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Medicine Science International Medical Journal

REVIEW ARTICLE

Medicine Science 2022;11(4):1738-44

Pharmacogenomics perspective for forensic toxicology: A mini review

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Received 10 August 2022; Accepted 24 October 2022
Available online 28.11.2022 with doi: 10.5455/medscience.2022.07.165

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Abstract

Pharmacogenetic and Pharmacogenomics are the study of the association between an individual's genotype and their response to xenobiotics Pharmacokinetic and pharmacodynamics variations can appear at the level of drug-metabolizing enzymes, drug transporters, drug targets or other biomarker genes. Pharmacogenomics can, therefore, be relevant in forensic toxicology. This review presents relevant aspects together with some examples from daily routines. Use of pharmacogenomics in forensic toxicology may add to the understanding of drug toxicity due to genetically predisposed impaired drug metabolism and may provide findings which may be back-extrapolated for the benefits of optimization of antemortem drug therapy.

Keywords: Forensic toxicology, pharmacogenomics, pharmacogenetics, toxicology

Introduction

Forensic toxicology has developed as a forensic science widely used to assist in death investigations, in civil and criminal matters involving drug use, in drugs of abuse testing in correctional settings and custodial medicine, in road and work-place safety, in matters involving environmental pollution, as well as in sports doping [1].

Personalised medicine originates from the necessity to tailor and accommodate the medical intervention to the patient. The core concept of personalised medicine is the consideration of patient-specific factors, ranging from mundane biological details such as age, gender, fitness, ethnicity etc. to much more unexpected variations such as single nucleotide polymorphisms (SNPs). Personalised medicine is one of many aspects of modern medicine that collectively pave the way for profound transformation of not only medicine, but also the likes of forensic sciences, as well as the justice system. These transformations will in turn produce marked effects, although what these effects may be, those involved are only barely beginning to understand and appreciate [2,3].

Although the first descriptions of inherited differences in drug metabolism date back to the 1950s, the advent of the field of pharmacogenomics and its transition from an area of nearly pure theoretical research to a promising medical tool with real-world applications in personalised medicine only became possible after the identification of an increasing number of genetic variations affecting genes that encode drug-metabolizing enzymes, drug transporters, and drug targets [4]. The relationship between personalised medicine and the justice system, especially in the modern times with the emergence of personalised medicin, in contingent on the interpretation of analytical results in both clinical and forensic settings, and this interpretation must take into consideration the genetic makeup of the patients, especially PGx [5-8]. The application of pharmacogenetics and pharmacogenomics allow the researchers to produce entirely new vantage points with which to examine the therapeutic landscape, allowing a priori

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determination of what the dosing and the expected patient response will be, as well as to produce a pre-determined estimate of whether nefarious effects ought to be observed as a result of such medical interventions [9].

In the same vein, a related concept that is also emerging by day is the field of molecular autopsy. Postmortem molecular autopsy focuses on how genetic features influence pharmacokinetics on the individual and population levels and can be used to predict pharmacodynamic outcomes related to cause and/or manner of death [10].

In the recent times, variability of biotransformation within and/or between individuals has been recognized to be as important as the sensitivity and specificity of the analytical methods, quality of the samples and their storage, inherent drug stability, and knowledge of the drug(s) disposition, all of which are now considered as the additional essential co-variables when interpreting drug toxicological data [1,4,11]. Most xenobiotics, such as drugs and toxins that enter the body have to be enzymatically modified before they can be excreted. This biotransformation is performed by drug-metabolising enzymes (DMEs) (e.g. CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) and by conjugation with polar groups (e.g. acetate, sulphate, glutathione, and glucuronic acid). The body's strategy to convert lipophilic substances into more water-soluble and, with that, more readily excreted polar products is either to add a polar, often oxygen-containing group, to the compound or to unmask a polar group on the compound, collectively known as the Phase I reactions (e.g. oxidation, reduction, hydrolysis, alkylation and dealkylation). These reactions can lead either to activation, or inactivation of the compound. Further increases in solubility are often achieved through the conjugation of the compound with a strongly polar conjugant, Phase II reactions (conjugation). There is great inter-individual variability in the response to different drugs. This variability is partly explained by genetic variations (polymorphisms) in DMEs, as well as in drug transporters and receptors [12-16] (Figure 1).

