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

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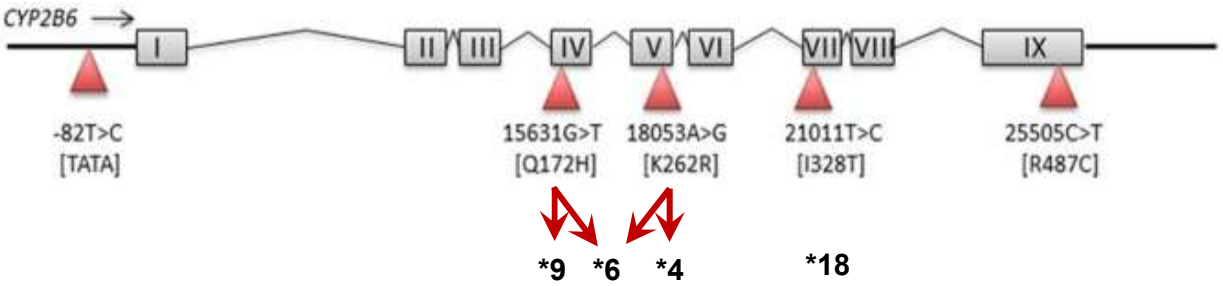
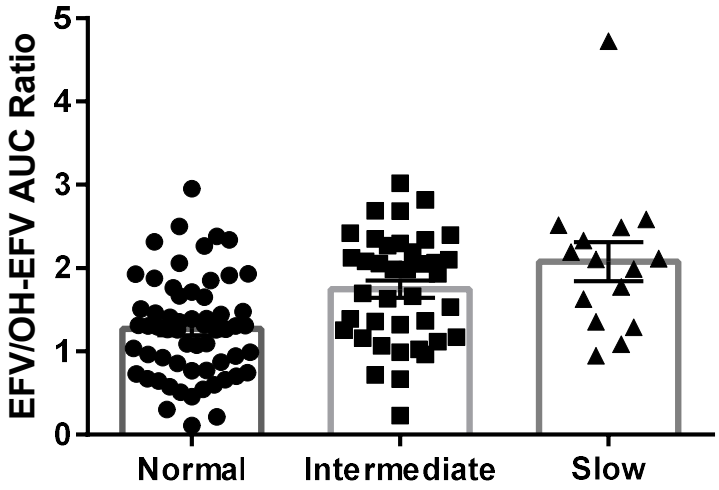
Background - CYP2B6

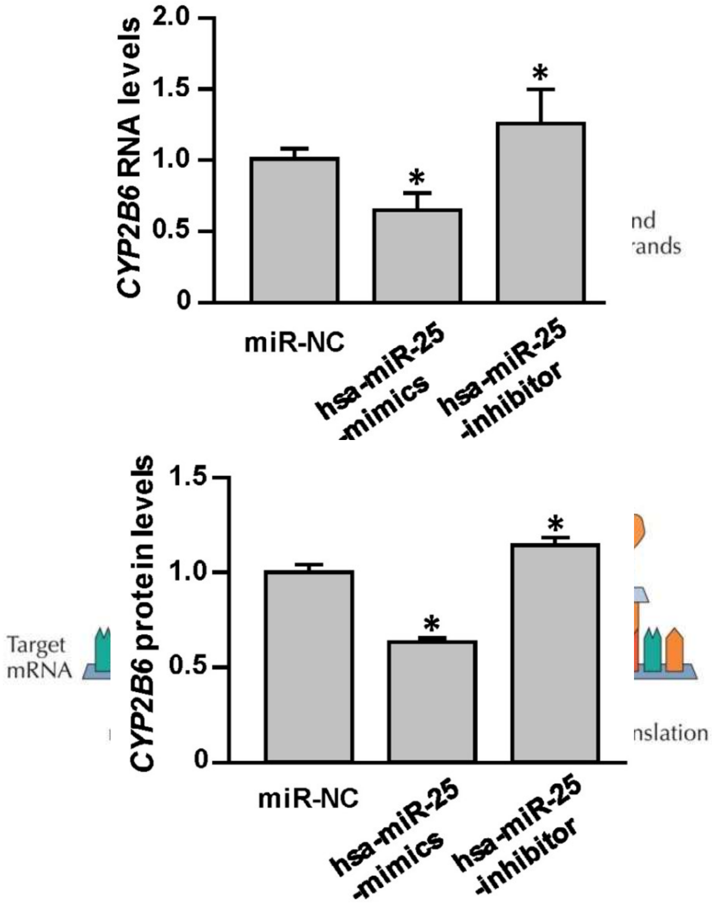


- Genetic variants in *CYP2B6* have been shown to alter enzyme activity.
  - *CYP2B6*6* and **18* lead to reduced 2B6 activity.
- Reduced metabolizer phenotypes are associated with increased efavirenz toxicity (sleep disorders, hallucinations).



