JARQ 54 (1), 7-12 (2020) https://www.jircas.go.jp

REVIEW

Sex and Breed Differences in the Constitutive Gene Expression of Hepatic Drug Metabolizing Enzymes in Meishan and Landrace Pigs: Testosterone-mediated Differences

Misaki KOJIMA*

Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization, Tsukuba, Japan

Abstract

Drug metabolizing enzymes (DMEs) that exist primarily in the liver are one of the critical factors determining the susceptibility of animals and humans to xenobiotics such as drugs, and also metabolize steroid hormones and other endogenous compounds. Although sex and/or species differences in the constitutive gene expression levels of hepatic DMEs have been reported to date, the causes of such differences have yet to be completely explained. The DME genes of pigs have recently been identified. Because pigs are valuable animals for human pharmacological and toxicological studies, research on the gene expression of DMEs in pigs is being promoted. We have found sex and/or breed differences in the constitutive gene expression of hepatic DMEs, including cytochrome P450s, sulfotransferases, and UDP-glucuronosyltransferases, in Meishan and Landrace pigs. We propose that physiological serum testosterone level is a critical host factor producing these differences. This review discusses the testosterone-mediated gene expression of hepatic DMEs.

Discipline: Animal Science
Additional key words: androgen, cytochrome P450s, liver, sulfotransferases, UDP-glucuronosyltransferases

Introduction

Hepatic drug metabolizing enzymes (DMEs) play an important role in the metabolism of xenobiotics such as drugs, and endogenous compounds such as steroid hormones (Almazroo et al. 2017). DMEs consist of phase I and phase II enzymes, responsible for the oxidation of xenobiotics (substrates) and the conjugation of substrates (including metabolites produced by the phase I DMEs), respectively. Incidentally, cytochrome P450s (CYPs), especially CYP1, CYP2 and CYP3 family isoenzymes, are representative phase I DMEs. Likewise, sulfotransferases (SULTs) and UDP-glucuronosyltransferases (UGTs) are representative phase II DMEs. These DMEs have different substrate specificities to each other, and their expression levels are often changed not only by physiological status but also by exposure to xenobiotics (Burkina et al. 2017, Graham & Lake 2008, Murray 2006, Parkinson et al. 2004). Furthermore, differences in the expression levels (activities) of the DMEs lead to differences in the clearance of drugs (Tanaka 1999) and the susceptibility to environmental pollutants, including carcinogens (Degawa et al. 1985, Shimizu et al. 2000). Therefore, studies on the expression of hepatic DMEs are important to understand the susceptibility of individual animals and humans to xenobiotics.

Domestic pigs (*Sus scrofa domesticus*) including mini pigs attract attention as a valuable animal model for humans in pharmacological and toxicological studies, due to the similarity of DME activities between humans and pigs (Burkina et al. 2017, Nielsen et al. 2017, Puccinelli et al. 2011, Skaanild 2006). The DME genes of pigs have recently been identified (Kojima & Morozumi

*Corresponding author: e-mail misaki@affrc.go.jp
Received 26 February 2018; accepted 29 May 2019.

2004, Puccinelli et al. 2011), and research on sex differences in the gene expression of pig DMEs is being rapidly developed (Kojima & Degawa 2013, Kojima & Degawa 2014, Kojima & Degawa 2016, Kojima & Morozumi 2004, Kojima et al. 2008, Kojima et al. 2010). In addition, pig DMEs are responsible for the elimination of boar taint in fat (Zamaratskaia & Squires 2009).

This review describes the sex and breed differences in the constitutive gene expression levels of DMEs (CYPs, SULTs and UGTs) in the pig liver, and proposes the serum testosterone level as being one of the critical factors producing these differences.

