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Novel Approaches to Discover Genes Linked to Drug Response
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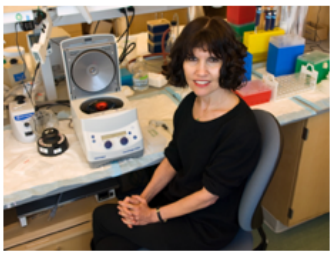

Kathy Giacomini

Membrane Transporters

Pharmacogenomics

Pharmacometrics

Data Science

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ARTICLES nature publishing group

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

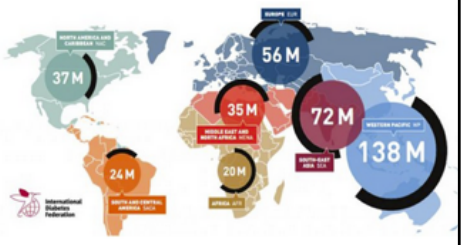
S Goswami[1], SW Yee[1], S Stocker[1], JD Mosley[2], M Kubo[3], R Castro[1], JA Mefford[1], C Wen[1], X Liang[1], J Witte[1], C Brett[4], S Maeda[3], MD Simpson[5], MM Hedderson[6], RL Davis[7], DM Roden[2], KM Giacomini[1] and RM Savic[1]

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

S Goswami[1], SW Yee[1], F Xu[2], SB Sridhar[2], JD Mosley[3], A Takahashi[4], M Kubo[4], S Maeda[4], RL Davis[5,6], DM Roden[3], MM Hedderson[2], KM Giacomini[1] and RM Savic[1]

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

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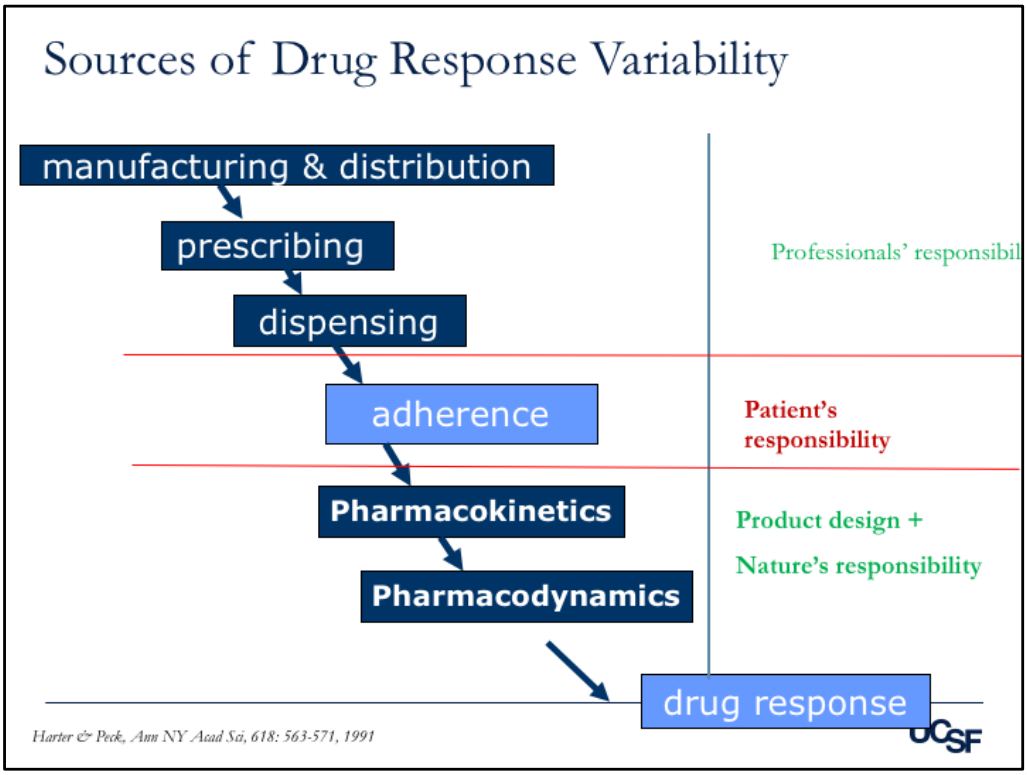
Sources of Drug Response Variability
manufacturing & distribution
prescribing
dispensing
adherence
Pharmacokinetics
Pharmacodynamics
drug response
Professionals' responsibil
Patient's responsibility
Product design +
Nature's responsibility
Harter & Peck, Ann NY Acad Sci, 618: 563-571, 1991
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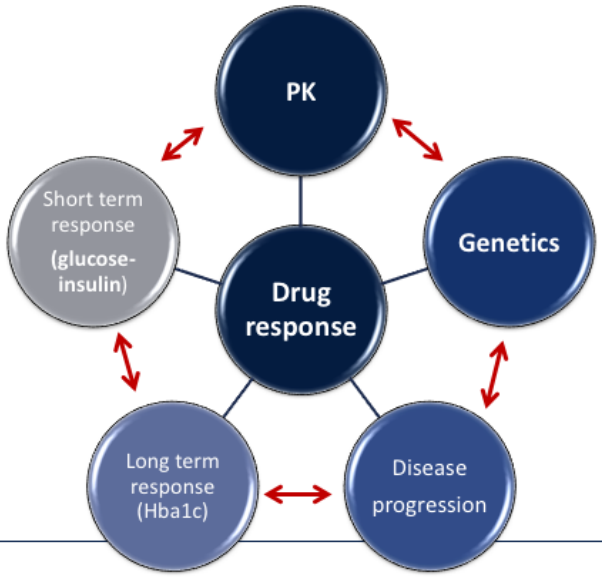
Goal: to understand variability in individual drug response to metformin



