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CYP3A4 Polymorphisms in Drug Metabolism: Implications for Hepatic, Endocrine, and Ocular Physiology

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Abstract

CYP3A4 is a key enzyme in drug metabolism, yet its clinical variability remains poorly understood. This review investigated the paradoxical behavior of the *1G allele, which displayed increased catalytic turnover *in vitro* but slower drug clearance *in vivo*. Reviewed studies demonstrated that CYP3A4 polymorphisms significantly influence fentanyl clearance, steroid metabolism, and susceptibility to steroid-induced ocular hypertension, underscoring the clinical importance of pharmacogenetic variability in therapeutic response and toxicity risk. Variants such as *1G and *22 were associated with altered enzymatic activity, inter-individual differences in pharmacokinetics, and clinically relevant variability in drug response. Current literature lacks integration across regulatory variants, protein-binding dynamics, and hepatic blood flow effects. To resolve this, targeted *in vitro–in vivo* comparison studies are recommended, alongside chromatin accessibility assays to explore transcriptional architecture. Reviewed studies consistently demonstrated that CYP3A4 polymorphisms significantly influence drug metabolism, affecting fentanyl clearance, steroid metabolism, and susceptibility to steroid-induced ocular hypertension. Demonstrating that CYP3A4 polymorphisms significantly influence drug metabolism, they underscore the growing clinical importance of genotype-systems. Physiologically-based pharmacokinetic modeling is proposed to deconvolute variant-specific clearance mechanisms. Genomic, transcriptomic, and metabolomic data offer a systems-level approach to understanding CYP3A4 function. Protein-binding dynamics and heme stabilization should be examined to prevent enzyme degradation. Additionally, *1G's interaction with co-medications and dietary modulators may influence clinical outcomes. Expanding existing studies to include CYP3A4 metabolizer phenotypes could improve risk stratification for steroid-induced ocular hypertension. Classifying metabolizer status based on allele combinations may bridge the gap between genomic complexity and clinical practicality, advancing personalized medicine.

Keywords: Cytochrome P450, Xenobiotics, Hepatic drugs, Drug metabolism

1. Introduction

Cytochrome P450 (CYP) is a superfamily composed of a vast array of enzymes that play pivotal roles in drug reactions. These enzymes are heme-containing monooxygenases. Monooxygenases are a class of enzymes that incorporate one atom of oxygen from molecular oxygen (O2) into a substrate. Meanwhile, the other oxygen atom is reduced to water. Heme is a molecule containing iron that's essential for oxygen transport. These cytochromes play pivotal roles in drug reactions and metabolism. Drug reactions vary considerably from person to person, and the outcomes of those reactions are primarily the result of drug metabolism. While found in many body tissues, CYP enzymes are predominantly present in the liver, intestines, and kidneys, specifically within the endoplasmic reticulum and mitochondria of cells. Of the total 57 isozymes–a group of two or more enzymes with identical functions but different structures discovered thus far, there are six major ones responsible for 90% of drug metabolism. The main isozyme this review investigates is CYP3A4. A wide variety of drugs work on different isozymes, and determining

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which isozymes are affected is critical in drug development. Cytochrome P450 enzymes are a specific type of isozyme, meaning they are enzymes that catalyze the same reaction but have different amino acid sequences. Figuring out which drugs are inducers or inhibitors of the isozyme CYP3A4 is crucial. Most drugs are deactivated, either directly or via facilitated excretion, through metabolism. That is why the CYP enzyme is so vital in understanding the metabolism of xenobiotics–or foreign substances–within the body. Xenobiotic metabolism is the general process by which living organisms break down and eliminate foreign compounds (xenobiotics), including drugs. Drug metabolism is a specific subset of xenobiotic metabolism that focuses on how the body processes pharmaceutical drugs. CYPs engage in the metabolism of xenobiotic compounds by using oxygen and electrons to modify substrates, such as Azelastine, via oxidation, the loss of electrons. CYPs found in bacteria and eukaryotic mitochondria are termed "type I," while those present in eukaryotic endoplasmic reticulum are termed "type II." CYP3A4 is a type II cytochrome P450 enzyme associated with the endoplasmic reticulum and responsible for the oxidative metabolism of over 50% of clinically used drugs.

1.1 Literature Review Methodology

This review synthesizes findings from peer-reviewed studies investigating CYP3A4 polymorphisms and their physiological implications. Relevant literature was identified using large online databases such as Google Scholar and PubMed. A special emphasis was placed on examining pharmacokinetics, enzyme activity, and clinical outcomes associated with CYP3A4 variations. Studies involving well-characterized alleles (such as the *1G variant) and clinically relevant substrates were selected to represent key interactions across hepatic, endocrine, and ocular systems, enabling a holistic analysis of CYP3A4-mediated variability in drug metabolism. Priority was given to studies integrating pharmacogenetics and physiological or therapeutic outcomes to provide analysis of CYP3A4-mediated variability in drug metabolism.