Sex and breed differences in the constitutive mRNA levels of hepatic DMEs

In domestic pigs and mini pigs, sex differences have been reported in the enzyme activities and protein levels of some CYPs, including CYP2A and CYP2E, and androgen has been considered a cause of those sex differences (Gillberg et al. 2006, Rasmussen et al. 2011, Skaanild & Friis 1999). More recently, sex and/or breed differences in the constitutive gene expression of hepatic DMEs, including CYPs, SULTs and UGTs, have been found in Meishan and Landrace pigs (Kojima & Degawa 2013, Kojima & Degawa 2014, Kojima & Degawa 2016, Kojima et al. 2008, Kojima et al. 2010).

As shown in Table 1, there are significant sex differences in the mRNA levels of phase I (CYP1A1, CYP1A2, CYP2A19, CYP2E1, CYP2B22, CYP2C33, CYP2C49, CYP3A22 and CYP3A29) and phase II enzymes (UGT1A1, UGT1A6 and UGT2B31) in the livers of five-month-old (mature) Meishan pigs (Kojima & Degawa 2013, Kojima & Degawa 2014, Kojima & Degawa 2016, Kojima et al. 2008, Kojima et al. 2010). The mRNA levels of CYP1A1, CYP1A2, CYP2A19, CYP2E1, CYP3A22, SULT1A1 and SULT2A1 in the liver are much lower in male Meishan pigs compared with females, whereas those of CYP2B22, CYP2C33, CYP2C49, CYP3A29, UGT1A1, UGT1A6 and UGT2B31

Table 1. Summary of sex and breed differences in the gene expression of hepatic DMEs in 5-month-old (mature) Meishan and Landrace pigs

DME genes	Meishan pigs				Landrace pigs			
	Sex differences	Castrated male	TP treatment*		Sex differences	Castrated male	TP treatment	
			Castrated male**	Intact female			Castrated male	Intact female
Phase I enzymes								
CYP1A1	♂ < ♀	↑	↓	↓	♂ = ♀	→	↓	↓
CYP1A2	♂ < ♀	↑	↓	↓	♂ = ♀	→	↓	↓
CYP2A19	♂ < ♀	↑	↓	↓	♂ < ♀	→	↓	↓
CYP2E1	♂ < ♀	↑	↓	↓	♂ = ♀	→	↓	↓
CYP2B22	♂ > ♀	↓	↑	↑	♂ = ♀	→	↑	↑
CYP2C33	♂ > ♀	↓	↑	↑	♂ = ♀	→	↑	↑
CYP2C49	♂ > ♀	↓	→	↑	♂ = ♀	→	→	→
CYP3A22	♂ < ♀	↑	↓	↓	♂ = ♀	→	→	→
CYP3A29	♂ > ♀	↓	↑	↑	♂ = ♀	→	→	→
CYP3A46	nd***	nd	nd	nd	♂ = ♀	→	↑	↑
Phase II enzymes								
SULT1A1	♂ < ♀	↑	↓	↓	♂ = ♀	→	↓	↓
SULT2A1	♂ < ♀	↑	↓	↓	♂ = ♀	→	↓	↓
UGT1A1	♂ > ♀	↓	↑	↑	♂ = ♀	→	↑	↑
UGT1A6	♂ > ♀	↓	↑	↑	♂ = ♀	→	↑	↑
UGT2B31	♂ > ♀	↓	↑	↑	♂ = ♀	→	↑	↑

The above data were published in our previous papers (Kojima & Degawa 2013, Kojima & Degawa 2014, Kojima & Degawa 2016, Kojima et al. 2008, Kojima et al. 2010). *Testosterone propionate (TP) dissolved in corn oil was injected intramuscularly five times, at a dose of 10 mg/kg body weight/injection, into the rear leg of each pig. Each individual injection was performed at 48 h intervals.
**Five-month-old male pigs castrated at the age of one month were used.
***nd: expression was not detected.
↑ and ↓: significant upregulation and downregulation, respectively, to the corresponding controls (castrated male *vs.* intact male or TP-treatment *vs.* no treatment)
→ : no significant change

are much higher in males than in females. However, no such sex differences are observed in Landrace pigs except for the level of CYP2A19 mRNA (Kojima & Degawa 2013). In addition, the *CYP3A46* gene in Landrace pigs is expressed with no sex difference, whereas no expression of the gene is observed in either sex of Meishan pigs (Table 1).