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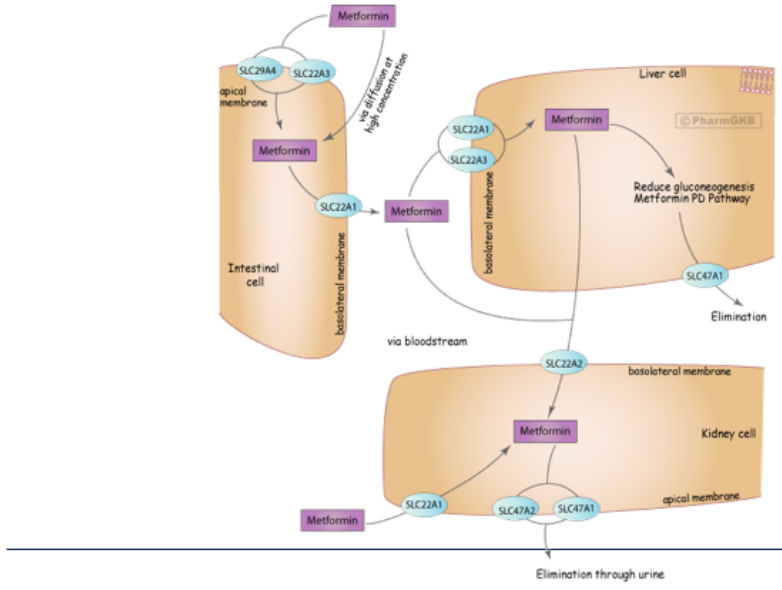
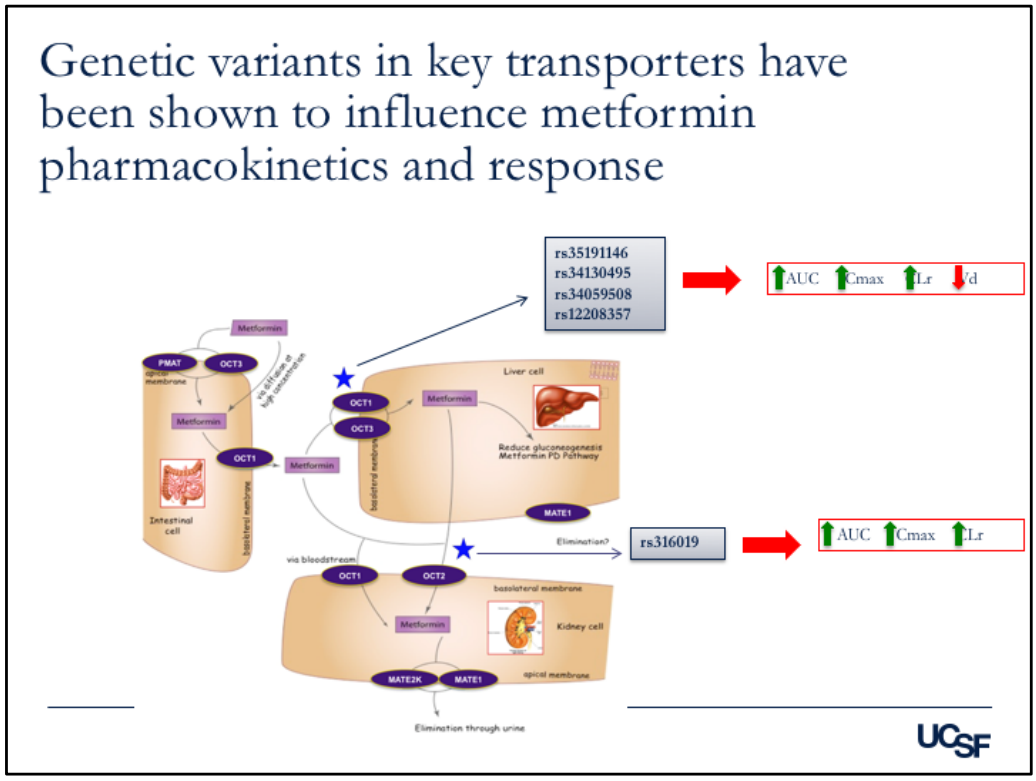
Research Focus

What are key genetic and non-genetic determinants of metformin response?