1.2 The Discovery of Cytochrome P450

CYPs were uncovered through research into the metabolism of steroids, drugs, and carcinogens in the 1950s. Research into drug metabolism expanded during the mid-twentieth century as the role of cytochrome P450 enzymes in drug metabolism and toxicity became established. The name "P450" was coined in 1958 when Marin Klingen observed that bubbling carbon monoxide (CO) through a preparation of rat liver microsomes resulted in a spectrum with maximum absorbance at 450 nm (Klingenberg, 1958; Omura, 2011). It was termed pigment "450" because of the unusual wavelength maximum at 450 nm for the Soret band, a light absorption band in the ultraviolet region of the electromagnetic spectrum.

The foundational characterization of cytochrome P450 was advanced by Ryo Sato and Tsuneo Omura, whose 1962 report and two follow-up papers in 1964 built upon Marin Klingen's original observation of a distinct absorbance peak at 450 nm—a spectral signature produced when carbon monoxide binds to the enzyme's reduced heme-iron center. Sato and Omura found that phospholipase A, specifically from heated snake venom, was effective at solubilizing the CO-binding hemoprotein, indicating a close association between the pigment and microsomal phospholipids. What's more, the unique Soret band feature, which later gave the P450 family its name, was subsequently linked to hydroxylation activity by Cooper-Rosenthal and Estabrook through studies on bovine adrenal microsomes (Cooper et al., 1965; Guengerich, 2020; OMURA & SATO, 1962, 1964).

Today, we understand vastly greater surrounding the metabolism of drugs and a plethora of aspects related to drug toxicity. A few of the factors involved consist of enzyme induction, a process in which exposure to certain substances, such as drugs or other chemical agents, increases the enzyme activity; enzyme inhibition, both reversible and irreversible, which reduces or eliminates ongoing enzyme activity; and pharmacogenetics, the study of genetic markers that influence the inter-individual variation in drug response (Figure 1). In general, reversible inhibition involves the temporary binding of an inhibitor to the enzyme, allowing for the restoration of enzyme activity upon inhibitor removal. Irreversible inhibition, conversely, involves a permanent inactivation of the enzyme, usually via covalent modification. Issues pertaining to drug toxicity include drug-drug interactions, drug-food interactions, and

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the roles of chemical moieties of drug candidates in drug discovery and development. The maturation of the field of P450 and drug toxicity has been brought about by key advancements in biochemistry and enzymology, computational capabilities, analytical chemistry, and molecular biology. Despite these advancements, problems continue to arise with P450s and drug toxicity in drug discovery and development in terms of drug interactions and lifespan. In the pharmaceutical industry, the interaction of scientists in medical chemistry, drug metabolism, and safety assessment is critical for success.

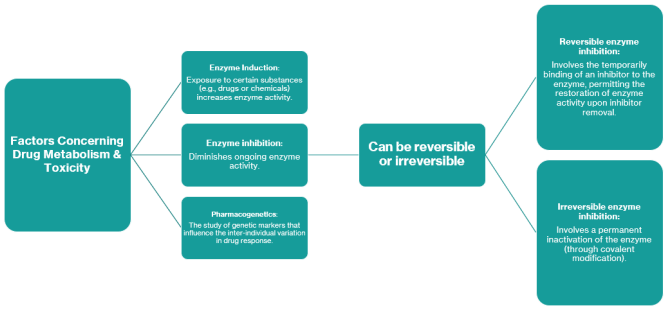
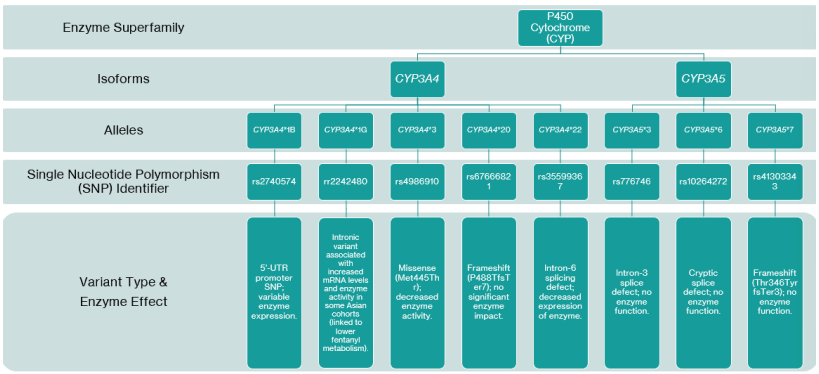


Figure 1. Factors Concerning Drug Metabolism & Toxicity.