Effects of castration and testosterone treatment on hepatic mRNA levels of DMEs

In five-month-old male Meishan pigs castrated at one month old, the levels of each DME mRNA are almost the same as those in the corresponding intact females, whereas no such castration effect is observed in Landrace pigs (Table 1). Additionally, in the treatment of castrated pigs of both breeds with testosterone propionate (TP), the mRNA levels of individual DMEs (except CYP2C49, CYP3A22 and CYP3A29 in Landrace pigs, and CYP2C49 in Meishan pigs) change to that of male Meishan pigs (Kojima & Degawa 2014, Kojima & Degawa 2016, Kojima et al. 2010, Kojima et al. 2013). Similar effects of TP treatment are observed in female pigs of both breeds (Table 1). These findings suggest that testosterone acts as an important host factor regulating the DME genes, except for *CYP2C49*, *CYP3A22*, *CYP3A29* and *CYP3A46*. For these four genes, unknown host factors are considered along with testosterone as the causes of sex and breed differences in gene expression.

Relation between serum testosterone amounts and hepatic DME mRNA levels

Age-dependent changes in the serum testosterone amount and hepatic expression level of individual DME mRNAs were comparatively examined using male Meishan and Landrace pigs (Fig. 1). Age-dependent increases were clearly observed in Meishan pigs, and the average amounts of serum testosterone in one-, two-, three-, four- and five-month-old pigs were 5, 15, 32, 41 and 49 ng/ml, respectively. In Landrace pigs, no such clear increases occurred, and the average amount of serum testosterone at the age of five months was 16 ng/ml. In addition, there was no significant difference in the amount of serum testosterone between Meishan and Landrace pigs at the age of one month. The average amounts of serum testosterone in three-, four- and five-month-old male Meishan pigs were more than twice as high as those in age-matched male Landrace pigs. Moreover, the serum testosterone amounts in male five-month-old cross breeds (ML, female Meishan × male Landrace; LM, female Landrace × male Meishan) were almost the same as those of age-matched male Meishan pigs, indicating that serum testosterone level is determined by autosomal dominant inheritance (Kojima & Degawa 2013).

The relationships between the amounts of serum testosterone and the expression levels of DME mRNAs were further examined using Landrace, Meishan, LM, and ML pigs (Kojima & Degawa 2013, Kojima & Degawa 2014, Kojima & Degawa 2016, Kojima et al. 2010). Negative correlations were shown between testosterone and the gene expression of *CYP1A1*, *CYP1A2*, *CYP2A19*, *CYP2E1*, *SULT1A1* and *SULT2A1*. As a typical example, the result for the *CYP1A2* gene is shown in Figure 2. However, there were positive correlations for the *CYP2B22*, *CYP2C33*, *CYP2C49*, *CYP3A29*, *CYP3A46*, *UGT1A1*, *UGT1A6* and *UGT2B31* genes. As a typical example, the result for the *CYP2C33* gene is shown in Figure 2. It is noteworthy that these testosterone-mediated effects on the expression of all the genes examined were only apparent in pigs with over 33 ng/ml of serum testosterone (Kojima & Degawa 2013, Kojima & Degawa 2014, Kojima & Degawa 2016, Kojima et al. 2010). This strongly supports a hypothesis that serum testosterone acts as a critical factor regulating the genes of many DMEs with a threshold amount. And in ML and LM pigs,

