Biochemical and Biophysical Research Communications 430 (2013) 833–838

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Serum androgen level is determined by autosomal dominant inheritance and regulates sex-related *CYP* genes in pigs

Misaki Kojima [a,*], Masakuni Degawa [b]

[a] *Animal Genome Research Unit, Agrogenomics Research Center, National Institute of Agrobiological Sciences, Kannondai 2-1-2, Tsukuba 305-8602, Japan*
[b] *Department of Molecular Toxicology, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan*

ARTICLE INFO

Article history:
Received 1 November 2012
Available online 28 November 2012

Keywords:
Androgen
Cytochrome P450
Sex difference
Strain difference
Pig

ABSTRACT

We have previously demonstrated differences between Meishan and Landrace pigs in their serum androgen levels (Meishan > Landrace) and the expression of genes encoding hepatic cytochrome P450 (CYP) 1A subfamily enzymes (Meishan < Landrace). In the present study, to clarify whether such differences are genetically controlled, we crossbred these pigs (female Meishan × male Landrace, ML; female Landrace × male Meishan, LM) and examined the expression levels of serum androgen and hepatic *CYP* family genes (*CYP1A1*, *CYP1A2*, *CYP2A19*, and *CYP2E1*) among ML, LM, and their parents. In sexually mature (5-month-old) male ML or LM pigs, not only the serum androgen level, but also the hepatic expression levels of all the *CYPs* examined were similar to those in male Meishan pigs. In addition, there were few breed differences among the females of Meishan, Landrace, ML and LM pigs in the expression of all the *CYP* genes examined. Furthermore, the expression levels of these *CYPs* in the females of Meishan and Landrace pigs could be decreased to the corresponding levels in male Meishan pigs by administration of testosterone propionate. The present findings demonstrate that serum androgen level is determined by autosomal dominant inheritance and that the level of serum androgen is one of the host factors regulating the constitutive expression of *CYP1A1*, *CYP1A2*, *CYP2A19*, and *CYP2E1* in the pig liver.

1. Introduction

Most xenobiotics, including drugs, are metabolized by hepatic cytochrome P450 (CYP), which consists of several subfamilies [1]. These CYP enzymes have different substrate-specificities [2,3], and their expression levels are influenced not only by physiological factors, but also by exposure to xenobiotics [4–7]. Therefore, studies on the expression of CYPs responsible for the metabolism of xenobiotics are important for understanding of the sex and species differences in the response to xenobiotics.

Androgen is one of the physiological factors that determine sexual dimorphism in the constitutive and/or xenobiotic-induced expression of CYPs, including Cyp1a2 [8–10] and Cyp2a/2b [11,12] in mice. In conventional pigs and minipigs, there are sex differences in the expression of several CYPs including CYP1A2, CYP2A, and CYP2E [13–16]. Recently, we have demonstrated that sex and breed differences in the constitutive expression levels of hepatic CYP1A subfamily enzymes, CYP1A1 and CYP1A2, in Meishan and Landrace pigs are closely correlated with differences in the level of serum androgen [17,18]. Incidentally, in sexually mature male Meishan pigs with high levels of serum androgen (around 50 ng/ml), the constitutive expression levels of hepatic CYP1A subfamily enzymes are much lower than those in female Meishan pigs and both sexes of Landrace pigs, whose serum androgen levels are less than 20 ng/ml [18]. However, the causes of the breed differences in the level of serum androgen and the expression levels of hepatic CYP1A subfamily enzymes between male Meishan and Landrace pigs remain unclear.

The pig is a mammalian species of particular interest in pharmacological and toxicological studies because it can be used as a laboratory model for human metabolism, without the requirement to induce the enzymes that carry out biotransformations [19,20]. Further analyses of the breed difference and the mechanism of androgen-associated gene expression of hepatic CYPs in Meishan and Landrace pigs will contribute to our understanding of sex and inter-individual differences in responses to xenobiotics, such as drugs and environmental chemicals, in humans.