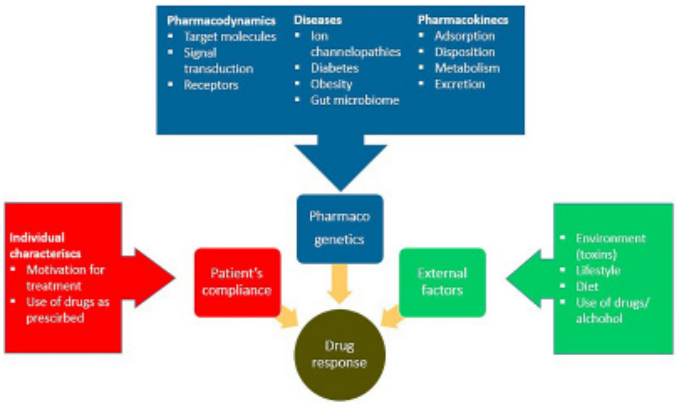

Figure 1. The factors modifying drug response differing individually

Even though the toxicokinetic and toxicodynamic characteristics of a xenobiotic, such as toxicity levels may be well-defined, the interpretation of toxicological data in a forensic toxicology setting is still a difficult undertaking. Such an undertaking may become even more of a challenge if one considers the fact that it is indispensable for the forensic pathologist and/or the forensic toxicologist to define as accurately as possible the cause and manner of death, taking into account numerous elements such as autopsy findings, medical history, and crime scene investigation for the forensic pathologist and, for the forensic toxicologist, co-variables that can affect the identified drug(s) pharmacokinetics and pharmacodynamics. In certain cases, pharmacogenetics can play an important role in the diagnosis, particularly if, for example, an aberrant metaboliser phenotype for the detected drug(s) is identified, leading to the accumulation to toxic levels or therapeutic failure. Thus, pharmacogenetic factors should be taken into consideration in the interpretation of post-mortem drug levels and can be an important new tool in the determination of the cause and manner of death.

The Pharmacogenetics Knowledgebase recognizes 66 very important pharmacogenes (VIPs) including channels/transporters, ABCs, solute carriers, voltage-gated potassium channels, phase I metabolism proteins, phase II metabolism proteins neurotransmitter receptors and opioid receptors. All of these proteins are considered VIPs, but the CYP family (namely CYP450 family 2 subfamily D polypeptide 6 [CYP2D6]), brain transporters, and opioid receptors are perhaps most applicable to the postmortem molecular autopsy [10,17].

In 2002 Jannetto et al. proposed an algorithm for the best use of pharmacogenetic data in forensic toxicology [18]. The algorithm they developed incorporates various co-variables such as acute or chronic toxicity, autopsy findings, site of sample collection, post-mortem interval, concomitant drug use, case/medical history, death scene investigation, and intent. Should a finding of elevated drug(s) and/or metabolite(s) be observed, these findings may then be compared with reference toxicological cases and further investigation may be conducted into the elaboration of additional co-variables, such as toxic drug concentration, high metabolic ratio, drug-drug interactions, and polymorphic drug metabolism [4]. Thus, this article aims to summarise and concatenate used pharmacogenetic strategies of forensic toxicology applications in order to promote understanding of the role of post-modern medicolegal practice. The reviewed data shall not only assist in the conduct of the aforementioned investigations, but also provide fresh insights into the participation of pharmacogenomics in clinical and forensic toxicology settings.

Fundamentals of Pharmacogenetics-Pharmacogenomics

Genetic variability in drug disposition arises due to the presence of molecular alterations in the genes encoding drug-metabolising enzymes, drug transporters and drug targets/receptors. Such aberrations in turn effect modifications in the specifics of drug dependency phenotypes, through the alterations of gene products and/or alteration of gene transcription. Genetic variation is a ubiquitous hallmark of the human genome and two major classes of such variation have been described to be of particular interest in genes of pharmacogenomic interest, the two classes being single nucleotide polymorphisms (SNPs) and complex genomic rearrangements [4,19].

According to the long-standing central paradigm of molecular genetics, the genetic information encoded in the DNA is transferred, through transcription, onto RNA, most prominently mRNA. The information in mRNA is transferred, in turn, through translation,

into biosynthesised proteins. These proteins may be drug-metabolising enzymes, transporters, and receptors. As a result, DNA variations partially determine enzyme activity, transporters, and receptor sensitivity. These genotypic variations, also known as polymorphisms, form the basic promise of pharmacogenetics. Although these polymorphisms exist independently of the drugs, they can (and in most cases do) constitute a major factor affecting drug response. The average human genome, comprising of 3 x10^9 (3 billion) base pairs, contains 10^7 (10 million) single nucleotide polymorphisms (SNPs), of which 10.000 are thought to be pharmacologically relevant. The American-led international Human Genome Project mapped in excess of 30.000 genes and 2.5 million SNPs. Among these SNPs, 0.2 to 0.5 million are thought to be in the exonic regions therefore present in the open reading frame (ORF) responsible for coding the RNA and/or peptide products translated from the said RNA. The remainder is situated in the intronic sequences, where they are not expected to have any significant effect, or in the promoter region of the gene, where they can result in changes of protein expression patterns. A growing volume of research data has increasingly linked the presence of polymorphisms to modified absorption, distribution and excretion of pharmaceutical agents [20,21].