1.3 Pivotal P450 Isoforms: Diversity, Redundancy, & Versatility

CYP3A4 is an isoform (variant of a protein encoded by the same gene but with a slightly different amino acid sequence) that is a component of the vast superfamily of CYP450 genetic family. The human cytochrome P450 3A (CYP3A) family is an essential member of the broader CYP superfamily of heme-containing enzymes. The CYP3A enzymes are vital for metabolizing both endogenous and exogenous compounds, and they have been reported to metabolize over half of all currently prescribed drugs (Zanger & Schwab, 2013). Variant members of the CYP3A family include CYP3A4, CYP3A5, CYP3A7, and CYP3A43. These variants are all located at the CYP3A locus. These enzymes catalyze various reactions and have remarkably broad substrate specificity, meaning that they can metabolize many of the same compounds. Because of their ability to interact with such structurally diverse compounds in their active site, CYP3A enzymes have a high versatility to alter responses to drugs. CYP3A4 and CYP3A5 are the best-characterized members of the family (Figure 2). There are a variety of alleles for each isoform, each with a single nucleotide polymorphism and downstream enzyme. Additionally, they are reported to be the most functionally redundant, but they do exhibit differences in their gene regulation and mRNA expression. CYP3A4 is considered the most important drug-metabolizing enzyme in the body and is the most abundant isoform in the liver. Moreover, CYP3A5 is the main source of CYP3A elsewhere in the body. CYP3A7 is the enzyme variant predominantly in the fetal liver (W. C. Wright et al., 2019).



The P450 cytochrome superfamily is depicted above, consisting of two key isoforms, CYP3A4 and CYP3A5.
Figure 2. CYP3A4 & CYP3A5 Variants: Key Variant Types & Enzyme Effects.

1.4 Connecting Genetic Variation of CYP3A4 Polymorphisms to Drug Metabolism in Physiology

One of the core isoforms in this review will be CYP3A4, which is found on chromosome 7q21.1. This chromosome also includes other CYP3A subfamily genes (Chen et al., 2016). Given its central role in drug metabolism, CYP3A4 stands out as a critical isoform of the cytochrome P450 superfamily, influencing the pharmacokinetics–the branch of pharmacology studying how drugs move through the body over time–of a broad range of compounds.

Despite extensive, in-depth characterization of its enzymatic function, emerging evidence suggests that genetic variations in CYP3A4 may significantly alter metabolic activity, yet the downstream consequences across major

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physiological systems remain an area of continued research and contention. Notably, variations in CYP3A4 activity have implications beyond hepatic drug metabolism, potentially impacting endocrine regulation and ocular health through altered steroid and xenobiotic processing. It is hypothesized that CYP3A4 polymorphisms, such as single-nucleotide polymorphisms in which a variation at a single point in a DNA sequence changes within a population, lead to differences in drug and hormone metabolism, contributing to system-specific variations in drug response, toxicity, and physiological outcomes. This review seeks to address the question: to what extent do CYP3A4 polymorphisms alter the metabolism of anesthetic and steroid drugs, and how do these changes impact hepatic, endocrine, and ocular physiology?

1.5 Hydroxylation as a Catalytic Mechanism

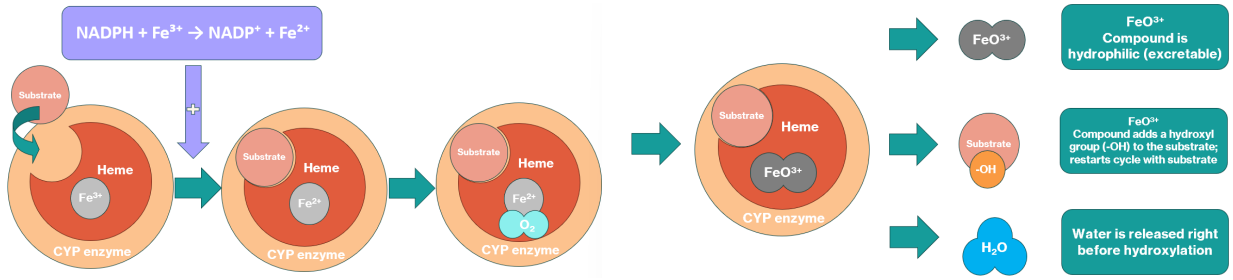
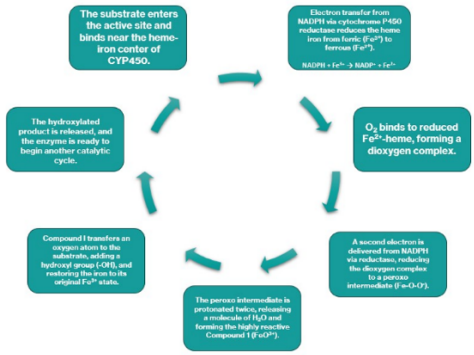


Figure 3. Enzyme-Substrate Activity of Cytochrome P450 Catalysis.

Cytochromes are redox-active proteins, proteins that transfer electrons, containing heme groups that play pivotal roles in electron transport chains within cells. Cytochrome P450 enzymes consist of an active site made from a heme-iron center. The iron compound is bound to the CYP enzyme (Figure 3). Substrates, such as the antihistamine drug Azelastine, bind to the active site at the heme group, which induces a three-dimensional change of the enzyme's active site. Following this, NADPH-cytochrome P450 reductases (PORs) are responsible for transferring the electrons from NADPH to cytochrome P450 enzymes (Figure 4). PORs act as a bridge, transferring electrons from NADPH to cytochrome P450. NAD(P)H represents both nicotinamide adenine dinucleotide (NAD^+) and its phosphorylated form, nicotinamide adenine dinucleotide phosphate ($NADP^+$), in their reduced forms, NADH and NADPH, respectively. In this system, the electron donor is either NADPH or NADH, depending on the organism, with NADPH predominating in mammals and NADH in some bacterial or mitochondrial systems. Nevertheless, NADPH is the more physiologically relevant and efficient electron donor in the context of P450. Oxygen binds to the ferrous-heme group following the reduction of iron. Reduction occurs once more, adding another electron to create a Fe-O_2 group known as a peroxide state. The peroxide group is short-lived as it gets protonated twice to yield water and the P450 Compound 1 (FeO_3^+). This activity permits the addition of a hydroxyl group (-OH), a necessary hydrophilic functional group, via hydroxylation to the compound (Gilani & Cassagnol, 2023).



