds, "but it's lost over time."

The study also looked at two other viruses in the same herpesvirus family as EBV — varicella zoster virus, which causes chickenpox and shingles, and cytomegalovirus — and found that levels of antibodies to these viruses were unaffected by vitamin D. "There is something special, something unique with EBV that we cannot explain," Røsjø concludes.

Røsjø found that even large doses of vitamin D do not help patients with established MS in the long term, but he thinks that it may have a role during initial EBV infection. "Vitamin D and EBV during adolescence might be some of the most important things when it comes to deciding MS risk," he says.

FAMILY FORTUNES

One of the big puzzles about MS is that it runs in some families, but it can also pop up sporadically in people with no family history of the disease. Meagan McEwen, who campaigns for people with MS in Ottawa and has the disease, and her boyfriend, who also has it, embody this puzzle. "In my boyfriend's family, there are many other people who have MS. That's not true in my family," she says. This paradox has fuelled hundreds of studies worldwide.

One such study focused on the risk factors associated with the onset of MS in Canadian children. A team of researchers, including Bar-Or, investigated several major risk factors, including EBV, vitamin D and a specific variant of the *HLA-DRB1* gene, which is one of the human leukocyte antigen (HLA) complex of genes that are involved in the immune response[13]. "One of the benefits of studying children is that there's a relatively short time between the time they are born and the time they develop disease," explains Ruth Ann Marrie, a neurologist at the University of Manitoba in Winnipeg and one of the co-authors. "There is less time for either extraneous or relevant events to occur."

> *"The genetic contribution doesn't explain even half the risk."*

Bar-Or and Marrie found that the genetic variant most strongly associated with MS around the world, *HLA-DRB1*15*, doubled the risk of children having MS. Since that study was published in 2011, the number of genetic variants known to affect MS risk has climbed from roughly 50 to about 200.

Bar-Or stresses that none of these MS-associated variants is abnormal — they are not 'disease genes' like the mutations that cause cystic fibrosis, for example. "There are many, many more healthy people who have one or more combination of those very same variants than there are people who develop MS," he says. It seems that environmental factors or epigenetics — changes to gene expression that do not alter the genetic code — may explain the different outcomes, even for people with the same genetic make-up. "Two individuals who may have the identical template end up manifesting their genes in different ways based on epigenetics," says Bar-Or.

Genetic susceptibility can also vary geographically, Marrie points out, which may help to explain some clusters. A study of genetic risk factors for MS in Ireland, for example, showed that the percentage of individuals with the genetic variants that put them at risk of MS was substantially lower in one county than in another one nearby[14]. "The genetic contribution doesn't explain even half the risk," cautions Bar-Or, so it may be possible to prevent the disease by targeting the environmental factors involved.

Marrie thinks that the future direction of MS research will be into how these factors interact. "Now that we have identified multiple potential factors, we will work on the biology of the relationship to understand how these factors work together, and whether order of exposure is important," says Marrie. But she and others admit that definitively proving the causal role of any of these factors may be impractical or even impossible.

None of the researchers are ready to say what factors might be causing the Canadian clusters. Could it be the long, dark winters? A genetic variant common in French Canadians? A local EBV outbreak? Some combination of all three?

Dutrisac hopes that information about his cluster can help researchers to find the answer. "There's something out there. We need to find the cause," he says. ■

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