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FACULTY OF VETERINARY MEDICINE

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PHARMACOGENETICS AND CANINE CYTOCHROME P450 POLYMORPHISMS

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Sarah SMETS

Promotors: Prof. Dr. Luc Peelman

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Literature Review

as part of the Master's Dissertation

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FOREWORD

The field of pharmacogenetics and cytochrome P450 in veterinary medicine are both areas that are only briefly touched upon during our education as clinical veterinarians. This literature review has given me the chance to research and learn more about both topics. I am convinced that both areas will be highly valuable in the future, and that even as a clinical practitioner you can benefit highly from understanding the basic concepts of pharmacogenetics and the mechanisms of cytochrome P450.

I would like to thank my family and especially my husband Anders Solberg for editing my drafts and helping me through the sometimes stressful moments of writing a master's dissertation.

Last but not least, I would like to thank my promotor Prof. Dr. Luc Peelman and co-promotor Prof. Dr. Alex Van Zeveren for editing my drafts, always being available for questions and guiding me through the complicated maze of genetic concepts and research.

ABSTRACT

Pharmacogenetics is the study of the impact of genetic variation on drug therapy and drug variations. The ultimate goal of this discipline is the creation of “personalized medicine”. In veterinary medicine, pharmacogenetics is still in the early stages of development but many advances have been made, especially with the completion of genome sequences of several species relevant to veterinary medicine. In particular, much focus is on the existence of different phenotypes due to genetic polymorphisms. In this dissertation, an overview of the evolution of pharmacogenetics will be given and some practical applications in both human and veterinary medicine will be explored.

Cytochrome P450 is an important topic of research within pharmacogenetics. The cytochrome P450 enzymes are an essential part in metabolization and elimination of numerous clinically used drugs. These enzymes are characterized by a large degree of inter-species and inter-individual variations in function and expressions due to differences in induction, inhibition and genetic polymorphisms. These altered functions can then lead to therapeutic failure or adverse drug reactions. This master’s dissertation gives an overview of the existing research and findings in regards to canine drug-metabolizing cytochrome P450 polymorphisms. The following genes in particular are discussed: *CYP1A1/CYP1A2, CYP2B11, CYP2C21/CYP2C41, CYP2D15, CYP2E1* and *CYP3A26*. The polymorphisms within these genes are well researched, but the effect of these variations on the drug metabolism on pharmacokinetics or the result *in vivo* is still unclear. More systematic research is needed to not only fully understand the impact of these genetic polymorphisms on drug therapy and adverse drug reactions, but also to elucidate the variations between and within different breeds.

Key Words: Canine - Cytochrome P450 - Drug metabolization – Pharmacogenetics - Polymorphisms.

SAMENVATTING

Farmacogenetica is de studie van overerfbare genetische variaties in geneesmiddelen metaboliserende mechanismen. Deze variaties kunnen ervoor zorgen dat de geneesmiddelen een veranderd therapeutisch effect hebben en zelfs een schadelijk effect verkrijgen. Hoewel noties van farmacogenetische principes terug gevonden kunnen worden ten tijde van Socrates, kende de discipline zijn echte start in de jaren '60. Initieel concentreerde de discipline zich voornamelijk op het onderzoek naar monogenische kenmerken. Dit evolueerde echter samen met de komst van verschillende nieuwe technieken en de verschillende genoom projecten. De focus nu ligt nu voornamelijk op multifactoriële en epigenetische eigenschappen.

De uiteindelijke doelstelling van farmacogenetica is om een geïndividualiseerde medicinale therapie te bekomen gebaseerd op de specifieke genetische samenstelling van een individu. Hierdoor kunnen dan schadelijke effecten van geneesmiddelen gereduceerd worden en kan ook een efficiëntere therapie bekomen worden. In de praktijk kent de farmacogenetica tot nu toe nog maar weinig toepassingen. In de humane geneeskunde bestaat er een genetische test, AmpliChip CYP450, die test voor variaties in *CYP2D6* en *CYP2C19* genen die zorgen voor verschillen in metabolisatie van enkele belangrijke psychofarmaca. In de diergeneeskunde bestaan er ook enkele toepassingen, de identificatie van variaties in het *ABCB1* gen is veruit de belangrijkste en ook de meest gebruikte. Polymorfisme in het *ABCB1* gen zorgt er namelijk voor dat ivermectine niet meer voldoende gemetaboliseerd wordt en dit kan leiden tot ernstige neurologische verschijnselen en zelfs tot sterfte van het dier.

Een belangrijk onderwerp binnen de farmacogenetica is het cytochroom P450 complex (CYP450). CYP450 is een superfamilie van proteïnes die wordt aangetroffen bij alle eukaryoten en prokaryoten. Om de grote hoeveelheid aan genen van elkaar te kunnen onderscheiden is er een strikte nomenclatuur ontwikkeld. CYP staat voor cytochroom, gevolgd door het nummer dat de familie aantoont, vaak wordt dit verder gevolgd door een letter die dan de subfamilie aanduidt en de naam eindigt met een nummer dat het polypeptide aangeeft. Bijvoorbeeld, CYP1A1 staat voor cytochroom uit familie 1, subfamilie A en polypeptide 1. Indien men het gen indiceert dan wordt de naam in het cursief geplaatst.

De CYP450 familie speelt een zeer belangrijke rol in onder andere geneesmiddelen metabolisatie, detoxificatie en activatie. CYP450 kan dan ook teruggevonden worden in hoofdzakelijk de lever en het gastro-intestinaal stelsel en in mindere mate in de longen, huid, nasaal epitheel en andere organen. Vooral de lever speelt een belangrijke rol in de fase I metabolisatie. Deze metabolisatie resulteert in een chemische modificatie van het substraat door het toevoegen van een functionele groep waardoor het substraat makkelijker geconjugeerd kan worden tijdens de fase II reacties. Hierdoor wordt het substraat dan geëlimineerd uit het lichaam.

Bij de hond zijn er tot nu toe 55 individuele genen en 9 pseudogenen geïdentificeerd en verscheidene CYP450 genen vertonen polymorfismen. Deze polymorfismen kunnen leiden tot overerfbare ziektes zoals adrenale hypoplasie, congenitale ichtyosis en spastische paraplegie 5A. Binnen het kader van de farmacogenetica zijn enkel de CYP450 genen belangrijk die te maken hebben met xenobiotica metabolisatie. Voor de hond zijn dit de volgende genen: *CYP1A1/CYP1A2, CYP2B11, CYP2C21/CYP2C41, CYP2D15, CYP2E1,* en *CYP3A26*. Er is weinig specifiek onderzoek gedaan over deze genen en de gekende informatie is vooral bekend doordat de hond vaak als proefmodel gebruikt wordt binnen de humane geneeskunde. De huidige kennis toont aan dat de polymorfismen

die gekend zijn geen grote implicaties hebben op therapeutisch gebied, maar men moet wel in het achterhoofd houden dat de meeste substraten van deze genen nog onvoldoende gekend zijn.

In conclusie kan gesteld worden dat er verder onderzoek nodig is over specifieke substraten en vooral ook over het *in vitro* en *in vivo* effect van deze polymorfismen. Dit is nodig opdat men de klinische implicaties van de polymorfismen in CYP450 genen bij de hond kan inschatten en zo ook praktische toepassingen kan creëren op basis van de farmacogenetische eigenschappen van canine CYP450. Daarbovenop zijn er verschillende factoren die het onderzoek bemoeilijken en die overwonnen moeten worden, zoals onder andere de variaties tussen niet alleen verschillende rassen , maar ook de variaties tussen verschillende lijnen binnen deze rassen.

INTRODUCTION

Pharmacogenetics is the study of inherited genetic variations in drug metabolic pathways which affect individual responses to drugs in regard to therapeutic and adverse effects. These individual responses are due to differences in genotypes, resulting in different phenotypes. Based on this principle, pharmacogenetics intention is to create a highly personalized pharmaceutical therapy. Although pharmacogenetics initially focused on human medicine, the discipline has expanded into veterinary medicine. One of the most researched topics in pharmacogenetics is Cytochrome P450. The cytochrome P450 enzymes are an essential part in drug metabolization and elimination of numerous clinically used drugs. These enzymes are characterized by a large degree of inter-species and inter-individual variations in function and expressions due to differences in induction, inhibition and genetic polymorphisms. These altered functions can then lead to therapeutic failure or adverse drug reactions.

This dissertation's main goal is to give a literature overview of two concepts, namely pharmacogenetics and the relevance of canine cytochrome P450 within this discipline. The objective is to provide a concise overview of the evolution of pharmacogenetics and its role in veterinary medicine. Furthermore, the research that has been conducted in regards to polymorphisms in drug metabolizing canine cytochrome P450's is reviewed.

This master's dissertation will provide a literature review of these two concepts. To fully understand the discipline of pharmacogenetics a brief outline of the history of pharmacogenetics is given, followed by an overview of the evolution of pharmacogenetics to pharmacogenomics. Then a few practical applications of pharmacogenetics within human and veterinary medicine are reviewed. Cytochrome P450 is explored. As mentioned, cytochrome P450 is an important topic within pharmacogenetics due to its importance in drug metabolizing. The focus of this dissertation is on canine cytochrome P450, but first a short, general overview of cytochrome P450 is given to provide a solid frame of reference for the rest of the literature review. Discussing all canine cytochrome P450 genes and polymorphisms would be beyond the scope of this dissertation. Therefore, the focus will be on genes that are important within drug metabolization. The following genes in particular are discussed: *CYP1A1/CYP1A2, CYP2B11, CYP2C21/CYP2C41, CYP2D15, CYP2E1* and *CYP3A26*.

LITERATURE STUDY

1. WHAT IS PHARMACOGENETICS?

The goal of offering drug therapy to a patient is to achieve a specific pharmacologic effect that is successful and without adverse effects. However, it is often difficult to predict the efficacy and safety of a specific drug in a patient. A variety of factors including age, disease, gender and species can influence the physiological effects of a specific drug (Mealey, 2006). Nevertheless, these factors often offer little explanation of the degree of individual variation in drug response. This is where pharmacogenetics comes in.