The P450 catalytic cycle depicts substrate binding, electron transfer, O_2 activation, oxygen insertion, product release, and then repeats using the substrate again.

Figure 4. Catalytic Cycle of Cytochrome P450-Mediated Hydroxylation.

2. From Hydroxylation to Clearance: Impacts of CYP3A4 Genetic Variation on Fentanyl Metabolism in the Hepatic System

Fentanyl, a prominent drug linked with many overdoses–even within medical settings–and prescribed for many

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common procedures, is a potent synthetic opioid analgesic (pain reliever). Pivotal to fentanyl metabolism in anesthetics, pharmacokinetic parameters (PK) essentially quantify how the body processes a drug. They follow a substrate's journey, encompassing how it is absorbed into the bloodstream, distributed through tissues, metabolized, and ultimately eliminated from the body. Underpinning these PK parameters is the cytochrome P450-catalyzed hydroxylation of fentanyl—carried out by CYP3A4 in hepatocytes in the liver. By inserting an oxygen atom, CYP3A4 converts lipophilic fentanyl into the more hydrophilic (excretable) norfentanyl metabolite. Consequently, any genetic variation that alters CYP3A4's hydroxylation activity will be reflected in PK measurements. By measuring peak blood levels, total exposure over time, and drug elimination rate, these parameters provide a means to adjust dosages for drug safety and efficacy, underscoring a way to avoid adverse physiological effects and prevent drug toxicity. Pharmacogenetic influence on PK can be quantified through the area under the curve (AUC) and clearance (Cl). AUC determines how much of the substrate (drug) that entered is present in the bloodstream over time; Cl measures how effectively said substrate can be removed from the bloodstream. This adjustment in drug dosage, or "precision medicine," utilizes tools from PK and molecular biology to improve the diagnosis and expand the treatment options of patients based on their biological and molecular profiles.

Fentanyl is primarily metabolized by the hepatic enzyme CYP3A4, and its clearance can vary depending on the presence of specific genetic polymorphisms. In Saiz-Rodríguez et al. (2013), carriers of reduced-function *CYP3A4* alleles (**3, *20,* and **22*) exhibited higher normalized AUC and lower clearance for CYP3A4-exclusive substrates, including fentanyl, suggesting diminished metabolic capacity. While these PK trends did not reach statistical significance across all ten drugs due to the small cohort size and inter-individual variability in CYP3A4 expression, they support the notion that genetic variation in CYP3A4 contributes to inter-individual differences in anesthetic response and recovery. Notably, the CYP3A4*22 allele has been associated with reduced enzyme expression and slower fentanyl metabolism, and in some Asian cohorts, CYP3A4*1G carriers required lower fentanyl doses in patient-controlled analgesia for postsurgical plans—findings that warrant further investigation.

Beyond single alleles, compound genotypes can produce even more pronounced effects. In Saiz-Rodríguez et al. (2013), the lone volunteer carrying the double CYP3A4*3/*22 genotype exhibited a 53% increase in normalized AUC and a 53% decrease in clearance for CYP3A substrates. This highlights how rare combined polymorphisms may significantly impair metabolic capacity. Yet the same study cautioned that CYP3A4's high inducibility and susceptibility to inhibition by co-administered drugs, herbal supplements, or dietary factors can mask these genetic effects, obscuring true genotype-phenotype relationships in clinical practice. Recent in vitro kinetic analyses further demonstrate that the *CYP3A4*1G* variant—prevalent in certain Asian populations—metabolizes CYP3A4 substrates, such as fentanyl, more rapidly under idealized conditions due to increased catalytic turnover, which refers to the efficiency with which an enzyme or catalyst converts substrate molecules into products. However, *CYP3A4*1G* carriers often display slower fentanyl clearance in vivo and require lower anesthetic doses to achieve comparable analgesia. Consequently, standard fentanyl infusion rates could potentially overdose **1G* carriers, raising the risk of adverse events such as respiratory depression or delayed recovery. Precision anesthesia protocols, especially in populations with high **1G* prevalence, could potentially benefit from genotype-guided dose adjustments to normalize drug exposure. Such protocols represent a crucial step toward individualized perioperative care (Saiz-Rodríguez et al., 2020).

These genotype-driven variations in CYP3A4 activity not only shape individual anesthetic response but also lay the groundwork for broader physiological differences, highlighting the need for targeted research into how SNPs (single-nucleotide polymorphisms) influence drug metabolism throughout physiological systems.

