Integrating experiments of man and nature to discover targets and direct therapy in metabolic disease

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7/25/19
Research Goals and Approach

• Diagnosing patients, giving them the right medicine

• Discovering therapeutic targets

Cellular assays
Type 2 Diabetes
Cardiovascular Disease
Fatty Liver Disease

mutations
patients
readouts
Talk outline

• The hammers:
  • Comprehensive mutagenesis
  • Disease-calibrated bioassays

• The nails...
  • Program 1. Genome-guided therapy

• Program 2. Adipocytes: Calibrated assays → TargetID
Genome sequencing is becoming easier
Genome interpretation is an unmet challenge
Rare, missense variants in PPARγ from 20,000 T2D case/controls

Majithia AR, et.al. PNAS, 111(36) 2014
Loss-of-function variants in PPARγ 7x increased risk T2D risk

Majithia AR, et.al. PNAS, 111(36) 2014
What if we could test in advance the function of every possible aa substitution in PPARγ?

1. 1:500 individuals have PPARG missense variant
2. 1:6 PPARG missense variants causes LOF
3. LOF missense variant = 7x T2D risk

PPARγ function “lookup table”
Functional testing of every possible PPARγ missense variant

Construct library containing 9,595 PPARG variants

1 construct per cell

Sort cells by PPARγ activity via CD36

Sequence cells, count PPARG variants

CD36 counts

<table>
<thead>
<tr>
<th>Variant</th>
<th>CD36-</th>
<th>CD36+</th>
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<tbody>
<tr>
<td>G2A</td>
<td>10</td>
<td>990</td>
</tr>
<tr>
<td>G2C</td>
<td>700</td>
<td>300</td>
</tr>
<tr>
<td>R165T</td>
<td>990</td>
<td>10</td>
</tr>
<tr>
<td>Y505V</td>
<td>650</td>
<td>350</td>
</tr>
<tr>
<td>Y505W</td>
<td>800</td>
<td>200</td>
</tr>
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</table>

raw function score

<table>
<thead>
<tr>
<th>Variant</th>
<th>G2A</th>
<th>G2C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+2.1</td>
<td>-1.2</td>
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<tr>
<td>R165T</td>
<td></td>
<td>-2.1</td>
</tr>
<tr>
<td>Y505V</td>
<td>-0.7</td>
<td></td>
</tr>
<tr>
<td>Y505W</td>
<td>-0.8</td>
<td></td>
</tr>
</tbody>
</table>

Majithia AR, et.al. Nature Genetics 2016
Functional testing of every possible PPARγ missense variant
Building a PPARγ classifier

Majithia AR, et.al. Nature Genetics 2016
Predicting clinical consequence of novel PPARγ missense variants

Majithia AR, et.al. Nature Genetics 2016
PPARγ classifier enables interpretation of any missense variant with respect to FPLD3/T2D

1. 1:500 individuals have PPARG missense variant

2. 1:6 PPARG missense variants causes LOF

3. LOF missense variant = 7x T2D risk

Majithia AR, et.al. Nature Genetics 2016
P1: Genome guided therapy: known genes
ID TZD responsive PPARG variants

1. PPARG variant carriers responsive to TZD
2. Lookup tables: TZD-responsive PPARG variant signatures
3. Predict and prospectively validate TZD-response

A Pharmacogenetic Approach to the Treatment of Patients with PPARG Mutations
Agostini M. et. al. Diabetes 2018
Monogenic diabetes mutations are clinically actionable

- HNF1A: Loss-of-function missense variants predict response to low dose SU

- KCNJ11: Gain-of-function missense variants predict response to high dose SU
Genome guided therapy: 
ID SU responsive HNF1A variant carriers

1. ~1.6% individuals have HNF1a missense variant

2. 1:3 HNF1a missense variants causes LOF (< 60% WT)

3. LOF missense variant = 6x T2D risk

Najmi et al., Diabetes 2018
Genome guided therapy:
ID SU responsive HNF1A variant carriers

1. Identify T2D cases with HNF1a missense variant
2. Categorize HNF1a missense variants benign vs LOF
3. Can HNF1a LOF variant carriers be free of insulin?
Genome guided therapy: future plans

• Lookup tables: rare missense variants
• Diagnose monogenic diabetes
• Recruit by “functional” genotype trials

![Genetic variants flowchart]

- Genetic variants
- Cellular function
- Disease status

- HNF1a
- HNF4a
- HNF1b
- PDX1
- PAX4
- PPARG
- KCNJ11
- ABCC8

- Transcriptional activation/FACS
- Genetically encoded voltage sensitive dyes

- General population
  - T2D,
  - Glycemic traits

- Clinically pre-selected
  - Lipodystrophy,
  - MODY,
  - Neonatal DM
Summary

• Individuals with large effects mutations that cause metabolic disease are hiding in plain sight