The lack and/or the presence of genetic variations in DME genes classify individuals into four different phenotypes within a population: poor metabolisers (PMs), who lack a functional enzyme, intermediate metabolisers (IMs), who are heterozygous for a defective allele orcarrying two alleles with reduced activity, extensive metabolisers (EMs), who carry wo active alleles, and ultra-rapid metabolisers (UMs), who carry more than two active alleles. Genetic variations might include SNPs, deletions, duplications, and other variations (Figure 2). This system of classification will be surpassed by a new quantitative system accounting more precisely for the individual allelic activity [22-26].

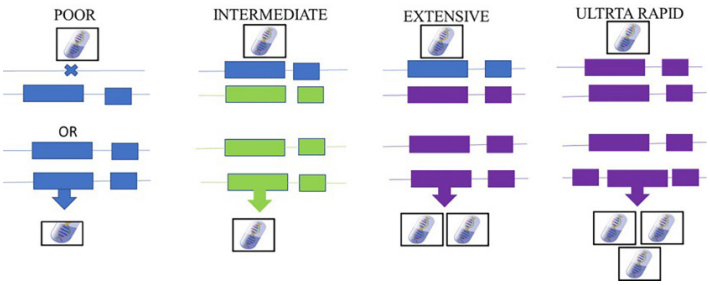

Figure 2. Schematic representation of adjusting dose for each patient's genotype and the types of metabolizer. X = whole gene deletion, blue = null variant, green = decreased-function variant, and purple = fully functional variant

Applications of Pharmacogenetics-Pharmacogenomics in Forensic and Clinical Toxicology Analysis

The analysis and elucidation of pharmacogenetic polymorphisms employ mainly two methods [27]. The phenotyping method deliberates on the determination of the phenotype by either direct measurement of enzyme activity or the monitoring of the time derivative concentration of a metabolite in a biological matrix, typically blood or urine. This method does not only produce real-time data on enzyme activity, but also naturally accounts for the environmental and physiological factors that my impact drug response, thus providing adirectly utilisable evaluation of the metabolic capacity of the individual. The genotyping method, on the other hand, analyses DNA on the molecular level, and aims for the specific detection of the presence of one or more genetic polymorphisms. Most current genotyping techniques employ PCR-based amplification of the target DNA, coupled with a target-specific consequent identification of the studies polymorphism(s).

In spite of well-defined reference drug toxicity levels, the field of forensic toxicology is often fraught with interpretive hurdles. Pharmacogenomics (PGx) is the study of the association between an individual's genotype and their response to xenobiotics. Pharmacogenetics (PGt) is often used to define the spectrum of inherited differences in drug metabolism and disposition, whereas pharmacogenomics refers to all the genes that determine drug behaviour and sensitivity. PGt and PGx, are used interchangeably [28,29]. Pharmacogenomics may play a part by enabling the forensic toxicologist to integrate data specific to the possible resolution of the toxicological puzzle. The use of PGx and PGt in the interpretation of forensic toxicology results is still sparse, but it is highly relevant because an individual's response to drug treatment varies due to genetic deficiencies, which can cause adverse drug reactions (ADRs) or even occasional deaths. Some studies show the importance of recognising poor metabolisers, who might have accumulated the drug to a sufficiently toxic level. Some other studies also show the importance of identifying individuals with extensive metabolism if the enzyme converts a pro-drug into an active drug [13,25,30-32].