3. Mitotane-Mediated CYP3A4 Induction and 5α-Reductase Inhibition in Endocrine System

Adrenocortical carcinoma (ACC) is a rare cancer that occurs in one to two people per million annually. ACC has a poor prognosis due to a high risk of recurrence and limited therapeutic treatment options. However, mitotane is a prominent drug to face this prognosis head-on. It is the first-line treatment for metastatic ACC. Additionally, hydrocortisone is a type of corticosteroid that is used to treat a variety of inflammatory, autoimmune, and hormonal conditions. Hydrocortisone works by suppressing the body's immune response and reducing inflammation. It also

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replaces low levels of steroid hormones in people with reduced adrenal gland function. This is especially critical in patients undergoing mitotane therapy for adrenocortical carcinoma, where accelerated cortisol inactivation necessitates significantly higher replacement doses of hydrocortisone. Because mitotane induces CYP3A4—a key enzyme in steroid metabolism—standard hydrocortisone regimens may be insufficient, prompting individualized dosing strategies guided by urine steroid profiles or plasma monitoring (Figure 5).

Left untreated, this rapid clearance can cause adrenal crises. In fact, the study by Chortis et al. (2013) investigated the effects of mitotane on in vivo steroid production, employing urinary steroid metabolomics for the analysis of 24-hour urine samples from patients with adrenocortical carcinoma (ACC) who received mitotane for adjuvant treatment or metastatic disease. Urinary samples were collected before and during mitotane therapy. Patients were divided into those receiving mitotane in the adjuvant setting or for metastatic ACC. Furthermore, specific steroid ratios were calculated to assess in vivo enzyme activities. Urinary steroid metabolite excretion was profiled using gas chromatography, the process of separating compounds in a mixture by injecting a gaseous or liquid sample into a mobile phase and passing the gas through a stationary phase, and mass spectrometry, an analytical tool used to measure the mass-to-charge ratio of the molecules present in a sample. CYP3A4 activity was measured by the ratio of 6α-hydroxycortisol to cortisol (6αOHF/F). Consequently, there was a strong induction of CYP3A4. The induction of CYP3A4 increased dramatically from a median of 2% before treatment to 56% during mitotane treatment (Table 1). This induction leads to rapid inactivation of more than 50% of the administered hydrocortisone. Mitotane was identified as one of the strongest inducers of CYP3A4, causing a 10- to 15-fold increase in 6αOHF/F excretion. These magnitude shifts indicate that standard glucocorticoid replacement regimens may be insufficient during mitotane therapy because accelerated steroid metabolism can substantially reduce circulating hydrocortisone levels. However, hydrocortisone replacement alone, even at high doses, did not account for this level of CYP3A4 induction.

Table 1. Enzyme Activity Marker Comparison of Steroid Concentrations and Ratios in Patients Before vs. During Mitotane Treatment for Metastatic ACC (MET; MET+M).

Enzyme	Activity Marker	Baseline (MET)	During Mitotane (MET+M)	Fold-Charge (MET+M / MET)
CYP3A4 induction Activity	6β-OHF / F	1.53	21.7	1318% ↑ (14.18× increase)
5α-Reductase inhibition	5α-THF / THF	0.58	0.02	96.55% ↓ (0.03448× decrease)
Androgen 5α→5β shift	An / Et	0.45	0.20	55.56% ↓ (0.4444× decrease)
Corticosteroid 5α→5β shift	5α-THB / THB	1.27	0.01	99.21% ↓ (0.007874× decrease)

In addition to this, computational analysis of the ratios of 5α- to 5β-reduced steroids revealed a distinct pattern of global 5α-reductase inhibition by mitotane compared with patients treated with a selective 5α-reductase type 2 inhibitor or patients with inactivating 5α-reductase type 2 mutations. These results point toward preferential inhibition of 5α-reductase type 1 by mitotane. 5α-reductase inhibition could also have beneficial consequences in the context of androgen-producing ACC, where it would be likely to help improve the clinical signs of androgen excess.

The CYP3A4 induction explains the necessity for doubling hydrocortisone replacement in mitotane-treated patients to prevent adrenal crisis. Thus, initiating and maintaining glucocorticoid replacement with at least 40-50 mg hydrocortisone per day (equal to 75 mg cortisone acetate), rather than the standard 20-25 mg hydrocortisone per day (equal to 37.5 mg cortisone acetate), is necessary. Glucocorticoids are compounds that belong to the corticosteroid family of compounds.

Longitudinal data showed a rapid onset of both CYP3A4 induction, with the full extent of the effect documented as early as 1-2 months after treatment initiation. These effects were also observed to be long-lasting, not returning to pretreatment levels even two years after treatment termination in one patient.

What's more, there was a significant correlation between plasma mitotane levels and CYP3A4 induction. Notably, significant effects on enzymatic activities were observed even at extremely low plasma mitotane levels, well below the suggested therapeutic range of 14-20 mg/L. Mitotane led to a significant down-regulation of overall steroidogenesis in metastatic ACC patients, as shown by a decrease in the sum of total androgen and mineralocorticoid metabolites, plausibly indicating an inhibition of another P450 enzyme, CYP11A1, the mitochondrial cholesterol side-chain cleavage enzyme that initiates steroid hormone biosynthesis.

