Novel Approaches to Discover Genes Linked to Drug Response

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Kathy Giacomin

Membrane Transporters

Pharmacogenomics

Pharmacometrics

Data Science
Genetic Variants in Transcription Factors Are Associated With the Pharmacokinetics and Pharmacodynamics of Metformin

S Goswami, SW Yee, S Stocker, JD Mosley, M Kubo, R Castro, JA Mefford, C Wen, X Liang, JWitte, C Brett, S Maeda, MD Simpson, MM Hedderon, RL Davis, DM Roden, KM Giacomini and RM Savic

A Longitudinal HbA1c Model Elucidates Genes Linked to Disease Progression on Metformin

S Goswami, SW Yee, F Xu, SB Sridhar, JD Mosley, A Takahashi, M Kubo, S Maeda, RL Davis, DM Roden, MM Hedderon, KM Giacomini and RM Savic
Metformin is the Major Drug In Treating Type 2 Diabetes

- 9% of world has Type 2 Diabetes
- Growing prevalence of disease
- HbA1c is a well established biomarker

- Wide variation in response to metformin
  - >1/3 of patients taking metformin do not respond sufficiently

  - The progression of HbA1c levels and metformin tolerance has not been well characterized
Sources of Drug Response Variability

- manufacturing & distribution
- prescribing
- dispensing
- adherence
- Pharmacokinetics
- Pharmacodynamics
- drug response

Professionals’ responsibility
Patient's responsibility
Product design + Nature’s responsibility

Harlow & Yok, Ann NY Acad Sci, 658: 565-571, 1992
Goal: to understand variability in individual drug response to metformin
Research Focus

What are key genetic and non-genetic determinants of metformin response?
1. Since metformin is not metabolized, membrane transporters in the intestine, kidney and liver play an even very important role in metformin disposition and response

2. Despite metformin’s peripheral glucose lowering effects in peripheral tissues such as fat and muscle, the primary site of action is in the liver, shown here

3. In the liver, metformin conducts many of its pharmacological actions, including increasing glucose uptake and decreasing gluconeogenesis
1. Genetic polymorphisms in these transporters have been shown to affect metformin’s pharmacological outcomes

2. A few small studies have shown the effect of genetic variants on metformin PK, fewer have looked at PD

3. For example, in the liver, there are 4 OCT1 variants, 1 of which is a deletion, that has been associated with an increase in AUC, CMAX, and renal clearance

4. There is an OCT2 coding variant, associated with an increase in exposure and renal clearance
1. Genetic polymorphisms in these transporters have been shown to affect metformin's pharmacological outcomes

2. A few small studies have shown the effect of genetic variants on metformin PK, fewer have looked at PD

3. For example, in the liver, there are 4 OCT1 variants, 1 of which is a deletion, that has been associated with an increase in AUC, CMAX, and renal clearance

4. There is an OCT2 coding variant, associated with an increase in exposure and renal clearance
Ethnic Differences in Metformin Response Have Been Reported

![Graph showing Reported Lower HbA1c in African Americans](image)

Integrative Pharmacokinetic, Genetic, Demographic and Clinical factors driving wide variation in long term response

Williams et al. “Differing Effects of metformin on Glucose Control by Race: Ethnicity, ICEM”
A Longitudinal HbA1c Model Elucidates Genes Linked to Disease Progression on Metformin Therapy

Rada Savic, PhD

Dept. Of Bioengineering and Therapeutic Sciences, UCSF
Research Aim

Characterize and predict treatment response to metformin using a linked bioinformatics and NLME approach in T2D patients
Identify patients likely not to respond

Questions to explore:
1. Can we identify and quantify disease progression in T2D patients?
2. At what point do HbA1c levels start to increase in T2D Patients?
3. What are the relative roles of genetic and non-genetic factors?
4. Which genes influence the dynamics of disease progression?
## Characteristics of Type 2 Diabetic Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1000</td>
</tr>
<tr>
<td>Males</td>
<td>415</td>
</tr>
<tr>
<td>Females</td>
<td>641</td>
</tr>
<tr>
<td>European Americans</td>
<td>376</td>
</tr>
<tr>
<td>African Americans</td>
<td>605</td>
</tr>
<tr>
<td>Asian Americans and Others</td>
<td>15</td>
</tr>
</tbody>
</table>

### Quantitative Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55 (23-80)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>96 (41-212)</td>
</tr>
<tr>
<td>Baseline HbA1c (%)</td>
<td>7.6 (5.6-17.9)</td>
</tr>
<tr>
<td>Metformin daily dose (mg)</td>
<td>1000 (200-2900)</td>
</tr>
<tr>
<td># HbA1c samples/patient</td>
<td>7.8 (1-48)</td>
</tr>
<tr>
<td>Years on study</td>
<td>2.78 (0.26-13.5)</td>
</tr>
</tbody>
</table>

- **Responding well**
- **Disease progression**
• The workflow for the research is as follows:

• Development of semi-mechanistic HbA1c model in a cohort of approximately 1100 patients with T2D with genomic data.