Pharmacogenetics is the study of inherited genetic variations in drug metabolic pathways which affect individual responses to drugs in regard to therapeutic and adverse effects. Traditionally, pharmacogenetics has focused on Mendelian monogenic variations, but it has expanded to include multifactorial influences (Kalow, 2002). Another term frequently used is pharmacogenomics, which focuses more on the detection of genes responsible for disease and using this knowledge to search for new drugs (Kalow, 2002).

Below, an introduction to the history and development of pharmacogenetics is given. Furthermore, a brief overview of current research topics and clinical applications in both human and veterinary medicine will be given before moving on to the pharmacogenetics aspect of the canine cytochrome P450.

1.1. THE ROOTS OF PHARMACOGENETICS

The origins of pharmacogenetics cannot be traced back to one specific point in time. Instead there are various key moments that have determined and defined its evolution.

Notions of individual variations in response to specific components can be found as early as 510 B.C. Pythagoras noticed that after ingesting fava beans, individual reactions ranged from no response at all to death (Nebert, 1999; Pirmohamed, 2001; Ingelman-Sundberg, 2004). These individual variations were later traced back to a glucose-6-phosphate dehydrogenase deficiency which causes an hemolytic anemic reaction (Canaparo, 2012). Despite some of these early notions, the foundations of pharmacogenetics can be found during the 20th century when the principles of genetics were established.

Sir Archibald Garrod is credited with first realizing that certain individuals are predisposed for disease, he researched phenylketonuria in regards to parental blood relation (Weber, 2001; Meyer, 2004; Gahl, 2008). Influenced by William Bateson, who first popularized the term genetics, and Mendel, who determined that this predisposition was recessively inherited. He also coined the term “chemical individuality of man”(Gahl, 2008). This term refers to deviations from expected, common pathways in the metabolism that take place when inborn metabolic errors alter the effects of drugs (Canaparo, 2012).

A second key moment came 30 years later with the research by L.H. Snyder. Snyder conducted one of the first pharmacogenetic and population-based studies when he tested the taste blindness for phenylthiocarbamide (PTC) in certain individuals. This study showed that the ability to not taste PTC was an autosomal recessive trait and that the disparities varied within populations of different ethnic origins (Meyer, 2004).

After a few decades of little progress, a new milestone was reached by Arno Motulsky in 1957. Motulsky is often called the father of pharmacogenetics because of his research detailed in his paper "Drug reactions, enzymes, and biochemical genetics" (Tauser, 2012). He laid the foundation for the hypothesis that variation among individuals in response to drugs were due to genetic differences (Canaparo, 2012).

Following Motulsky's publication a rapid expansion of the discipline began. In 1959 Friedrich Vogel used the term pharmacogenetics for the first time in his publication "Moderne problem der humangenetic" (Meyer, 2004; Botillo et al., 2013). He defined it as "the study of the role of genetics in drug response" (Nebert, 2008). In 1962, Werner Kalow reviewed all previously published research on drug response in connection to genetic factors and bundled them into the first published book solely dedicated to pharmacogenetics "Pharmacogenetics: Heredity and the Response to Drugs" (Kalow, 2002; Meyer, 2004; Tauser, 2012). After these publications, pharmacogenetics got its start as a real discipline.

This short overview shows that pharmacogenetics has come a long way and was constantly changing and adapting to new research methods and technologies. However, it was still primarily a study of the biological consequences of mutations and Mendelian-based genetics (Kalow, 2002). One of the most important events in genetics is the Human Genome Project from 1990-2003; this shaped and changed the field of pharmacogenetics drastically.

1.2. THE EVOLUTION OF PHARMACOGENETICS TO PHARMACOGENOMICS AND BEYOND

The human genome project determined the further evolution of the field of pharmacogenetics. This project, completed in 2003, mapped the full genetic make-up contained in the genome which encompasses the full set of DNA (Hartl, 2014). Since the human genome project the field of pharmacogenetics has expanded rapidly.

Throughout the past two decades, several approaches within the field have been developed; these approaches often appeared concurrently with new developments and technological advances. A brief overview of some of the most significant approaches is given below.

The first approach to emerge is the candidate gene approach, this concentrates on associations between genetic variation in predetermined genes and phenotypes (Tauser, 2012). These candidate genes are selected based on previous knowledge of the gene's function, and building on the hypothesis that a variation within that particular region will have an effect on its function. This way a genetic drug-profile can be developed. The main focus is on single nucleotide polymorphisms (SNPs). An SNP is a variation in a single nucleotide in the DNA sequence; they are the main source of genetic and phenotypic variation (Canaparo, 2012). This was the main focus, mainly because rapid sequencing and genotyping of these SNPs could easily be done. An advantage of this approach is that it is hypothesis-driven and that it generates sufficient statistical power (Sissung, 2010). The drawbacks are that the results are difficult to interpret and that the identification of genetic markers is inconsistent (Tauser, 2012; Sissung, 2010).

A second approach is the pathway-based approach. It is based on prior knowledge of variations in genes and the pathways used and it tests whether a particular pathway is associated with a phenotype (Sissung, 2010; Tauser, 2012). This approach is mostly suitable for studies that want to test the interaction between genes (Tauser, 2012). A drawback of the approach is that it requires

very complex studies using a large sample size and there often is an insufficient understanding of the pathway interactions (Sissung, 2010; Tauser, 2012).

As the above explained approaches show, the focus of the field of pharmacogenetics was shifting from monogenic to multi-factorial and epigenetic characteristics. When the mapping of the human genome was finalized, several studies showed a poor link between SNPs in candidate genes and phenotypes (Canaparo, 2012). In fact, other functional SNPs and even synonymous or silent SNPs are now suspected to have a functional impact (Komar, 2007; Canaparo, 2012). This caused a switch within pharmacogenetics from studying the effects of single genes to studying the effect of multiple genes on drug response (Canaparo, 2012). These genome-wide association studies (GWAS) scan the entire genome for a common genetic variation. Furthermore, advances in technology and techniques enabled researchers to scan millions of SNPs simultaneously using genome-wide SNP arrays (Foroud and Koller, 2013). This switch directed the change from pharmacogenetics to pharmacogenomics.

The GWAS has multiple advantages over the candidate gene approach; one advantage is that it is not hypothesis-driven and thus can reveal unexpected SNPs (Sissung, 2010; Canaparo, 2012; Tauser, 2012). A disadvantage is that the human genome counts more than 10 million SNPs. In order to overcome this vast challenge several strategies have been developed such as advancing the technology, but also applying genome-wide haplotype pharmacogenomics association studies (Canaparo, 2012; Tauser, 2012). Due to these developments, the HapMap project was realized in 2005. This project intends to generate a complete haplotype of the human genome through creating an extensive library of tagging SNPs (tSNPs) (Tauser, 2012). tSNPs are SNPs that represent an entire haplotype, using these tSNPs vastly expands the genomic coverage of variability (Tauser, 2012). This means that with identifying one tSNP, one also automatically identifies the associated SNPs in that particular haplotype region.

Recent developments in the field of genomics and epigenetics are changing the direction of research once again. Epigenetics is defined as the study of inheritable changes that are not caused by variations in nucleotide sequences (Canaparo, 2012; Tauser, 2012). For example, a gene function can be altered in a reversible way through DNA methylation and histone modification (Kalow, 2002; Canaparo, 2012). This is an important concept because in addition to the effect a gene has on drug efficacy, one now also needs to take into account the effect a certain drug can have on the way the gene is expressed (Kalow, 2002). Pharmacoepigenetics and pharmacogenomics try to foresee drug response and potential adverse reactions based on the epigenetic variations of an individual (Canaparo, 2012; Tauser, 2012).

In the future, it is very likely that the studies on drug response will expand beyond genomics and become inter-disciplinary to include disciplines such as proteomics, transcriptomics, metabolomics and advanced mathematical models to become a systeomics or systemic biology approach (Witkamp, 2005; Nebert, 2004).

1.3. PRACTICAL APPLICATIONS OF PHARMACOGENETICS

The goal of pharmacogenetics is to ultimately provide individualized drug therapy and to recognize which drugs need an individual dosage in order to improve the drug treatment (Ingelman-Sundberg, 2004). Mealey states in her article about pharmacogenetics that the individualization of drug therapy

has two clinical applications. First, patients with higher risk for adverse drug reactions can be recognized. Second, one can predict the patients that are most likely to benefit from a particular drug therapy (Mealey, 2006).

Individual variation and genetic variation can be found in several aspects of pharmacology, such as pharmacokinetics and pharmacodynamics. More specifically, the following aspects of pharmacology can greatly be affected by genetic polymorphism. Drug absorption is the ability of a drug to be absorbed into the systemic circulation. This bioavailability is often determined by the physiochemical properties of the drug and its ensuing hepatic metabolization. Besides these two factors it is also dependent on intestinal phase I drug metabolism enzymes and efflux transporters (Mealey, 2006; Tauser, 2012). Furthermore, drug distribution, drug metabolization and drug elimination can be affected. Genetic variations in these mechanisms can produce a significant change in the absorption of a drug. In addition to the genes involved in drug metabolization or drug transport, polymorphism in drug targets and nuclear receptors are also a significant source of individual responses to drugs (Sissung, 2010; Tauser, 2012).

1.3.1 Pharmacogenetics in human medicine

Pharmacogenetics in human medicine focuses primarily on genes encoding drug transporters, drug-metabolizing enzymes and drug-targets (Ingelman-Sundberg, 2004). Variations in these genes cause adverse drug reactions (ADRs). ADRs are very important; they are reported to be the 4th to 6th leading cause of death in the USA (Nebert, 1999). In particular, cytochrome P450 polymorphisms account for 86% of phase-I metabolic enzymes which in turn account for 56% of all ADRs (Ingelman-Sundberg, 2005).