Candidate Genes that Modulate Metformin Pharmacokinetics

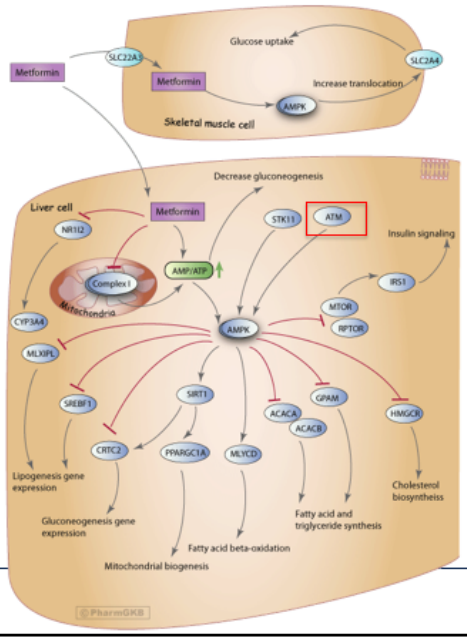


1. Since metformin is not metabolized, membrane transporters in the intestine, kidney and liver play an even very important role in metformin disposition and response
2. Despite metformin's peripheral glucose lowering effects in peripheral tissues such as fat and muscle, the primary site of action is 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

Candidate Genes that Modulate Metformin Pharmacodynamics



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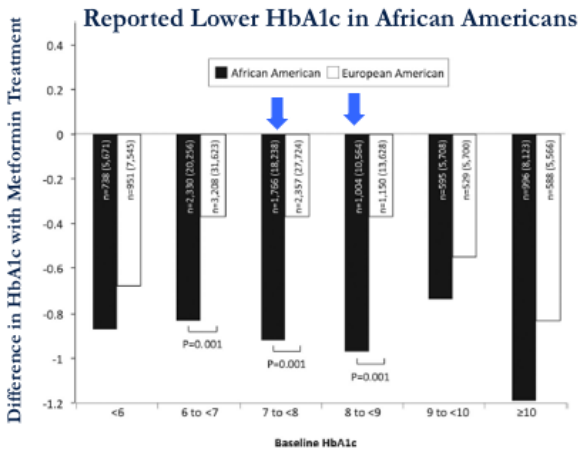
Ethnic Differences in Metformin Response Have Been Reported



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

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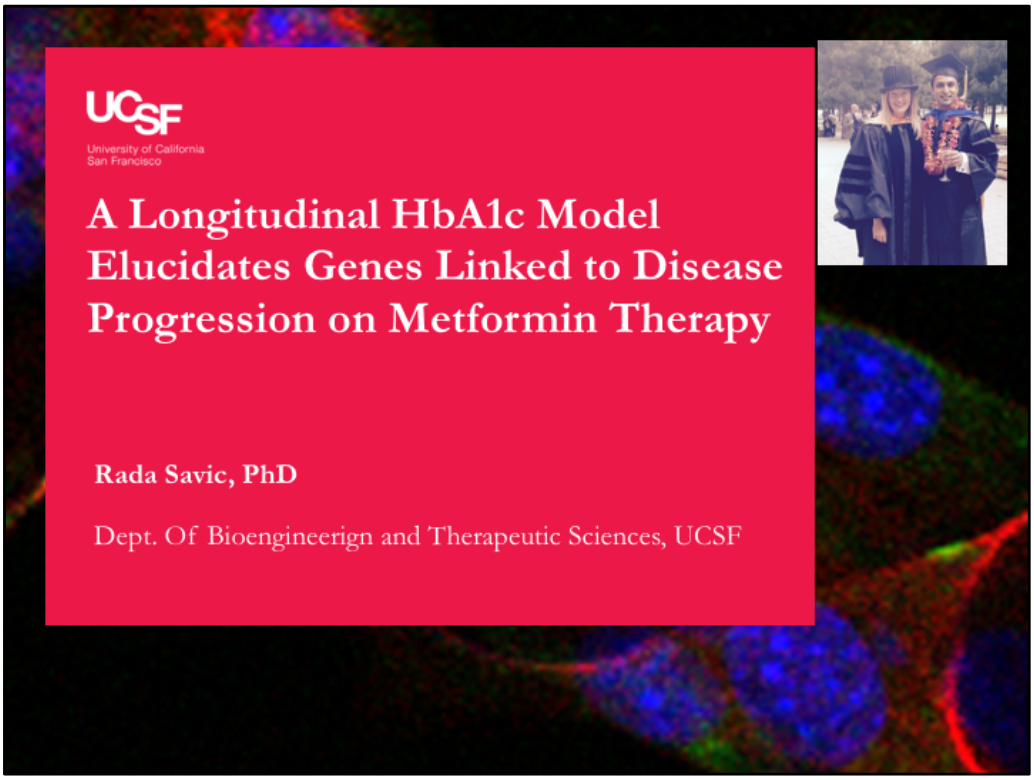
Williams et al, "Differing Effects of metformin on Glycemic Control by Race-Ethnicity, JCEM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A Longitudinal HbA1c Model Elucidates Genes Linked to Disease Progression on Metformin Therapy
Rada Savic, PhD
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Research 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?**

