# Science Advances from NIDDK

## *Advances for January 2010*


- *New Insights into Pancreatic Development*
- *Metabolic Regulation by the Immune System*
- *Genetic Risk Factor Identified for Liver Disease Development in Cystic Fibrosis*
- *Deaf1 Gene May Play a Role in Type 1 Diabetes*
- *Improved Treatment for Diabetes Related to Cystic Fibrosis*
- *A New Chemical Bond in Collagen IV Networks*
- *Master Regulator of Intestinal Development Identified*


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NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

## *New Insights into Pancreatic Development*

New studies are providing key insights into the progenitor cells that give rise to different cell types in the pancreas. Both type 1 and type 2 diabetes are characterized by loss of functional beta cells, the pancreatic cells that produce the hormone insulin. Strategies to repopulate beta cells, either by transplant or by induction of new beta cell formation, show promise in treating diabetes. Toward successful therapeutic strategies, scientists are making progress in identifying, characterizing, and understanding the factors and mechanisms that underlie pancreatic development. In addition to the insulin-producing beta cells, the pancreas is composed of multiple other cell types. Some of these—like beta cells—produce hormones released into the blood to regulate the body's metabolism; these cell types are termed "endocrine." Other cell types produce proteins that aid in the digestion of food; these cell types are termed "exocrine." In order to promote the formation of new beta cells, scientists, including members of the NIDDK-supported Beta Cell Biology Consortium, are determining when and how certain pancreatic progenitor cells become "committed" to developing into specific endocrine or exocrine cell types.

In one study, scientists investigated the role of a group of proteins, called presenilins, in specifying pancreatic cell types in mice. They studied cells that have Ngn3, a well-established marker of embryonic pancreatic progenitor cells, and discovered that the activity of presenilins was needed to block the cells from becoming exocrine cells. This showed that Ngn3 was not sufficient to commit pancreatic progenitor cells to endocrine cell types. Rather, cells that have Ngn3 can become endocrine or exocrine cells—a flexibility of Ngn3 cells that was previously unknown. In a second study, using elegant labeling techniques to mark a single mouse pancreatic progenitor cell and monitor its progression, another group of scientists focused on the Ngn3 cells that become endocrine cells and found that these cells are "unipotent" precursors. That is, Ngn3 is a marker shared in common by cells destined to develop into different endocrine cell types, but each individual Ngn3-containing cell is committed to becoming only one of the endocrine cell types. In fact, unlike other progenitor cells that proliferate to generate many mature cells, Ngn3-containing cells often appeared not to proliferate at all, with each one simply morphing into its final endocrine cell type. These findings are important because understanding the characteristics of progenitor cells that can turn into beta cells can help inform new strategies toward generating new beta cells to replace those impaired by diabetes.

In a final study, scientists uncovered additional plasticity in a pancreatic endocrine cell type—the alpha cell. Using genetic techniques in mice, the researchers increased the levels of a protein called Pax4, which is known to be involved in promoting cells to develop into endocrine cell types. They found that mice with high levels of Pax4 had oversized clusters of beta cells, which resulted from alpha-beta precursor cells and established alpha cells being induced to form beta cells. In addition, in a mouse model of diabetes, the high levels of Pax4 promoted generation of new beta cells and overcame the diabetic state. The discovery that alpha cells have the potential to convert to beta cells, and the additional insights on pancreatic progenitor cells made in the other studies, generate a fuller picture of pancreatic development. These advances may pave the way toward new cell-based therapies for diabetes.

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*Cras-Méneur C, Li L, Kopan R, and Permutt MA: Presenilins, Notch dose control the fate of pancreatic endocrine progenitors during a narrow developmental window. Genes Dev 23: 2088-2101, 2009.*

*Desgraz R and Herrera PL: Pancreatic neurogenin 3-expressing cells are unipotent islet precursors. Dev 136: 3567-3574, 2009.*

## *Metabolic Regulation by the Immune System*

New research may help break the link between obesity and diabetes. Although overweight and obesity confer significant risk for developing type 2 diabetes, the precise mechanisms underlying the relationship between body fat and diabetes remain unclear. An accumulation of research over the last several years, however, implicates the chronic, low-grade inflammation which typically accompanies obesity, in promoting insulin resistance, which is a precursor to type 2 diabetes. Earlier work found a type of immune system cell, the macrophage, is abundant in the fat tissues of obese mice (and humans). Macrophages are responsible for producing some of the chemical signals that trigger the inflammatory response. Now, new research highlights a role for a different type of immune system cell, called the $CD4^{+}Foxp3^{+}$ Regulatory T ($T_{reg}$) cell. Such $T_{reg}$ cells are found in the fat of lean mice, but not in those that are overweight. Experimentally depleting $T_{reg}$ cells from lean mice increased their resistance to insulin. Importantly, the researchers found that the $T_{reg}$ cells produce chemical signals which attenuate the inflammatory response when experimentally introduced into fat mice, helping restore the animals to a more normal metabolic state. This suggests that restoring normal immune regulatory signals in people at risk for type 2 diabetes may one day interrupt the development of insulin resistance and help them prevent the disease.