Zanger et al, *Front Genet* (2013), unpublished data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.

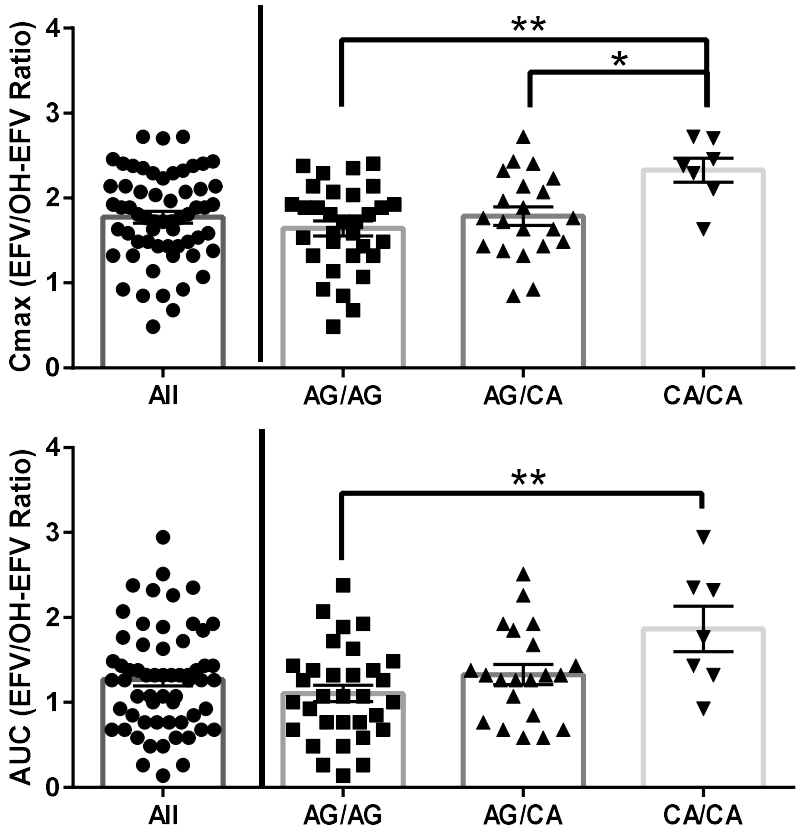
The Cell, Fourth Edition, ASM (2006)
Lin et al, *Biochem Pharm* (2016)

Hypothesis

- Variability in efavirenz pharmacokinetics are associated with genetic variants that alter miRNA regulation of *CYP2B6.*

3'UTR variants are associated with CYP2B6 activity *in vivo*

- Retrospectively sequenced the *CYP2B6* 3'UTR of 200 healthy human volunteers administered a singe dose of efavirenz (100/600 mg).
- CYP2B6 activity for 114 volunteers: Cmax and $AUC_{0\text{-}48hr}$ (EFV/8OH-EFV)
- Two variants, rs12979270 and rs12979898 variants were in perfect LD in our population.
  - rs70950385

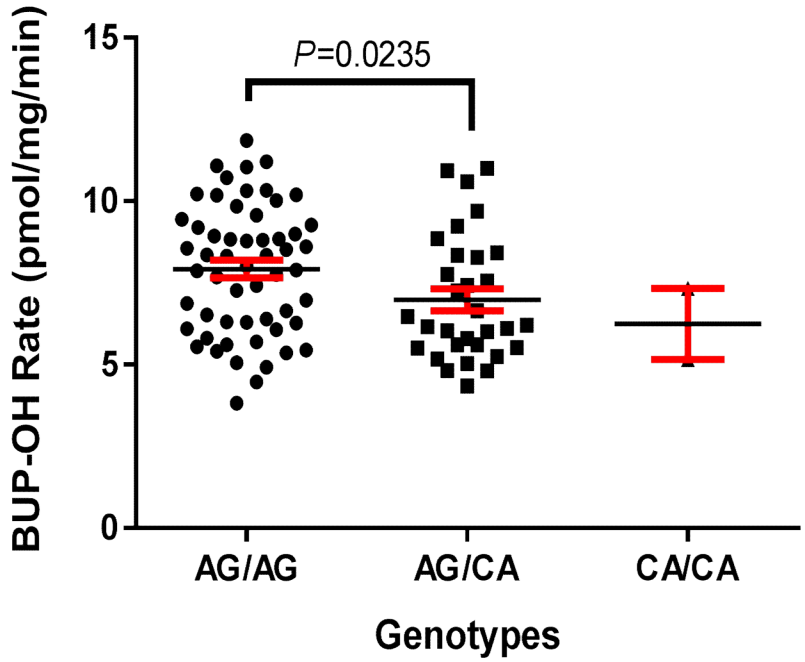


Among normal CYP2B6 metabolizers, the rs70950385 variant is associated with decreased CYP2B6 activity *in vivo*.