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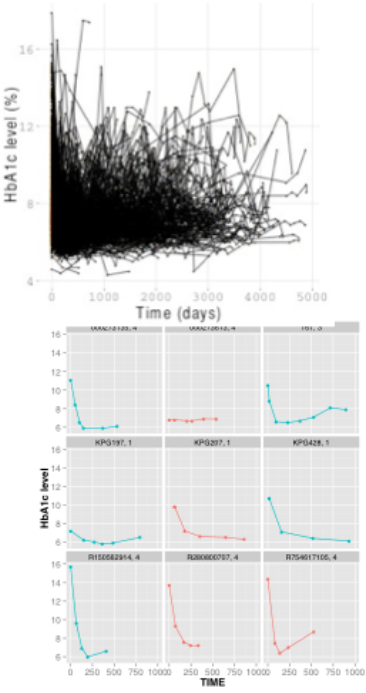

Characteristic	Type 2 Diabetic Patients
N	1056
Males	415
Females	641
European Americans	376
African Americans	665
Asian Americans and Others	15
Quantitative Variable	**Median (range)**
Age (years)	55 (23-90)
Body weight (kg)	96 (34-212)
Baseline HbA1c (%)	7.6 (5.6-17.9)
Metformin daily dose (mg)	1000 (200-2500)
# HbA1c samples/patient	7.5 (1-45)
Years on study	2.78 (0.28-13.5)

Responding well

Disease progression

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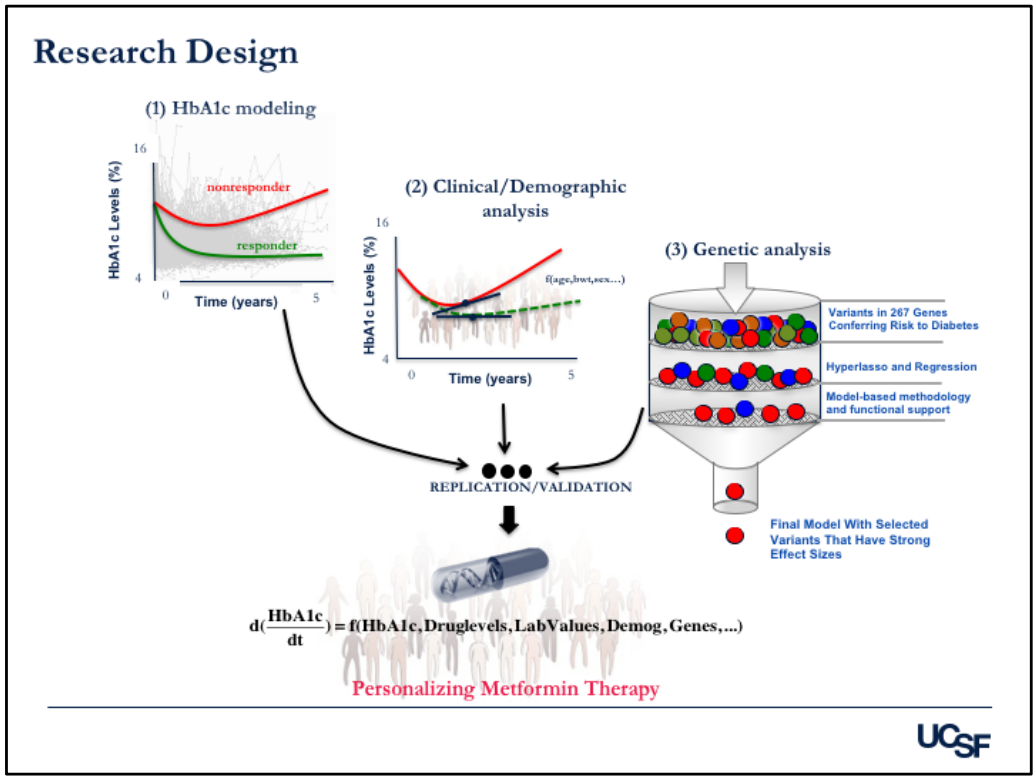
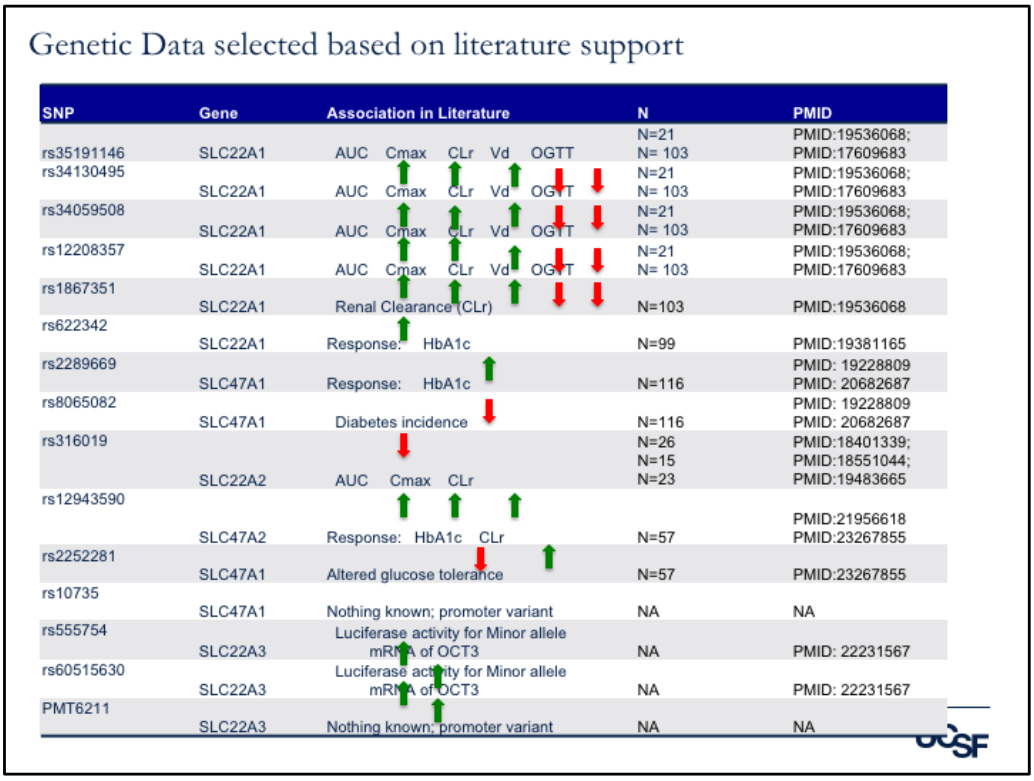


