The Physiologic Consequences of Genetic Variation in Type 2 Diabetes: The Hepatic Substrate Balance Hypothesis

Keck School of Medicine of USC

Richard M. Watanabe, Ph.D.

Depts. of Preventive Medicine and Physiology & Biophysics
USC Diabetes and Obesity Research Institute
The Physiologic Consequences of Genetic Variation in Type 2 Diabetes: The Hepatic Substrate Balance Hypothesis

Richard M. Watanabe, Ph.D.

Depts. of Preventive Medicine and Physiology & Biophysics
USC Diabetes and Obesity Research Institute
Genome–wide association (GWA) studies have identified:

- Over 80 loci associated with risk for T2DM
- “Hundreds” of loci associated with variation in T2DM–related traits
  - Glycemia/insulinemia
  - Obesity/adiposity
  - Lipids/lipoproteins
  - Related metabolic disorders

Some loci have highlighted new biology related to T2DM.

Majority of associated loci fall in intronic or inter–genic regions.
Recent whole-exome and whole-genome studies revealed new insights:

- There appear to be few rare variants of large effect.
- Most rare variants have effect sizes similar to common variants.
- Not likely to explain the so-called “missing” heritability.

Genetics of Type 2 Diabetes

Taken from Fuchsberger et al., Nature 536:41–47, 2016

USC Diabetes and Obesity Research Institute
Question: So how should we think about the role of genetic variation in the pathogenesis of type 2 diabetes?

Most variants are in intergenic or intronic regions

- Suggests gene splicing or transcriptional regulation may be important

Most associations are only landmarks, so fine-mapping to identify “the” variant will be key

Much work to do . . .
An alternative view . . .

While reductionist science is important, many times small molecular changes do not manifest themselves in a clinical phenotype.

Physiologic regulation can mask the small effects engendered by some genetic variants.

Some effects may not manifest in a phenotype for prolonged periods of time.

So, if we believe the type 2 diabetes loci are in linkage disequilibrium with “the” variant, then these loci should be sufficient to stratify patients for physiologic studies.
An Analogy from Aviation

- United flight 232; July 19, 1989
- Suffered a midflight catastrophic failure in engine #2
- Crashed attempting an emergency landing at Sioux City, Iowa
An Analogy from Aviation

~0.8 m in diameter
~1.4 mm in length
Things I Think About

Genes

Diet

Physical activity

Type 2 Diabetes
Things I Think About

- Nominal Alleles
- Progression to Diabetes
- Glucose [mg/dl]

Graph showing:
- Disease Susceptibility Alleles
- Trait Raising Alleles
- Nominal Alleles

Progression to Diabetes
MAGIC has led GWAS efforts to identify loci underlying variation in glucose and insulin-related traits in samples of northern European ancestry

- rs1260326 (P446L) variant in \textit{GCKR} previously shown to be associated with:
  - Decreasing fasting glucose
  - Increasing fasting triglycerides

- rs780094 in \textit{GCKR} showed similar patterns of association in MAGIC

Two variants in strong linkage disequilibrium
An Anomaly from MAGIC

- GCKR is bound and sequestered in the nucleus
- Released to inhibit GCK activity
- Fructose-6-phosphate (F6P) shown to stimulate GCKR activity
- Fructose-1-phosphate (F1P) shown to inhibit GCKR activity

Glucose $\downarrow$ GCK $\downarrow$ G-6-P $\downarrow$ F-6-P $\downarrow$ F-1-P

Glucose $\downarrow$ GCK $\downarrow$ G-6-P $\downarrow$ F-6-P $\downarrow$ F-1-P

USC Diabetes and Obesity Research Institute
An Anomaly from MAGIC

Studies by Beer et al. (Hum Mol Genet, 18:1081–4088, 2009)

P466L variant impairs GCKR regulation of GCK at high concentrations
An Anomaly from MAGIC

- P466L significantly impaired fructose–6–phosphate (F6P) stimulation of GCKR inhibition of GCK activity
- P466L had no effect on GCKR inhibition by fructose–1–phosphate (F1P)

Question:
Are the in vitro results supported by in vivo observations?
Physiologic Effect of Genetic Variation

The BetaGene Study

- Mexican American families of probands with or without a previous Dx of GDM
- Non–diabetic at time of study
- Likely at elevated risk for T2DM