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Thus, inter-individual differences in CYP3A4 activity, amplified by mitotane's potent induction (Figure 5), drive up to 14-fold acceleration of cortisol clearance and near-complete inhibition of 5α-reductase pathways. Recognizing these enzyme shifts, as seen in Table 1, underscores the need for genotype-guided glucocorticoid and androgen replacement to prevent adrenal crises and optimize therapeutic outcomes in ACC patients (Chortis et al., 2013). As illustrated in Figure 5, Mitotane docks at the cytochrome P450 heme-iron and, through sequential NAD(P)H-driven electron transfers that reduce Fe^{3+} to Fe^{2+}, enables O_2 binding to form a ferric-peroxo intermediate. Protonation of this intermediate yields hydroxylated mitotane, water, and the reactive FeO^{3+} (Compound I) species. Thus, Mitotane dramatically induces CYP3A4 (6β-OHF/F rising from 2% to 56%) and concurrently inhibits 5α-reductase, shifting steroid 5α/5β ratios. This enzyme shift accelerates hydrocortisone clearance by over 50%, necessitating at least double the standard glucocorticoid replacement (40–50 mg/day). These findings highlight the need for genotype-guided dosing to prevent adrenal crisis and optimize therapy in ACC patients.

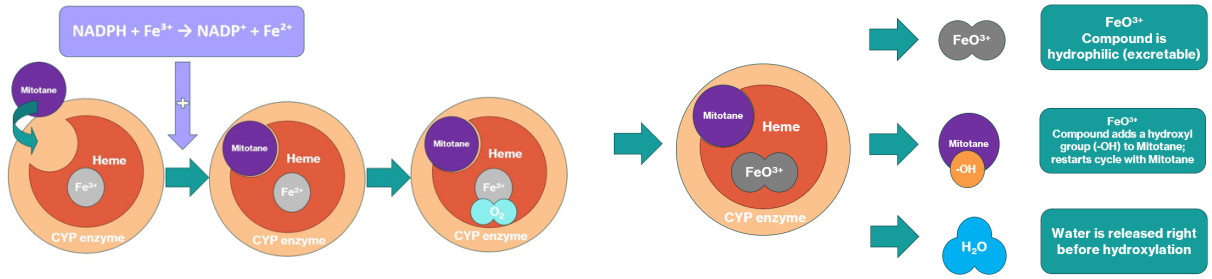

Figure 5. Mitotane Drug Substrate Activity within Cytochrome P450 Catalysis.

4. Bridging the Connection between CYP3A4 and Steroid-Induced Ocular Hypertension (SIOH)

Steroid-induced ocular hypertension (SIOH) was described in the 1950s. McLean was the first to observe an increase in intraocular pressure (IOP) associated with the administration of adrenocorticotropic hormone, marking the first recognition of SIOH. In a follow-up study by Wright et al., a relationship between the CYP3A4 phenotype, the function activity of the CYP3A4 enzyme's metabolism, and steroid-induced IOP response was examined. This underscores critical importance because there could be a relationship between the CYP3A4 phenotype and the rate of steroid-induced intraocular pressure (IOP) response based on existing genetic data. Some genetic variants, found via genetic testing, are correlated with reduced CYP3A4 enzyme function, and these CYP3A4 metabolizer phenotypes can be classified as CYP3A4 poor metabolizer, poor to intermediate, intermediate, and intermediate to normal phenotypes (Figure 6). CYP3A4 poor metabolizers tend to have dramatically reduced to no function, while intermediate metabolizers are expected to have moderately reduced function. Specifically, the study by Wright et al. hypothesized that patients with poor or intermediate metabolizer phenotypes of CYP3A4 may have an increased risk for steroid-induced IOP response. Additionally, the study by Wright et al. found that the percutaneous route of steroids is associated with an onset of SIOH within 2-5 years of treatment, with a higher incidence of approximately 77% when compared to inhalation or the nasal route of administration. The location of the percutaneous route affects risk with the application of steroids to the eyelids, conferring an increased risk of glaucoma. Risk factors of SIOH include family history of glaucoma, topical steroid route, younger children, advanced age, and pre-existing glaucoma. It is pertinent that the inhibition of the CYP3A4 enzyme by other medications can lead to increased concentrations of the steroid and side effects. Additionally, CYP3A4 genetic variants may also affect exogenous corticosteroid exposure.

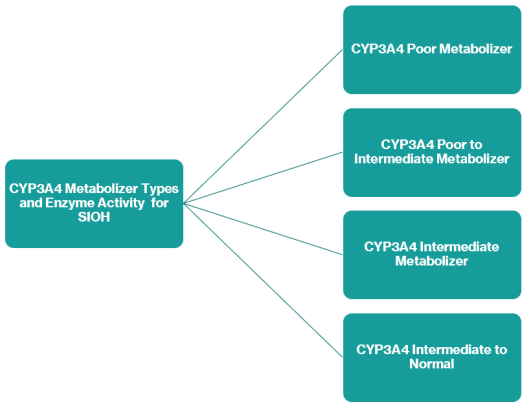

Figure 6. CYP3A4 Metabolizer Phenotype Distribution in SIOH Patients.