In the present study, we crossbred Meishan and Landrace pigs and used their F1 pigs together with the parent pigs, to examine whether the differences between male Meishan and Landrace pigs in the expression levels of serum androgen and hepatic *CYP* genes, including *CYP1A1* and *CYP1A2*, are genetically determined.

* Corresponding author. Fax: +81 29 838 8610.
E-mail address: misaki@affrc.go.jp (M. Kojima).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.11.060

2. Materials and methods

2.1. Animals

Meishan, Landrace, and their crossbred F1 (ML, Meishan × Landrace; LM, Landrace × Meishan) pigs were kept at the National Institute of Livestock and Grassland Science, Tsukuba, Japan. These F1 pigs were produced by mating between one sire of each breed and two dams of the other breed. All the pigs used were fed a commercial grain diet and provided with water *ad libitum*. Pigs were killed between 10:00 and 11:00 am at the age of 1, 2, 3, or 5 months. Some of male Meishan and Landrace pigs were castrated at the age of 1 month and killed at 5 months of age. After each animal in the experimental groups was killed, a portion of the liver was quickly removed, frozen in liquid nitrogen and kept at −80 °C for subsequent analyses.

All animals were handled humanely under the guidelines of the National Institute of Agrobiological Sciences and National Institute of Livestock and Grassland Science (Tsukuba, Japan).

2.2. Treatment with androgen

Testosterone propionate (TP) was purchased from Sigma Chemical Co., St Louis, MO, USA. TP (50 mg/ml) 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. The pigs were killed 24 h after the final injection.

2.3. Serum testosterone level

Blood samples were collected from individual male pigs between 10:00 and 11:00 am. After clotting at room temperature, the serum was separated from each blood sample by centrifugation at 1500*g* for 15 min at 4 °C and stored at −80 °C until use. The levels of serum testosterone were measured using the Correlate-EIA Testosterone Enzyme Immunoassay Kit (Assay Designs, Inc., Ann Arbor, MI, USA) according to the manufacturer's instructions.

2.4. Expression of hepatic CYP mRNAs

Total RNA was prepared from individual livers using TRIzol Reagent (Invitrogen Corp., Carlsbad, CA, USA) and used to determine the levels of *CYP1A1*, *CYP1A2*, *CYP2A19,* and *CYP2E1* mRNAs. The amounts of these mRNAs were measured by real-time RT-PCR method using an 7500 Real Time PCR System with SYBR green master mix (PE Applied Systems, Tokyo, Japan) as described previously [18]. The primer sets and their concentrations used in the present study were indicated in Table 1. 18S was used as an internal standard. The amount of each cDNA was assessed using the relative standard curve method, as described in PE Applied Biosystems User Bulletin #2 (1997). Standard curves to determine the gene expression levels were generated using an RT-reaction mixture with total RNA from the liver of 5-month-old female Landrace pigs.

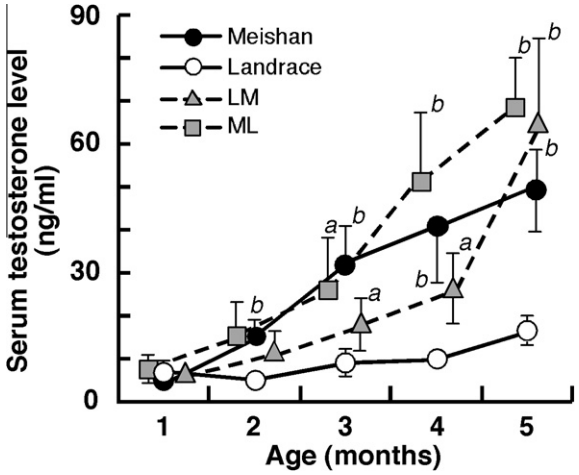

Fig. 1. Age-dependent changes in the levels of serum testosterone in the male Meishan pigs, Landrace pigs and their F1 (ML and LM) pigs. Blood samples were collected from individual males (n = 6, 7, 7, 9 and 8 for 1-, 2-, 3-, 4- and 5-month-old Meishan pigs, respectively; n = 5, 2, 5, 2 and 7 for 1-, 2-, 3-, 4- and 5-month-old Landrace pigs, respectively; n = 7 for each F1 (ML or LM) pig examined at each indicated age). [a,b]Significant differences from the age-matched male Landrace pigs: [a]$P < 0.05$, [b]$P < 0.01$.