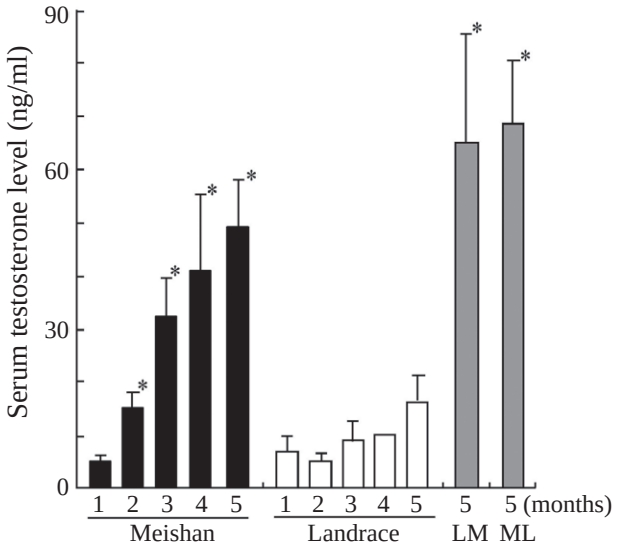

Fig. 1. Age-dependent changes in the amounts of serum testosterone in male Meishan, Landrace, and their F_1 (ML and LM) pigs

Blood samples were collected from individual male pigs. The above data were published in our previous paper (Kojima & Degawa 2013). Each column shows the mean value for each experimental group, and each bar represents standard deviation from the mean. *Significant differences from the age-matched male Landrace pigs: $*P < 0.01$.

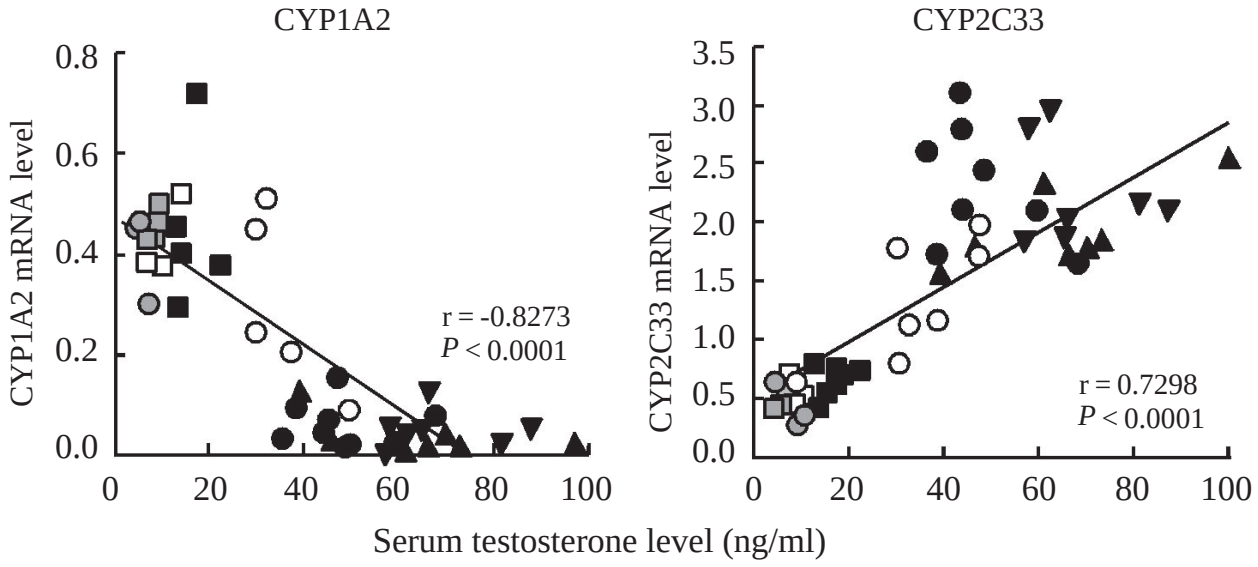

Fig. 2. Relation between the amounts of serum testosterone and the expression levels of CYP1A2 and CYP2C33 mRNA in male pigs

The above data were published in our previous papers (Kojima & Degawa 2013, Kojima & Degawa 2016, Kojima et al. 2010). The correlations were determined by regression analysis, where r is the correlation coefficient. Gray squares and circles denote one-month-old Landrace and Meishan pigs, respectively; open squares and circles denote three-month-old Landrace and Meishan pigs, respectively; closed squares and circles denote five-month-old Landrace and Meishan pigs, respectively; and closed triangles and reverse triangles denote five-month-old LM and ML pigs, respectively.