The study further aimed to evaluate whether a relationship could be established between the CYP3A4

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phenotype and steroid-induced IOP response. Lymphocyte-derived DNA sequencing of CYP3A4 from a sample of 10,073 patients was completed using the PGRN-Seq assay (Pharmacogenomics Research Network Sequencing assay). The patient population with reduced CYP3A4 enzyme activity phenotypes were identified and included based on the following criteria. Patients must have received at least three eye exams with IOP measurements where at least one of these eye exams occurred while on a systemic or intranasal steroid applied to the skin, or a steroid injected to a part of the body other than the eye. Patients with glaucoma, glaucoma suspects, pseudoexfoliation syndrome, and those younger than eighteen years of age were excluded. In fact, reduced CYP3A4 metabolizer phenotypes may contribute to slower corticosteroid metabolism, potentially increasing intraocular steroid exposure and elevating the risk of SIOH or glaucoma. Of the participants in the study, the controls with CYP3A4 normal metabolizer phenotype were identified based on matching age within 5 years, race, and sex. Steroid-induced IOP response was defined as an increase of three or more mmHg.

The main correlation between SIOH and CYP3A4 was the increase in the incidence of steroid-induced IOP response for the reduced CYP3A4 phenotype group when compared with controls. In addition, the study by Wright et al. found that some genetic variants are closely correlated with increased adverse effects. In a case-control study, the CYP3A4 variant rs2242480, formerly known as *CYP3A4*1G*, was associated with an increased risk of steroid-induced osteonecrosis of the femoral head, a condition in which the blood supply to the bone in the hip joint (femoral head) is disrupted, leading to bone cell death and potential collapse of the femoral head. It is important to note that all patients in this study were of Caucasian descent, thereby limiting the application of its findings to other racial groups. This is in part due to the low frequency of CYP3A4 reduced phenotypes in the general population. Another limitation of the study is its exclusion of other genes that could influence SIOH risk; such genes associated with SIOH include HCG22, among others. What's more, differing routes of steroid administration could have introduced variability between the study group and the control group due to the differences seen in the literature regarding the time to onset of SIOH with various routes of steroid administration (J. A. Wright et al., 2025).

5. Discussion

In retrospect, the studies by Saiz-Rodríguez et al. (2020), Chortis et al.(2013), and Wright et al. (2025) were all fraught with limited sample sizes. Specifically, the Saiz-Rodríguez et al. (2020) study had a relatively small cohort, limiting generalizability across ethnic groups with different allele frequencies. Fentanyl was the main drug analyzed, so findings may not extrapolate to other CYP3A4 substrates. While pharmacokinetic changes were observed, the study didn't directly correlate these with clinical outcomes like analgesia or adverse effects. Genotyping was used, but actual enzyme activity (phenotype) can be influenced by non-genetic factors such as diet, co-medications, or liver function.

Moreover, the study by Chortis et al. (2013) was substantiated on a lack of longitudinal data, as the study did not assess long-term consequences of altered steroid metabolism or adrenal insufficiency. In the study by Chortis et al. (2013), several important limitations were evident. The work lacked longitudinal follow-up, leaving the long-term consequences of altered steroid metabolism and potential adrenal insufficiency unaddressed. Although a marked induction of CYP3A4 activity was demonstrated, the molecular pathways driving this effect and the downstream physiological impacts weren't elucidated. Particularly, the mechanism by which mitotane activates CYP3A4 at the transcriptional level remains unknown, as no mRNA or protein-level analyses were performed to confirm enzyme upregulation. Similarly, while the urinary steroid profile suggested inhibition of 5α-reductase—mirroring patterns seen in genetic type 2 deficiency—the study did not investigate the underlying mechanism. These gaps highlight the need to examine CYP3A4 regulation across multiple layers: transcriptional, translational, and post-translational, rather than focusing solely on enzyme activity.

What's more, the study by Wright et al. (2025), which was on steroid-induced intraocular pressure response, solely included patients of Caucasian descent, thereby limiting the application of its findings to other racial groups. This is in part due to the low frequency of CYP3A4 reduced phenotypes in the general population. The study also excluded other genes that could influence steroid-induced hypertension. Additionally, concomitant medications, diet, herbal supplements, and lifestyle factors (including smoking and alcohol) were incompletely recorded, leaving potential inducers/inhibitors uncontrolled. The endpoints were limited to AUC and clearance; there was no assessment

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of clinical outcomes like efficacy measures or adverse-event rates.