The clinical applications of PGx are classified according to the drug group, specialities, and diseases and include opioids, pain management, nicotine addiction HIV treatment, immunosuppressives, and thiopurine S-methyltransferase for acute lymphoblastic leukaemia, and psychiatry [33,34]. Polymorphisms of the neurotransmitter transporters (serotonin, norepinephrine (noradrenaline), dopamine and P-glycoprotein, and serotonin transporter show little effect on serotonin active antidepressants selective serotonin reuptake inhibitor (SSRI) response, suggesting that the current scientific literature showing transporter genotypes is not yet contributory to predictive therapy [29,35]. There exists evidence to support the use of genotype-based dosing for drug transporters such as P-glycoprotein, organic anion transporting polypeptide C (OATP-C) and other CYP enzyme genes. In daily routine, concentrations of drugs have to be compared with the same kind of reference values for both therapeutic and toxic levels. Ratios of parent drug to metabolite concentrations can be useful the decision of whether an intake was acute or the result of, chronic use. However, other variables also influence the ratio of parent drug to metabolite concentrations, including interactions between different drugs and genetic variations described above. For these reasons, genotyping has become more common in forensic toxicology.

Recently, pharmacogenomics as a tool of the molecular autopsy has been used for the assessment of genetic contribution to drug toxicity in post-mortem forensic toxicology. The findings, as well as other applications of clinical and scientific findings in forensic science, might add to the understanding of disease mechanism, and optimisation of treatment including drug therapy. In addition, the use of pharmacogenomics in forensic toxicology may add to the understanding of drug toxicity due to genetically linked impairment of drug metabolism and may provide findings that

may be back-extrapolated for the benefits of optimisation of ante-mortem drug therapy. Pharmacogenomics in forensic toxicology would thus provide a better interpretation of the obtained results, indirectly enabling the emerging personalised medicine [33]. The full use of post-mortem PGx is only possible by integrating forensic pathology, toxicology and genetics. Pathophysiological condition(s) and the concentrations of all drugs and their relevant metabolites in the body at the time of death, combined with the genotype, could shed light on the prediction of individual responses. The forensic toxicological findings (typically polypharmacy) can be combined with detailed pathological data to form a reservoir of material for systematic post mortem pharmacogenetic investigations for particular drugs or case-control studies [29,36-38]. There are interesting possibilities and objectives for systematic post-mortem pharmacogenetic studies. All the data collected in this manner should be considered along with the fact that an individual's pathophysiological phenotype affecting drug efficacy (the ability of a drug to produce the desired therapeutic effect) depends to some degree on the genetic constitution of an individual and several other factors. These additional factors include developmental stage, physiological and environmental factors, associations with disease states or specific conditions, such as ion channelopathies (e.g. long QT syndrome, diabetes, obesity and the gut microbiology [29].

The more common pharmacogenomics methods are readily performed either by custom-developed assays or commercially available tests or assay platforms. The treatments included nonamplification, e.g., real-time PCR, signal amplification, signal amplification methods including endpoint polymerase chain reaction (PCR) detection, fluorescent in-situ hybridization (FISH), target and, allele-specific primers, length analysis using restriction fragment length polymorphism (RFLP) and oligonucleotide ligation assay (OLA), and new methods including solid phase microarrays and fluorescent-based bead assay (liquid microarray) [26,39,40].

Determining an individual's genotype is easier, with several advantages over phenotyping. It only has to be performed once in a lifetime and requires a small amount of DNA-bearing material, such as blood or a buccal swab. This sample can be obtained at any time and with the individual on current medication. Another advantage is that genotyping can be carried out in post mortem samples. A disadvantage of genotyping is the need to be aware of the possible presence of unknown sequence variants, which can affect the enzyme activity.

The CYP family of enzymes is known to be strongly affected by epigenetic regulation. Therefore, the presence of a particular genotype does not correlate directly with the presence of the phenotype represented by that particular genotype [41,42]. Additional factors can be of substantial importance for the specific drug metabolism and this also needs to be taken into account. The possibility of predicting an individual's phenotype on the basis of the genotype can be used clinically to individualise a patient's drug therapy, also known as personalised medicine.

Pharmacogenetics-Pharmacogenomics Perspective of Clinical and ForensicToxicology Pharmacogenomic variations and/or interactions must be accounted for when elucidating potential impact on drug response by an individual [43]. Such a case may be the application of pharmacogenomics to the determination of the presence or absence of drug toxicity. Such input is crucial, because pharmaceutical agents are one of the most commonly identified causes of adverse events, resulting significant morbidity and mortality [18].

The role of pharmacogenomics analysis in forensic investigations has already been emphasized as a holistic approach of molecular analysis in conjunction to macroscopic, microscopic and toxicological observations, constituting an integral part of the modern medico-legal study of death [38]. Nevertheless, the area of medico-legal investigation also involves occupational medicine due to the consequences of toxic-exposed workers.