the CYP3A46 mRNA detected in Landrace pigs but not in Meishan pigs is also expressed, suggesting that expression of CYP3A46 mRNA is determined by autosomal dominant inheritance (Kojima & Degawa 2016). The levels of CYP3A46 mRNA in ML and LM pigs were higher in males than in females, although no such sex difference was observed in Landrace pigs. The expression levels of CYP3A46 mRNA in castrated male and intact female Landrace pigs were increased by TP treatment (Table 1) to levels almost the same as those in male ML and LM pigs (Kojima & Degawa 2016).

Involvement of serum testosterone in sex and breed differences in DME gene expression

It has been reported that sex differences are present in the constitutive levels (gene expression, protein levels, and/or activity) of hepatic DMEs, including CYPs, SULTs and UGTs, in rodents and humans (Alnouti & Klaassen 2011, Buckley & Klaassen 2009, Klaassen et al. 1998, Scandlyn et al. 2008, Shelby et al. 2003, Tanaka 1999, Waxman & Hollway 2009). To date, the secretion profile of growth hormone (GH) has been considered as one of the physiological factors responsible for these sex differences (Alnouti & Klaassen 2011, Buckley & Klaassen 2009, Klaassen et al. 1998, Shelby et al. 2003, Waxman & Hollway 2009). However, despite sex differences in the GH secretion profile in Landrace pigs (Arbona et al. 1988), there are no sex differences in the constitutive hepatic gene expression levels of CYPs (*CYP1A1*, *CYP1A2*, *CYP2B22*, *CYP2E1*, *CYP2C33*, *CYP2C49*, *CYP3A22*, *CYP3A29* and *CYP3A46*), SULTs (*SULT1A1* and *SULT2A1*) or UGTs (*UGT1A1*, *UGT1A6* and *UGT2B31*) (Kojima & Degawa 2013, Kojima & Degawa 2014, Kojima & Degawa 2016, Kojima et al. 2008, Kojima et al. 2010). Accordingly, it is thought that the differences in the gene expression levels of these DMEs in the liver are not dependent on only the difference in the GH secretion profile. As another critical physiological factor determining sex and breed differences in the constitutive gene expression of CYPs, SULTs and UGTs in pig liver, the serum testosterone level is strongly proposed. In addition, clear testosterone effects are seen at a threshold level (about 33 ng/ml of serum), as shown in Figure 2. The testosterone hypothesis is supported by evidence that: 1) sex differences in hepatic constitutive mRNA levels of DMEs are only observed in pig breeds such as Meishan, ML, and LM, whose males have high levels of serum testosterone (over 33 ng/ml of serum); 2) the mRNA levels of individual DMEs in castrated male Meishan pigs are almost the same as those in the corresponding intact females; and 3) TP treatment of castrated Meishan and Landrace pigs, and of female pigs of both breeds, results in an increase of their serum testosterone levels to 92-299 ng/ml (Kojima & Degawa 2013) and changes the expression

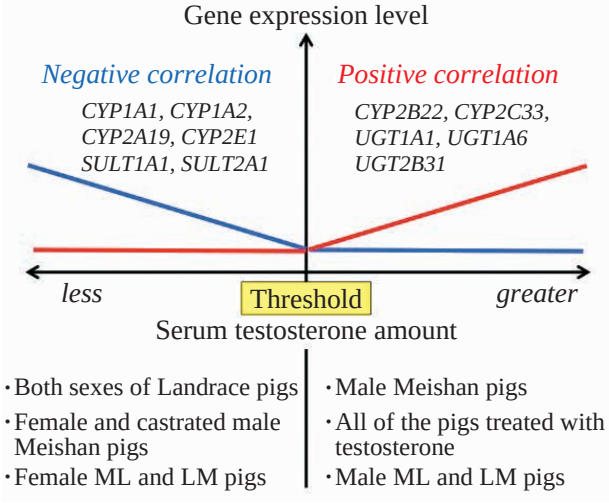

Fig. 3. Effect of serum testosterone on DME gene expression in pig liver

patterns of individual DME genes to those of intact five-month-old male Meishan pigs. However, since modification of the GH secretion profile by androgen has been reported (Dubreuil et al. 1989, Giustina & Veldhuis 1998), it has also been suggested that the castration- and/or TP treatment-mediated changes in the mRNA levels of hepatic CYPs, SULTs and UGTs might partially occur through conversion of the GH secretion pattern.