2.5. Statistical analysis

Significant differences were assessed by Student's *t*-test.

3. Results

3.1. Serum testosterone levels in male Meishan, Landrace, and their F1 pigs

The levels of serum testosterone in sexually mature (5-month-old) male Meishan pigs were approximately 3-fold higher than those in the corresponding Landrace pigs (Fig. 1). The levels of serum testosterone (45–85 ng/ml) in 5-month-old F1 (ML and LM) pigs were similar to those (40–60 ng/ml) in Meishan pigs. However, age-dependent increases in the testosterone levels during the 2–4 months after the birth in LM pigs were slightly low compared with those in the age-matched ML and Meishan pigs. In addition, the levels of serum testosterone in male Landrace pigs were only about 20 ng/ml even at 5 months.

Table 1
Primer pairs used in the present study.

Gene name	Primer	Concentration of each primer (nM)	Reference or accession no.
CYP1A1	cagagctgcttagccttatcaacc (forward)	200	18
	ctggatgctgggatttgtcaccag (reverse)	200	18
CYP1A2	gtgaggagatgttcagcatcgtgaag (forward)	200	18
	cttctgtatctcaggatatgtcaca (reverse)	200	18
CYP2A19	gacttcatcgactccttcctcatc (forward)	200	AB052255
	Catcgttgtgctgaccgtctc (reverse)	200	AB052255
CYP2E1	ccaaacagaatccctgccatcaa (forward)	100	AB052259
	Ggagtccagtgtcggaattacca (reverse)	100	AB052259
18S	cggctaccacatccaaggaag (forward)	100	18
	gctggaattaccgcggctg (reverse)	100	18

