GENOMIC IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF METFORMIN-RESPONSIVE REGULATORY ELEMENTS

Marcelo R. Luizon, W. L. Eckalbar, Y. Wang, S. L. Jones, R. P. Smith, L. Lin, S. Yee, K. M. Giacomini, N. Ahituv

Department of Bioengineering and Therapeutic Sciences and Institute for Human Genetics, University of California San Francisco (UCSF), CA, USA
**Metformin** is the first-line therapy for Type 2 Diabetes, but its mechanisms of action in the liver are not fully known.

Transporters are major determinants of the PK, and their genotypes explain part of the variance in metformin response.

Gong et al., Pharmacogenet Genomics. 2012
Gene regulatory elements can have a major effect on interindividual differences in drug response.

Reviewed findings from 108 Pharmacogenomic GWAS
Hypothesis

• Gene regulatory elements may explain a subset of the variation in metformin response.

General AIM

• We carried out RNA-seq and ChIP-seq on human hepatocytes treated with and without metformin in order to better characterize the mechanisms of action of metformin.
Methods

Primary human hepatocytes

Vehicle (Non-Treated), 8h → RNA-seq

2.5mM Metformin, 8h → RNA-seq
We identified novel metformin-induced transcriptional regulators.
Methods

Primary human hepatocytes

Vehicle (Non-Treated), 8h
- RNA-seq
- ChIP-seq
  - ATF3
  - CBP
  - H3K27ac
  - H3K27me3

2.5mM Metformin, 8h
- ChIP-seq
- RNA-seq
  - ATF3
  - CBP
  - H3K27ac
  - H3K27me3

Candidate regions bearing a conditional ATF3 or CBP occupancy and the H3K27ac active enhancer mark
Results

ChIP-seq for H3K27ac identified metformin-induced peaks near genes associated with *positive regulation of glucose metabolic processes*.

**GREAT analysis:**
FDR=1.44e-2
Methods

Primary human hepatocytes

Vehicle (Non-Treated), 8h
- RNA-seq
- ChIP-seq
  - ATF3
  - CBP
  - H3K27ac
  - H3K27me3

2.5mM Metformin, 8h
- ChIP-seq
  - ATF3
  - CBP
  - H3K27ac
  - H3K27me3
- RNA-seq

Metformin-treated or NT liver cells (Huh-7)

Enhancer sequences? Minimal Promoter Luciferase
Results & Discussion

Enhancer assays for candidate sequences which were **induced** or **repressed** by metformin treatment on liver cells.

Potential molecular mechanisms for the metformin action on gluconeogenesis

Results & Discussion

Metformin induced peak in an intron of the ataxia telangiectasia mutated (ATM) gene near the GWAS lead SNP rs11212617

The minor allele (C) of the most strongly associated SNP, rs11212617 was associated with treatment success in a GWAS for glycemic response to metformin (P=1.9x10^{-7})

Zhou et al., Nat Genet. 2011
Results

rs277070 and rs277072 in the ATM intron are linked to rs11212617

The first and more common haplotype in CEU population has the associated C allele of the GWAS lead SNP

01 - rs227070, T>G
03 - rs227072, A>C
93 – rs11212617, C>A
**Results & Discussion**

Enhancer assays for *sequences with the associated haplotype* showed increased enhancer activity upon metformin response, suggesting that it might lead to elevated expression of *ATM*.

Possible mechanisms of the anti-diabetic effects of ATM

Conclusions

• We found several metformin up-regulated genes, including novel transcription factors using RNA-seq.

• We identified putative enhancer sequences induced by metformin using ChIP-seq.

• We found a metformin treatment success-associated haplotype in the ATM locus that showed increased enhancer activity following metformin treatment.

• Our findings provide for an increased understanding of mechanisms of action of metformin, and for the identification of novel candidates for T2D treatment.
Acknowledgements

Ahituv Lab
• Nadav Ahituv
• Walter L. Eckalbar (Bioinformatics)
• Yao Wang (Functional assays)

Giacomini Lab
• Sook Wah Yee

PMT Grant

Funding (Brazil)