- The workflow for the research is as follows:
- Development of semi-mechanistic HbA1c model in a cohort of approximately 1100 patients with T2D with genomic data.
- SCM, clinical/demographic analysis of model parameters prior to the investigation of genetics.
- Genetic analysis
  - Selection of candidate 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 patters 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 %

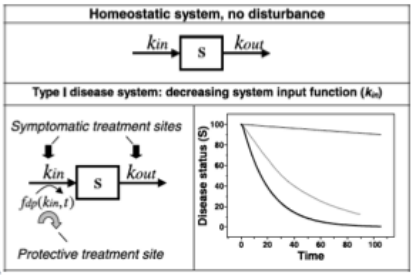
Base Disease Progression Model Development

1.) Turnover HbA1c Model

$$\frac{d(HbA1c)}{dt} = \boldsymbol{Kin} * (1 - Metf\_Effect) - Kout * HbA1c$$

2.) Synthesis rate of HbA1c time dependent

$$\frac{\boldsymbol{d(Kin)}}{\boldsymbol{dt}} = Ksyn * (1 + dispr) - Kloss * Kin$$



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

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Post et al. Disease System Analysis: Basic Disease Progression Models in Degenerative Disease

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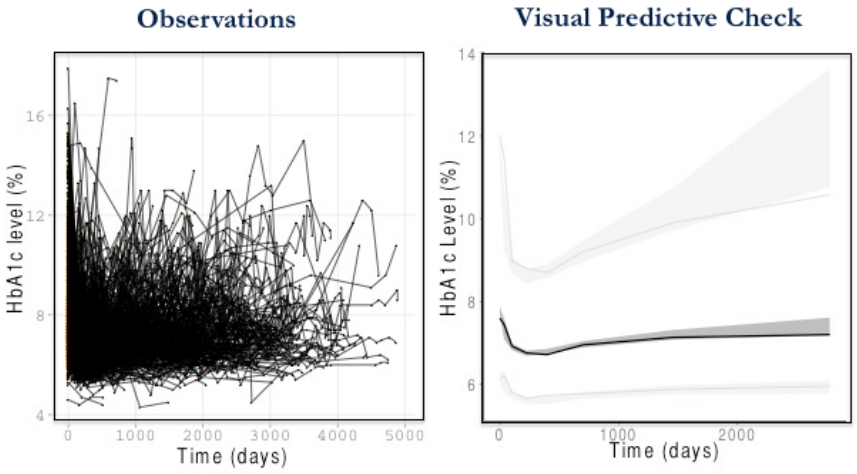
Longitudinal Model Captured Upward HbA1c Trends