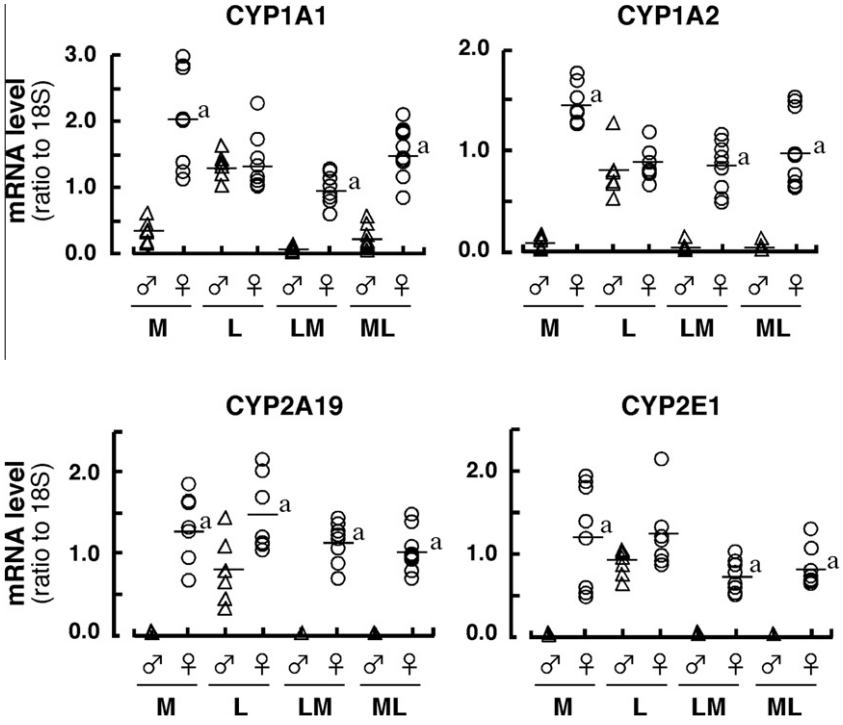

Fig. 2. Expression levels of hepatic *CYP* mRNAs in 5-month-old Meishan (M) pigs, Landrace (L) pigs, and their F1 (ML and LM) pigs. Expression levels of *CYP1A1*, *CYP1A2*, *CYP2A19*, and *CYP2E1* mRNAs were determined by real-time RT-PCR. The levels were normalized to that of 18S as an internal standard. Triangles and circles show individual males and females, respectively, in each breed (n = 8 each for male and female Meishan pigs; n = 7 and 8 for male and female Landrace pigs, respectively; n = 7 and 9 for male and female LM pigs, respectively; n = 7 and 10 for male and female ML pigs, respectively). Bar indicates the average in each group. [a]Significant difference between males and females in each breed: [a]$P < 0.01$.

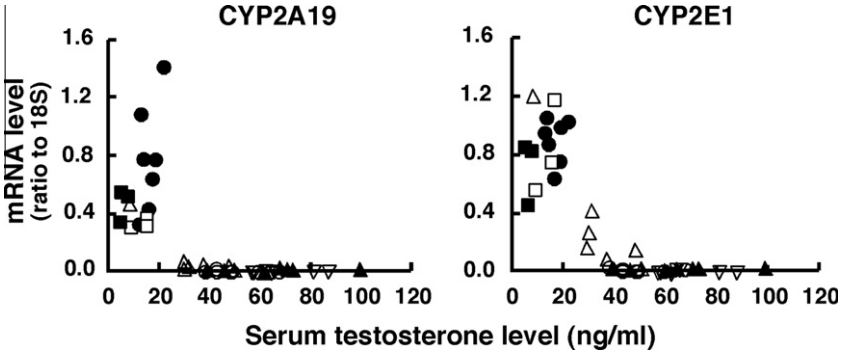

Fig. 3. Relationships between the serum testosterone level and the gene expression level of *CYP2A19* and CYP2E1 in male pigs. The relationships were assessed on the basis of the data of Figs. 1 and 2. ■; 1-month-old Meishan pigs (n = 3), □; 2-month-old Meishan pigs (n = 3), Δ; 3-month-old Meishan pigs (n = 7), ○; 5-month-old Meishan pigs (n = 8), ●; 5-month-old Landrace pigs (n = 7), ▲; 5-month-old LM pigs (n = 7), ▽; 5-month-old ML pigs (n = 7).

3.2. Expression of hepatic CYP mRNAs in Meishan, Landrace, and their F1 pigs

Hepatic gene expression levels of the sex-related CYPs, *e.g.* CYP1A1, CYP1A2, *CYP2A19*, and CYP2E1, which are higher in female pigs than in males [13,14,16–18], were examined among the 5-month-old Meishan, Landrace, and their F1 (ML and LM) pigs. In Meishan, ML, and LM pigs, significant sex differences in the constitutive levels of the mRNAs of all the CYPs examined were observed. In the three breeds of pigs, the levels of these *CYP* mRNAs were significantly greater in females than in males (Fig. 2). In particular, the mRNAs of *CYP2A19* and CYP2E1 were barely detected in Meishan, ML, and LM male pigs. On the other hand, no such sex-differences, with the exception of *CYP2A19* mRNA, were observed in Landrace pigs, and *CYP2A19* and *CYP2E1* mRNAs were definitely detected in both sexes of the pigs (Fig. 2).

