THE FUTURE OF THE *CYP2D6* MOLECULAR AUTOPSY USING TRAMADOL-EXPOSED INDIVIDUALS

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In situations where traditional medico-legal autopsy is negative (i.e., cause (CoD) and/or manner of death (MoD) is undetermined) genotypes of the highly polymorphic pharmacogene *CYP2D6* and routine toxicology data may provide better resolution of CoD/MoD. The *CYP2D6* genotype is converted to an activity score (AS; a qualitative measure of enzyme activity) and a metabolizer phenotype: poor (PM), intermediate (IM), extensive/normal (EM/NM), or ultrarapid (UM). Studies characterizing the associations between specific *CYP2D6* star (*) alleles (i.e., the collection of causal single nucleotide [SNPs] and/or insertion/deletion [INDELs] polymorphisms within the gene region) and idiosyncratic responses typically rely on targeted genotyping of polymorphisms known *a priori*. While insightful for many cases, Genome Wide Association Studies and SNP-targeted massively parallel sequencing (MPS) lack the ability to identify novel and/or rare polymorphisms. Recent studies characterized many additional polymorphisms in *CYP2D6* at the population level that are ignored for * allele characterization but are flagged as damaging by variant effect predictors. It has been shown *in silico* that inclusion of these polymorphisms may decrease the AS of 11% of the healthy population. Herein, molecular autopsy of tramadol-exposed Finns was performed to evaluate the association between previously overlooked *CYP2D6* polymorphisms, and full-gene haplotypes, and toxicological observations. Long-range PCR was performed using KAPA Biosystems Fragment A, B, and H primers, custom primers targeting the 3' and 5' untranslated and promoter regions, and KAPA HiFi HotStart polymerase. Fragments A, B, and H were analyzed on the Agilent 2200 Tape Station to identify copy number variation and hybrid genes. Libraries of the custom PCR product were made using the Nextera XT DNA Library Preparation Kit and sequenced on the Illumina MiSeq Desktop Sequencer. Associations are reported between previously unidentified polymorphisms and the toxicologically-determined concentration of O-desmethyltramadol. Empirical data comparing targeted and full-gene haplotypes confirm *in silico* predictions of decreased AS for a subset of individuals when considering more comprehensive genotyping. These data serve as a foundation for empirical evaluation of the full-gene region for more comprehensive molecular autopsies. This presentation will describe the molecular autopsy process and how using *CYP2D6* and toxicological data may provide greater resolution of CoD and/or MoD.