Metabolic Syndrome

Exocrine or Endocrine? A circulating pancreatic elastase which regulates glucose homeostasis

Anna L Gloyn¹

¹Oxford Centre for Diabetes Endocrinology & Metabolism & Wellcome Centre for Human Genetics, University of Oxford, UK & Oxford NIHR Biomedical Research Centre, Churchill Hospital, UK

Anna.Gloyn@drl.ox.ac.uk

15 A recent study by *Esteghamat et al.*, published in *Nature Genetics* has reported
16 *CELA2A*, a pancreatic enzyme, to be a novel genetic cause for Metabolic Syndrome and
17 atherosclerosis.

18 Metabolic Syndrome is a cluster of conditions that occur together, increasing risk of
19 heart diseases, stroke and type 2 diabetes (1). These conditions include increased blood
20 pressure, high blood sugar levels, excess body fat around the waist, and abnormal
21 cholesterol or triglyceride levels (1). Metabolic Syndrome increases the risk of damage to
22 major blood vessels that supply the heart with blood, oxygen and nutrients causing a build-
23 up of cholesterol-containing deposits (plaques) in the coronary arteries (atherosclerosis)
24 which eventually leads to coronary artery disease (CAD) (1). Both genetic and
25 environmental factors (in particular, dietary habits) are common risk factors for metabolic
26 syndrome (1). Human genetic disorders have an unrivalled ability to pinpoint key pathways
27 and proteins which are critical for human metabolism and glucose homeostasis (2). In some
28 instances, these proteins have been shown to be amenable to therapeutic manipulation,
29 fostering interest in the translational opportunities of new genetic discoveries (3, 4).

30

31 In this study (5) the authors analysed individuals that had a rare and extreme
32 phenotype combining Metabolic Syndrome with early-onset atherosclerosis which it
33 appeared could be the result of a major defect in a single gene. Using next-generation
34 sequence technologies, the authors surveyed the entire protein coding sequence (the
35 “exome”), and found that the *CELE2A* (*Chymotrypsin-like elastase family member 2A*) gene
36 harboured several novel protein-coding variants only seen in individuals with this extreme
37 phenotype. In a large family, they demonstrated that a protein-coding variant in *CELE2A*
38 was only present in individuals with the Metabolic Syndrome and not in any unaffected
39 family members, providing compelling evidence for causality. Finding a single genetic
40 explanation for this cluster of conditions identifies a unifying cause for diverse abnormalities
41 in multiple clinically-relevant traits including elevated levels of triglycerides, glucose and
42 blood pressure levels, all of them risk factors for CAD. Through *in vitro* studies, the authors
43 were able to establish that the disease-associated non-conservative amino acid changes in
44 the *CELE2A* protein led to a loss of function, thereby connecting reduced *CELE2A* protein
45 activity to increased CAD risk.

46 Finding a novel genetic disorder is exciting, in itself, but in this instance, it is what the
47 discovery tells us about the protein encoding by *CELE2A* that is truly fascinating. The protein
48 *CELE2A* is produced by the acinar cells of the exocrine pancreas (6) and released into the
49 gut, where, as a member of a family of proteins implicated in digestion, it contributes to the
50 breakdown of food (7). Individuals with one of the disease-causing mutations in *CELE2A*
51 were found not only to have reduced activity of the enzymes (elastases) which break down
52 proteins, in their plasma but also to have higher levels of glucagon, a glucose-raising
53 hormone, secreted from the pancreatic islets, and with effects largely antagonistic to
54 insulin. This encouraged the authors to investigate the relationship between circulating
55 levels of *CELE2A* and blood glucose following a meal. *CELE2A* levels were observed to rise in
56 parallel with the glucose lowering hormone insulin, with glucagon levels showing an inverse
57 relationship. This established an intriguing relationship between this digestive enzyme,
58 *CELE2A*, from the exocrine pancreas, and key metabolic hormones, insulin and glucagon,
59 from the endocrine pancreatic islets. This observation is fascinating because the exocrine
60 and endocrine pancreas are considered to be functionally unconnected despite their
61 intimate physical connection and shared embryological origin. To explore how *CELE2A*
62 might orchestrate these effects, the authors turned to a mouse model of diabetes, showing
63 that intravenous administration of recombinant *CELE2A* protein increased circulating insulin
64 and reduced blood glucose concentrations. The authors found evidence to suggest that
65 *CELE2A* achieves this both through enhancing insulin secretion and increasing insulin
66 degradation/clearance.

