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Entitled

MOLECULAR AND BIOCHEMICAL ANALYSIS OF CYTOCHROME P450 2C19 AND 2D6

by

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Abstract

Pharmacogenomics (PGx) is a relatively new field of study. It links genetics to pharmacology since it deals with the influence of the genetic makeup of the individual on their ability to respond to specific medications. Some of the most important genes in this field, dubbed very important pharmacogenes (VIPs), belong to the cytochrome P450 (CYP) superfamily of drug metabolizing enzymes. The two members of this family that are the main focus of this thesis are CYP2C19 and CYP2D6. They play major roles in the metabolism of numerous medications, and it is therefore imperative that variations within those genes in various populations are detected in an efficient and timely manner, as well as establishing their impact on the function of the encoded enzymes.

In this study, seven variants in CYP2C19 that have been detected in the Emirati population were analysed to determine their potential effects on the enzyme's activity. Major aims of this thesis were to perform in silico analysis, generate the seven target variants in mammalian expression vectors, express them in mammalian cell lines, and measure the resulting enzyme's activities. The variants were successfully generated by site-directed mutagenesis using two expression vectors as templates and were expressed in COS and Hek293T cells. However, the enzymatic activity tests using lysates from those cells were inconclusive and, therefore, further analysis, perhaps using different kits, are needed to further establish the impact of those variants.

For CYP2D6, a long-range PCR-based technique was optimized and utilized to detect gene copy numbers using DNA extracted from blood samples isolated from psychiatric patients to determine their CYP2D6 metabolic status. In particular, this test was used to detect if the patients have a deletion star allele (CYP2D6*5) or duplication of CYP2D6. The results indicate that this approach could be used to implement genetic testing to determine CYP2D6 copy numbers in patients requiring medication metabolized by this enzyme.

Keywords: Pharmacogenomics (PGx), cytochrome P450s (CYP), CYP2C19, CYP2D6, enzyme activity, personalized medicine.