3.3. Androgen-dependent downregulation of the CYP2A19 and CYP2E1 genes

We have previously demonstrated the androgen-dependent downregulation of the *CYP1A1* and *CYP1A2* genes in Meishan and Landrace pigs [18]. The previous report and the present results (Fig. 2) on the female-dominant expression of the *CYP2A19* and *CYP2E1* genes in Meishan, ML, and LM pigs suggest that androgen also downregulates the *CYP2A19* and *CYP2E1* genes. Therefore, the relationship between the expression of these *CYP* genes and the level of serum androgen was examined using male Meishan, Landrace, and their F1 (ML and LM) pigs. The constitutive expression levels of *CYP2A19* and *CYP2E1* mRNAs were lower in the pigs (5-month-old Meishan, ML, and LM) with more than 30 ng/ml of serum testosterone, as compared with those in the pigs (5-month-old Landrace and 1–3-month-old Meishan pigs) with the lower concentration of the androgen (Fig. 3).

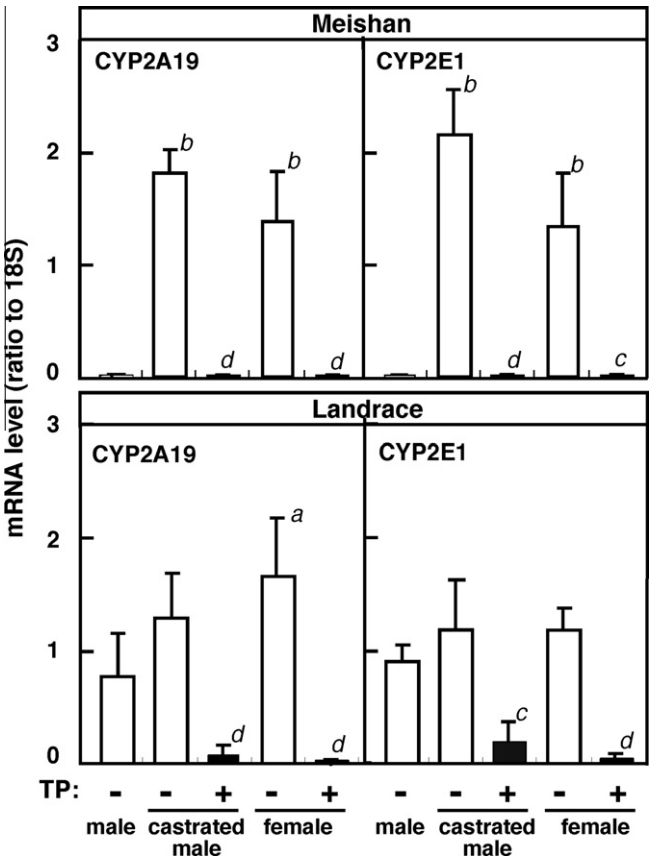

Fig. 4. Effects of castration and/or testosterone propionate (TP)-treatment on the gene expression of hepatic *CYP2A19* and CYP2E1 in 5-month-old Meishan and Landrace pigs. TP was injected intramuscularly into 5-month-old castrated male and intact female Meishan and Landrace pigs, as described in "Section2". Expression levels of *CYP2A19* and *CYP2E1* mRNAs were determined by real-time RT-PCR. The levels were normalized to that of 18S as an internal standard. Each column indicates the mean in each experimental group, and each bar represents the standard deviation of the mean ($n = 8$ and 7 for intact males of Meishan and Landrace pigs, respectively; $n = 3$ for other groups). [a,b]Significant differences from the corresponding male pigs assessed by Student's *t*-test: [a]$P < 0.05$, [b]$P < 0.01$. [c,d]Significant differences between TP-treated and untreated pigs in each breed assessed by Student's *t*-test: [c]$P < 0.05$, [d]$P < 0.01$.