(Higher ratio=less metabolism)

rs70950385 variant is associated with CYP2B6 activity *in vitro*

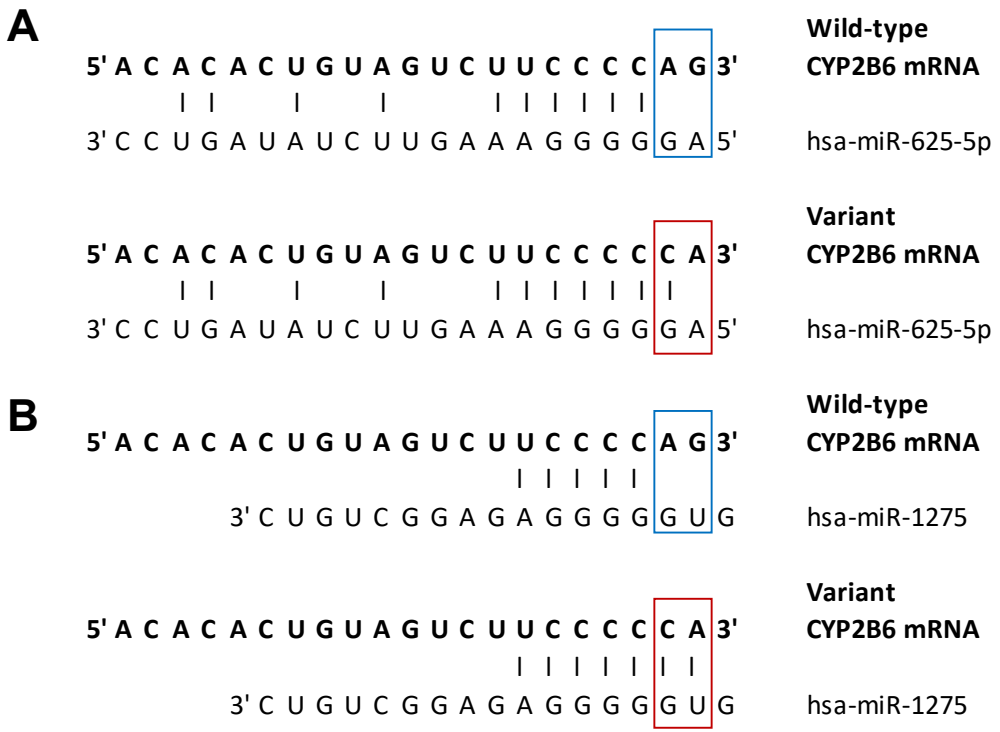
- Sequenced the 3'UTR of 90 liver tissue samples; CYP2B6 activity was determined in microsomal preparations using bupropion as a probe.



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

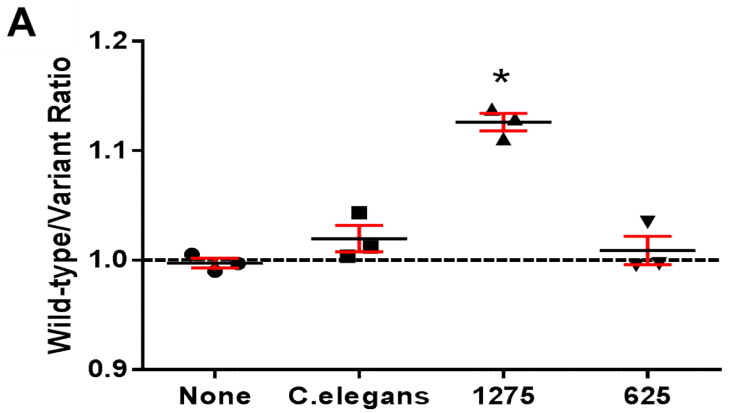
Proposed Mechanism

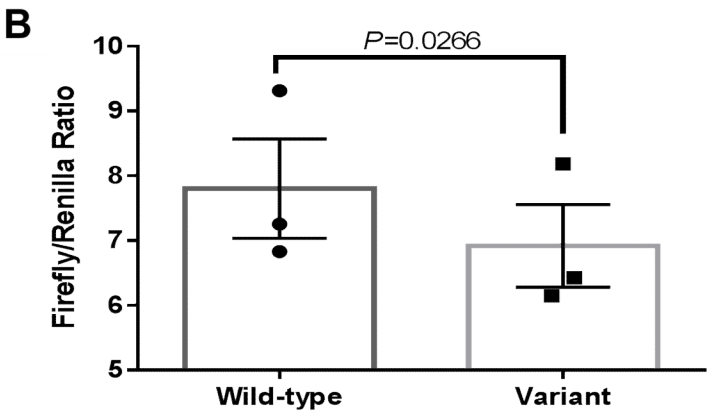
- rs70950385 (AG→CA) variant predicted to create a miRNA binding site for miR-625-5p and miR-1275.



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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