67 Glucose-stimulated insulin release from the pancreatic islet beta-cells is triggered by
68 increased intracellular calcium levels with L-type calcium channels playing a central role
69 (Figure 1). Activation of the L-type calcium channels involves proteolytic cleavage and the
70 authors indicate that *CELE2A* is involved in this process, explaining how the protease could
71 modulate insulin secretion capacity. Similarly, the authors demonstrate that *CELE2A* also
72 degrades insulin through proteolytic cleavage (Figure 1). To extend the role of *CELE2A* to
73 other metabolic processes and pathways underlying the metabolic syndrome, the authors
74 screened for other proteins with extracellular motifs capable of binding *CELE2A*. This
75 uncovered, integrin A2B, a component of a receptor complex that binds fibrinogen and
76 triggers platelet aggregation, a process which contributes to atherosclerosis. The naturally-
77 occurring disease-causing *CELE2A* mutations, by reducing protease activity, lead to a

78 reduced ability to augment insulin secretion and degradation and to increased platelet
79 aggregation (Figure 1).

80 Although the authors present a compelling argument for a role for CELE2A in glucose
81 homeostasis, further work is required to provide a complete picture of how changes in
82 activity of circulating CELE2A influence the risk of CAD. The authors note that the glucose
83 lowering effects of human CELE2A protein in the mouse were less pronounced than those of
84 recombinant rat CELA2A protein: this might indicate that other factors not captured in the
85 *in vitro* assays are required for human CELE2A function. Although the mutations resulted in
86 a loss of elastase activity, mutation-carriers had higher circulating CELE2A protein levels
87 than non-carriers, which could have unknown physiological consequences if there is any
88 gain of function associated with the protein.

89 This discovery has wider implications because it adds to growing interest in links
90 between defects in the exocrine pancreas and the pathogenesis of diabetes. It has been
91 known for some time that certain subtypes of diabetes (so called Type 3C, or
92 pancreatogenic diabetes), occur secondary to disorders which affect the structure and
93 function of the exocrine pancreas. One well-studied example is cystic fibrosis: mutations in
94 the *CTFR* gene which lead to the profound pulmonary dysfunction in this condition also lead
95 to loss of exocrine pancreas function (8) But up to 50% of those with cystic fibrosis also
96 develop diabetes by adulthood (9). Although there is no consensus on the mechanism for
97 this, there is evidence to suggest that pancreatic exocrine insufficiency leads to progressive
98 damage to the pancreatic islets (8). Another link between exocrine and endocrine function
99 comes from evidence that common genetic variation in the *CTRB2* gene, which encodes a
100 second pancreatic digestive enzyme, chymotrypsinogen B2, influences insulin levels via its
101 effects on the secretion from the gut of the metabolically-active peptide, Glucagon-Like
102 Peptide 1 (10).

103 The findings from this study suggests that population-level analyses of the relationships
104 between circulating CELE2A protein levels and glycemic phenotypes could be instructive to
105 understand disease aetiology. With growing numbers of genetic signals for type 2 diabetes
106 emerging from large-scale association studies, there will be opportunities to determine
107 whether any of these might be acting through effects on exocrine pancreatic function. It
108 appears that the exocrine pancreas is involved in more than digestion alone.

109

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115

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Figure 1

(A) The pancreas consists of both exocrine (acinar & ductal cells) and endocrine (pancreatic islets of Langerhans). The exocrine pancreas releases elastases into the pancreatic duct and then into the gut, whilst the endocrine pancreas releases hormones into the circulation. Although produced in the acinar cells of the exocrine pancreas, CELE2A is released into the circulation and has effects on both cells in the endocrine pancreas. CELE2A activates the L-type calcium channels of the pancreatic beta cells which control intracellular Calcium levels which trigger insulin exocytosis. Circulating CELE2A also breaks down insulin in the circulation. **(B)** Mutations in the CELE2A protein identified in patients with CAD have a reduced ability to activate the L-type calcium channels resulting in less intracellular calcium concentrations and diminished insulin secretion. Reduced circulating CELE2A activity also results in reduced insulin degradation.

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Healthy Individual

A

Whole pancreas

Cross Section of Pancreas

Pancreatic Duct

Acinar & ductal cells

Pancreatic islet

CELE2A

Pancreatic Beta Cell

Glucose

K_{ATP} channel

L-type Calcium Channel

↑ Insulin

Ca^{2+}

CELE2A

Insulin ↑ Degraded Insulin

Individual with Coronary Artery Disease

B

Whole pancreas

Cross Section of Pancreas

Pancreatic Duct

Acinar & ductal cells

Pancreatic islet

Mutant CELE2A

Pancreatic Beta Cell

Glucose

K_{ATP} channel

L-type Calcium Channel

↓ Insulin

Ca^{2+}

Mutant CELE2A

Insulin ↓ Degraded Insulin