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*Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelson S, and Mathis D: Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 15: 930-939, 2009.*

## *Genetic Risk Factor Identified for Liver Disease Development in Cystic Fibrosis*

An international scientific collaboration has resulted in the discovery of the first genetic risk factor for severe liver disease development in some people with cystic fibrosis (CF). CF is an inherited disease that affects mainly the lungs, pancreas, and sweat glands. However, many individuals with CF also develop abnormal liver function and fibrosis, with a some progressing to severe liver disease. The major genetic defects responsible for CF are known to be mutations in the gene called *CFTR*, which result in mucus accumulation in airways and abnormal function of other organs. However, the process by which liver disease develops in individuals with CF is unclear, and patients with the mutated *CFTR* gene exhibit a wide range of disease severity. Based on these observations, additional genetic factors likely play a role in determining susceptibility to liver disease development in patients with CF. Currently, no diagnostic test exists to identify which individuals with CF are at high-risk of developing severe liver disease. In order to study a sufficiently large number of individuals with CF, researchers at sites around the globe worked together to identify genetic risk factors associated with severe liver disease development in CF. They increased their chances of finding these genetic factors by performing two sequential studies of patients with CF and healthy controls: an initial study examining five genes suspected of contributing to CF liver disease, followed by a second study to confirm these genetic associations. In the initial study, two genetic variants, one in the *SERPINA1* gene, referred to as the *SERPINA1 Z* allele, and another in the *TGFB1* gene, were associated with CF liver disease; however, the second study confirmed only *SERPINA1 Z* allele as a risk factor. When the data from both studies were combined for greater statistical power, the *SERPINA1 Z* allele was shown to have a strong association with CF liver disease. The *SERPINA1 Z* allele is known to cause proteins to fold incorrectly and to accumulate in liver cells; however the mechanism by which it contributes to CF liver disease requires further study.

This research identifies the *SERPINA1 Z* allele as the first genetic factor that increases the risk of developing severe liver disease in individuals with CF. This discovery allows for the possibility of future infant screening programs that follow up on diagnosis of CF by testing for their genetic susceptibility to develop severe liver disease later in life, so that appropriate preemptive action can be taken.

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*Bartlett JR, et al. for the Gene Modifier Study Group: Genetic Modifiers of Liver Disease in Cystic Fibrosis. JAMA 302: 1076-1083, 2009.*

## *Deaf1 Gene May Play a Role in Type 1 Diabetes*

Scientists identified a gene that may play a role in the development of type 1 diabetes. Examining genes from a mouse model of type 1 diabetes, the scientists found that cells in the animals' pancreatic lymph nodes make two forms of the same gene called deformed epidermal autoregulatory factor 1 (*Deaf1*). One form of this gene encodes full-length, functional Deaf1 protein, while the other encodes a shorter, nonfunctional variant form. The research suggests that the full-length, functional form of Deaf1 may control the production of molecules needed to eliminate immune cells that can destroy insulin-producing cells in the pancreas, causing type 1 diabetes. Moreover, the presence of the Deaf1 variant was found to prevent the full-length Deaf1 protein from functioning normally. Additional experiments showed that the variant form inhibited turning on genes needed to produce certain molecules involved in immune regulation. Researchers also found that levels of the variant form of Deaf1 were higher in people with type 1 diabetes compared to levels in people without the disease. In addition, the human variant form inhibited the full-length form from functioning normally. The research suggests that the development of type 1 diabetes may in part be due to increased levels of the Deaf1 variant protein in pancreatic lymph nodes. Increased levels of Deaf1 variant may, in turn, lead to reduced production of molecules that are required to educate the immune system not to attack the body's own cells, including the insulin-producing cells of the pancreas. Furthermore, the findings suggest that the Deaf1 variant form may predict risk for type 1 diabetes and be a target for therapy.

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*Yip L, Su L, Sheng D, Chang P, Atkinson M, Czesak M, Albert PR, Collier AR, Turley SJ, Fathman CG, and Creusot RJ: Deaf1 isoforms control the expression of genes encoding peripheral tissue antigens in the pancreatic lymph nodes during type 1 diabetes. Nat Immunol 10: 1026-1033, 2009.*

## *Improved Treatment for Diabetes Related to Cystic Fibrosis*

A new study has revealed that a diabetes treatment can benefit many patients with an increasingly common complication of cystic fibrosis. Cystic fibrosis (CF), a genetic disorder that leads to chronic lung infections, once led inevitably to childhood death from scarring of the lungs. New treatments are helping people with CF live much longer—often into their 30s and 40s. However, as they age, an increasing number of people with CF are developing CF-related diabetes (CFRD), which has been associated with reduced survival. CF severely damages the pancreas, affecting first its vital role in producing digestive enzymes needed for food absorption from the intestine, and later its production of insulin needed to transport glucose fuel into cells. Replacement of lost digestive enzymes improves growth and nutrition, but sufficient insulin is also needed to maintain body weight and muscle mass. Still, because patients with CFRD may not be at risk for many of the serious complications associated with other forms of diabetes, the benefit of adding insulin therapy to the already burdensome CF treatment regimen has been uncertain. A recent clinical trial has now shown that aggressive insulin therapy, begun earlier in the course of their diabetes than previously recommended, can help many people with CFRD maintain their body weight and potentially avoid the excess mortality associated with CFRD.

