Searching for the $G_{516}T$ Polymorphism on the CYP2B6 gene in HIV-1Patients

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Gene CYP2B6

The CYP2B6 $G_{516}T$ polymorphism has a major impact on the level of hepatic metabolism.

Objectives

Identify the $G_{516}T$polymorphism located at exon 4 of the CYP2B6 gene in HIV-1 or HVC/HBV co-infected patients.

Review

The CYP2B6 gene has been mapped in the context of chromosome 19, and encodes a member of the enzyme superfamily, cytochrome P450 monooxygenase (CYP450), which is located in the endoplasmic reticulum.

This protein catalyses several reactions involved in drug metabolism.

Many genetic disorders have been reporte as having influence on management, metabolism, distribution and elimination of drugs used in HIV/AIDS therapy (1).

The $G_{516}T$ polymorphism in the CYP2B6 gene, present in exon 4, shows a major impact with clinical relevance on the level of hepatic metabolism, with consequently translates into changes in the respective plasmatic concentrations of drugs (2).

This genetic variant results in the substitution of a guanine for a thymine at nucleotide 516 of the coding sequence wich leads to the amino acid substitution at position 172, glutamine for histidine (Q172H) and whose role in enzyme activity is still not clear.

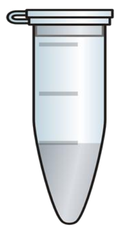
This has been described as a "non-sense" polymorphism, affecting the metabolic activity by alteration of substrate binding or aberrant splicing, leading to a decreased amount of normal mRNA and thus reducing the levels of functional protein (3).

Materials and Methods

Sample

1ml blood of 27 patients infected with HIV1 or co-infected with HBV/C

Extraction of Genomic DNA



Cell lysis
↓
Binding
↓
Wash
↓
Elution

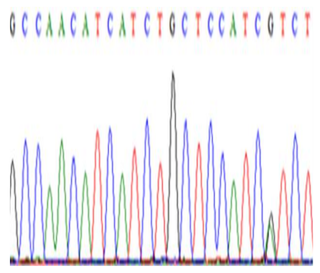
100µl DNA

PerfectPure DNA Blood Kit (5Prime)

PCR Amplification

- Primer Forward 5´TGTTGTAGTGAGAGTTCTG3´
- Primer Reverse 3´GATAGGGACAGAGATGGCAG5´

Sequencing Analyses



- BLAST (Basic Local Alignment Search Tool)
- BioEdit

Results

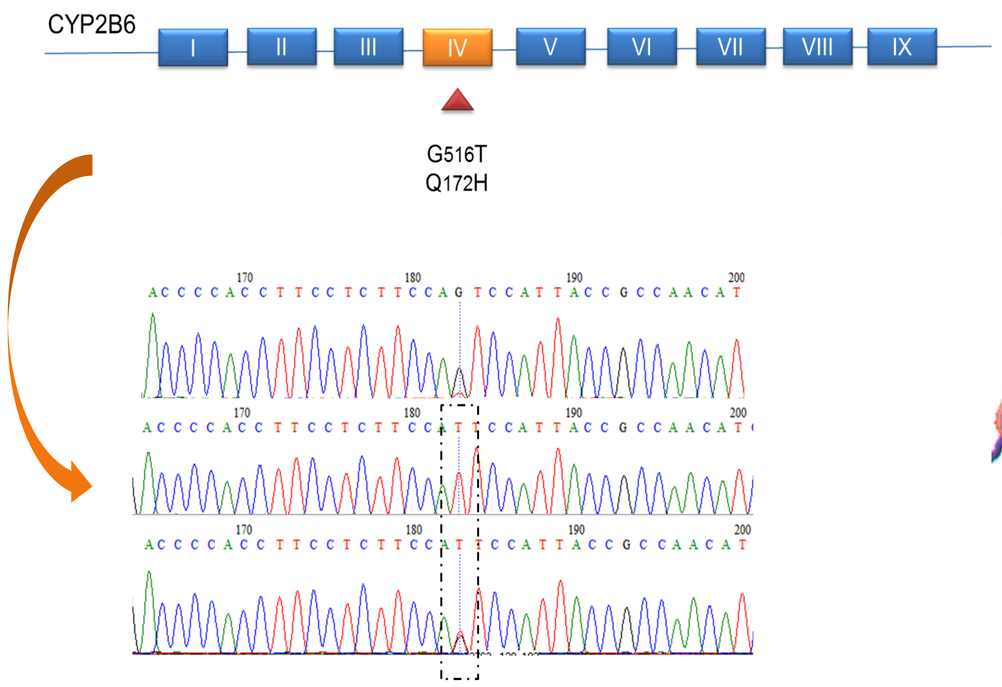


Fig. 1 - Gene CYP2B6 represented by exons and introns, the red triangle indicates the location of the SNP $G_{516}T$, the substitution leads to $Glu_{172}His$. The results of sequence alignment reveal the site of nucleotide substitution.

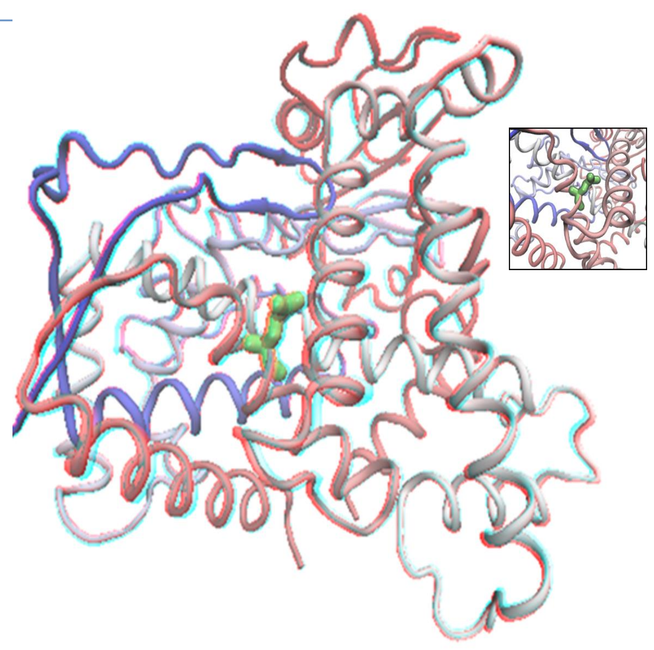


Fig. 2 - 3D structure of native CYP2B6 protein, the SNP G516T is illustrated in green. Image acquired by VMD.

Patients	CYP2B6 Polymorphism G516T
2	G – G
8	G – T
17	T – T

25 patients present the $G_{516}T$ polymorphism on the CYP2B6 gene.

Conclusion

The G516T genetic variant can occur in heterozygosity (GT) or homozygosity (TT). Subjects with homozygous T allele (TT) exhibit enzymatic activity at lower levels when compared to individuals homozygous for the G allele (GG). Individuals heterozygous (GT) have intermediate levels (4).

Our results allow us to identify the presence of polymorphic mutation CYP2B6 $G_{516}T$ in 25 patients, 8 of them carry the heterozygous genotype GT and 17 carry homozygous genotype TT.

In the future, therapeutics for HIV can be set in compliance with the patient's genetic information, leading to reduced toxicity levels and improved patient compliance to treatment.

References

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2. Aberrante Splicing Caused by Single Nucleotide Polymorphism c.516G_T [Q172H], a Marker of CYP2B6*6, Is Responsible for Decreased Expression and Activity of CYP2B6 in Liver. Hofmann *et al*, 2008. *The Journal of Pharmacology and Experimental Therapeutics*
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