Pharmacogenetics plays an important role in patient response to drug regimen, some examples of which are detailed below. Such details are well-established in previous literature, but constitute only a small minority of the overall drug-pharmacogenetics landscape. Such literature extends from the likes of drug pharmacogenetics, as described in the remainder of this article, to novel fields such as the determination and evaluation of treatment for drug addiction.

Methadone is metabolized through the liver by cytochrome P450 enzymes CYP3A4, CYP2D6 and CYP1A2 and buprenorphine is mainly metabolized by CYP3A4 enzyme. A recent review reported that Caucasians who lack CYP2D6 function appear to be protected from oral opioid dependence since this genotype is underrepresented in the opiate-addicted population and these poor metabolizers are satisfied with the withdrawal and anti-craving relief provided by methadone treatment. Ultra-rapid metabolizer heroin-dependent patients have felt dissatisfied with methadone therapy and can thrive using buprenorphine due to a lack of significant CYP2D6-mediated metabolism [44]. In a limited number of cases of methadone toxicity, Wong et al. (2003) showed that the prevalence of poor metabolizers was higher but not significantly different from that of a control group (n=23). They concluded that CYP2D6 mutations may not yet be directly associated with methadone toxicity, and pharmacogenomics, complementing other case findings in the molecular autopsy, is considered an adjunct in interpreting the methadone toxicity of poor and intermediate metabolizers [45].

Most opioid drugs are partly or extensively metabolized by the highly polymorphic CYP2D6 enzyme to more potent analgesic metabolites, leading to CYP2D6 PMs being more prone to therapeutic inefficiency in contrast to CYP2D6 UMs who can experience life-threatening toxicity [46]. Moreover, many drugs are inhibitors of CYP2D6, resulting in the possibility of opioid intoxication due to drug-drug interactions [47]. Therapeutic opioids (etc. codeine, tramadol, oxycodone, hydrocodone, ethylmorphine) used for acute and chronic pain and dependency are commonly implicated in severe adverse effects, as well as drug-related deaths [48-52]. Fentanyl is another powerful synthetic opioid analgesic that is similar to morphine but is 50 to 100 times more potent. Pharmaceutical fentanyl was developed for pain management treatment of cancer patients, applied in a patch on the skin. Because of its powerful opioid properties, Fentanyl is also diverted for abuse. Forensic cases and deaths caused by illicit use of fentanyl today have become the problem of many countries [53-55].

Codeine, used for its weak narcotic analgesic effects, is well-absorbed via the gastrointestinal route and is partly metabolized

to its major active metabolite, morphine, via O-demethylation by CYP2D6 [56,57].

Although the morphine-generating metabolic pathway only accounts for approximately 10% of total codeine metabolism, this bioactivating pathways is deemed essential for the emergent narcotic and/or analgesic effects of codeine. As a result, codeine may fail to demonstrate therapeutic effect at normal doses (Type F adverse drug reaction) in the 5-10% of Caucasians that are CYP2D6 PMs [58]. In the same vein, CYP2D6 Ums, who constitute 1-10% of the Caucasian population, may experience a codeine-precipitated morphine overdose [59,60]. In addition, codeine-induced morphine overdose may be exacerbated in CYP2D6 UM individuals when polypharmacy with macrolides and/or azole derivatives, the latter class of which are inhibitors of another enzyme in codeine metabolism, namely CYP 3A4 [59].

Oxycodone (14-hydroxy-7,8-dihydrocodeinone) is a semi-synthetic opioid agonist frequently prescribed for the relief of moderate to severe pain. Oxycodone is also O-demethylated via CYP2D6 into its active metabolite oxymorphone [61]. Jannetto et al. (2002) showed that in the presence of CYP2D6 metabolic deficiency, oxycodone toxicity can be induced. The author hence suggested that post-mortem oxycodone concentration should be evaluated in conjunction with the medical history, death scene investigation, autopsy findings, and post- mortem interval as well as pharmacogenetics [18]. Due to CYP2D6 polymorphisms, the genotyping of patients assists in the optimisation of oxycodone therapy for the patient, with dose reductions or switchover to CYP2D6-independent alternative pharmaceuticals for CYP2D6 [62].

