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MODELING OF THREE-DIMENSIONAL CHROMOSOME STRUCTURE IN BETA CELLS TO IDENTIFY GENETIC MECHANISMS UNDERLYING TYPE 2 DIABETES

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1. Project Summary

Type 2 diabetes is the leading form of diabetes, affecting more than 400 million people. In 2019 diabetes killed more than one million people. Despite its importance, the molecular mechanisms involved in type 2 diabetes are still largely unknown. The main consequence of this fact is that we do not have treatments that are capable of correcting the molecular defects that cause the disease, and therefore we cannot use treatments to slow its progression, nor to prevent or cure the disease. Over the last 12 years genetic studies have been carried out with hundreds of thousands of samples from patients and controls, and this has led to the identification of more than 400 parts of the genome that contain genetic variants that individually have a small but unequivocal effect on the susceptibility for type 2 diabetes. These genetic findings open avenues to understand which molecular processes might determine why different people exposed to the same environmental settings either develop diabetes or are relatively protected. The interpretation of these genetic signals, however, has been complicated by the fact that most variants involved are not located in genes, which are the genomic regions whose functions tend to be understood, but are instead predominantly found in parts of the genome that initially had no known function.

The CRG group, which coordinated this project, created charts of the genome that pinpointed DNA sequences that do not encode genes, yet act as switches to turn on genes that need to be active in insulin-producing beta cells. These parts of the genome, called enhancers, decipher the DNA sequence in beta cells, allowing them to use the right genes to make and secrete insulin. These genome maps enabled demonstrating that a large fraction of the genetic variants involved in diabetes alter the function of this type of switches, even though they do not directly affect the DNA sequence of any gene. In some cases, it was possible to show how they alter the function of these switches. These findings create new opportunities to understand at a molecular level why some people are more prone to develop type 2 diabetes.

The CRG-CNAG and CABD-Sevilla groups have pioneered the development of methods and strategies to understand the function of these non-coding parts of the genome. The application of these approaches to disease models such as type 2 diabetes has obvious potential.

The objective of the project funded by the TV3 Marathon has been to use new gene editing tools ("CRISPR-Cas9") and pioneering methods developed by the CRG-CNAG and CABD-Sevilla groups to dissect the impact of genetic variants associated with type 2 diabetes, establish with certainty which are the causal variants and gene targets, and evaluate how they affect the function of our genome.

2. Results

During this project, three-dimensional maps of the genome were completed, which allowed us to connect genetic variants to genes involved in diabetes susceptibility (Miguel Escalada et al., Nature Genetics 2019). Genome editing tools were used to dissect the function of a dozen regions of the genome that contain disease-risk variants and affect specific target genes in human beta cells, thereby establishing cause-effect relationships relevant to the genetic mechanisms of the disease. In collaborative work by the groups in this proposal, three-dimensional genome models of these areas were created, which allowed understanding why genomic regions apparently very distant from their target genes in the linear genome sequence are actually very close in three-dimensional space.

Despite the importance of insulin-producing beta cells for diabetes, other tissues are also important (shoulder, liver, fat, brain). It is known that different people develop diabetes by visibly different defects, although in practice no objective tools are available to separate these forms of diabetes. This study showed that a large part of the genetic variants implicated in type 2 diabetes are located in three-dimensional genome “hubs” that connect genetic "switches" to genes important for beta-cell function. This enabled creating genetic models that predict risk of type 2 diabetes mediated through genes that act on beta cells, as opposed to other sets of genetic variants that affect the risk of diabetes through other mechanisms. The models derived from this study open avenues to stratify people not simply by their absolute genetic risk for diabetes, but more specifically through mechanisms. This work was published in Nature Genetics, Irene Miguel Escalada et al., 2019.

In other collaborative studies, gene editing tools were used to modify the human genome in pancreatic islet cells from organ donors, rather than in cell lines, and

identified targets implicated in type 2 diabetes. This study, which represents a transformative methodological shift, was published in Nature Communications, Romina Bevaqua, 2021.

Other work from this period has generated more than 10,000 mutations in a region of the genome important for diabetes, which enabled dissecting its genomic function. The work has not yet been published, but is part of a doctoral thesis deposited by Berta Font Conill.

The grant has allowed the group led by Prof. Marti Renom to develop models to study the three-dimensional structures of the genome that regulate the genome in pancreatic beta cells, where genetic variants act. This work has resulted in the collaborative publications mentioned above, and additionally in important methodological work in the journals described below as well as in the doctoral thesis of Dr. Julen Mendieta Esteban.

The Seville CABD group, led by the late Professor Jose Luis Gomez Skarmeta, generated and characterized regulatory maps in human fetal tissues, including the pancreas, which have been used by the La Marató project to identify genetic variants that act during fetal development. In addition, the group has developed and collaborated in the application of new methods to detect three-dimensional changes, and to study the epigenome in pancreatic tissues relevant to the genetic mechanisms of type 2 diabetes (Nature Communications, Gerrard et al. 2020). The group also identified regions important for diabetes that can be studied in zebrafish, thus opening up new possibilities for analysis that are not available in human cell- or tissue-based models.

3. Relevance to potential future implications

These results establish molecular links between genetic variants and risk mechanisms for type 2 diabetes. This type of knowledge is needed to develop future therapies targeting the genetic mechanisms involved in T2D. The CRG group is currently using such information for this purpose.

Type 2 diabetes is a very common, but very heterogeneous disease. There are currently no tools to distinguish which molecular mechanisms lead to type 2 diabetes in different people. The studies published by Miguel-Escalada et al. demonstrate how it is possible to use epigenomic information to generate predictive models to identify people at risk for developing the disease through different mechanisms. This type of tool will be important for the implementation of precision medicine models.

4. Scientific bibliography generated

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