The RHAPSODY project: Biomarkers in type 2 diabetes

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Development of type 2 diabetes: A stepwise process

Normal glycemic control → Prediabetes → Type 2 diabetes → Diabetes deterioration

- Prediabetes: Impaired Glucose tolerance / Impaired Fasting glycemia

Rate of glycemic deterioration in T2D patients from the GoDarts cohort (HbA1c/year)

Donelly LA, et al., Diabetologia, 2018
Development of type 2 diabetes: A stepwise process

Normal glycemic control ➔ Prediabetes ➔ Type 2 diabetes ➔ Diabetes deterioration

A

- Normal glycemic control: 93.6%
- Prediabetes: 5.2%
- Type 2 diabetes: 1.2%

- Impaired Glucose tolerance
- Impaired Fasting glycemia

F

- Cluster 1 (SAID): 19.8%
- Cluster 2 (SIDD): 14.0%
- Cluster 3 (SIRD): 10.6%
- Cluster 4 (MOD): 41.0%
- Cluster 5 (MARD): 14.7%

Ahlqvist E et al., The Lancet Diabetes Endocrinology, 2018
Identification of biomarkers

Normal GT → Pre-T2D → T2D → Glycemic deterioration

Specific Questions:

• Can we identify biomarkers that are prognostic of T2D susceptibility and T2D deterioration?

• Can such biomarkers predict dysfunctions in β-cells or in insulin target tissues?

• Can we identify the tissues and metabolic pathways controlling the production of these biomarkers?
Biomarkers

- Molecules that can be objectively measured and evaluated as an indicator of normal biological or pathogenic processes, as well as pharmacological response to a therapeutic intervention

http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm
Plasma Biomarkers

- Lipids
- Polar metabolites
- Proteins
- RNAs
Plasma Biomarkers

Requirements for biomarker discovery:

• Technological platforms for quantitative measurements of molecular species

• Patient cohorts with detailed phenotyping and biosamples (plasma)

• Preclinical models of prediabetes/diabetes

• Federated database for all data storage and analysis
RHAPSODY Plasma Biomarkers Strategy

Biomarkers for:
- Disease stratification
- Diagnosis
- Disease monitoring
- Innovative clinical trials

Legal & Economics
Cohorts
Insulin resistance biomarkers
Beta-cell biomarkers
Omics
Federated Database
Use of preclinical models to identify biomarkers of diabetes susceptibility and of β-cell dysfunction

- Use of preclinical models to identify candidate biomarkers for the progression to type 2 diabetes and validation in human cohorts

- Use of preclinical models to identify plasma biomarkers predictive of β-cell function and to identify the metabolic pathways involved in biomarker production
Use of preclinical models to identify biomarkers of diabetes susceptibility and of β-cell dysfunction

6 mouse strains

- C57Bl/6
- A/J
- Balb/c
- AKR
- 129S2
- DBA/2J

High Fat

Weight

Regular

2, 10, 30 and 90 days

Insulin secretion

Glucose Tolerance

Blood Glucose (mg/dL)

Time (hour)

Insulin Tolerance

Time after insulin addition (minutes)

Islet morphology

Lipidomics

RNA-Seq

Islets, liver, fat, muscle

10 September 2021
Integrative analysis of plasma lipidomics with mouse physiological traits

Lipid profiles across all conditions (6 strains, 4 time points, 2 diets)

- Correlation, hierarchical clustering
- Modules of lipids with similar profiles
- Correlation of module profiles with physiological traits
- Investigation of trait-associated lipid modules
Ceramides are correlated to glucose intolerance and insulin sensitivity in metabolically challenged mouse strains.
Analysis of ceramides in the plasma of individuals from the DESIR cohort

- Prospective cohort of > 5000 people followed for > 9 years
- **Summary of plasma analysis**

<table>
<thead>
<tr>
<th>Recruitment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yr 0</td>
<td>Yr 3</td>
</tr>
<tr>
<td>Group 1: 82 ND</td>
<td>82 T2D</td>
</tr>
<tr>
<td>Group 2: 48 ND</td>
<td>48 ND</td>
</tr>
<tr>
<td>Group 3: 62 ND</td>
<td>61 ND</td>
</tr>
<tr>
<td>Control: 105 ND</td>
<td>102 ND</td>
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</tbody>
</table>
Dihydroceramides are enriched in the plasma of T2D patients up to 9 years before diagnostic

The DESIR cohort

Wigger L et al., Cell Reports, 2017
Use of preclinical models to identify biomarkers of diabetes susceptibility and of β-cell dysfunction

• Use of preclinical models to identify candidate biomarkers for the progression to type 2 diabetes and validation in human cohorts

• Use of preclinical models to identify plasma biomarkers predictive of β-cell function and to identify the metabolic pathways involved in biomarker production
A systems biology analysis of the crosstalk between liver and pancreatic β-cell function through plasma lipids

• Progressive loss of β-cell secretion capacity over time

• Questions:
  – Can we find plasma biomarkers predictive of β-cell function?
  – Can we identify the tissue and metabolic pathway that produce the biomarker?
  – Can we establish a link between a tissue metabolic pathway – plasma biomarkers – and β-cell function?
Search for plasma lipids as potential biomarkers: experimental design

- C57BL/6
- BALB/cJ
- DBA/2J

Days: 5, 13, 33

Basal glycemia, Basal insulinemia, OGTT, ITT

RNA-seq, Lipidomics

Regular, High Fat
Plasma Triglycerides (TAGs) correlate with β-cell insulin secretion genes and liver fatty acid degradation pathway

Fatty acid degradation (β-oxidation)

Glucose-stimulated insulin secretion (GSIS)
IN HUMANS: Same correlation between plasma TAGs and insulin secretion genes as in mice

- Islets from partially pancreatectomized patients
- Plasma lipids from the same patients

Wigger L. (...) Solimena, M., Nat. Metabolism, 2021

Identification of PITPNC1 as a novel regulator of insulin secretion
Conclusions

• The use of preclinical models allowed to identify plasma biomarkers for type 2 diabetes susceptibility

• Such biomarkers were found to be prognostic biomarkers also in humans

• These biomarkers could be demonstrated to correlate with the function of pancreatic β-cells

• Comparative analysis in mice and humans of the correlation between plasma TAGs and islet gene expression allowed to characterise a so far unknown regulator of insulin secretion
RHAPSODY biomarker identification in humans
(See presentation by R. Slieker)

- Biomarker discovery was assayed for:
  - Proteomics
  - Lipids
  - Polar metabolites

- **Phase 1: Discovery** of biomarkers of T2D progression using samples from the three RHAPSODY discovery cohorts (NT = 9900)

- **Phase 2: Replication** of biomarkers of T2D progression (NT= 4000)

- **Phase 3: Establishment of a biomarker shortlist**
Biomarker shortlist includes the same lipids identified in preclinical models

Lipidomics:

- TAG class (namely 50.1.0, 46.1.0, 46.2.0, 48.1.0, 51.1.0, 48.2.0, 48.3.0 and 49.1.0)

- Sphingomyelin (SM 42.2.2)
Next steps

Evaluate the utility of the biomarkers in a **prospective clinical trial** to assess their utility for:

- **Stratification of diabetic patients** - to more precisely identify high-risk subjects at baseline more likely to respond to a specific intervention

- **Monitoring of diabetes progression** - to improve understanding of the course of the disease, or specific symptoms of the disease
Thank you for your attention!

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