Recently, our preliminary results have indicated that the gene expression level of the hepatic androgen receptor (AR) responsible for testosterone-mediated signal transduction is significantly higher in male five-month-old Meishan pigs compared with corresponding Landrace pigs, and that there is no difference in the amino acid sequence of AR between Meishan and Landrace pigs. Accordingly, not only serum testosterone levels but also the hepatic *AR* gene expression level might be important physiological factors determining the breed differences between Meishan and Landrace pigs, in DME gene expression in the liver. However, the exact mechanisms of androgen- and/or GH-mediated regulation of the *CYP*, *SULT* and *UGT* genes remain unclear.

Conclusion

Serum testosterone level, which is determined by autosomal dominant inheritance (Kojima & Degawa 2013), is herein proposed to act as a critical host factor determining constitutive gene expression levels of DMEs responsible for metabolizing xenobiotics such as drugs (Fig. 3). If so, it might be possible to predict the expression levels of hepatic DMEs in individual animals and humans using the serum testosterone level as an indicator. Moreover, measurement of the serum testosterone amount could contribute to the development of individual drug treatments, as there are large individual variations in blood testosterone level in humans. Since DMEs such as CYP2A19, CYP2E1 and SULT1A1 in pigs are responsible for the elimination of skatole in fat, one of the compounds causing boar taint (Zamaratskaia & Squires 2009), further studies concerning the genetic regulation of these DMEs, polymorphism of DME genes, and the dietary supplements affecting expression and/or activity of the DMEs, would assist the production of meat without boar taint.

Acknowledgment

We wish to thank Catherine Perfect, MA (Cantab), from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

References

Almazroo, O. A. et al. (2017) Drug metabolism in the liver. *Clin. Liver Dis.*, **21**, 1-20.

Alnouti, Y. & Klaassen, C. D. (2011) Mechanisms of gender-specific regulation of mouse sulfotransferases (Sults). *Xenobiotica*, **41**, 187-197.

Arbona, J. R. et al. (1988) Secretory patterns and metabolic clearance rate of porcine growth hormone in swine selected for growth. *J. Anim. Sci.*, **66**, 3068-3072.

Buckley, D. B. & Klaassen, C. D. (2009) Mechanism of gender-divergent UDP- glucuronosyltransferase mRNA expression in mouse liver and kidney. *Drug. Metab. Dispos.*, **37**, 834-840.

Burkina, V. et al. (2017) Comparison of xenobitotic-metabolising human, porcine, rodent, and piscine cytochorome P450. *Toxicology*, **375**, 10-27.

Degawa, M. et al. (1985) Sex-dependent induction of hepatic enzymes for mutagenic activation of a tryptophan pyrolysate component, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole, by feeding in mice. *Cancer Res.*, **45**, 96-102.

Dubreuil, P. et al. (1989) Castration and testosterone effects on endogenous and somatocrinin-induced growth hormone release in intact and castrated male pigs. *Dom. Anim. Endocrinol.*, **6**, 15-24.

Gillberg, M. et al. (2006) Regulation of gender-dependent CYP2A expression in pigs: Involvement of androgens and CAR. *Basic Clin. Pharmacol. Toxicol.*, **98**, 480-487.

Giustina, A. & Veldhuis, J. D. (1998) Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human, *Endocrine Rev.*, **19**, 717-797.

