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Genomics Confirms “Inflammatory Signature” In New-Onset, At-Risk Diabetic Patients

Researchers at the Medical College of Wisconsin have used a sensitive genomics technique to show that individuals recently diagnosed with Type 1 diabetes have a unique “proinflammatory signature.” The study, which appears in a recent issue of *The Journal of Immunology*, also found that in a group of at risk patients who eventually developed Type 1 diabetes, this inflammatory signature was present well before diagnosis and the emergence of islet cell autoantibodies.

In autoimmune diseases like Type 1 diabetes, a proinflammatory signature refers to the presence of various proteins and cellular molecules involved in signaling the immune cells to take action, suggesting initiation of disease. It will therefore be important to determine the value of these signatures in predicting the possible onset of Type 1 diabetes, and as inclusion criteria or endpoint measures in clinical trials aimed at preventing it.

Elevated blood serum levels of proteins called cytokines and chemokines have previously been reported in recent-onset Type 1 diabetes. These elevations likely signal the activation of the immune system attack on insulin-

producing cells in the pancreas, and thus represent an important clinical tool for predicting disease onset. However, the levels of these factors in the blood are often too low to measure directly, or reliably.

In this study, led by Xujing Wang in the laboratories of Martin J. Hessner, researchers used a genomics strategy to test for the presence of proinflammatory factors in the serum of Type 1 patients. They collected serum samples and then used them to induce gene expression in cultured blood cells from healthy donors; this approach provides a more sensitive test for identifying proinflammatory serum factors, on the premise that even low levels of immune regulatory factors should be sufficient to induce the expression of genes under their influence. After culture, gene expression was measured using automated microarray analysis—a technique in which a microscopic collection of individual genes is combined with labeled DNA from study samples, allowing measurement of the expression levels of thousands of genes simultaneously.

Nearly 200 unique genes

Blood serum from each of the patients with recent-onset diabetes induced the expression of 192 genes, including genes responsible for numerous protein receptors and signaling molecules, key among them interleukin-1 (IL-1).

The 192-gene molecular signature was not seen in patients with long-standing diabetes, or in those in the control group without diabetes. The absence of a signature in patients with long-standing diabetes is consistent with their being at an immunologically inactive stage, the authors stated, and supports the hypothesis that recent-onset diabetes is a stage of active autoimmunity.

Perhaps most interesting, a long-term investigation of three at-risk siblings who eventually developed Type 1 diabetes revealed that the inflammatory signature

appears years before onset, and in one patient, even before the emergence of islet cell autoantibodies. In this patient, autoantibodies were absent as much as five years before the onset of Type 1 diabetes, whereas a serum sample taken at this time induced the proinflammatory signature.

It will be important to know if the inflammatory signature described in this study, which was shown to resolve at variable times after disease onset, coincides with the end of the honeymoon period, an immunologically active period that follows the initiation of insulin therapy. During the honeymoon period, patients often experience a restoration of beta cell function lasting from months to years, providing a window of opportunity for potential therapeutic intervention.

While Juvenile Diabetes Research Foundation (JDRF) did not fund this study, one important extension of this research might be to investigate the utility of the inflammatory signature as a marker of disease progression and in predicting good drug targets. For example, JDRF will soon launch a trial to establish whether therapy with a drug that blocks the proinflammatory pathway specifically mediated by IL-1—using an IL-1 receptor antagonist—might prolong the honeymoon period in newly diagnosed patients.

Certain Patients Less Likely to Develop Diabetic Kidney Disease

A large genetics study has shown that persons with Type 1 diabetes carrying a particular gene variant are protected to some degree from diabetic nephropathy, a progressive and life-threatening kidney disease that develops in about one of three individuals with Type 1 diabetes.

Individuals who have inherited the “allele,” or alternative form of the gene in question—called DRB1*04—appear to be able to tolerate, or defer, the harmful effects of hyperglycemia. DRB1*04, an established marker for Type 1 diabetes, thus appears to act as both a risk factor for the disease, and a protective factor against nephropathy,

the investigators concluded.