Personalized medicine is the ultimate goal, but in clinical circumstances pharmacogenetics has not been widely used as a means to implement a proper therapy. One of the reasons for this underutilization is that clinical practitioners are not educated appropriately. In addition, the availability of the genotype information and the time-consuming and often expensive tests in the lab are also cited (Wilffert et al., 2013).

One of the first clinical applications in human medicine is the use of the AmpliChip CYP450 test. This is the first FDA approved pharmacogenetic test and genotypes 27 alleles in *CYP2D6* and three alleles in *CYP2C19* genes (Nebert, 2004; Fleischer et al., 2008). The test can be used to identify ultra-rapid and poor metabolizers and is intended for the dosing of antipsychotic drugs and is now extensively used in psychiatry (Spina and de Leon, 2014).

In the future, pharmacogenetics could lead to using genotyping to personalize drug treatment and thus decrease the cost of treatment while increasing the efficacy of drugs. Ingelman-Sunderberg predicts that about 10-20% of all drug therapies could benefit from personalized P450 gene-based medicine. Major drug companies and the Federal Drug Authority take the pharmacogenetic aspects into account (Ingelman-Sundberg, 2004).

1.3.2 Pharmacogenetics in veterinary medicine

Although the field of pharmacogenetics is expanding rapidly in human medicine, the application in veterinary medicine is lagging. This is often due to the high costs and complexity of the research projects. Most research within veterinary medicine is based on human medicine or conducted with the intent to transfer it to human medicine. Furthermore, in veterinary medicine the pharmacogenetic differences between species and even breeds needs to be taken into account. Despite a slower development, significant achievements have been made within veterinary pharmacogenetics. So far the majority of the focus has been on the polymorphism in the p-glycoprotein membrane transporter. Identifying this polymorphism is the most used clinical application of pharmacogenetics in veterinary medicine (Fleischer et al., 2008; Martinez et al., 2008).

In clinical practice Dowling states that veterinarians use the following proverb: “white feet, don’t treat” (Dowling, 2006). Dogs with this phenotype often display a severe neurotoxic reaction to macrocyclic lactones. This is a classic example of pharmacogenetics. During the 1990’s it was discovered that this sensitivity in dogs involved the drug transporter P-glycoprotein (P-gp), a product of the *ABCB1* gene, formerly known as the *MDR1* gene (Dowling, 2006; Mealey, 2006; Mosher and Court, 2010). P-gp is a transmembrane protein expressed in the luminal border of intestinal epithelial cell, bile canaliculi, renal tubular epithelial cells, the placenta and brain capillary endothelial cells (Dowling, 2006; Mealey, 2006). Its main activity is to regulate the drug concentration in tissues and its main function is to limit the drug uptake by pumping the drugs out of the body and away from sensitive tissues such as the brain, fetus and other tissues and consequently expediting the drug elimination in these systems (Martinez et al., 2008; Mosher and Court, 2010).

In dogs, the polymorphism of the *ABCB1* gene has been researched extensively. The polymorphism is caused by a frame shift due to a four base-pair deletion mutation that generates several premature stop codons (Dowling, 2006; Mealey et al., 2001). This results in a substantially shortened and non-functional protein; only 10% of the product is synthetized (Mealey, 2006). In research with knock-out mice it was concluded that P-gp was not necessary for basic functions, but that it caused severe problems when certain drugs were administered (Dowling, 2006).

A good example of P-gp protein deficiency is ivermectin sensitivity in collies. Homozygous dogs with two alleles that contain the deletion, experience severe neurological symptoms when receiving a single dose. Heterozygous dogs, with one normal and one mutation allele, show neurological symptoms when receiving higher doses or daily doses of the drug (Mealey, 2006). This sensitivity is prevalent in herding breeds such as Collies (75%) and Australian Shepherds and present in German Shepherds and also some non-herding breeds such as the Silken windhound and the long-haired Whippet according to samples collected in Germany and the United States (Dowling, 2006; Mosher and Court, 2010). Another example of P-gp polymorphism can be seen in drug elimination. P-gp’s can be found in the bile canaliculi and renal tubular epithelial cells. A deficiency causes myelosuppression and gastrointestinal toxicity after treatment with certain anticancer agents (Martinez et al., 2008). Several DNA tests to detect this polymorphism are available and are currently one of the most frequently used in clinical practice.

As mentioned before within veterinary medicine a pharmacogenetic difference between species and breeds needs to be taken into account. As the above example of P-gp shows, there are significant variations between individuals and also between breeds of dogs. A reasonable amount of research has been done on this topic.

2. CYTOCHROME P450 PHARMACOGENETICS

2.1. WHAT IS CYTOCHROME P450

2.1.1 Introduction

Cytochrome P450 (CYP450) is a superfamily of proteins found in every single organism. This enzyme complex plays a significant role in drug metabolization, detoxification, and activation; and also in steroid hormone and sterol biosynthesis, vitamin biosynthesis, and enzymatic activation of carcinogens and other toxins.

Since its first discovery in 1962, knowledge and research in regards to CYP450 has increased exponentially (Omura, 2014). As of 2014, 18.000 P450 proteins have been discovered in eukaryotes and prokaryotes, and it is very likely many more will be discovered as technology progresses (Omura, 2014). Because of the essential role of CYP450, it is important to provide more information on its nomenclature, structure, location, functions and species differences.

2.1.2 General Classification and Nomenclature

Before an overview of the functions of CYP450 can be given, it is necessary to give an outline of the complex nomenclature system. Due to the large volume of *CYP450* genes across species, a clear method of naming these genes is necessary. A nomenclature system founded on evolutionary relationships, based on phylogenetic trees derived from P450 protein sequences, was first created in 1987 by D. Nelson (Nelson, 1998). However, due to the large amount of *CYP450* genes still being discovered during genome projects and advances in genomic sequencing of several species, an adjustment of nomenclature was deemed necessary. These adjustments are overseen by the Committee on Standardized Cytochrome P450 Nomenclature headed by D. Nelson (Nelson, 2006).

In short, CYP represents cytochrome P450 followed by a number that designates the family, and sometimes a letter indicating the sub-family, and ending in a number representing the actual polypeptide (Nelson et al., 1996). The name is italicized when indicating the actual gene and not italicized when representing the cDNA, mRNA or enzyme. To better understand the entire nomenclature system, more detail is provided about the division into clans, families and sub-families.

The division into clans is one the of the most recent changes to the CYP450 nomenclature. Due to the increasing number of *CYP450* genes and families, an overarching organization was deemed necessary. Through tracing the evolutionary history of CYP450, it was discovered that the *CYP450* gene is very old. David Nelson's research showed that all *CYP* clans started in a "cytochrome P450 genesis locus, where one progenitor *CYP* gene duplicated to create a tandem set of genes that were precursors of the 11 animal clans" (Nelson et al., 2014). Based on this information, the concept of clans was created. "A *CYP* clan is a clade of genes. Clans are relatively deep branchings on phylogenetic trees. There are eleven total clans of animal *CYP*s but only four clans in most arthropods and nematodes and five in ticks. Plants have a different set of *CYP* clans, except for *CYP51* and *CYP74* that are in common" (Nelson et al., 2014). The eleven animal clans are *CYP* clans 2, 3, 4, 7, 19, 20, 26, 46, 51, 74 and mitochondrial.

The next level of division is a family. A family is a group of *CYP* genes that have ≥ 40% protein sequence in common (Nelson, 2006). Initially, there were less than 100 P450 families known for eukaryotes and a numerical naming system was created as follows: animals *CYP* 1-49, lower eukaryotes *CYP* 51-69, plants *CYP* 71-99 (Nelson, 2006). This system has been revised several times, and has now been expanded to a four-digit system for newly discovered genes: bacteria *CYP*

1001-2999, animals *CYP* 3001-4999, lower eukaryotes *CYP* 51-69, and plants *CYP* 7001-9999 (Nelson, 2006). Overall, in the animal kingdom 14 different CYP families can be found CYP1, CYP2, CYP3, CYP4, CYP5, CYP7A, CYP7B, CYP8, CYP11, CYP17, CYP19, CYP21, CYP24, CYP27, and CYP51.

The last major division is that of sub-families. A sub-family has ≥ 55% of their protein sequence in common. These groups are identified by the use of letters, the first 24 had single letters and now double letters are being used, allowing for up to 576 letters or subfamilies. The letters I and O are not used to avoid confusion with 1 and 0. The Arabic number at the end of the name indicates the individual polypeptide (Nelson, 2006).

When a new *CYP450* sequence is discovered, it has to undergo a name process by the Committee on Standardized Cytochrome P450 Nomenclature. First, a BLAST search is performed and the submitted sequence is compared to a database containing all discovered *CYP450* sequences (Nelson, 2006). This search tries to match up the new gene with existing families. If no match of ≥ 40% is discovered, it means the gene might be part of a new family. In recent years this only occurred with fungal or bacterial *CYP450* genes; the vast majority of vertebrate *CYP450* genes have been discovered (Nelson, 2006). The next step is to identify the sub-family. Unlike new families, new sub-families are found frequently. In order to assign a new sub-family, additional subsets of sequences are compared and both a UPGMA and neighbor-joining tree is derived (Nelson, 2006).

An additional difficulty to the naming of *CYP450* genes is the existence of pseudogenes and polymorphisms within genes. Generally a P is placed at the end of the gene name to indicate that it is a pseudogene (Nelson, 2014). However, several types of pseudogenes were discovered and each type is indicated in a specific way now. A pseudogene that is virtually intact is designated the letter P after the gene name. A solo exon pseudogene is assigned an extension –*sel*[x-y]; sel stands for solo exon and [x-y] is the number of exons the pseudogene differs from the functional gene (Nelson, 2004). A detritus exon, a duplication of one or more exons, is named by the extension –*de* (Nelson, 2004). Last, a duplication of an internal exon is indicated by–*ie* (Nelson, 2004). Polymorphism can occur through point mutations, when these mutations are identified, they are indicated by X###Y; X is the wild type and Y is the mutant amino acid (Nelson et al., 1996).