• Human genetics can be used to calibrate bioassays in vitro to predict clinical consequence in vivo

• Next step: recruit by genotype and do a clinical trial
Talk outline

• The hammers:
  • Comprehensive mutagenesis
  • Disease-calibrated bioassays

• The nails...
  • Program 1. Genome-guided therapy

  • Program 2. Adipocytes: Calibrated assays → TargetID
Approaches to targetID

- OF nature
  - GWAS

- ON man
  - treatment + omics

- BY man
  - model system
Approaches to targetID: challenges

OF nature
• GWAS
• What genes?

ON man
• treatment + omics
• Causality?

BY man
• model system
• Human relevance?
P2: Adipocytes – Calibrated assays, TargetID Mapping IR genes: adipocytes are key

New genetic loci link adipose and insulin biology to body fat distribution

Genetic Association of Waist-to-Hip Ratio
With Cardiometabolic Traits, Type 2 Diabetes,
and Coronary Heart Disease


eTable 5. Association of WHRadjBMI polygenic risk score with potential confounders in UK Biobank.

<table>
<thead>
<tr>
<th></th>
<th>Quartiles of WHRadjBMI Polygenic Risk Score</th>
<th>Test for Trend</th>
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<tbody>
<tr>
<td>Quartile Range</td>
<td>Q1 0.73 to 1.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q2 &gt;1.12 to 1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q3 &gt;1.2 to 1.28</td>
<td></td>
</tr>
<tr>
<td>Number of Participants</td>
<td>27997</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27996</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27998</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27997</td>
<td></td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>3426 (12%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3366 (12%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3368 (12%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3451 (12%)</td>
<td></td>
</tr>
<tr>
<td>Past smoker, n (%)</td>
<td>10155 (40%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10188 (40%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10193 (40%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10227 (41%)</td>
<td></td>
</tr>
<tr>
<td>Moderate exercise ± SD, mean number of days per week</td>
<td>3.60 ± 2.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.63 ± 2.34</td>
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<td></td>
<td>3.61 ± 2.33</td>
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</tr>
<tr>
<td></td>
<td>3.60 ± 2.35</td>
<td></td>
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<tr>
<td>Intense exercise ± SD, mean number of days per week</td>
<td>1.81 ± 1.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.60 ± 1.96</td>
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</tr>
<tr>
<td></td>
<td>1.80 ± 1.94</td>
<td></td>
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<tr>
<td></td>
<td>1.81 ± 1.96</td>
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<tr>
<td>Daily alcohol consumption, n (%)</td>
<td>6052 (22%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6032 (22%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6056 (22%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6020 (22%)</td>
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<tr>
<td>Six tablespoons of vegetables or more per day, n(%)</td>
<td>8175 (32%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8203 (32%)</td>
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</tr>
<tr>
<td></td>
<td>8322 (32%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8201 (32%)</td>
<td></td>
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<tr>
<td>Red meat consumption three or more times per week, n(%)</td>
<td>6215 (22%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6191 (22%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6223 (22%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6269 (23%)</td>
<td></td>
</tr>
<tr>
<td>Breastfed as baby, n(%)</td>
<td>15117 (71%)</td>
<td></td>
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<td></td>
<td>15052 (71%)</td>
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<tr>
<td></td>
<td>14976 (71%)</td>
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<tr>
<td></td>
<td>14971 (71%)</td>
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Abbreviations: SD = standard deviation

Nutritional SD = standard deviation
Approaches to targetID: the genes under GWAS

- **OF nature**
  - GWAS – T2D, FI, BMI

- **ON man**
  - Adipocyte expression

- **BY man**
  - LOF → adipocyte differentiation
Approaches to targetID: the genes under GWAS

1. Gene selection: 133 genes
   - Traits: Mendelian, Lipodystrophy / Insulin resistance
   - GWAS: T2D, BMI / WHR, Fasting Insulin
   - Known regulators: Insulin transduction, Adipocyte, Cell essential
   - Expression filter: > 0.1 FPKM in SGBS adipocytes
   - Lentiviral construct array: 424 unique sgRNA
     - 3 sgRNA / gene
     - 25 non-targeting

2. LOF perturbations: 4800 wells
   - Lentiviral infection pre-adipocytes
   - Gene deletion (10 days)
   - Adipocyte differentiation (11 days)
   - Mutation efficiency