This research, published in the journal *Diabetes*, is the first to report an association between an inherited variation in the DRB1 gene and susceptibility to diabetic kidney disease. The finding offers an important clue about the genetics of nephropathy, and may contribute to the development of targeted preventive therapies as well as new therapeutic targets.

Previous studies have identified four major inherited risk factors for Type 1 diabetes: the HLA genes DRB1, DQA1, and DQB1 located on chromosome 6, which play an important role in the immune system; and variants of the insulin gene on chromosome 11. To determine whether specific genetic factors play a role in the development of diabetic nephropathy, the researchers analyzed these genes using DNA samples from the GoKinD collection, an initiative supported by JDRF, the NIH, and the CDC. The Genetics of Kidneys in Diabetes Study (GoKinD) was created as a resource for investigators studying the genetics of diabetic nephropathy, by providing them with DNA samples as well as extensive clinical information from patients with diabetes with and without kidney disease. In this study, researchers analyzed 829 diabetic patients with nephropathy and 904 without this complication.

The most striking finding was that Type 1 patients carrying two copies of allele DRB1*04 were 50 per cent less likely to have diabetic kidney disease, regardless of how long they had diabetes.

The authors also found a correlation between the number of copies of DRB1*04 and A1C levels, a marker of blood glucose control. They reported that, in the subset of patients who did not have kidney disease despite long-term diabetes, patients' A1C levels increased from an average of 7.2 to 7.3 if they had, respectively, zero or one copy of the gene variant, to 7.7 if they had two copies of the allele. This suggests that DRB1*04 confers some sort of protection from nephropathy, perhaps via a mechanism involving tolerance for hyperglycemia.

Further investigations will be necessary to understand the biology underlying DRB1*04's protective effect.

The research was led by Suzanne K. Cordovado in the laboratory of Patricia W. Mueller, at the National Center for Environmental Health, Centers for Disease Control and Prevention in Atlanta, Georgia.

Elusive Pancreatic Progenitor Cells Found in Mice

Researchers in Belgium have identified a bona fide pancreatic progenitor cell—a cell that has the capacity to generate new insulin-producing beta cells. The recent discovery significantly advances the potential of beta cell regeneration as a cure for Type 1 diabetes.

The researchers, at the JDRF Center for Beta Cell Therapy in Diabetes in Brussels, Belgium, noted in the January 25 issue of the journal *Cell* that if the finding made in mice holds for humans, the newfound progenitor cells may represent “an obvious target for therapeutic regeneration of beta cells in diabetes.”

“One of the most interesting characteristics of these [adult] progenitor cells is that they are almost indistinguishable from embryonic progenitor cells,” said Harry Heimberg, lead investigator of the new report. “In terms of their structure and gene expression, there are no major differences. They look and behave just like embryonic beta cell progenitors.”

Patricia Kilian, director of JDRF's regeneration program, said: “We at JDRF believe this new research provides novel insights that may provide therapeutic potential to regenerate beta cells in Type 1 diabetes.”

Previous studies have suggested the existence of a beta cell progenitor in the pancreas after birth, but the identification and characterization of such a cell has not yet been fully achieved. Other studies have shown that replication of adult beta cells can account for beta cell

turnover and the expansion of insulin-producing beta cells under normal physiologic conditions.

Together, these findings made scientists question the role or existence of a progenitor cell in regeneration. “Most people gave up looking because the cells are so few and so hard to activate,” Heimberg said.

In this study, Heimberg's team tied off a duct that drains digestive enzymes from the pancreas, an event that led to a doubling of beta cell mass in the injured part of the pancreas within two weeks. The animals' pancreases also began producing more insulin, evidence that the new beta cells were fully functional. Using a genetic labeling technique, the researchers found that the new beta cells were derived from precursor cells that expressed Neurogenin 3, a master gene in embryonic progenitor cells; and that production of the new beta cells depended on the activity of this gene. Heimberg suspects that the regenerative process is sparked by an inflammatory response in the enzyme-flooded pancreas.

“The most important challenge now is to extrapolate our findings to patients with diabetes,” he reported, adding that further investigations will be required before any potential diabetes treatment stemming from this research might be developed. ■