2.1.3 Location and Mechanisms of Drug Metabolization

CYP450 is a group of enzymes that plays a role in not only drug metabolization, detoxification, and activation, but also in steroid hormone and sterol biosynthesis, vitamin biosynthesis, and enzymatic activation of carcinogens. To describe all the functions and mechanisms of CYP450 would lead beyond the scope of this thesis. Therefore, only the mechanisms related to drug metabolism will be discussed.

CYP450's are located in different organs, including the liver, lungs, intestines, nasal epithelia, skin and others. However, the majority is located in the liver and intestines and these are the main locations for drug metabolization (Martinez et al., 2013). Heikkinen et al. quantified the amount of CYP450 enzymes in intestines and liver in Beagles through mass spectrometry. In this study the following observations were made. First, the intestines showed a much lower amount of protein "per mass of whole tissue" than the liver. Second, the activity of enzymes decreased from small intestines to the colon, indicating that the small intestines are more important in metabolizing drugs than the rest

of the GI tract. Third, CYP3A12 and CYP2B11 enzymes amounts were higher in intestines than in the liver, while other enzymes were higher in the liver than in the intestines (Heikkinen et al., 2012).

CYP450 is involved in 2 important phases of pharmacokinetics. First, it plays a role in oral drug absorption through intestinal metabolism. Second, it plays a key role in drug metabolism mainly through metabolization in the liver. The main goal of metabolizing xenobiotics is to inactivate them and increase the polarity of the drug to ensure a better renal or biliary excretion (Trepanier, 2006). Besides inactivation, CYP450 enzymes can also bioactivate pro-drug components into their active form. The enzymes can also produce a toxic metabolite out of an otherwise harmless component.

A few important concepts in regards to CYP450 pharmacogenetics are substrates, phase I reactions, induction, and inhibition. A substrate can be defined as "The chemical entity whose conversion to a product or products is catalyzed by one or several enzymes" (McNaught and Wilkinson, 1997). CYP450 substrates can be exogenous, such as pharmaceuticals and other xenobiotics. The substrate can also be endogenous, for example steroid hormones. Eukaryotic CYP450's show a substrate specificity that is not rigorous, they can bind a large variety of substrates of different chemical properties, size and structure (Poulson, 2014).

Xenobiotic metabolization occurs in 2 phases. Phase I metabolization results in a chemical modification of the substrate through the addition of a functional group, usually a hydroxyl group for the majority of CYP450 enzymes, which can then be used as a location to attach a conjugate (Ando, 2014). Hydroxylation is the most important reaction for CYP450, through mono-oxygenases, an atom of molecular oxygen is incorporated into the substrate and one atom is added to a water molecule (Ionescu and Caira, 2005). The reaction can be summarized as follows: $R\text{-}H + O_2 + 2e^- + 2H \rightarrow R\text{-}OH + H_2O$. Phase II of drug metabolization is initiated when phase I metabolization is not sufficient to clear the substrate; the most important pathway here is glucuronidation, which also occurs in the liver microsomal system, but is not related to CYP450 (Ionescu and Caira, 2005).

An induction of a CYP450 enzyme occurs when a substrate gives a chemical signal to a nuclear receptor, leading to a transcriptional activation of the gene, and to a higher activity and synthesis of enzymes (Trepanier, 2006). Through induction the metabolism can be enhanced, leading to reduced plasma concentrations of xenobiotics (Ando, 2014).

A CYP450 enzyme can be inhibited when there is a drug-drug interaction of 2 substrates in competition for the same CYP enzyme. Three different inhibition mechanisms can be detected. First, a competitive inhibition occurs when 2 substrates compete for the same catalytic binding site on the CYP450 enzyme. Second, a non-competitive inhibition happens when a substrate binds on a site, but also changes the binding site of another substrate, rendering it inactive. Last, a mechanism-based or suicide inhibition happens when a reactive metabolite is created that binds irreversibly onto a binding site (Trepanier, 2006).

2.2. CANINE CYP450 PHARMACOGENETICS

2.2.1 Introduction

Most research about *CYP450* genes is conducted in regards to human genes. Canine *CYP450* genes were initially researched due to canines being used as experimental models for human pharmaceutical experimants. This is due to a large degree of homogeneity between human and canine *CYP450* genes. Nevertheless, more and more research is being conducted specifically about canine *CYP450* genes. In the future, the research and knowledge about canine *CYP450* genes should increase.

2.2.2 Canine CYP450 Classifications

The canine CYP450 complex exists out of 10 clans, 18 families, 45 subfamilies, 55 individual genes and 9 pseudogenes. This compared to humans, that have 57 active *CYP450* genes and 58 pseudo-genes (Ingelman-Sundberg, 2005). A full overview of all the currently identified genes can be found in table VIII. This table gives a brief outline of what is currently known about the genes, their location, activities and pathologies.

2.2.3 Canine CYP 450 genes and polymorphisms

As mentioned, polymorphisms in genes can cause differences in genotypes and phenotypes. This is also the case with CYP450. Polymorphisms in *CYP450* genes can cause inter-individual variations in the metabolization of not only xenobiotics, but also endogenous compounds such as hormones, lipids and vitamins. When variations occur in *CYP450* genes that catalyze endogenous compounds, hereditary diseases including adrenal hypoplasia, congenital ichtyosis and spastic paraplegia 5A can occur. These variations will not be discussed here. The focus will be on the polymorphisms that affect *CYP450* genes that regulate drug metabolism. Due to the limited information available, this section will start with a general description of the gene's function, location and other relevant information, which is generally derived from knowledge about human *CYP450* genes. When available, a detailed description of the canine *CYP450* gene and polymorphism is given and the differences with human *CYP450* genes are highlighted.

2.2.3.1. CYP1A2

The CYP1A family contains 2 members, *CYP1A1* and *CYP1A2*. *CYP1A2* is well documented in humans; it metabolizes around 4% of pharmaceuticals and several important endogenous compounds, including steroids. It also plays a role in activating a number of pro-carcinogenic compounds (Martignoni et al. 2006; Zhou et al., 2010). The enzyme can metabolize xenobiotics and endogenous compounds through a large range of metabolic pathways including hydroxylation, oxidation, sufloxidation and N-demethylation (Zhou et al., 2010).

The canine *CYP1A2* gene is 81% identical to the human gene. There are distinct differences between both species despite the large overlap in amino acids. In humans, the gene represents about 13% of the total CYP content in the liver and the extra-hepatic tissues have no or very low levels of expression (Martignoni et al., 2006). In dogs however, CYP1A2 is expressed at a lower level

in the hepatic tissue, accounting for about 4% of the total hepatic CYP expression (Martinez et al., 2013). Additionally, Mise et al. characterized the substrate specificity of canine *CYP1A2* (Mise et al., 2008). The results of this study showed that there is a significant difference in substrate specificity between dogs and humans. For example, melatonin is only partially metabolized in dogs and estradiol is not metabolized at all in dogs, while these two are specific substrates for the human CYP1A2 enzyme (Mise et al., 2008; Zhou et al., 2010). This should be considered when using dogs as an experimental model for human pharmaceuticals. More examples can be found in table I.

In humans, *CYP1A2* is characterized by a significant inter-individual variation due to polymorphisms in the gene, but also due to epigenetic and environmental factors (Zhou et al, 2010). A significant polymorphism has also been discovered in canines. In 2004, Mise et al. were one of the first to study the inter-individuality in dogs in regards to *CYP1A* (Mise et al., 2004a and Kamimura, 2006). Through an experiment using 5-(3-Methoxyphenyl)-3-(5-methyl-1,2,4-oxo=adiazol-3-yl)-2-oxo-1,2-dihydro-1,6-naphthyridine (AC-3933), dogs with a phenotype of poor metabolizers and a phenotype of extensive metabolizers were discovered. The poor metabolizers showed a much lower clearance of AC3933 than extensive metabolizers. Mise et al. concluded that this lower clearance was a direct result of a polymorphic expression of *CYP1A2* (Mise et al., 2004a).

A follow-up study identified this polymorphism as a nonsense mutation in the cDNA where a premature stop codon was inserted instead of an arginine at position 373 (Mise et al., 2004b). This mutation results in an amino acid sequence that is 140 amino acids shorter than the normal gene with a lack of a heme-binding site; this results in a non-functional enzyme (Mise et al., 2004b; Mosher and Court, 2010). The polymorphism is consequently caused by an SNP in the coding region and it is a non-functional allelic variant of the wild-type gene. Generally, the mutation is referred to as *CYP1A2 C1117T* or *CYP1A2 R373X*. This study also revealed an allele frequency of 37% and a prevalence of the homozygous mutant genotype in more than 10% of 149 Beagles genotypes (Mise et al., 2004b). This shows that *CYP1A2* polymorphism can play a significant role in veterinary pharmacogenetics.

Further research has shown that besides Beagles, the polymorphism is also present in several other breeds (Court, 2013). The highest allele frequency was found in Irish Wolfhounds (42%), Japanese Beagles (39%), and Berger Blanc Suisse (28%). All other breeds had a much lower allele frequency, around 10% or less. Herding dogs are well presented within this list and Court even suggests that there might be a common ancestry between *CYP1A2 R373X* and the *MDR-1* deletion mutation. He also concludes that the gene polymorphism frequency is about 1% in the general population, which indicates that it is a relatively rare mutation (Court, 2013).

Although the polymorphism in *CYP1A2* is well researched, for now it appears that this cytochrome is not used to metabolize any clinically relevant veterinary drugs. However, its importance lays in its use in human clinical studies regarding specific types of cancers, such as colon cancer induced by cigarette smoke (Mealey, 2006; Zhou et al., 2010).

2.2.3.2. CYP2B11

The CYP2B family is known for its induction by baribiturates (Martignoni et al., 2006). In humans, the CYP2B family contains *CYP2B6* and *CYP2B7. CYP2B6* is a very important drug metabolizing gene; it metabolizes about 25% of clinically available pharmaceuticals (Martignoni et al., 2006). It is also responsible for metabolizing the following important drugs: efavirenz, nevirapine, bupropion, methadone, and cyclophosphamide (Martignoni et al., 2006; Martinez et al., 2013). But it also

metabolizes environmental toxins such as aflatoxin B1 (Martignoni et al., 2006). Besides these xenobiotics, it is also responsible for the oxidative hepatic metabolism of steroids and it metabolizes endogenous compounds including progesterone (Shou et al., 2013). *CYP2B6* accounts for 2-10% of the CYP450 content in the liver; it has not been detected in the intestines (Martignoni et al., 2006; Martinez et al., 2013).