• SCM, clinical/demographic analysis of model parameters prior to the investigation of genetics.

• Genetic analysis
  • Selection of candidate genes: 2 approaches were used to prioritize genes for analysis on disease progression. 1) Pulling disease linked genes from an online GWAS database called HuGE navigator. 2) Pulling all relevant PK/PD genes from PharmGKB. The reason these two sources were preferred is because we hypothesize that the long term trajectory of HbA1c may be influenced by both T2D disease genes as well as metformin pharmacological genes. Drug resistance and a patient’s underlying disease progression may play a role here.
  • Selection of SNPs within 50 kilobases of the genes of interest (both pharmacological and disease based).
  • Hyperlasso methodology. Considers correlation patterns across SNPs being tested. Corrects for number of SNPs as well as number of phenotypes tested.
  • Top HL SNPs are then investigated using a model based approach: A full model of HL identified SNPs was created. SNPs with low effect sizes and not statistically significant were removed.
1. Collection of variants in literature associated with metformin phenotypic outcomes

2. In addition to transporter variants, there has been 1 Genome wide association study that identified an ATM locus associated with response. This study however was not replicated and the phenotype that was looked at was a binary stratification of HBA1C.

3. Limitations anchoring these studies include: sample size, and methodology

4. Also, a couple of these variants, although not much known, are promoter variants with minor allele frequencies >10 %
Base Disease Progression Model Development

1) Turnover HbA1c Model

\[
\frac{d(HbA1c)}{dt} = Kin \times (1 - Metf\_Effect) - Kout \times HbA1c
\]

2) Synthesis rate of HbA1c time dependent

\[
\frac{d(Kin)}{dt} = Ksyn \times (1 + dispr) - Kloss \times Kin
\]

3) Symptomatic metformin effect

4) Exposure - response built in
   - serum creatinine surrogate for the exposure

5) Full variance - covariance block + Cox-Box transformation for 2 ETAS
   - baseline
   - disease progression
   - effect

Post et al. Disease System Analysis: Basic Disease Progression Models in Degenerative Disease
Longitudinal Model Captured Upward HbA1c Trends

Observations

Visual Predictive Check

- Source Files:
  1) PD_Datasumm.R
  2) Vpc_ron.R
  3) Further modification illustrator etc.
Research Design

(1) HbA1c modeling

Standard SCM

(2) Clinical/Demographic analysis

(3) Genetic analysis

HbA1c Levels (%) vs Time (years)

Personalizing Metformin Therapy

UCSF
Genetic Analysis

1. Initial GWAS Approach
   Machine learning on individual disease progression rates
   > 10,000 SNPs

2. Selection of 267 physiologically meaningful genes
   12,000 variants within a 50-kilobase region around each gene
   Pharmacogenomics – PKPD genes
   HuGE Navigator – disease-limited genes
   > 12,000 SNPs

3. Hyperlasso and regression on disease progression
   SNP correlation patterns accounted for
   Corrected for number of phenotypes and SNPs
   21 SNPs identified significant
   21 SNPs

4. NLME Model based approach – full model established
   21 SNPs

5. Parsimonious model (9 SNPs)
   SNPs with low effect sizes and no significance removed
   9 SNPs
## Significant associations

### Table 3: Summary of top genetic variants included in the population pharmacodynamic model of eNOS

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Chr</th>
<th>Gene</th>
<th>Minor allele</th>
<th>Major allele</th>
<th>Feature</th>
<th>MDR Score</th>
<th>MDR Score CEU</th>
<th>MDR Score HSD</th>
<th>Game Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs130678356</td>
<td>19</td>
<td>MLKG2d - unknown</td>
<td>G</td>
<td>A</td>
<td>Proximal</td>
<td>0.6003</td>
<td>-0.147 (G)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>rs2685022</td>
<td>6</td>
<td>HOMC - hBAAT</td>
<td>G</td>
<td>A</td>
<td>Proximal</td>
<td>0.4609</td>
<td>0.39 (G)</td>
<td>0.48</td>
<td>0.20</td>
</tr>
<tr>
<td>rs2661342</td>
<td>6</td>
<td>MYPK - unknown</td>
<td>G</td>
<td>A</td>
<td>Proximal</td>
<td>0.6229</td>
<td>0.23 (G)</td>
<td>0.22</td>
<td>0.40</td>
</tr>
<tr>
<td>rs2081403</td>
<td>6</td>
<td>SUCSD2 - FAM109C</td>
<td>T</td>
<td>C</td>
<td>Interm</td>
<td>0.64</td>
<td>0.84 (T)</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>rs2279467</td>
<td>6</td>
<td>SNX14 - unknown</td>
<td>A</td>
<td>G</td>
<td>Interm</td>
<td>0.65</td>
<td>-0.42 (G)</td>
<td>0.14</td>
<td>0.21</td>
</tr>
<tr>
<td>rs5382250</td>
<td>6</td>
<td>SLURF - unknown</td>
<td>T</td>
<td>C</td>
<td>Interm</td>
<td>0.6003</td>
<td>-0.30 (T)</td>
<td>0.17</td>
<td>0.38</td>
</tr>
</tbody>
</table>
## Significant associations