Tricyclic antidepressants (TCAs) can similarly precipitate mortality as a result of their severe cardiotoxic effects. Amitriptyline and nortriptyline are metabolised by a variety of CYP enzymes. CYP2C19 is of major importance in N-demethylation of these drugs, whereas CYP2D6 has been shown to participate in trans-hydroxylation of the benzylic C-10 in both amitriptyline and nortriptyline [63]. In a study by Koski et al. (2006), 202 post-mortem toxicological cases were studied, along with genotype determination of CYP2D6 and CYP2C19 in these cases and the determination of blood amitriptyline and nortriptyline, as well as their metabolites[37]. CYP2D6 and CYP2C19 genotypes were demonstrated to be correlated with amitriptyline metabolite patterns in post-mortem materials, with amitriptyline metabolism being more dependent on CYP2D6 than on CYP2C19 genotype. The authors also reported an unusually high peripheral blood concentration of amitriptyline in one suicidal case with a CYP2D6 PM genotype. Doxepin is another psychotropic agent with TCA and anxiolytic action reported a case of fatal doxepin poisoning coinciding with a CYP2D6 PM phenotype due to a completely non-functional CYP2D6 genotype [38].

Tramadol is a centrally-acting analgesic used in the treatment of moderate to severe pain and has a wide range of applications, including treatment for acid reflux and fibromyalgia. Tramadol possesses weak μ-opioid agonist action and displays additional antinociceptive actions via the inhibition of the neuronal norepinephrine and serotonin re-uptake. The main metabolic pathways of tramadol are O- and N-demethylation, catalyzed by CYP2D6 and CYP2B6, respectively [64]. O-desmethyltramadol (ODT) is considered to be the metabolite most responsible for analgesic action with about 300-fold higher affinity than the parent compound for μ-opioid receptors. Consequently, therapeutic efficiency and toxicity of tramadol are expected to vary significantly between individuals depending on their CYP2D6 genotypes. In particular, the use of tramadol can be expected to be more problematic in CYP2D6 UMs than PMs, because of the possibility of an ODT overdose. Stamer et al. reported the case of an opioid-related respiratory depression in a patient receiving tramadolfor analgesia [65].

Another recent study Nunnu et al.'s 2021 125 articles on pharmacogenetics and forensic oxicology has been reviewed and all studies highlighted the importance of a pharmacogenetics study in drug-related deaths, especially in cases of non-overdose of drugs of abuse. Based on the results of this systematic review study, in the forensic field, gene investigation should be performed in relation to the hypothesis of the drug assumption: the most investigated genes are CYP2C19, CYP2D6, and CYP3A4 [66].

Conclusion

Personalized justice is the umbrella term used to describe any and all applications of personalized medicine in antemortem, perimortem and postmortem settings in medico-legal investigations. Moreover, the ramifications personalized medicine will have on forensic toxicology can be considered self-evident, especially in the development of personalized justice, developed in analogy to personalized medicine. The potential of pharmacogenomics in the employ of forensic toxicology shows immense potential and promise.

Moreover, the role of the genetic make-up of a person in toxic response is still under-studied, especially concerning the enormous effect it can have on the variance of response to an intoxicating agent. The emergence of pharmacogenomics in a clinical setting will not only greatly improve the healthcare quality received by the patient, chiefly due to the tailoring of a drug regimen (TDM) implementation and consequent reduction of adverse drug reactions that may arise due to the utilization of the pharmaceutical with no regard to the pharmacogenetic and pharmacogenomic confounding variables, but also reduce hospitalization time and overall cost, both to the patient and to the healthcare system in general. Moreover, routine genotyping in drugs with known pharmacogenomic parameters will allow collection of a large volume of data, paving the way for extensive evaluation in the age of "big data" and elucidation of common or uncommon metabolic features that may be difficult to observe in the absence of pharmacodynamics. This not only makes the development of a toxicity profile for any agent more difficult but also restricts the treatment options available to the toxicologist, be it in a clinical setting or a forensic setting.

A great promise of the incorporation of pharmacogenomics into forensic toxicology could be the development of novel agents that not only acknowledge the underlying great genetic variance in human populations but also take advantage of such heterogeneity to improve the pharmaceutical effect while simultaneously reducing or outright eliminating potential toxicity concerns. Although the incorporation of these principles into a field such

as forensic toxicology will be undoubtedly challenging, future studies will help determine whether such a tool will serve as a remarkable and veritable complement for the forensic toxicologist in the determination of the cause and the manner of death. The government and other funding agencies should give more incentive for adopting the practices related to personalized medicine. Faster, reliable and cost-effective approaches for sequencing, screening, and diagnosis of diseases should be developed which will bring the use of personalized medicine in real practice. In addition, pharmacogenomics methods are a well-established approach that should be included in the routine applications of the forensic field and used actively and effectively.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

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