In canines the *CYP2B6* gene is not present. However, a paralog named *CYP2B11* is present and is 78% identical to the human gene is. The associated enzyme was first discovered in 1987 as a major phenobarbital-induced liver enzyme, and was initially named PBD-2 (Duignan et al., 1987). Despite the similarity in amino acid sequence, the dog gene shows several differences in respect to its closest related human paralog. First, *CYP2B11* catalyzes the N-demethylation of dextromethorphan, which is metabolized by *CYP3A* in humans. Second, it catalyzes mephenytoin through 4'-hydroxylation of mephenytoin; this is mediated by *CYP3A* in humans. Third, *CYP2B11* also plays a role in S-warfarin-hydroxylation together with *CYP3A12* (Martignoni et al., 2006). Fourth, rifampicine a major *CYP2B6* inducer, does not induce *CYP2B11*. This is caused by a difference in activation through gene regulation (Graham, 2006). Additionally, the dog seems to be the only species where polycyclic aromatic hydrocarbons are metabolized through the CYP2B family (Guengerich, 1997). Last, *CYP2B11* accounts for about 11% of the total hepatic CYP450 content (Baratta et al., 2009).

It is now known that *CYP2B11* is responsible for the metabolization of important veterinary drugs including propofol, diazepam, midazolam, medetomidine and cyclophosphamide (Shou et al., 2003; Trepanier, 2006; Martinez et al., 2013). Additionally, *CYPB11* is greatly influenced by drugs that act as inducers including phenobarbital which causes a 9.9 times increase in activity (Graham et al., 2002). It is also influenced by inhibitors including chloramphenicol, an antibiotic, that prolongs the anesthesia induced by propofol (Martinez et al., 2013). Other drugs such as propofol, medetomidine and atipamezole can be selective inhibitors *in vitro*, but their effect *in vivo* is unsure (Baratta et al., 2009; Martinez et al., 2013). A more expanded list of *CYP2B11* substrates, inductors and inhibitors can be found in table II.

The *CYP2B6* gene is characterized by a very high level of variation in humans; around 60 allelic variants have been found and many of these polymorphisms cause a decreased or increased expression and activity (Martinez et al., 2013; Rettie and Thummel, 2014). In canines, polymorphisms in *CYP2B11* have not been researched well. In a University of Utrecht project, student A. Wenker studied potential polymorphisms in *CYP2B11* (Wenker, 2009). She conducted a sequencing study through PCR amplification, found several polymorphisms and analyzed them through PolyPhen, an application used to predict the effect of SNPs on the enzyme's function. A first polymorphism was found in exon 2; this variant resulted in a change in amino acid sequence. Her PolyPhen search predicted that this change likely has a negative effect on the enzyme function (Wenker, 2009). A second polymorphism was found in exon 7 in the second base pair and was present in a large part of the tested population. This variation causes a change in the splice site and results in an abnormal protein with a decrease in effectiveness (Wenker, 2009). Furthermore, she found 2 putative intronic SNP's that appear to have no effect on the amino acid coding. However, intronic SNPs have been found in the human *CYP2B6* and they do alter the enzyme function of this gene (Wenker, 2009).

To date no polymorphism has been recognized with a proven, phenotypic change in metabolization. However, it is very likely that *CYP2B11* can affect a variety of clinically important veterinary drugs. A brief overview of some of the most important examples is given.

An important interbreed variation was noticed in regards to greyhounds. Greyhounds are known to recover slowly after thiobarbiturate or propofol anesthesia. Several studies in the 1980's showed that this slow recovery was mainly due to a slower drug metabolism (Mosher and Court, 2010). Court et al. and Hay Kraus et al. conducted *in vitro* studies that showed the rate of propofol hydroxylation was much slower and this is due to lower expression of *CYP2B11* (Court, 1999; Hay Kraus et al., 2000). However, several authors have stipulated that it is still uncertain whether this low expression is due to an unrecognized polymorphism in *CYP2B11* and if this low expression is also the cause of decreased clearance of thiobarbiturates or other pharmaceuticals in Greyhounds (Mosher and Court, 2010; Martinez et al., 2013).

Ketamine and midazolam are also two anesthetic substrates that are frequently used. Medetomidine, also an anesthetic, is an inhibitor of CYP2B11. Combinations of these substrates and the inhibitor is often administered at the same time. In several studies by Baratta et al. and Shou et al. the potential to have pharmakinetic drug interactions when both types of drugs are administered was explored. These *in vitro* studies showed that medetomidine does alter the pharmacokinetics when administered together with ketamine or midazolam. However, other factors probably minimize the effect of this interaction, such as the short half-time of anesthetics and the rigid anesthesia regiments (Baratta et al, 2009; Shou et al., 2013).

Cyclophosphamide is often used to treat immune mediated diseases such as Immune Mediated Hemolytic Anemia and is part of a chemotherapy protocol to treat lymphoma (Burgess et al., 2000; Macdonald et al., 2005). Due to the serious side-effect of this drug, it is important to have a good understanding of the pharmacogenetics that can interfere in the working of this drug.

As shown above, *CYP2B11* research in regards to polymorphisms and potential drug variability has been limited. In order to make further conclusions in regards to the effect of a polymorphism on Greyhound drug metabolism and important veterinary drugs, more research into molecular pharmacogenetics and *in vivo* clinical effects need to be done.

2.2.3.3. CYP2C21 / CYP2C41

The CYP2C family in canines has 2 members: *CYP2C21* and *CYP2C41*. *CYP2C21* is present in all dogs and is primarily found in the liver, but low levels can also be found in skin, the mammary gland, lymph nodes and bone (Martinez et al., 2013). The ortholog of *CYP2C21* is *CYP2C19* in humans; they have 70% of their amino acid sequence in common (Mosher and Court, 2010). *CYP2C19* is an important drug metabolizing gene in humans and is responsible for metabolizing about 10% of human drugs (Lee and Shin, 2014). The gene is also characterized by a lot of inter-individual variation, especially among different races (Lee and Shin, 2014). The differences between the human ortholog and the canine are extensive. For example, in humans, the CYP2C family is responsible for the warfarin, tolbutamide, and S-mephenytoin metabolism, while the metabolization of these substrates is severely impaired in dogs (Martignoni et al., 2013).

Little is known about canine *CYP2C21*; this is due to the fact that many of the substrates metabolized by *CYP2C21* are also metabolized by other CYP450 enzymes. To date only a limited amount of specific substrates have been found that are metabolized by *CYP2C21* and little is known about CYP2C21 interactions. A list of some of the substrates metabolized by *CYP2C21* is shown in table III.

CYP2C41 was discovered during an attempt to clone *CYP2C21* from liver RNA by Blaisdell et al. in 1998 (Blaisdell et al., 1998). Back then, it was the first polymorphic variation found within canine

CYP450 (Blaisdell et al., 1998). However, this gene is not expressed in every dog; Blaisdell's study discovered that only 16% of the Beagles and mixed breeds examined had the gene and that this variation is not sex or breed determined (Blaisdell et al., 1998). This polymorphism was confirmed in another study by Graham et al., but they found that 5 out of 11 Beagles were positive for this gene (Graham et al, 2003). The gene is 70% identical to *CYP2C21*, but differs due to a partial gene deletion in exon 4 to exon 7 (Blaisdell et al., 1998; Mosher and Court, 2010). These findings indicate that the *CYP2C41* gene is partially or completely deleted in most dogs. The impact of this deletion is limited, and research by Shou et al. showed that *CYPC41* metabolizes the same substrates as *CYP2C21*, but with a lesser efficiency (Court, 2013; Shou et al., 2013).

In conclusion, it is unclear whether *CYP2C41* can have an impact on canine drug metabolization. In order to determine its effect more research needs to be done to discover specific and high turnover substrates of this gene (Mosher and Court, 2010).

2.2.3.4. CYP2D15

The canine CYP2D15 is an ortholog of the human CYP2D6 enzyme. In humans the CYP2D6 has a low expression level in the liver, about 5% of total CYP450. However, the enzyme metabolizes about 30% of used drugs including anti-arrhythmica, adrenoreceptor antagonists, and tricyclic depressants (Roussel et al., 1998; Mosher and Court, 2010; Martignoni et al,, 2006).

The canine gene is mainly expressed in the liver. About 3 to 20% of the canine CYP450 expression in the liver can be attributed to this gene. The enzyme can also be found in the bladder, kidney, lung and brain, but not in the intestines (Roussel et al., 1998; Martinez et al., 2013). The enzymatic activities of the canine gene are very similar to those of the human gene (Martignoni et al., 2006). A list of common substrates, inductors and inhibitors can be seen in table IV.

Polymorphisms are common within the CYP2D family. In humans, *CYP2D6* is one of the most researched CYP450 genes due to its many polymorphisms which alter drug metabolization significantly between individuals and racial groups (Martignoni et al., 2006). These polymorphisms are often characterized by extensive metabolizer and poor metabolizer phenotypes. These polymorphisms can lead to several serious adverse drug reactions and interactions (Roussel et al., 1998). Additionally, the gene has been linked to Parkinson's Disease, liver cancer, lung cancer and melanoma (Martignoni et al., 2006).