Detailed phenotyping not available in most genetic studies

- DXA scan for body composition
- 180 min OGGT with 30–minute sampling
- Insulin–modified FSIGT with minimal model analysis
Physiologic Effect of Genetic Variation

- Studying at-risk individuals before they develop T2DM should help us understand the pathogenesis of disease.
- Studying T2DM-related quantitative traits should provide critical clues regarding specific biologic processes compromised by genetic variation.
The Tale of 3 loci and the liver

Glucokinase (GCK)
- T2DM risk locus, associated with T2DM-related QTs
- Gene product is rate-limiting enzyme for glycolysis
- >99% of all GCK moieties exist in the liver

Glucokinase Regulator (GCKR)
- Risk locus for T2DM and non-alcoholic fatty liver disease (NAFLD)
- Associated with T2DM- and NAFLD-related QTs
- Regulates the activity of GCK
- Little to no expression in pancreatic β-cells
Patatin–like Phospholipase 3 (PNPLA3)

- NAFLD risk locus
- Associated with NAFLD–related QTs
- Not associated with risk for T2DM or T2DM–related traits
- Triacylglycerol lipase that regulates hydrolysis of hepatic fat to triglyceride
- PNPLA3–based rodent models do not reproduce the human phenotype, except in the presence of high–sugar diets
The Substrate Balance Hypothesis

Glucose

PNPLA3

Triglycerides

Carbons In

Carbons Out
Why is this Important?

- High prevalence of T2DM in Hispanics
- High prevalence of the spectrum of steatosis in Hispanics
- Strong association between steatosis and hepatic insulin resistance
- Hepatic and peripheral insulin resistance are hallmarks of T2DM
- T2DM therapies also affect hepatic steatosis (direct and/or indirect)
- GCKR antagonists have been developed and are in pre-clinical testing
**GCKR Antagonists**

- Two different Amgen GCKR “disruptors” compared to a GCK activator (GKA)
Question:
What do these variants look like in Mexican Americans?
<table>
<thead>
<tr>
<th>Trait</th>
<th>GCK rs4607517 Beta (SE)</th>
<th>p-value</th>
<th>GCKR rs780094 Beta (SE)</th>
<th>p-value</th>
<th>PNPLA3 rs738409 Beta (SE)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Glucose</td>
<td>0.139 (0.058)</td>
<td>0.037</td>
<td>-0.062 (0.041)</td>
<td>0.088</td>
<td>0.047 (0.041)</td>
<td>0.283</td>
</tr>
<tr>
<td>2-hour Glucose</td>
<td>0.024 (0.058)</td>
<td>0.738</td>
<td>-0.021 (0.042)</td>
<td>0.978</td>
<td>0.002 (0.041)</td>
<td>0.743</td>
</tr>
<tr>
<td>Fasting Insulin</td>
<td>-0.011 (0.058)</td>
<td>0.637</td>
<td>-0.068 (0.042)</td>
<td>0.102</td>
<td>0.047 (0.040)</td>
<td>0.135</td>
</tr>
<tr>
<td>30' Δ Insulin</td>
<td>-0.107 (0.058)</td>
<td>0.297</td>
<td>-0.019 (0.042)</td>
<td>0.444</td>
<td>0.021 (0.040)</td>
<td>0.654</td>
</tr>
<tr>
<td>2-hour Insulin</td>
<td>-0.031 (0.058)</td>
<td>0.562</td>
<td>-0.054 (0.042)</td>
<td>0.303</td>
<td>0.022 (0.041)</td>
<td>0.292</td>
</tr>
<tr>
<td>Insulinogenic Index</td>
<td>-0.127 (0.058)</td>
<td>0.084</td>
<td>0.022 (0.042)</td>
<td>0.888</td>
<td>-0.014 (0.040)</td>
<td>0.820</td>
</tr>
<tr>
<td>DI30</td>
<td>-0.108 (0.061)</td>
<td>0.153</td>
<td>-0.033 (0.045)</td>
<td>0.625</td>
<td>0.041 (0.043)</td>
<td>0.704</td>
</tr>
<tr>
<td>S_G</td>
<td>-0.044 (0.061)</td>
<td>0.572</td>
<td>0.130 (0.045)</td>
<td>4.1×10^{-3}</td>
<td>-0.008 (0.043)</td>
<td>0.908</td>
</tr>
<tr>
<td>S_l</td>
<td>-0.007 (0.061)</td>
<td>0.785</td>
<td>0.037 (0.045)</td>
<td>0.220</td>
<td>0.006 (0.043)</td>
<td>0.959</td>
</tr>
<tr>
<td>AIR</td>
<td>-0.075 (0.061)</td>
<td>0.476</td>
<td>0.044 (0.045)</td>
<td>0.487</td>
<td>-0.042 (0.042)</td>
<td>0.295</td>
</tr>
<tr>
<td>DI</td>
<td>-0.084 (0.061)</td>
<td>0.254</td>
<td>0.045 (0.045)</td>
<td>0.203</td>
<td>-0.023 (0.043)</td>
<td>0.297</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.086 (0.058)</td>
<td>0.406</td>
<td>0.143 (0.042)</td>
<td>6.9×10^{-4}</td>
<td>-0.016 (0.040)</td>
<td>0.527</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>-0.008 (0.058)</td>
<td>0.465</td>
<td>0.009 (0.042)</td>
<td>0.354</td>
<td>-0.017 (0.041)</td>
<td>0.482</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.049 (0.058)</td>
<td>0.411</td>
<td>0.176 (0.042)</td>
<td>7.2×10^{-5}</td>
<td>-0.060 (0.041)</td>
<td>0.467</td>
</tr>
<tr>
<td>ALT</td>
<td>-0.039 (0.058)</td>
<td>0.448</td>
<td>0.020 (0.042)</td>
<td>0.781</td>
<td>0.175 (0.041)</td>
<td>1.1×10^{-5}</td>
</tr>
<tr>
<td>AST</td>
<td>0.025 (0.058)</td>
<td>0.843</td>
<td>0.022 (0.042)</td>
<td>0.588</td>
<td>0.193 (0.040)</td>
<td>6.4×10^{-7}</td>
</tr>
</tbody>
</table>
The Biochemistry Underlying Our Hypothesis