Graham, M. J. & Lake, B. G. (2008) Induction of drug metabolism: species differences and toxicological relevance. *Toxicology*, **254**, 184-191.

Klaassen, C. D. et al. (1998) Regulation of sulfotransferase mRNA expression in male and female rats of various ages. *Chem-Biol. Interact.*, **109**, 299-313.

Kojima, M. & Degawa, M. (2013) Serum androgen level is determined by autosomal dominant inheritance and regulates sex-related CYP genes in pigs. *Biochem. Biophys. Res. Commun*., **430**, 833-838.

Kojima, M. & Degawa, M. (2014) Sex differences in the constitutive gene expression of sulfotransferases and UDP-glucuronosyltransferases in the pig liver: Androgen-mediated regulation. *Drug Metb. Pharmacokinet*., **29**, 192-197.

Kojima, M. & Degawa, M. (2016) Sex differences in constitutive mRNA levels of CYP2B22, CYP2C33, CYP2C49, CYP3A22, CYP3A29 and CYP3A46 in the pig liver: Comparison between Meishan and Landrace pigs. *Drug Metb. Pharmacokinet*., **31**, 185-192.

Kojima, M. & Morozumi, T. (2004) Cloning of six full-length cDNAs encoding pig cytochrome P450 enzymes and gene expression of these enzymes in the liver and kidney. *J. Health. Sci*., **50**, 518-529.

Kojima, M. et al. (2008) A novel gender-related difference in the constitutive expression of hepatic cytochrome P4501A subfamily enzymes in Meishan pigs. *Biochem. Pharmacol*., **75**, 1076-1082.

Kojima, M. et al. (2010) Androgen-mediated down-regulation of *CYP1A* subfamily genes in the pig liver. *J. Endocrinol*., **207**, 203-211.

Murray, M. (2006) Altered CYP expression and function in response to dietary factors: potential roles in disease pathogenesis. *Curr. Drug Metab*., **7**, 67-81.

Nielsen, S. D. et al. (2017) Constitutive expression and activity of cytochrome P450 in conventional pigs. *Res. Vet. Sci*., **111**, 75-80.

Parkinson, A. et al. (2004) The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes. *Toxicol. Appl. Pharmacol*., **199**, 193-209.

Puccinelli, E. et al. (2011) Xenobitotic metabolizing cytochrome P450 in pig, a promising animal model. *Curr. Drug Metab*., **12**, 507-525.

Rasmussen, M. K, et al. (2011) Gender-related differences in cytochrome P450 in porcine liver - Implication for activity, expression and inhibition by testicular steroids. *Reprod. Dom. Anim*., **46**, 616-623.

Scandlyn, M. J. et al. (2008) Sex-specific differences in CYP450 isoforms in humans. *Expert Opin. Drug Metab. Toxicol*., **4**, 413-424.

Shelby, M. K. et al. (2003) Tissue mRNA expression of the rat UDP-glucronosyltransferase gene family. *Drug Metab. Dispos*., **31**, 326-333.

Shimizu, Y. et al. (2000) Benzo[a]pyrene carcinogenicity is lost lacking the aryl hydrocarbon receptor. *Proc. Natl. Acad. Sci. USA*, **87**, 779-782.

Skaanild, M. T. (2006) Porcine cytochrome P450 and metabolism, *Curr. Pharm. Des*., **12**, 1451-1427.

Skaanild, M. T. & Friis, C. (1999) Cytochrome P450 sex differences in minipigs and conventional pigs, *Pharmacol. Toxicol*., **85**, 174-180.

Tanaka, E. (1999) Gender-related differences in pharmacokinetics and their clinical significance. *J. Clin. Pharm. Ther*., **24**, 339-346.

Waxman, D. J. & Holloway, M. G. (2009) Sex differences in the expression of hepatic drug metabolizing enzymes. *Mol. Pharmacol*., **76**, 215-228.

Zamaratskaia, G. & Squires, E. J. (2009) Biochemical, nutritional and genetic effects on boar taint in entire male pigs. *Animal*, **3**, 1508-1521.