The canine *CYP2D15* also has several polymorphisms; six variants of the normal gene were found (Paulson et al., 1999; Roussel et al., 1998). First, the *CYP2D15*2* and *CYP2D15*3* polymorphisms have substitutions in 3 places: G186S, I250F and I307V. These variants exhibit about 80% of the regular enzyme activities (Martinez et al., 2013). Second, there is a splice variant, CYP2D15del, with a deletion of 51 amino acids in exon 3 (Paulson et al., 1999; Mosher and Court, 2010). This allele has no enzymatic activity resulting in a poor metabolizer and extensive metabolizer phenotype. Third, CYP2D15WT2 also has substitutions in 3 places: G186S, I250F and I307V. The enzymatic activity varies depending on the substrates. For example the mutant enzyme has a 38% activity on bufuralol and a 75% activity on celecoxib compared to the normal enzyme (Martinez et al., 2013). Last, there is CYP2D15V1 and CYP2d15V2 variants. CYP2D15V1 has 5 substitutions: S186G, I250F, I307V, I338V, and K407E. CYP2D15V2 is a splice variant with a deletion of G121-A172 in exon 3 and substitutions in 3 places: G186S, I250F and I307V. The substitution and splice variation lead to a change in activity, but it is almost undetectable (Roussel et al., 1998; Martinez et al., 2013).

Little research has been done to connect the variations within this enzyme to actual variations with the metabolism, but it is highly likely that the variations result in a decreased activity of the enzyme (Mosher and Court, 2009). In his 2013 paper on CYP450 polymorphisms, Court speculates that the polymorphic changes have little impact on enzyme activity with the exception of CYP2D15WT2 which has a 50% lower bufuralol hydroxylation (Court, 2013). Furthermore, he challenges Paulson's conclusions in regards to CYP2D15 polymorphic clearance of cerecoxib. He concludes that the celecoxib polymorphism is still unexplained (Court, 2013).

2.2.3.5. CYP2E1

CYP2E1 plays a role in both drug metabolization and in nutritional support. The gene is expressed in multiple tissues, such as the liver, the lung, nose, and oropharynx (Martignoni et al., 2006). It is an enzyme that is expressed at a relatively constant rate, but it can also be induced by ethanol, fastening and in diabetes patients (Lankford et al., 2000; Martignoni et al., 2006). The most important mechanism of CYP2E1 is oxidation. In addition to oxidizing a limited amount of drugs, the enzyme oxidates a significant amount of carcinogens, such as benzene, nitrosamines, and styrenes (Martignoni et al., 2006). Furthermore, the enzymes play a large role in the intermediary metabolism of endogenous products such as ketones and fatty acids (Lankford et al, 2000). Furthermore, in humans, CYP2E1 enzymes make up about 6% of the total CYP450 in the liver and metabolize about 2% of pharmaceuticals (Martignoni, et al., 2006).

In humans, there are several polymorphisms in this gene, but no significant changes in drug metabolization have been found. The canine *CYP2E1* is 77% identical to the human gene. However, Langford et al. tested the enzymatic activity of *CYP2E1* in regards to chlorzoxazone and found that "the canine CYP2E1 is less efficient in metabolizing this substrate than human CYP2E1" (Lankford et al., 2000). So care should be taken when using canines as a model when using CYP2E1 specific substrates. However, more substrate studies are needed for the canine CYP2E1 gene. An overview of current substrates, inducers and inhibitors of *CYP2E1* is seen in table V.

In 2000, Lankford et al. discovered a polymorphism in CYP2E1. A SNP at position 485, causes an amino acid substitution from tyrosine to histamine (Lankford et al., 2000). This variant is named CYP2E1 T485H. The SNP is not near the heme binding region, but is part of the beta-sheet structure close to "substrate recognition site 6as predicted by Gotoh" (Lankford et al., 2000). In addition, Lankford et al. researched the frequency of this variant by genotyping 100 mixed-breed dogs and 13 purebred beagles. The SNP was found in 15% of the mixed breed dogs and 19% of the Beagles (Lankford et al., 2000).

2.2.3.6. CYP3A Sub-family

The CYP3A family is the most important human drug metabolizing family. Enzymes from this family metabolize about 50% of all available human pharmaceuticals available (Martignoni et al., 2006; Lee and Shin, 2014). Besides drugs, the gene is also involved in the oxidation of steroids, bile acids and retinoic acid (Rettie and Thummel, 2014). In humans, four enzymes are expressed: CYP3A4, CYP3A5, CYP3A7 and CYP3A34 (Rettie and Thummel, 2014). In canines the CYP3A family exists out of CYP3A12 and CYP3A26 (Martignoni et al., 2006). This family is primarily found in the liver, it constitutes out of 30% of the CYP450 liver content in humans and about 14% of the CYP450 liver content in canines. The subfamily also plays a large role in the first-pass drug metabolization in the

intestines (Martignoni et al., 2006). Inducers activate the enzymes through transcriptional activation and some of the most important inhibitors are ketoconazole and itraconazole (Martignoni et al., 2006).

In canines, most research in regards to the 3A subfamily involves *CYP3A12*, which is an ortholog of the human *CYP3A4* and has 79.8% of the sequence in common (Martinez et al., 2013). *CYP3A12* is the main CYP3A enzyme in the duodenum, accounting for 99.8% of the total CYP3A content. In the liver, it only constitutes 24.8% of the total hepatic CYP3A content (Chen et al., 2009; Heikkinen et al., 2012; Martinez et al., 2013). As with many of the canine CYP450 genes, little information is known about specific substrates for *CYP3A12*. A list of some of the known substrates, inducers and inhibitors is shown in table VI. Furthermore, extrapolation from *CYP3A4* information to the canine gene is not recommended (Martinez et al., 2013).

Little is known about *CYP3A26*, and usually the literature discusses both enzymes together as a subfamily (Martinez et al., 2013). *CYP3A26* accounts for 75.2% of the total CYP3A hepatic pool in canines and little is expressed in the duodenum unlike *CYP3A12* (Chen et al., 2009; Martinez et al., 2013). *CYP3A26* is 96% identical to *CYP3A12* and differs only in 22 of 503 amino acids (Fraser et al., 1997). Despite the high degree of identity, 9 of the different amino acids are located in the substrate binding site, which greatly affects the metabolizing capacity of *CYP3A26*. *CYP3A26* has a much lower capacity to metabolize steroids then *CYP3A12* (Locuson et al., 2009). Some examples of the substrates, inducers and inhibitors of *CYP3A26* are shown in table VII.

A polymorphism has been identified for *CYP3A12* . The variant has five amino acid changes at positions 309, 421, 422, 423, and 425, this results in an “amino acid change in exon 10 and four amino acid changes in exon 11” (Paulson et al., 1999). This variant is generally referred to as *CYP3A12*2*. Despite these differences compared to the wild type, *in vitro* experiments have shown that the variant has about the same metabolic capacity as the regular *CYP3A12* in regards to testosterone-6-beta-hydroxylation (Court, 2013).

DISCUSSION

In this dissertation an overview of the concept of pharmacogenetics is given and the available research on the pharmacogenetics of canine CYP450 is summarized. Although the field of pharmacogenetics is expanding quickly, the practical applications are limited. In veterinary medicine the field has taken off since the start of several genome projects. Pharmacogenetics has one important practical application in veterinary medicine and that is the detection of the *ABCB1* deletion.

Most of the knowledge about canine CYP450 is derived from studies about human CYP450 because canines are also often used as a model for human pharmaceutics. Although the amount of canine CYP genes varies in the literature, about 55 individual CYP450 genes and 9 pseudogenes have been identified. Of those genes the following are important for drug metabolizing: *CYP1A1/CYP1A2, CYP2B11, CYP2C21/CYP2C41, CYP2D15, CYP2E1,* and *CYP3A26*. In all these genes polymorphisms have been identified. Some of them show a lower capacity to metabolize xenobiotics, but none of the research shows them having a significant clinical or therapeutic impact.

From this literature review we can conclude that further research is necessary in several areas. One of the most challenging parts will be to identify all the polymorphisms in the drug metabolizing CYP450 genes. This is challenging because of the large variety of breeds within canines. Because not only do these polymorphisms only appear in certain breeds, often they also only appear in certain lines within the breed. Therefore, it is necessary to make sure that the pool of researched animals and breeds is large. In addition, more research needs to be done to find specific substrates for each of the CYP450 genes. This is important to not only better understand the mechanisms of drug metabolization, but it is necessary to identify the genes using probes based on the specific substrates. As the overview of the available research shows, more *in vitro* and *in vivo* studies need to be done in order to fully understand the clinical impact of these polymorphisms.

With more research the pharmacogenetic significance of canine CYP450 can increase. Veterinary medicine is a growing branch within the pharmaceutical industry, and the pharmacogenetics of CYP450 can play a significant role in the development of new therapies and making existing therapies more efficient. A true practical application, such as personalized medicine for canines, will most likely not happen in the near future.

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APPENDICES

Table I: Substrates, Inductors, and Inhibitors of the canine *CYP1A2* in comparison to human substrates.

Substrates	Inductors	Inhibitors	Human Substrates
Phenacetin	Polychlorated biphenyl	Alfa-naphtoflavone	Aminopyrine
Ethoxyresorufin	Beta-Naphtoflavone	Ondansetron	Aniline
Tacrine	Omeprazole	Furafylline	Bufuralol
Melatonin		Fluvoxamine	Midazolam
Theophylline		Enrofloxacin	caffeine
Caffeine (very limited)			9-cis-Retinol
Fluoroquinolones			Estradiol

Adapted from: Mise, 2004b, 2008; Martignoni et al., 2006; Martinez et al., 2013.

Table II: Substrates, Inductors, and Inhibitors of the canine *CYP2B11*

Substrates	Inducers	Inhibitors
Diazepam	Phenobarbital	Chloramphenicol
Temazepam	Bergamottin	S-Medetomidine
Testosterone		Atipamezole
Diclofenac		Ketoconazole
Propofol		Miconazole nitrate
Midazolam		Propofol
Ketamine		Loperamide
Atipamezole		Diazepam
Medetomidine		Griseofulvin
CPA		Piroxicam
Methadone		Sulfadimethoxine
Pentobarbital		Ivermectine
Warfarin		Fluoxetine HCl
Phenytoin		Deracoxib
Progesteron		Cyclosporine A
Cyclophosphamide		

Adapted from: Shou et al., 2003; Trepanier, 2006; Aidasani et al., 2008; Martinez et al., 2013.

