In vitro and in vivo functional testing of SNPs in the 3’UTR of CYP2B6

Kimberly S. Burgess

J. Ipe, M. Swart, IF Metzger, J. Lu, N. Thong, Z. Desta, Y. Liu, R. Pearce, A. Gaedigk, TC Skaar

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Indiana University School of Medicine
Department of Pharmacology & Toxicology, Division of Clinical Pharmacology
Background - CYP2B6

- Genetic variants in CYP2B6 have been shown to alter enzyme activity.
  - CYP2B6*6 and *18 lead to reduced 2B6 activity.

- Reduced metabolizer phenotypes are associated with increased efavirenz toxicity (sleep disorders, hallucinations).

Zanger et al, Front Genet (2013), unpublished data
Background - microRNA

- **microRNA Length**: 17-22 nucleotides
- **Mechanism of action**: bind to the 3’UTR of target mRNA
  - Seed sequence critical for miRNA targeting
- **Scope**: 2588 mature miRNAs identified in humans.
- miRNAs have been predicted and experimentally validated to target many genes, including CYPs.
Hypothesis

• Variability in efavirenz pharmacokinetics are associated with genetic variants that alter miRNA regulation of CYP2B6.
3’UTR variants are associated with CYP2B6 activity \textit{in vivo}

- Retrospectively sequenced the CYP2B6 3’UTR of 200 healthy human volunteers administered a single dose of efavirenz (100/600 mg).

- CYP2B6 activity for 114 volunteers: $C_{\text{max}}$ and $AUC_{0-48\text{hr}}$ (EFV/8OH-EFV).

- Two variants, rs12979270 and rs12979898 variants were in perfect LD in our population.
  - rs70950385

Among normal CYP2B6 metabolizers, the rs70950385 variant is associated with decreased CYP2B6 activity \textit{in vivo}.

(Higher ratio=less metabolism)
rs70950385 variant is associated with CYP2B6 activity *in vitro*

- Sequenced the 3'UTR of 90 liver tissue samples; CYP2B6 activity was determined in microsomal preparations using bupropion as a probe.

Among all human liver samples tested, rs70950385 variant is associated with decreased CYP2B6 activity.
Proposed Mechanism

- rs70950385 (AG→CA) variant predicted to create a miRNA binding site for miR-625-5p and miR-1275.
rs70950385 (CA allele) creates a miRNA binding site

- Created firefly luciferase plasmids containing either wild-type or variant miRNA binding sites.
- Transfected ± predicted miRNA or control miRNA in HepG2 cells.
- Firefly luciferase activity normalized to Renilla luciferase control.

The rs70950385 variant creates miR-1275 binding site.
Conclusions

• The rs70950385 variant decreases CYP2B6 activity in vitro and in vivo.
• Genetic variants in the 3’UTR have the ability to alter enzyme activity by interfering with miRNA binding.
• Genetic variants in the 3’UTR may explain variation in metabolism and drug response.
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Division of Clinical Pharmacology

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