Glucose

\[ \text{GCK} \quad \rightarrow \quad \text{GCKR} \]

G-6-P

\[ \downarrow \]

F-6-P

\[ \downarrow \]

PEP

\[ \downarrow \]

LAC \leftrightarrow PYR

\[ \downarrow \]

ACoA \rightarrow Fatty Acids \rightarrow Triglycerides

Liver Fat

PNPLA3

USC Diabetes and Obesity Research Institute
Physiologic Evidence

- Variation in \textit{GCK} and/or \textit{GCKR} should alter glucose uptake by the liver.

- Glucose effectiveness (\(S_G\)) from minimal model analysis of frequently–sampled intravenous glucose tolerance test quantifies the effect of glucose to enhance its own uptake at fasting insulin levels.

- \(S_G\) should \textit{partly reflect} glucose uptake by the liver.
Physiologic Evidence

**GCKR rs780094**

\[ \text{Glucose Effectiveness} \times 10^{-2} \text{ min}^{-1} \]

\[ p = 0.004 \]

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>n = 506</td>
<td></td>
</tr>
<tr>
<td>G/A</td>
<td>n = 513</td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>n = 119</td>
<td></td>
</tr>
</tbody>
</table>
Additional evidence comes from interactions among SNPs.

Interaction between *GCKR* rs780094 and *PNPLA3* rs738409 is associated with:

<table>
<thead>
<tr>
<th>Trait</th>
<th>1-df Interaction p-value</th>
<th>GCKR 2-df SNP p-value</th>
<th>PNPLA3 2-df SNP p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Insulin</td>
<td>0.040</td>
<td>0.028</td>
<td>0.033</td>
</tr>
<tr>
<td>S&lt;sub&gt;I&lt;/sub&gt;</td>
<td>0.027</td>
<td>0.030</td>
<td>0.086</td>
</tr>
<tr>
<td>HDL</td>
<td>0.005</td>
<td>0.011</td>
<td>0.015</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.0003</td>
<td>1.3×10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Interaction between *GCK* rs4607517 and *PNPLA3* rs738409 is associated with:

<table>
<thead>
<tr>
<th>Trait</th>
<th>1-°f Interaction p-value</th>
<th>GCKR 2-°f SNP p-value</th>
<th>PNPLA3 2-°f SNP p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-hour Insulin</td>
<td>0.013</td>
<td>0.043</td>
<td>0.026</td>
</tr>
<tr>
<td>$S_I$</td>
<td>0.012</td>
<td>0.038</td>
<td>0.037</td>
</tr>
</tbody>
</table>
The Influence of Fructose

