Researchers show how mutations in 'dark genome' cause pancreatic malformations

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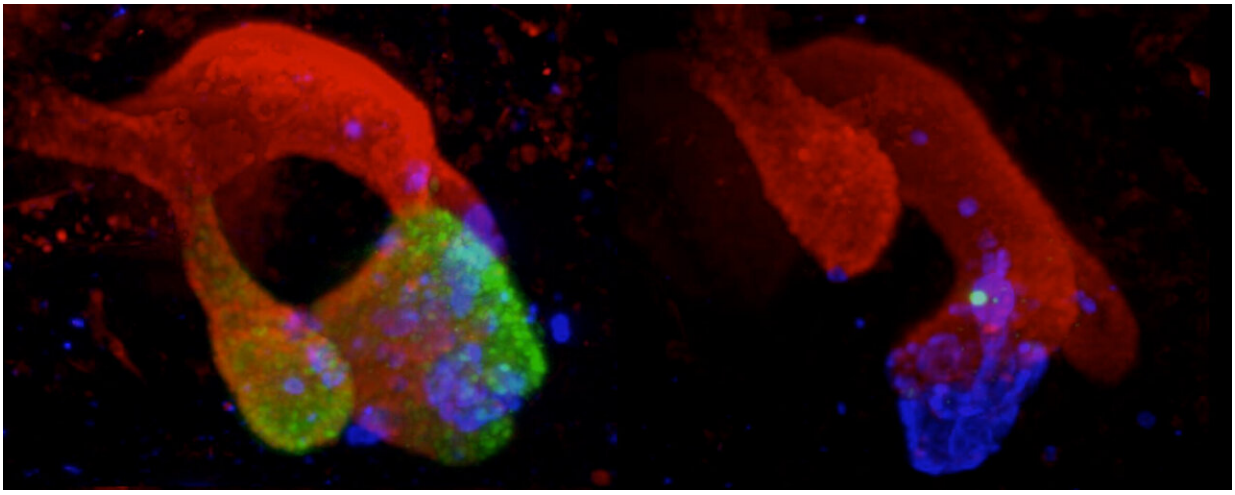

Mice with a deletion in the pancreas agenesis enhancer do not express PTF1A (green) in embryonic multipotent pancreatic progenitors, leading to underdeveloped pancreas and insulin-deficient diabetes. Immunofluorescence of embryonic pancreatic buds from control (left) and enhancer-deleted (right) mice stained for PTF1A (green), PDX1 (red), glucagon (blue). Credit: Miguel Angel Maestro/*Developmental Cell*.

Researchers at the Center for Genomic Regulation (CRG) have identified a DNA sequence that is crucial for pancreatic differentiation and function—and for the first time—describe how it works.

Patients with mutations in a DNA sequence—which they coin

EnhP—develop pancreas malformations. It is the most clear-cut example to date of an inherited disease that is caused by mutations that do not disrupt the DNA sequence of a gene.

Disorders caused by mutations in a single DNA sequence, for example Huntington disease or sickle cell anemia, are known as monogenic diseases. In the vast majority of cases, such mutations disrupt a protein-coding gene. In this case, the mutations in EnhP disrupt a single "enhancer" instead of a single gene.

Our genomes contain hundreds of thousands of DNA elements that are thought to act as enhancers. These enhancer DNA sequences act as switches to turn on the transcription of their target genes in the right tissues.

According to the authors of the study, published today in *Developmental Cell*, EnhP is by no means the only enhancer defect to cause disease. Mutations in enhancers may be the cause of a monogenic disease in many patients in which laboratory tests have failed to disclose causal gene mutations.

Understanding the role of enhancers in disease could change how we practice medicine. "Clinical genetics is shifting from a focus on sequencing protein-coding genes to sequencing whole genomes. It is now theoretically possible to discover disease-causing mutations that lie outside of traditional areas of the genome, although it is still challenging to discern which parts of the genome are truly vulnerable to mutations," explains Dr. Jorge Ferrer, senior author of the study, coordinator of the Medical Genomics Transversal Program at the CRG and group leader at CIBERDEM.

The researchers had previously discovered EnhP when studying developmental disorders across ten different families. In collaboration

with a team in Exeter, United Kingdom, they had found that mutations in the enhancer were the most common cause of pancreatic agenesis, a rare congenital disorder causing loss of pancreatic tissue and neonatal diabetes.

In this study, researchers developed on their previous work to explain why this particular enhancer is vulnerable to disease-causing mutations. Using CRISPR, the team genetically-engineered mouse models to study the effects of the enhancer. Mice lacking both copies of EnhP were born with a severely underdeveloped pancreas and insulin-deficient diabetes. They also studied human stem cells in vitro.

They show that EnhP works by increasing rates of transcription of a nearby gene known as the pancreas associated transcription factor 1a (PTF1A). More specifically, the study revealed that EnhP's only role is to activate a whole cluster of enhancers that also regulate PTF1A in the very first cells that form the pancreas during fetal development. When these other enhancers are activated and PTF1A transcription is turned on, this sets in motion a cascade of molecular events that lead to the formation of normal pancreatic cells.

"We show that enhancers operate in a hierarchical manner, and this one sits straight at the top, says Dr. Ferrer. This is a new concept, and it solves a paradox of how mutations in a single enhancer can be catastrophic despite the existence of multiple other enhancers regulating the same gene. This is not just about this particular enhancer or disease, there are probably many other enhancers with this particular function in the human genome. Finding them will help us understand which enhancers are vulnerable to mutations that cause various other monogenic diseases."

The discovery also has ramifications for efforts to generate insulin-producing beta cells in laboratory conditions. Cell transplantation is a

feasible option for diabetic patients, but the demand for functional cells from dead donors far outstrips supply.

Growing beta cells in culture would be one way of addressing this challenge, but these rarely share the same functional features as normal human beta cells. This is in part because we don't know the mechanisms required for proper differentiation.

"EnhP sparks a molecular program that is needed for proper formation of human beta cells. This knowledge can be harnessed to improve laboratory conditions to create beta cells," concludes Dr. Ferrer.

More information: Jorge Ferrer, Pancreas agenesis mutations disrupt a lead enhancer controlling a developmental enhancer cluster, *Developmental Cell* (2022). DOI: 10.1016/j.devcel.2022.07.014. www.cell.com/developmental-cel … 1534-5807(22)00531-7

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