3. Image acquisition: ~77,000 images
   - 3840 wells
   - 5 sites / well, 20x magnification
   - 4 channels / site

4. Data analysis: ~3.2x10^7 measurements
   - Morphologic feature extraction:
     - nuclei, adipocyte, droplets...
     - 425 features
   - Data integration, QC, dimensionality reduction
   - Clustering:

Jiao Y., et.al. Molecular Metabolism 2019
Approaches to targetID: the genes under GWAS

-log(p value)
differentiation (fraction of total cells/well)

Class
- adipocyte differentiation /lipid content
- human lipodystrophy
- insulin signaling
- novel hits

Jiao Y., et.al. Molecular Metabolism 2019
Approaches to target validation: credentialing a specific gene/hypothesis

OF nature
- Protein-coding variation

ON man
- TG:HDL ratio

BY man
- Genes that alter adipocyte diff
Approaches to target validation:
Using nature’s experiments, protein-coding variation

30 variants / gene
90% of genes have 1 or more variants

T2D genes: Protein-altering variants in exome sequencing of 5000 individuals

Tennessen J Science 2012
Flannick J Nature 2019
Human genetics for target validation:
H$_1$: LOF $\rightarrow$ ↑adip diff $\rightarrow$ ↑ insulin sensitivity

<table>
<thead>
<tr>
<th>Gene</th>
<th>protein isoform</th>
<th>Variant type</th>
<th># amino acids</th>
<th>anomAD missense</th>
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<tr>
<td></td>
<td></td>
<td>missense</td>
<td>protein</td>
<td>saturation</td>
</tr>
<tr>
<td>PPARG</td>
<td>NP_056953.2</td>
<td>98</td>
<td>505</td>
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<tr>
<td>PTEN</td>
<td>NP_000305</td>
<td>48</td>
<td>403</td>
<td>7657</td>
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</table>

Table 1: Unique protein-altering variants in 53,520 sequenced with TG/HDL

PTEN Mutations as a Cause of Constitutive Insulin Sensitivity and Obesity
Human genetics for target validation:
$H_1$: LOF $\rightarrow$ ↑adip diff $\rightarrow$ ↑ insulin sensitivity

PPARG

PTEN

A Saturation Mutagenesis Approach to Understanding PTEN Lipid Phosphatase Activity and Genotype-Phenotype Relationships

Taylor L. Mighell, Sara Evans-Dutson, and Brian J. O’Roak

Functional score averaged normalized log(TG:HDL)

Functional score averaged normalized log(TG:HDL)
Human genetics for target validation: 
\( \text{H}_1 \): Cancer and diabetes are separable in PI3K

### Table 1. Variants in PIK3CA from 49,997 sequenced individuals and 10,202 tumors

<table>
<thead>
<tr>
<th>Type</th>
<th>UKBiobank</th>
<th>TCGA</th>
<th>Overlap</th>
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<tbody>
<tr>
<td>synonymous</td>
<td>67</td>
<td>45</td>
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<tr>
<td>missense</td>
<td>77</td>
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<td>indel</td>
<td>3</td>
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<tr>
<td>stop</td>
<td>1</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>splice site</td>
<td>14</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>UTR</td>
<td>40</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>201</strong></td>
<td><strong>394</strong></td>
<td><strong>10</strong></td>
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Human genetics for target validation
Genome scale adipocyte differentiation assay
Human genetics for target validation
Genome scale adipocyte differentiation assay
Human genetics for target validation
Genome scale adipocyte differentiation assay
Human genetics for target ID/validation: future directions

**OF nature**
- GWAS
- Exome sequencing

**Ukbiobank**
- 500,000 longitudinally phenotyped
- 2020: full exome sequencing

**Hepatic lipid accumulation**
- cell type credentialing
- image-based assay credentialing

**ON man**
- insulin sensitizing perturbations + genomic measurement
- RNAseq, ATACseq, ssSeq

**BY man**
- genomewide XPR screens
- saturation mutagenesis

**UK Biobank**
- 500,000 longitudinally phenotyped
- 2020: full exome sequencing
Discovering targets and directing therapy

- Rare-protein coding variation that arose recently in human evolution exists in most genes

- Understanding functional impact is the key to both:
  - interpreting the consequence for human health
  - leveraging “experiments of nature” to identify validate therapeutic targets

- Massively parallel, disease calibrated assays can systematically characterize function of genes and variants
Acknowledgements

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Gina Peloso
Jerry Olefsky

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NIDDK KO8
NIDDK R03
UCSD/UCLA DRC P&F (P30 DK063491)
P1: Genome guided therapy: known genes

- Lookup tables: rare missense variants
- Diagnose monogenic forms of insulin resistance
- Recruit by “functional” genotype trials