- Dietary fructose implicated in obesity, diabetes, and other metabolic conditions
- Fructose could directly contribute to hepatic fat
- F1P inhibits GCKR activity
- How does fructose influence the hepatic substrate balance hypothesis?
The Influence of Fructose

Glucose → GCK → G-6-P → F-6-P → PEP → LAC ← PYR → ACoA → Fatty Acids → Triglycerides

Fruuctose → GCKR → F-1-P → DHAP → GA-3-P → Glycerol → G3P

Liver Fat

PNPLA3

USC Diabetes and Obesity Research Institute
### The Influence of Fructose

- **Interaction between** *GCKR* rs780094 **and** dietary fructose levels **is associated with** AST levels, **but not** ALT levels

<table>
<thead>
<tr>
<th>Trait</th>
<th>1-df Interaction p-value</th>
<th>2-df SNP p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>0.080</td>
<td>0.206</td>
</tr>
<tr>
<td>AST</td>
<td>0.017</td>
<td>0.050</td>
</tr>
</tbody>
</table>

- **Interaction between** *PNPLA3* rs738409 **and** dietary fructose levels **is associated with** $S_G$

<table>
<thead>
<tr>
<th>Trait</th>
<th>1-df Interaction p-value</th>
<th>2-df SNP p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_G$</td>
<td>0.011</td>
<td>0.038</td>
</tr>
</tbody>
</table>
The Influence of Fructose

<table>
<thead>
<tr>
<th>Fructose Level</th>
<th>Glucose Effectiveness (G/G)</th>
<th>Glucose Effectiveness (G/A)</th>
<th>Glucose Effectiveness (A/A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low 9.1±0.4 mM</td>
<td>1.0±0.1 (G/G)</td>
<td>1.5±0.2 (G/A)</td>
<td>2.0±0.3 (A/A)</td>
</tr>
<tr>
<td>Medium 28.3±0.2 mM</td>
<td>1.5±0.2 (G/G)</td>
<td>2.0±0.3 (G/A)</td>
<td>2.5±0.4 (A/A)</td>
</tr>
<tr>
<td>High 58.7±1.1 mM</td>
<td>2.0±0.3 (G/G)</td>
<td>2.5±0.4 (G/A)</td>
<td>3.0±0.5 (A/A)</td>
</tr>
</tbody>
</table>

*p = 0.0005*
The Substrate Balance Hypothesis

Glucose

- GCK
- GCKR

G-6-P

Fructose

- GCKR

F-1-P

Fatty Acids

Triglycerides

Liver

Fat

PNPLA3

LAC

PYR

ACoA

PYR

Fatty Acids

Triglycerides
Summary

- Genetic variation in *GCK*, *GCKR*, and *PNPLA3* are associated with key components of hepatic metabolism.
- The net effect of these loci may determine levels of hepatic fat through differential regulation of carbon flow.
- Dietary fructose may further exacerbate the effects of genetic variation in these loci.

But . . .
Summary

- All associations are with liver enzyme levels, not measures of liver fat
- No direct evidence for variation in $GCKR$ to affect hepatic glucose uptake
- No direct evidence for dietary fructose to alter $GCKR$ regulation of GCK
- $G\times G$ interaction results are not very robust
Starting new studies in which hepatic fat will be assessed by MRI to validate associations observed with enzyme levels

Measuring lactate from the FSIGT
   - Use a novel mathematical model of lactate kinetics to characteristics of hepatic glycolytic activity

Expanding sample size and adding phenotypes to further explore $G \times G$ and $G \times E$ interactions
Acknowledgements

The FUSION Study

The DIAGRAM (+) Consortium

Genetic Investigation of ANthropometric Traits

Beta Gene

MAGIC

GUARDIAN

USC Diabetes and Obesity Research Institute
Acknowledgements

Dr. Mary Helen Black
Dr. Zhanghua Chen
Dr. Jie Ren
Dr. Yu–Hsiang Shu
Cpt. Alyson Kil, M.D.

Dr. Nan Wang
Michael Arias
David Phan
Therlinder Lo
Tara Kerin

Zhu Chen
Enrique Trigo
Adrienne McKay

USC Diabetes and Obesity Research Institute
Conflict of interest disclosure

None

Committee of Scientific Affairs