These findings both reinforce and expand upon established knowledge on CYP3A4-mediated drug metabolism. It was found that the *CYP3A4*22* allele is consistently linked to reduced hepatic enzyme activity and slower drug clearance; the findings from the studies by Chortis et al. (2013) and Saiz-Rodríguez et al. (2020) reinforce this in both anesthetic and steroid metabolism. Additionally, the *CYP3A4*1B* promoter variant depicts increased transcriptional activity, aligning with earlier endocrine studies on elevated steroid turnover. Gender-stratified ocular drug response revealed significant CYP3A4-mediated clearance differences in women, diverging from male-dominant cohorts in earlier studies. Untargeted metabolomic profiling identified novel biomarkers (oxysterols) of CYP3A4 activity not captured in previous targeted assays in the study by Chortis et al. (2013). The study by Chortis et al. (2013) suggests the possibility that in vivo cortisol metabolite ratios diverged from in vitro predictions, indicating tissue-specific regulatory mechanisms that are not captured by cell-based models.

Genetic variation in CYP3A4, notably SNPs like **1G, *22*, and compound genotypes, plays a pivotal role in drug metabolism across multiple physiological systems. *CYP3A4*1G* is specifically linked to increased risk of steroid-induced osteonecrosis of the femoral head (hip joint), underscoring the clinical relevance of pharmacogenetic screening. Overall, this emphasizes the need for genotype-guided precision medicine to: tailor dosing strategies for anesthetics, steroids, and hormone therapies; minimize adverse effects; and enhance therapeutic efficacy.

Moreover, possible gaps in the literature have been uncovered throughout this investigation, such as the *1G paradox. In vitro assays show increased catalytic turnover, yet clinical studies report slower drug clearance. There has been scarce exploration of linked regulatory variants, protein-binding dynamics, or hepatic blood flow effects. Moving forward, it is recommended that targeted in vitro-in vivo comparison studies resolve the *1G paradox. It is also recommended to perform chromatin accessibility assays that will discern whether DNA around the CYP3A4 gene is available for transcription machinery to explore the regulatory architecture around *1G. Physiologically-based pharmacokinetic modeling must be performed to deconvolute variant effects on clearance. Multi-omic datasets (genomic, transcriptomic, and metabolomic) must be integrated for systems-level insight. Protein-binding dynamics and heme stabilization mechanisms should be explored to prevent enzyme degradation also. Future studies should investigate *1G's interaction with common co-medications and dietary modulators to determine whether these factors amplify or mitigate its effects on CYP3A4 activity and drug clearance. Additionally, it could be valuable to expand the study by Wright et al. to include CYP3A4 metabolizer phenotypes for SIOH risk stratification. Classifying metabolizer status based on allele combinations could also bridge the gap between genomic complexity and clinical practicality in personalized medicine.

A context-based predictive framework for drug safety could serve as a real-time, adaptive system that synthesizes multiple layers of patient-specific information to anticipate and mitigate adverse drug outcomes. This model could enable the immediate interpretation of pharmacogenomic variants of CYP3A4. This model would be able to continuously evaluate drug-drug interactions, enzyme competition, and cumulative pharmacodynamic effects. In the future, this model incorporates known CYP inducers and inhibitors from dietary sources and adjusts its predictions based on individual intake patterns. This algorithmic framework would be able to simulate pharmacokinetic curves and flags when predicted concentrations exceed safety thresholds. Additionally, adrenal suppression risk modeling tracks corticosteroid exposure, tapering schedules, and stress events to predict HPA (hypothalamic-pituitary-adrenal) axis vulnerability. IOP Spike Prediction could represent a precision-specific model designed to forecast the likelihood of a patient experiencing a sudden rise in intraocular pressure; this predictive framework integrates multiple variables, including steroid potency, ocular bioavailability, and genetic predisposition, to generate personalized monitoring timelines in future practice. This model would rely on machine learning and probabilistic modeling to generate individualized risk scores that could be embedded into electronic health records and prescribing platforms to deliver context-aware alerts, dose adjustments, and monitoring recommendations. By combining genotype-informed insights with personalized medicine frameworks and looking toward future integration of AI-driven platforms, multi-omic data, and context-aware clinical tools, static pharmacogenetics has the potential to be transformed into a dynamic ecosystem of anticipatory care. Ultimately, CYP3A4 polymorphisms contribute significantly to inter-individual variability in drug metabolism across the hepatic, endocrine, as well as ocular systems. Moreover, it was surmised that variants such as *1G and *22 influence anesthetic clearance, steroid metabolism, and susceptibility to steroid-induced

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ocular hypertension, illustrating the clinical importance of pharmacogenetic variations in therapeutic response and toxicity risk. Collectively, the review supports the growing role of genotype-guided precision medicine in improving patient outcomes.

6. Conclusion

CYP3A4 polymorphisms markedly alter the variability in drug metabolism across hepatic, endocrine, and ocular systems. Variants such as *1G and *22 influence anesthetic clearance, steroid metabolism, and susceptibility to steroid-induced ocular hypertension, demonstrating the vitality of pharmacogenetic variability in therapeutic responses and toxicity risks. Together, these investigative studies aid the growing role of genotype-guided precision medicine in optimizing drug dosing, improving patient outcomes, and minimizing adverse effects. Continued combination of pharmacogenomics and physiologically based pharmacokinetic modeling may further advance individualized therapeutic strategies while enhancing the understanding of metabolism. Overall, the review points toward how CYP3A4 polymorphisms play a crucial role in deciding the inter-individual variability in drug metabolism and therapeutic response across multiple physiological systems.

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