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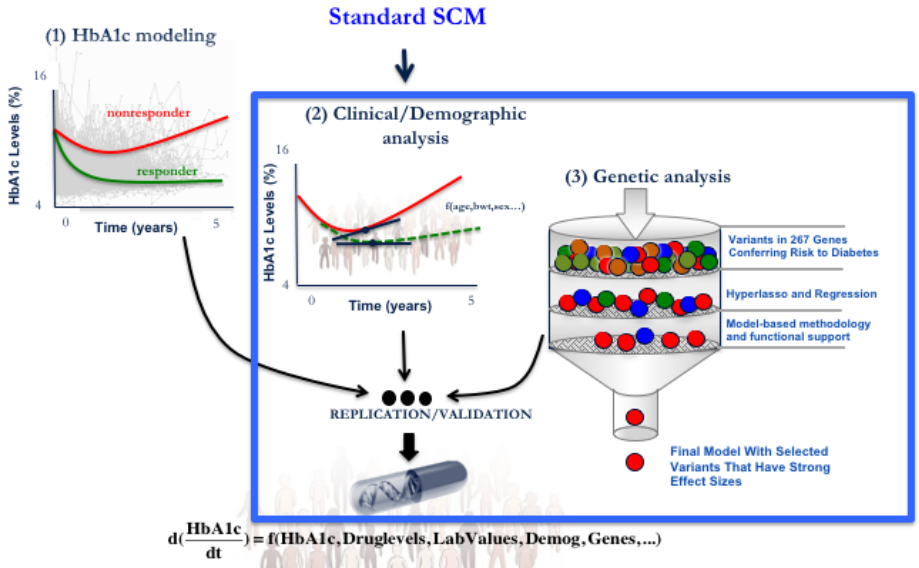
- Source Files:

1) PD_Datasumm.R
2) Vpc_ron.R
3) Further modification illustrator etc.

Research Design



Personalizing Metformin Therapy

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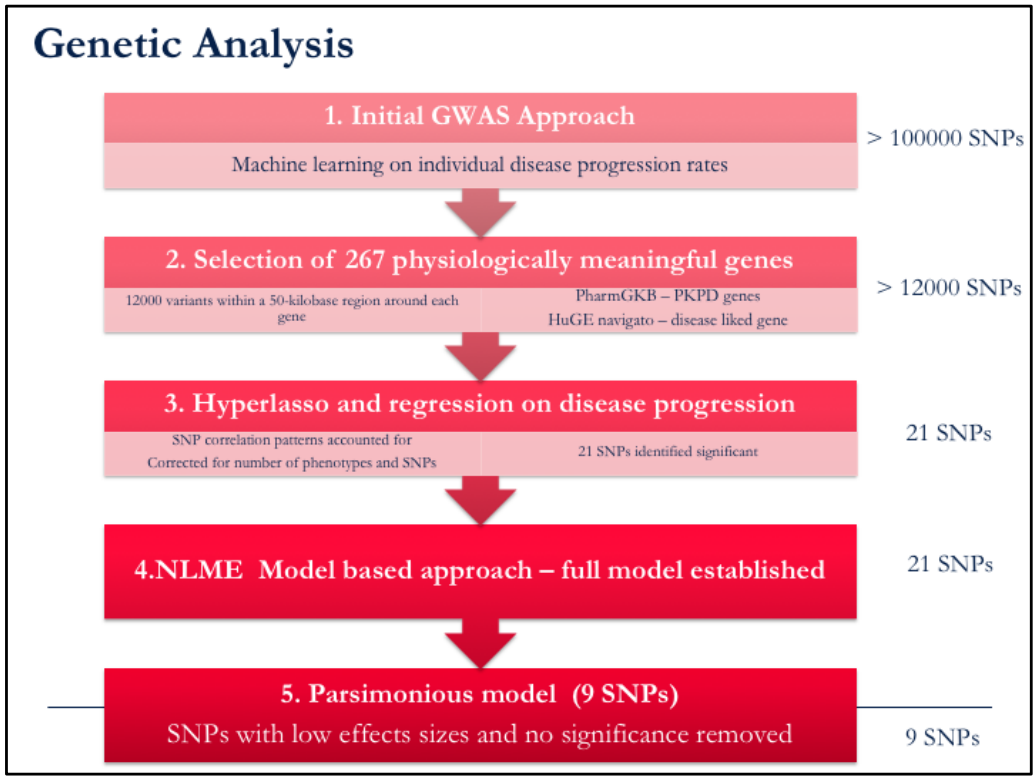

Genetic Analysis
1. Initial GWAS Approach
Machine learning on individual disease progression rates
> 100000 SNPs
2. Selection of 267 physiologically meaningful genes
12000 variants within a 50-kilobase region around each gene
PharmGKB – PKPD genes
HuGE navigato – disease liked gene
> 12000 SNPs
3. Hyperlasso 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 effects sizes and no significance removed
9 SNPs

