## K32 The Role of Cytochrome P450 2B6 (CYP2B6) Genetic Polymorphisms in Unexpected Methadone Fatalities

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After attending this presentation, attendees will understand the potential role of cytochrome P450 2B6 (CYP2B6) genetic polymorphisms in methadone metabolism and overdose. CYP2B6 is one of the key enzymes involved in the metabolism of methadone. The research presented explores the association of specific Single Nucleotide Polymorphisms (SNPs) in the CYP2B6 gene to methadone metabolism and whether the presence of such SNPs increases the likelihood of fatal methadone intoxication in the Caucasian population. This research may provide insight as to why some individuals succumb to methadone intoxication.

This presentation will impact the forensic science community by serving as a key aspect for the development of genetic testing to be used for screening patients placed on methadone therapy. The hypothesis of this study is that one or more SNPs located within the CYP2B6 gene contributes to or is linked to the methadone poor metabolizer phenotype and identifying such individuals before treatment may decrease the number of fatalities due to methadone intoxication.

West Virginia and Kentucky ranked in the top ten states for increases in fatal methadone overdoses in 1999-2005. CYP2B6 plays a significant role in stereo-selective metabolism of (S)-methadone to 2-ethyl-1,5-dimethyl-3,3-diphenylpyrolidine (EDDP), an inactive methadone metabolite. Elevated (S)-methadone can cause cardiotoxicity by prolonging the QT interval of the heart's electrical cycle. Large inter-individual variability in the pharmacokinetics of methadone causes ambiguity in the relationship between dose, plasma concentrations, and side effects. While other pharmacogenetic studies have been conducted involving methadone-related intoxications, these studies did not include methadone-only cases. Rather, the studies used cases involving mixed drug intoxications and/or had small sample sizes.

The current study examines 228 cases involving fatal methadone intoxications, 136 of which are attributed to methadone alone. A control group of 268 cases without methadone detected was also studied. Genomic DNA was extracted from blood stain cards prepared during autopsies performed at the West Virginia and Kentucky Offices of the Chief Medical Examiner using the QIAamp® DNA Micro DNA Kit following the manufacturer's protocol for dried blood spots with modification for greater DNA concentration. SNP genotyping was achieved using TaqMan® SNP Genotyping kits from Life Technologies™ following the manufacturer's protocols for real-time polymerase chain reaction and allelic discrimination analyses. Allelic and genotypic frequencies were determined for the six SNPs on the CYP2B6 gene (rs2279344, rs3211371, rs3745274, rs4803419, rs8192709, and rs8192719) in the fatalities attributed to methadone intoxication (n=228) and the control (n=268) cases.

The frequency distributions for each of the six SNPs genotyped were in Hardy-Weinberg equilibrium based on a Chi-squared goodness of fit test with two degrees of freedom. The frequency of minor allele carriers was significantly different between the observed genotypic frequencies and general population ($p < 0.05$) for all the SNPs, except rs8192709, in the West Virginia/Kentucky population for all of the methadone overdose cases and the methadone-only overdoses. A Tukey test showed there was a significant difference in the mean blood methadone concentrations (mg/L) between the variants for SNP rs3211371 in the methadone only fatalities ($p=0.003$). This was not observed in the other SNPs genotyped, but there was an apparent enrichment of the minor allele in the methadone cases.

The results of this study indicate that SNP rs3211371 on the CYP2B6 gene is likely linked with a slow-metabolizer phenotype for methadone and may contribute to unexpected methadone fatality. An individual carrying at least one copy of the minor allele for any of the SNPs studied could have a poor methadone-metabolizer phenotype leading to an increased risk of fatal methadone intoxication.

**Methadone, Genetic Polymorphisms, CYP2B6**