Table III: Substrates, Inductors, and Inhibitors of the canine *CYP2C21*

Substrates	Inducers	Inhibitors
(S)-Mephenytoin	Phenobarbital	Ketoconazole
Diclofenac		Vincristine
Midazolam		Miconazole nitrate
Sulfaphenazole		Loperamide
		Amoxicilline
		Ivermectin
		Praziquantel
		Ranitidine HCl
		Piroxicam
		Prednisone

Adapted from: Shou et al, 2003; Aidasani et al., 2008; Martinez et al., 2013.

Table IV: Substrates, Inductors, and Inhibitors of the canine *CYP2D15*

Substrates	**Inducers**	**Inhibitors**
Celecoxib		Clomipramine
Propanolol		Fluoxetine
Buforalol		Ketoconazole
Dextromethorphan		Loperamide
Bunitrolol		Quinidine
Imipramine		Ritonavir
Metoprolol		Metoclopramide HCl
Desipramine		Ondansetron HCl
Maropitant		Trimethoprim
Tramadol		Doxorubicin
		Vincristine
		Clomipramine HCl
		Fluoxetine HCl
		Diphenhydramine
		Omeprazole
		Prednisolone

Adapted from: Shou et al, 2003; Martignoni et al., 2006; Aidasani et al., 2008; Martinez et al., 2013.

Table V: Substrates, Inductors, and Inhibitors of the canine *CYP2E1*

Substrates	**Inducers**	**Inhibitors**
Acetaminophen	Ethanol	
Chloroxazone	Acetone	
Theophylline		
Ethanol		
Enflurane		
Halothane		

Adapted from: Martignon et al., 2006.

Table VI: Substrates, Inductors, and Inhibitors of the canine *CYP3A12*

Substrates	**Inducers**	**Inhibitors**
Diazepam	Phenobarbital	Ketoconazole
Nordiazepam	Rifampicin	Dexamethasone
Testosterone	Efavirenz	Bergamottin
Dextromethrophan	Lacidipine	Loperamide
Midazolam	Clotrimazole	Miconazole nitrate
Eplenerone	Felodipine	Vincristine
Diclofenac	Phenytoin	Ivermectine
Erythromycine	Ritonavir	Cyclosporin A
Cyclosporine	Netredipine	
	Sulfinpyrazole	
	Lansoprazole	
	Phenobarbital	

Adapted from: Shou et al., 2003; Trepanier, 2006; Aidasani et al., 2008.

Table VII: Substrates, Inductors, and Inhibitors of the canine *CYP3A26*

Substrates	**Inducers**	**Inhibitors**
Diazepam	Phenobarbital	Ketoconazole
Nordiazepam	Rifampicin	Dexamethasone
Testosterone	Efavirenz	Bergamottin
Dextromethrophan	Lacidipine	
Midazolam	Clotrimazole	
Eplenerone	Felodipine	
Diclofenac	Phenytoin	
Erythromycine	Ritonavir	
Cyclosporine	Netredipine	
	Sulfinpyrazole	
	Lansoprazole	
	Phenobarbital	

Adapted from: Shou et al., 2003; Trepanier, 2006.

Table VIII: Overview of Canine CYP450 genes

CLAN	FAMILY	SUBFAMILY	GENE	VARIANT	CHROMOSOME	SYNONYMS	ACTIVITY (uniprot.org)	PROCESSES (uniprot.org)	METABOLISM (Reactome.org)	PATHOLOGIES directly associated with defect / deficiency
CLAN 2	CYP1	A	1A1	46 Variants	30		Aromatase activity; oxidoreductase activty; vitamin D 24-hydroxylase activity; iron ion binding; heme binding.	Amine metabolic process; drug metabolic process; hydrogen peroxide biosynthetic process; toxin metabolic process; cellular response to organic cyclic compound; heterocycle metabolic process; response to toxic substance.	Synthesis of epoxy (EET) and dihydroxyeicostrienoic Acids (DHET); Synthesis of (16-20)-hydroxyeicosatetraenic acids (HETE); Xenobiotics.	
			1A2	140 Variants	30		Aromatase activity; demethylase activity; caffeine oxidase activity; iron ion binding; heme binding.	Alkaloid metabolic process; cellular respiration; cellular response to cadmium ion; dibenzo-p-dioxin metabolic process; exogenous drug catabolic process; hydrogen peroxide biosynthetic process; lung development; monocarboxylic acid metabolic process; monoterpenoid metabolic process; oxidative deethylation; oxidative demethylation; porphyrin-containing compound metabolic process; post-embryonic development; regulation of gene expression; steroid catabolic process; toxin biosynthetic process.	Synthesis of epoxy (EET) and dihydroxyeicosatrienoic acids (DHET); Synthesis of (16-20)-hydroxyeicosatetraenoic acids (HETE); Aflatoxin activation and detoxification.; Methylation; Aromatic amines can be N-hydroxylated or N-dealkylated by CYP1A2.	
		B	1B1	19 Variants	17		Oxireductase activity, Iron Ion Binding, heme binding.	Angiogenesis, arachidonic acid metabolic process, cellular aromatic compound metabolic process, cellular response to hydrogen peroxide, cellular response to organic cyclic compound, collagen fibril organization, endothelial cell-cell adhesion, endothelial cell migration, estrogen metabolic process, intrinsic apoptotic signaling pathway in response to oxidative stress, membrane lipid catabolic process, negative regulation of cell adhesion mediated by integrin, negative regulation of cell migration, negative regulation of cell proliferation, negative regulation of NF-kappaB transcription factor activity, nitric oxide biosynthetic process, positive regulation of angiogenesis, positive regulation of apoptotic process, positive regulation of JAK-STAT cascade, positive regulation vascular endothelial growth factor production, regulation of reactive oxygen species metabolic process, response to toxic substance, retinal blood vessel morphogenesis, retinal metabolic process, retinol metabolic process, toxin metabolic process, trabecular meshwork development, xenobiotic metabolic process.	Synthesis of epoxy (EET) and dihydroxyeicosatrienoic acids (DHET); synthesis of (16-20)-hydroxyeicosatetaenoic acids (HETE); Endogenous sterols.	Glaucoma
		D	1D1P		1					
	CYP2	A	2A13	88 Variations	1	CYP2A7, CYP2A6	Arachidonic acid epoxygenase activity, steroid hydroxylase acitivity, Oxireductase activity, oxygen binding, iron ion bindng, heme binding.	Coumarin metabolic process, exogenous drug catabolic process, xenobiotic metabolic process, epoxygenase P450 pathway, oxidation-reduction process.	Aflatoxin activation and detoxification; Xenobiotics.	
			2A25				Oxireductase activity, Iron Ion Binding, heme binding.			
		B	2B11	23 Variations	1	CYP2B6, PBD-2	Arachidonic acid epoxygenase activity, steroid hydroxylase acitivity, Oxireductase activity, oxygen binding, iron ion bindng, heme binding.	Cellular ketone metabolic process, exogenous drug catabolic process, steroid metabolic process, epoxygenase P450 pathway, oxidation-reduction process, xenobiotic metabolic process.	Xenobiotics	
			2BP							
		C	2C21		28	CYP2C8	Aromatase activity, iron ion biding, heme binding.		Synthesis of (16-20)-hydroxyeicosatetraenoic acids (HETE); Synthesis of epoxy (EET) and dihydroxyeicosatrienoic acids (DHET); xenobiotics.	
			2C21-IE5B							

CLAN	FAMILY	SUBFAMILY	GENE	VARIANT	CHROMOSOME	SYNONYMS	ACTIVITY (uniprot.org)	PROCESSES (uniprot.org)	METABOLISM (Reactome.org)	PATHOLOGIES directly associated with defect / deficiency
			2C41				Aromatase activity, iron ion biding, heme binding.			
			2C94P		28					
		D	2D15	7 Variants			Aromatase activity, monoxygenase activity, oxidoreductase activity, iron ion binding, heme binding.			
		E	2E1	5 Variants	28		Arachidonic acid epoxygenase activity, steroid hydroxylase acitivity, Oxidoreductase activity, oxygen binding, iron ion bindng, heme binding.	Exogenous drug catabolic process, monoterpenoid metabolic process, steroid metabolic process, heterocycle metabolic procss, epoxygenase P450 pathway, oxidation-reduction process, xenobiotic metabolic process.		
		F	2F1	11 Variants	1	CYP2F	Monoxygenase activity, oxidoreductase activity, oxygen binding, iron ion binding, heme binding.		Xenobiotics	
			2FP		1					
		G	2G2		1					
		J	2J2	22 Variants			Arachidonic acid 11,12-epoxygenase activity, Arachidonic acid 14,15-epoxygenase activity, arachidonic acid epoxygenase activity, linoleic acid epoxygenase activity, steroid hydroxylase acitivity, Oxireductase activity, oxygen binding, iron ion bindng, heme binding.	Arachidonic acid metabolic process, exogenous drug catabolic process, epoxygenase P450 pathway, linoleic acid metabolic process, oxidation-reduction process, xenobiotic metabolic process.	Synthesis of epoxy (EET) and dihydroxyeicosatrienoic acids (DHET).	
		R	2R1	66 variants	21		Vitamin D3 25-hydroxylase activity, oxireductase activity, oxygen binding, steroid hydroxylase activity, iron ion binding, heme binding.	Exogenous drug catabolic process, oxidation-reduction process, organic acid metabolic process, xenobiotic metabolic process.		Rickets vitamin D-dependent 1B
		S	2S1	33 Variants	1		Arachidonic acid epoxygenase activity, steroid hydroxylase acitivity, Oxireductase activity, oxygen binding, iron ion bindng, heme binding.	Epoxygenase P450 pathway, oxidation-reduction process, exogenous drug catabolic process, xenobiotic metabolic process.	Miscellaneous substrates	
		T	2T2		1					
		U	2U1	20 Variations	32	SPG49	Oxireductase activity, oxygen binding, steroid hydroxylase activity, iron ion binding, heme binding.	Exogenous drug catabolic process, oxidation-reduction process, organic acid metabolic process, xenobiotic metabolic process.	Synthesis of (16-20)-hydroxyeicosatetraenoic acids; miscellaneous substrates.	Spastic paraplegia 56
		W	2W1	6 Variants	6	FLJ20359, MGC34287	Oxireductase activity, oxygen binding, steroid hydroxylase activity, iron ion binding, heme binding.	Exogenous drug catabolic process, oxidation-reduction process, organic acid metabolic process, xenobiotic metabolic process.		
		AB	2AB1P							
		AC	2AC1							
	CYP 17	A	17A1	24 Variants	28		Steroid 17-alpha-monooxygenase activitu; iron ion binding; heme binding.	Glucocorticoid biosynthetic process; progesterone metabolic process; hormone biosynthetic process.	Androgen biosynthesis; glucocorticoid biosynthesis; endogenous sterols.	Adrenal Hyperplasia 5
	CYP 21	A	21A2	42 Variants	12		Steroid 21-monooxygenase activity; steroid binding; iron ion binding; heme binding.	Steroidogenesis; 21-hydroxylation of steroids; Oxidation 17HPROG	Glucocorticoid biosynthesis;Mineralocorticoid biosynthesis; Endogenous sterols.	Adrenal Hyperplasia 3
CLAN 3	CYP 3	A	3A12	167 Variants	6		Oxidoreductase activity, iron ion binding, heme binding.		Aflatoxin activation and detoxification; Xenobiotics; Miscellaneous substrates.	
			3A26	223 Variants	6					
			3A98							
			3A99		15					
	CYP 5	A	5A1		15					