Significant associations

Table 3 Summary of top genetic variants included in final population pharmacodynamic model of metformin

SNP	Chr	Gene	Minor allele	Major allele	Feature	Model-based *P*-value	Effect size of minor allele on DP parameter	MAF CEU	MAF YRI	Gene functions
rs12907856	15	*VPS13C* - vacuolar protein sorting-associated 13 gene family	G	A	Proximal	0.0003	-0.147 (GA)	0.30	0.30	This gene encodes a member of a vacuolar protein. SNP in this gene was associated with glucose-stimulated insulin response.[41,43]
rs2815022	6	*KCNK16* - Potassium Channel, Two Pore Domain Subfamily K, Member 16	G	A	Proximal	0.009	0.39 (GA)	0.48	0.26	This gene encodes the TALK-1 channel, the most abundant potassium channel in human beta-cells and it modulates beta-cells electrical excitability, second-phase insulin secretion and glucose homeostasis.[44,45]
rs2617102	8	*CSMD1* - CUB And Sushi Multiple Domains 1	C	A	Intron	0.02	0.717 (CA)	0.19	0.20	This gene encodes an integral membrane protein with unknown molecular function. SNP in this gene was associated with congenital hyperinsulinism of infancy.[46]
rs2954625	8		T	C	Intron	0.029	0.23 (TC)	0.21	0.46	
rs316009	6	*SLC22A2* - Solute Carrier Family 22 (Organic Cation Transporter), Member 2	T	C	Intron	0.04	-0.44 (TC)	0.10	0.08	This is a transporter in the kidney that secretes metformin into the urine. This SNP is in linkage disequilibrium to a non-synonymous variant, A270S (rs316019), which was associated with metformin disposition.[47,48]
rs642887	18	*EMILIN2* - Elastin Microfibril Interfacer 2	A	G	Intron	0.05	-0.42 (AG)	0.14	0.21	An extracellular matrix glycoprotein associated with thrombosis. EMILIN2 is involved in regulating platelet activation important for cardiovascular development,[49,50] whereas EMILIN1 may be involved in regeneration of islets, which could play a role in blood glucose lowering.[51]
rs6982250	8	*SULF1* - Sulfatase 1	T	C	Intron	0.0023	-0.37 (TC)	0.17	0.28	This gene encodes an enzyme, which is involved in modulating growth factor signaling. Data from sulfatase knockout mice showed that it plays a role in diabetic nephropathy.[52]