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Gene</th>
<th>Minor allele</th>
<th>Major allele</th>
<th>Feature</th>
<th>Model-based P-value</th>
<th>Effect size of minor allele on SF parameter</th>
<th>MAF</th>
<th>IAF</th>
<th>Gene function</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10995524</td>
<td>14</td>
<td>PDK4-10 Protein Kinase 4</td>
<td>T</td>
<td>G</td>
<td>Proximal</td>
<td>0.006</td>
<td>-0.28 (TG)</td>
<td>0.27</td>
<td>0.39</td>
<td>This is a transcriptional repressor, which plays an important role in cell cycle regulation and is located in chromosome 14q24.3 (35), which is a locus associated with insulin-dependent diabetes mellitus susceptibility.26</td>
</tr>
<tr>
<td>rs7600549</td>
<td>55</td>
<td>IRS1-405K Domain Containing 2</td>
<td>C</td>
<td>T</td>
<td>Intron</td>
<td>0.029</td>
<td>-0.16 (CT)</td>
<td>0.05</td>
<td>0.20</td>
<td>This SNP is associated with reduced insulin secretion.68</td>
</tr>
</tbody>
</table>

**P-value** - The significance level that resulted from adjusted t-test of the differences between the two groups (SNP) and the control group (subset). SNP, minor allele frequency (MAF), disease progression (DP), northern European (5), African population.
Top Variants From Hyperlasso and Model Based Methodology Were More Influential on HbA1c Levels than Demographic Covariates

Simulated median (95% CI) of HbA1c levels

<table>
<thead>
<tr>
<th>Normal</th>
<th>Poorer response</th>
<th>Improved response</th>
<th>Poorer response</th>
<th>Improved response</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12057866(G)</td>
<td>1.28 [1.07, 1.19]</td>
<td>1.08 [1.07, 1.19]</td>
<td>1.05 [0.83, 1.15]</td>
<td>1.03 [0.82, 1.16]</td>
</tr>
<tr>
<td>rs6111695(G)</td>
<td>0.85 [1.08, 1.06]</td>
<td>0.85 [1.08, 1.06]</td>
<td>0.85 [1.08, 1.06]</td>
<td>0.85 [1.08, 1.06]</td>
</tr>
<tr>
<td>rs499602(G)</td>
<td>0.94 [1.08, 0.95]</td>
<td>0.94 [1.08, 0.95]</td>
<td>0.94 [1.08, 0.95]</td>
<td>0.94 [1.08, 0.95]</td>
</tr>
<tr>
<td>rs2945532(T)</td>
<td>0.87 [1.05, 0.88]</td>
<td>0.87 [1.05, 0.88]</td>
<td>0.87 [1.05, 0.88]</td>
<td>0.87 [1.05, 0.88]</td>
</tr>
<tr>
<td>rs1119982(A)</td>
<td>1.21 [1.11, 1.31]</td>
<td>1.21 [1.11, 1.31]</td>
<td>1.21 [1.11, 1.31]</td>
<td>1.21 [1.11, 1.31]</td>
</tr>
<tr>
<td>rs436357(A)</td>
<td>1.15 [1.04, 1.25]</td>
<td>1.15 [1.04, 1.25]</td>
<td>1.15 [1.04, 1.25]</td>
<td>1.15 [1.04, 1.25]</td>
</tr>
<tr>
<td>rs3586655(T)</td>
<td>1.15 [1.04, 1.25]</td>
<td>1.15 [1.04, 1.25]</td>
<td>1.15 [1.04, 1.25]</td>
<td>1.15 [1.04, 1.25]</td>
</tr>
<tr>
<td>rs1161655(G)</td>
<td>1.17 [1.07, 1.28]</td>
<td>1.17 [1.07, 1.28]</td>
<td>1.17 [1.07, 1.28]</td>
<td>1.17 [1.07, 1.28]</td>
</tr>
<tr>
<td>Age (&gt;9 years)</td>
<td>1.05 [1.04, 1.15]</td>
<td>1.05 [1.04, 1.15]</td>
<td>1.05 [1.04, 1.15]</td>
<td>1.05 [1.04, 1.15]</td>
</tr>
<tr>
<td>Body Weight (140 kg)</td>
<td>1.05 [1.04, 1.15]</td>
<td>1.05 [1.04, 1.15]</td>
<td>1.05 [1.04, 1.15]</td>
<td>1.05 [1.04, 1.15]</td>
</tr>
<tr>
<td>Serum Creatinine (0.6 mg/dL)</td>
<td>0.98 [1.07, 1.09]</td>
<td>0.98 [1.07, 1.09]</td>
<td>0.98 [1.07, 1.09]</td>
<td>0.98 [1.07, 1.09]</td>
</tr>
</tbody>
</table>