CLAN	FAMILY	SUBFAMILY	GENE	VARIANT	CHROMOSOME	SYNONYMS	ACTIVITY (uniprot.org)	PROCESSES (uniprot.org)	METABOLISM (Reactome.org)	PATHOLOGIES directly associated with defect / deficiency
CLAN 4	CYP4	A	4A36		15					
			4A37	126 Variants	15		Oxidoreductase activity, monooxygenase activity, iron ion binding, heme binding.		Synthesis of (16-20)-hydroxyeicosatetraenoic acids; fatty acids.	
			4A38	120 Variants	15		Oxidoreductase activity, monooxygenase activity, iron ion binding, heme binding.		Synthesis of (16-20)-hydroxyeicosatetraenoic acids; fatty acids.	
			4A39	219 Variants	15	CYP4A11	Oxidoreductase activity, monooxygenase activity, iron ion binding, heme binding.	12-hydroxylates DDCX	Synthesis of (16-20)-hydroxyeicosatetraenoic acids; fatty acids.	
		B	4B1	73 Variants	15		Oxidoreductase activity, monooxygenase activity, iron ion binding, heme binding.	12-hydroxylation of ARA	Fatty Acids	
		F	4F8	43 Variants	20		Oxidoreductase activity, monooxygenase activity, iron ion binding, heme binding.	19-Hydroxylates PGH2	Fatty Acids	
			4F22	52 Variants	20	FLJ39501	Oxidoreductase activity, monooxygenase activity, iron ion binding, heme binding.	20-hydroxylates TrXA3		Congenital ichtyosis.
		V	4V2	28 Variants	16	CYP4AH1	Oxidoreductase activity, monooxygenase activity, iron ion binding, heme binding.	Fatty Acid omega-oxidation	Fatty Acids	
		X	4X1	113 Variants	15	MGC40051	Oxidoreductase activity, monooxygenase activity, iron ion binding, heme binding.			
CLAN 7	CYP 7	A	7A1	101 Variants	29	CYP7	Oxidoreductase activity, monooxygenase activity, iron ion binding, heme binding.	Cellular response to glucose stimulus.	Synthesis of bile acids and bile salts via 7alpha-hydroxycholesterol; endogenous sterols; orphan transporters.	
		B	7B1	173 Variants	29	SPG5A	Oxysterol 7-alpha-hydroxylase activity; iron ion binding; heme binding.	Negative regulation of intracellular estrogen receptor signaling pathway; Positive regulation of epithelial cell proliferation; Prostate gland epithelium morpogenesis.	Synthesis of bile acids and bile salts via 27-hydroxycholesterol; synthesis of bile acids and bile salts; endogenous sterols, oprhan transporters.	Spastic paraplegia 5A; Congenital bile acid synthesis defect 3.
	CYP 8	A	8A1							
		B	8B1	22 Variants	23	CYP12	Sterol 12-alpha-hydroxylase activity; iron ion binding; heme binding.	Lipid Metabolic Process	Synthesis of bile acids and bile salts via 24-hydroxycholesterol; Synthesis of bile acids and bile salts via 27-hydroxycholesterol; Synthesis of bile acids and bile salts via 7alpha-hydroxycholesterol; 12-hydroxylation of sterols.	
	CYP 39	A	39A1	99 Variants	12		Oxysterol 7-alpha-hydroxylase activity; steroid 7-alpha-hydroxylase activity; iron ion binding; heme binding.	Bile acid biosynthesis process; cholesterol catabolic process.		
CLAN MITOCH	CYP 11	A	11A1	93 Variants	30	CYP11A; P450SCC	Cholesterol monooxygenase activity; iron ion binding; heme binding.	C21-steroid hormone biosynthetic process.	Pregnenolone biosynthesis; endogenous sterols.	Congenital adrenal insufficiency
		B	11B1						Endogenous sterols; glucocorticoid biosynthesis	Adrenal hyperplasia 4;
			11B2	51 Variants	13		Oxidoreductase activity, monooxygenase activity, iron ion binding, heme binding.		Glucocorticoid biosynthesis; mineralocorticoid biosynthesis; endogenous sterols.	Corticosterone methyloxidase 1 deficiency
	CYP 24	A	24A1	59 Variants	24	CP24, CYP24, P450-CC24.	1-alpha,25-dihydroxyvitamin D3 24-hydroxylase acitivty; 25-hydroxycholecalciferol-24-hydroxylase activity; iron ion binding; heme binding.	Osteoblast differentiation; Vitamin D metabolic process, Response to Vitamin D.	Vitamins, Vitamin D (Calciferol) Metabolism.	Infantile hypercalcemia.

CLAN	FAMILY	SUBFAMILY	GENE	VARIANT	CHROMOSOME	SYNONYMS	ACTIVITY (uniprot.org)	PROCESSES (uniprot.org)	METABOLISM (Reactome.org)	PATHOLOGIES directly associated with defect / deficiency
	CYP 27	A	27A1	80 Variants	37	CP27, CTX, CYP27.	Monooxygenase activity, oxidoreductase activity, heme binding, iron ion binding.		Synthesis of bile acids and bile salts via 24-hydroxycholesterol; Synthesis of bile acids and bile salts via 27-hydroxycholesterol; Synthesis of bile acids and bile salts via 7alpha-hydroxycholesterol.	Cerebrotendinous Xanthomatosis.
		B	27B1	4 variants	10	CYP1, P450c1, PDDR, VDD1.	Calcidiol 1-monooxygenase activity, iron ion binding, heme binding.	Bone mineralization; Calcium ion homeostasis; Calcium ion transport; decidualization; G1 to G0 transition; negative regulation of calcidiol 1-monooxygenase activity; negative regulation of cell growth; negative regulation of cell proliferation; positive regulation of keratinocyte differentiation; positive regulation of vitamin D 24-hydroxylase activity; positive regulation of vitamin D receptor signaling pathway; regulation of bone mineralization; response to estrogen; response to interferon-gamma; response to lipopolysaccharide; lresponse to vitamin D; vitamin D catabolic process.	Vitamins; Vitamin D metabolism.	Rickets Vitamin D-dependent 1A
		C	27C1	66 variants	19	FLJ16008	Mono-oxygenase activity; oxireductase activity; iron ion binding; heme binding.			
CLAN 19	CYP 19	A	19A1	44 Variants	30		Aromatase activity; iron ion binding; heme binding.	Androgen metabolic process; prostate gland growth.	Estrogen biosynthesis; endogenous sterols.	Aromatase excess syndrome.
CLAN 20	CYP 20	A	20A1	29 Variants	37	CYP-M	Heme binding; iron ion binding; oxidoreductase activity.			
CLAN 26	CYP 26	A	26A1	16 Variants	28	CP26, CYP26, P450RAI, P450RAI1	4-hydroxylate atRA		Vitamins	skeletal and craniofacial anomalies.
		B	26B1	31 Variants	17	P450RAI-2	Retinoic acid 4-hydroxylase activity; retonoic acid binding; iron ion binding; heme binding.	Bone morphogenesis, cellular response to retonoic acid, embryonic limb morphogenesis, male meiosis, negative regulation of retinoic acid receptor signaling pathway; positive regulation of gene expression; positive regulation of tongue muscle cell differentiation; proximal/distal pattern formation; retinoic acid catabolic process; retinoic acid receptor signaling pathway; spermatogenesis, tongue morphogenesis; cell fate determination; cornification; establishment of skin barrier.	Vitamins	
		C	26C1				Retinoic acid 4-hydroxylase activity; retonoic acid binding; iron ion binding; heme binding.	Anterior/Posterior pattern specification; central nervous system development; retinoic acid catabolic process; cellular response to retinoic acid; neural crest cell development; Retinoic acid receptor signailing pathway.	Vitamins	Focal facial dermal dysplasia
CLAN 46	CYP 46	A	46A1	46 Variants	8	CYP46	Cholesterol 24-hydroxylase activity; Iron ion binding; Heme binding.	Cholesterol catabolic Process; Xenobiotic Metabolic Processes	Synthesis of bile acids and bile salts via 24-hydroxycholesterol	
CLAN 51	CYP 51	A	51A1	48 Variants	14	CP51, CYP51, CYPL1, LDM, P450-14DM, P450L1	sterol 14-demethylase activity; Iron ion binding; heme binding	Steroid biosynthetic processes	Cholesterol biosynthesis; endogenous sterols	

Adapted from: Croft D., 2015; Cunningham F. et al., 2015; Nelson D., 2015; The UniProt Consortium, 2015.