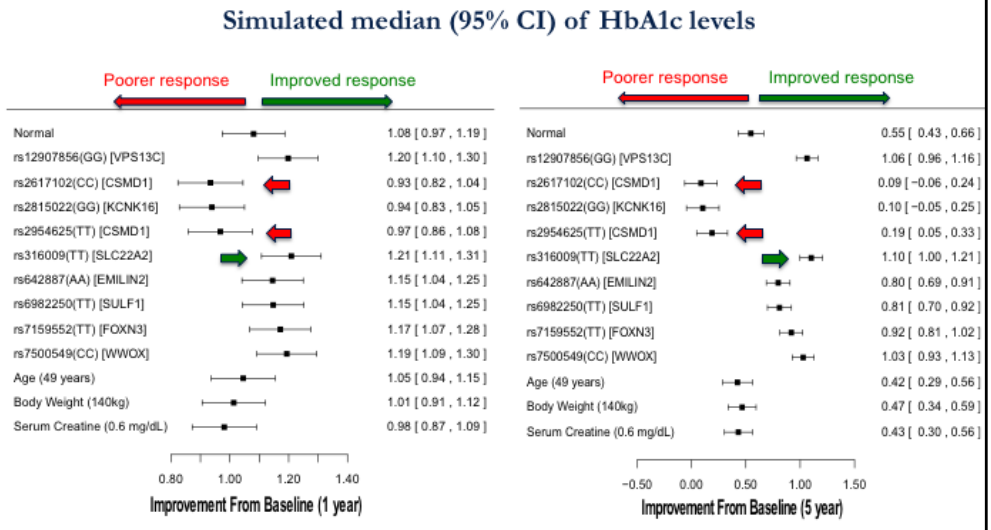
Significant associations

Table 3 Continued

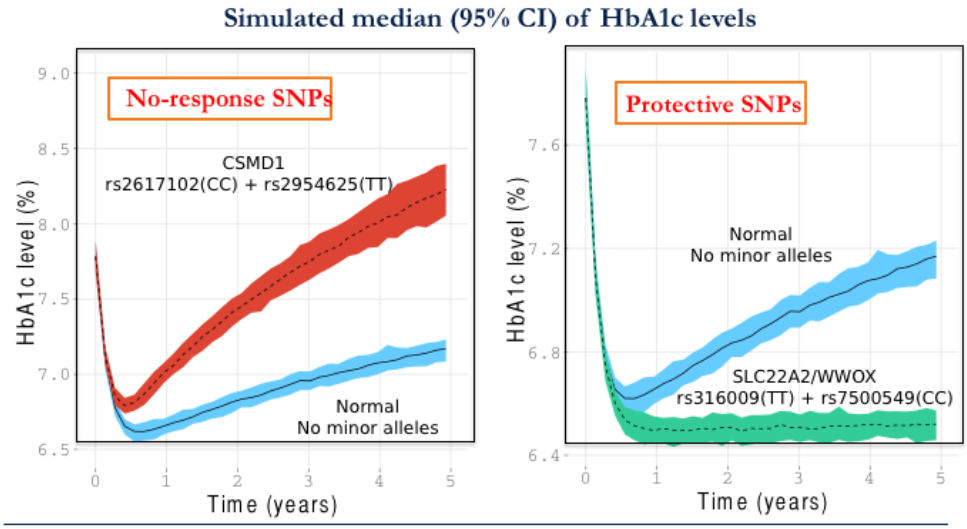
SNP	Chr	Gene	Minor allele	Major allele	Feature	Model-based *P*-value	Effect size of minor allele on DP parameter	MAF CEU	MAF YRI	Gene functions
rs7159552	14	*FOXN3* - Forkhead Box N3	T	G	Proximal	0.009	-0.25 (TG)	0.27	0.35	This is a transcriptional repressor, which plays an important role in cell cycle arrest. This gene is localized in chromosome 14q24.3-q31, which is a locus associated with insulin-dependent diabetes mellitus susceptibility.[53]
rs7500549	16	*WWOX* - WW Domain Containing Oxidoreductase	C	T	Intron	0.029	-0.16 (CT)	0.55	0.20	SNP in this locus was associated with reduced insulin secretion.[54]

P-value = The significance level that resulted from objective function value changes after SNP addition to the demographic corrected base model. Genetic variant rs3160009 significance threshold was determined through model-based analysis of the SNP effect of carriers of either 1 or 2 alleles. MAF, minor allele frequency; DP, disease progression; CEU, Northern Europeans; YRI, African population.

Top Variants From Hyperlasso and Model Based Methodology Were More Influential on HbA1c Levels than Demographic Covariates



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



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 alleles of labeled gene(s) with 5th and 95th confidence interval of median.

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Description		BASE MODEL	FULL MODEL	FINAL MODEL
Objective function value		6212.14	6071.49	6134.225
OFV Difference from Base Model		0	-140.65	-77.915
Base Model Parameters	Baseline	7.74	7.74	7.74
	Half Life of Effect	40.9	42.9	42
	Proportional Error	0.0977	0.0974	0.0975
	Additive Error	0.1 Fixed	0.1 Fixed	0.1 Fixed
	Effect	0.131	0.136	0.135
	Disease Progression	82	234	172
	Scale on Effect	2.38	2.31	2.37
	Kloss	0.204	0.25	0.232
	Scale on Disease Progression	-0.246	-0.408	-0.31
Covariates on Effect Magnitude	Body Weight	-0.00268	-0.00263	-0.00262
	Average Serum Creatinine	0.276	0.26	0.273
	Clinical Site Effect (Vanderbilt)	-0.165	-0.162	-0.165
	Clinical Site Effect (Marshfield Clinic)	-0.303	-0.28	-0.285
	Clinical Site Effect (Kaiser Georgia)	-0.0251	-0.0301	-0.0333
Covariates on Disease Progression	Age	-0.0281	-0.029	-0.029
	rs2815022 (GA)	NA	0.272	0.389
	rs2815022 (GG)	NA	0.966	0.936
	rs316009 (TC)	NA	-0.552	-0.442
	rs316009 (TT)	NA	-0.894	-0.73
	rs2954625 (TC)	NA	0.0342	0.234
	rs2954625 (TT)	NA	0.735	1.13
	rs2617102 (CA)	NA	0.517	0.717
	rs2617102 (CC)	NA	1.06	1.07
	rs6982250 (TC)	NA	-0.357	-0.312
	rs6982250 (TT)	NA	-0.276	-0.559
	rs7159552 (TG)	NA	-0.337	-0.248
	rs7159552 (TT)	NA	-0.595	-0.576
	rs12907856 (GA)	NA	-0.363	-0.147
	rs12907856 (GG)	NA	-0.765	-0.745
	rs7500549 (CT)	NA	-0.217	-0.162
	rs7500549 (CC)	NA	-0.815	-0.588
	rs642887 (AG/AA)	NA	-0.506	-0.429
Parameter Variability	Baseline	16.90%	16.90%	16.90%
	Disease Progression	324%	180%	225.40%
	Effect Magnitude	76.40%	74.80%	74.90%

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, CSMD1, 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 pateints

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Acknowledgements

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