Do Epigenetic Differences Contribute to CRPS Risk?

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One of the mysteries of CRPS is why one person develops the condition whereas another does not, despite both experiencing similar injuries. While risk factors for developing CRPS are only poorly understood at present, knowledge of such risk factors might permit earlier intervention or even prevention of CRPS after injury in high risk individuals. Studies in both CRPS patients and in experimental animal models of CRPS have increasingly highlighted a role for immune alterations and inflammatory processes in CRPS[1]. We might therefore expect that differences between individuals in the immune and inflammatory systems could contribute to CRPS risk following injury.

One likely source of immune and inflammatory differences would be genetic, deriving from differences from person to person coded in the individual's DNA. Genetic factors are known to contribute to risk for a variety of diseases, such as cancer and Alzheimer's disease. To date, evidence for genetic risk factors in CRPS comes from a handful of studies, and is limited in part by lack of replication from one study to the next (i.e., finding the same genetic risk factors across studies). One genetic finding that has been replicated suggests a role for differences in the human leucocyte antigen (HLA) system in determining risk for CRPS[2,4]. The HLA system produces proteins that are responsible for regulation of the human immune system, and a role for this system in CRPS fits with other recent evidence for immune mechanisms in CRPS.

It is often believed that DNA "hardwires" a person for risk, that is, a person with a genetic risk factor will develop the condition whereas a person without the risk factor will not. The emerging field of epigenetics indicates that this view is incorrect. Just as important as the genetic code in a person's DNA is whether or not specific genes are "turned on." Epigenetics addresses this key issue of whether genetic risk factors are turned on and whether protective genetic factors may be turned off. In genetic language, such epigenetic differences are referred

to as gene expression differences. To those not familiar with the area, it may come as a surprise that gene expression can be influenced by environmental factors, and that these gene expression changes can be passed down to offspring, just as the actual DNA code is inherited by offspring. A key way in which gene expression is altered is by the process of DNA methylation, a chemical change occurring at what are referred to as CpG sites (places in the DNA code where the amino acids Cytosine and Guanine occur in sequence linked by a phosphate).

My colleagues and I at Vanderbilt have recently completed the first study of differences in DNA methylation in CRPS patients compared to non-CRPS pain patients, a study funded in part by a generous research grant from the RSDSA. Taking advantage of DNA methylation data collected as part of a larger Department of Defense funded study, we compared 9 patients meeting the Budapest criteria for CRPS with 38 patients experiencing persistent pain who did not meet CRPS criteria. Although this particular CRPS sample was somewhat unusual in that all patients (in both groups) had persistent limb pain following a post-traumatic amputation resulting from military service in Iraq, the study did allow us to compare DNA methylation between individuals with pain plus typical CRPS features and individuals experiencing pain without CRPS features. We hypothesized that differential patterns of DNA methylation might account for why some individuals studied developed features diagnostic of CRPS and others did not, despite the fact that all underwent a similar type of injury.

Our results, not yet published in a peer-reviewed scientific journal, are intriguing. We examined over 450,000 CpG sites, and identified all sites for which DNA methylation differed between CRPS and non-CRPS patients. We employed methods that adjusted both for the small number of patient studied and for the large number of CpG sites examined. We found that 250 CpG sites were differentially methylated between the two patient groups, with 5 of those sites

highly significant in the statistical sense. One of the 5 sites showing the largest group difference was in the HLA-DRB6 gene (a gene in the immune regulatory HLA system described above). Interestingly, this is exactly the same gene identified as a top hit in the only other available gene expression study in CRPS[3], which used alternative methods not examining the DNA methylation targeted in our study. The similarity of these two findings using different methods represents an important replication, highlighting the likely importance of epigenetic differences in the immune system in determining CRPS risk. This conclusion is supported by the fact that 5 of the 250 CpG sites differing between groups in terms of DNA methylation were in genes known to be involved in the HLA immune pathway. Also of interest was the finding that 5 of the CpG sites differing between CRPS and non-CRPS pain patients were in genes known to be involved in the system regulating inflammation. These findings indicating gene expression differences (via DNA methylation) in multiple immune- and inflammation-related genes is entirely consistent with animal work and other human studies supporting a role for immune and inflammatory mechanisms in CRPS. Other notable findings revealed DNA methylation differences between CRPS and non-CRPS pain patients in genes impacting on oxidative stress responses, the renin-angiotensin system, blood vessel formation, skin resiliency, and bone turnover. Each of these findings fit with theoretically plausible roles of these diverse systems in CRPS (e.g., bisphosphonate drugs target bone turnover mechanisms and have shown some efficacy for treating CRPS).

Our DNA methylation data were examined not only in terms of individual CpG sites, but also in terms of known gene *networks* reflecting common underlying biological functions. These analyses indicated that CRPS patients displayed significantly different patterns of DNA methylation (compared to non-CRPS pain patients) in five functional categories reflecting

immune system function, three hormone-related categories, and two categories related to differences in cation and ion transport (i.e., ability to move molecules across cell membranes in the body). These latter findings hint that novel CRPS risk factors related to differences in hormone regulation and transport across cell membranes may deserve further investigation.

In summary, our results for the first time suggest that risk for CRPS following injury may derive in part from differences in whether or not genes are expressed (i.e., turned on or off) through the process of DNA methylation. Consistent with known CRPS mechanisms and limited available genetic studies, the strongest finding was for an association between CRPS risk and expression of immune-related genes, with results also highlighting the likely importance of inflammatory-related genes. We have partially replicated our results in terms of the genetically-determined component of gene expression, finding differences in 5 of the same genes identified in the study detailed above between a broad "limb pain" group (1,564 patients) and a "no limb pain" group (3,070 patients). Nonetheless, determining the ultimate clinical value of these findings must await true replication. If future studies find similar results, these findings may help guide research into novel mechanisms contributing to CRPS (e.g., hormone-related) and would highlight the need to further develop interventions that target immune and inflammatory-related mechanisms contributing to CRPS.

References

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