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## *A New Chemical Bond in Collagen IV Networks*

Researchers have discovered a new kind of chemical bond in biological tissue, a fundamental discovery in structural biology that may provide insights into several human diseases. The bond connects two of the subunits that make up collagen IV, a protein that is an important component of the extracellular matrix. Collagen provides structural support to tissues, serves as a scaffold upon which other complexes are assembled, and mediates cellular signaling. Scientists have long known that collagen subunits are linked to one another through intermolecular bonds that lend strength and structural integrity to the matrix; however, the precise nature of these bonds had eluded them. Using advanced techniques to study protein structure, the researchers discovered a novel chemical bond between a sulfur atom of the amino acid methionine on one subunit and a nitrogen atom of a modified form of the amino acid lysine on another. This is the first time that a "sulfilimine" bond – a direct bond between sulfur and nitrogen atoms – has been found in a native biomolecule.

Collage IV networks have been implicated in a number of human diseases. Two collagen subunits are defective in the inherited kidney disorder Alport syndrome, a condition in which waste filtering by the kidney is impaired due to disruption of the extracellular matrix. Furthermore, one collagen chain is involved in the rare, autoimmune kidney disease, Goodpasture syndrome. In this disease, the misguided antibody attack on the kidney's extracellular matrix is thought to be due to exposure of a usually-hidden part of the collagen molecule, which may be made accessible as a consequence of the absence or breakage of the sulfilimine bond. Discovery of this novel chemical bond therefore represents not only an important advance in our knowledge of collagen structure, but also may identify causes of disease and possible treatment approaches.

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*Vanacore R, Ham AJ, Voehler M, Sanders CR, Conrads TP, Veenstra TD, Sharpless KB, Dawson PE, Hudson BG: A sulfilimine bond identified in collagen IV. Science 325: 1230-1234, 2009*

## *Master Regulator of Intestinal Development Identified*

Scientists have found that a single gene plays an essential role in controlling normal intestinal development. *In utero*, the gastrointestinal (GI) tract develops from the foregut, which becomes the esophagus, stomach, and upper part of the small intestine; the midgut, which gives rise to the small intestine; and the hindgut, which forms the cecum and colon. The formation of the intestine is directed by a complex network of precisely timed signals passing between and within cells of these developing tissues, including layers of cells that will form the lining of the intestine (epithelial cells) and cells that will form intestinal smooth muscle and other intestinal tissues (mesenchymal cells). Some of these signals are so essential for life that, when mutated, the resulting deficiency causes death *in utero*. For example, mutation of a mouse gene important to intestinal development, called *Cdx2*, is lethal for mice at the embryonic stage. Humans also carry a form of this gene, referred to as *CDX2*.

To investigate the role that the *Cdx2* gene plays specifically in development of the intestine, scientists created a mouse model in which the *Cdx2* gene is only mutated in developing intestinal cells; with this restricted Cdx2 deficiency, the developing mice do not die *in utero* and can thus be studied. When the scientists compared this mutant mouse model with normal mice, they observed some dramatic and surprising changes in the development of the intestine. For example, the colon in the mutant mouse failed to develop correctly, mimicking a disorder in humans known as colonic atresia. Also, epithelial cells on the inner layer of the intestine did not differentiate to form the correct cell types and structures, such as villi with brush border membranes needed for nutrient absorption. Instead, the mutant epithelial cells more closely resembled cell types found in the upper GI tract, specifically the esophagus, rather than those found in a normal intestine. Analyses of the "transcriptome"—the collection of genes turned on (expressed)—in the small intestine confirmed that the *Cdx2*-deficient small intestine had converted to a more esophageal type by turning on genes typically seen in the esophagus but not the intestine. As the intestine develops, Cdx2 production normally becomes restricted to epithelial cells. However, the researchers showed that cells of the other tissue layers within the intestine were also altered to be more esophagus-like, suggesting that *Cdx2* deficiency-related epithelial modifications subsequently caused changes in adjacent tissue layers, converting them to an esophageal program as well.

These experiments demonstrate the importance of *Cdx2* to proper intestinal development by characterizing the repercussions of its removal, including abnormal colon formation and a switch to an upper GI tract phenotype. Based on these findings, Cdx2 is now recognized as playing a critical role in programming tissues along the length of the GI tract to have specific cell types and features to suit their different functional needs. Beyond informing our understanding of intestinal development, these studies have implications for human conditions in which intestinal programming is altered, such as colonic atresia.

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