Improvement From Baseline (1 year)

0.80 1.00 1.20 1.40

Improvement From Baseline (5 years)

-0.50 0.00 0.10 0.20

UCSF
Genetic Interactions between CSMD1, SLC22A2, and WWOX Impact the Dynamics of HbA1c

Simulated median (95% CI) of HbA1c levels

- No-response SNPs
  - CSMD1 rs2617102(CC) + rs2954625(TT)
  - Normal No minor alleles

- Protective SNPs
  - SLC22A2/WWOX rs316009(TT) + rs7500549(CC)
  - Normal No minor alleles

Blue shade with solid line: Simulated median for patients carrying no minor alleles with 5th and 95th confidence interval
Red/green shade with dashed line: Simulated median for patients carrying minor allele(s) of labeled gene(s) with 5th and 95th confidence interval of median
<table>
<thead>
<tr>
<th>Description</th>
<th>BASE MODEL</th>
<th>FULL MODEL</th>
<th>FINAL MODEL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objective function value</strong></td>
<td>0.212.14</td>
<td>0.01948</td>
<td>0.0134.225</td>
</tr>
<tr>
<td><strong>OFV Difference from Base Model</strong></td>
<td>0</td>
<td>-15.58</td>
<td>-27.91</td>
</tr>
</tbody>
</table>

### Base Model Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>BASE MODEL</th>
<th>FULL MODEL</th>
<th>FINAL MODEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate</td>
<td></td>
<td>2.12</td>
<td>2.12</td>
<td>2.12</td>
</tr>
<tr>
<td><strong>Half-life of Effect</strong></td>
<td>40</td>
<td>42</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Proportional Error</td>
<td>0.097</td>
<td>0.097</td>
<td>0.097</td>
<td></td>
</tr>
<tr>
<td><strong>Additive Error</strong></td>
<td>0.1 Fixed</td>
<td>0.5 Fixed</td>
<td>0.5 Fixed</td>
<td></td>
</tr>
<tr>
<td><strong>Effect</strong></td>
<td>0.133</td>
<td>0.16</td>
<td>0.135</td>
<td></td>
</tr>
<tr>
<td>Disease Progression</td>
<td>8.2</td>
<td>2.34</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Scales on Effect</td>
<td>2.38</td>
<td>2.34</td>
<td>2.37</td>
<td></td>
</tr>
<tr>
<td>Chisq</td>
<td>0.204</td>
<td>0.25</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

### Covariates on Effect Magnitude

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight</strong></td>
<td>-0.00264</td>
</tr>
<tr>
<td>Average Serum Creatinine</td>
<td>0.274</td>
</tr>
<tr>
<td><strong>Covariates on Disease Progression</strong></td>
<td>-0.0161</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td>0.326</td>
</tr>
</tbody>
</table>

### Parameter Variability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BASE MODEL</th>
<th>FULL MODEL</th>
<th>FINAL MODEL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>16.90%</td>
<td>16.90%</td>
<td>16.90%</td>
</tr>
<tr>
<td>Disease Progression</td>
<td>2.58%</td>
<td>100%</td>
<td>222.45%</td>
</tr>
<tr>
<td>Effect Magnitude</td>
<td>76.00%</td>
<td>74.90%</td>
<td>74.90%</td>
</tr>
</tbody>
</table>
Summary & Next steps

1. The onset of observable disease progression is approximately 1 year.

2. HbA1C levels increase at a rate of 0.1% [0.04%-0.16%] vs 0.16% [0.08%-0.22%] HbA1c/year for responders/non-responders.

2. **Joint PKPD, Pharmacogenomics and Bioinformatics** approaches revealed 9 variants that accounted for approximately 1/3 of the estimated variability in long term response.

   -> GWAS approaches usually account for up to max 3% of the variance.

3. Two known genes, GSMD1, WWOX, that were previously identified to confer risk to Type 2 Diabetes, and a well known transporter gene SLC22A2 candidates for potential stratified diabetes therapy.

4. **Next step**: Validation on the database from 10000 patients.
Acknowledgements

Kathy Giacomini
Sook Wah Yee
Ron Keizer
Les Benet

Savic Lab @ UCSF

Funding: FDA ORISE, RAP Diabetes Award
NIH Training Grant