To further confirm the androgen-dependent downregulation of *CYP2A19* and *CYP2E1* genes, we examined the effects of castration and/or administration of TP on their expressions in Meishan and Landrace pigs. The constitutive expression levels of *CYP2A19* and *CYP2E1* mRNAs in male Meishan pigs were much lower than those in the females (Fig. 4). However, castration of male Meishan pigs led to dramatic increases in the levels of *CYP2A19* and *CYP2E1* mRNAs, and reaching levels almost the same as those in females. On the other hand, in Landrace pigs, no such effect of castration was observed. Furthermore, in either Meishan or Landrace pigs, the administration of TP to the castrated males and to the intact females led to dramatic reductions of the levels of *CYP2A19* and *CYP2E1* mRNAs (Fig. 4). Serum testosterone levels in the TP-treated pigs were in the range of 92 ~ 299 ng/ml.

4. Discussion

We have previously demonstrated that there are sex and/or breed differences in the levels of serum testosterone and the constitutive expression levels of hepatic CYP1A1 and CYP1A2 in Meishan and Landrace pigs [17,18]. Incidentally, constitutive expression levels of hepatic *CYP1A* and *CYP1A2* mRNAs were considerably lower in the pigs with high levels (more than 33 ng/ml) of serum testosterone compared with those in the pigs with lower levels (*e.g.* the mean concentrations of serum testosterone were 50 ng/ml and 18 ng/ml in 5-month-old male Meishan and Landrace pigs, respectively).

In the present study, we first examined whether the serum androgen level is genetically controlled, using Meishan, Landrace, and their F1 (ML and LM) pigs. The levels of serum testosterone in the 5-month-old males of ML and LM pigs were almost the same as those in the age-matched male Meishan pigs (40–85 ng/ml). These results indicate that the causal gene(s) determining serum androgen level exist in autosome (non-sex chromosomes), but not in sex chromosomes. Furthermore, the breed difference in serum androgen level is thought to be dependent on that in expression balance of the genes encoding the biosynthetic and metabolic enzymes of androgen because there were breed differences between male Meishan and Landrace pigs at the age of 5 months not only in the gene expression levels of hepatic androgen-metabolizing enzymes, such as CYP1A1, CYP1A2, *CYP2A19*, and CYP2E1 [21,22], but also in those of testicular androgen-biosynthetic enzymes, such as CYP11A1 and CYP17A1 [23] (unpublished data).

In 5-month-old Meishan, ML, and LM pigs, but not Landrace pigs, significant sex differences (males < females) were observed in the expression levels of hepatic CYP1A1, CYP1A2, *CYP2A19*, and CYP2E1. Such sex differences agreed with previous reports [13,14,16–18]. Castration of male Meishan pigs changed the expression pattern of *CYP2A19* and *CYP2E1* mRNAs to a female-type. On the other hand, treatments with TP of the castrated males and intact females of Meishan and Landrace pigs changed the *CYP* mRNA expression pattern to a male-type of Meishan pigs. These effects of TP and/or castration agreed with previous reports [18,24]. Accordingly, serum androgen level is strongly suggested to be one of the critical factors leading to the sex and breed differences in the constitutive gene expression of hepatic CYPs, such as CYP1A1, CYP1A2, *CYP2A19*, and CYP2E1, in pigs.

The secretion profile of growth hormone (GH) might be also considered as one of physiological factors responsible for the sex difference in the constitutive expression level of several CYPs [25–27]. However, the androgen theory proposed in the present study is strongly supported by the findings that in spite of sex differences in secretion profile of GH in Landrace pigs [28], there are no sex differences in the constitutive levels of *CYP1A1*, *CYP1A2*, and *CYP2E1* mRNAs in the pigs. Furthermore, it has been reported that the secretion profile of GH could be modified by the administration of androgen [29–32]. Accordingly, the drastic decreases in the expression levels of hepatic *CYP* mRNAs observed in the female and castrated male pigs treated with androgen are thought to occur, at least in part, through changes in secretion profile of GH, especially to a male-type secretion profile. However, exact mechanisms for the androgen- and GH-mediated regulations of the *CYP* genes remain unclear yet.

Androgen-mediated downregulation of constitutive gene expression of hepatic CYPs, might occur through the reduction and/or inactivation of common transcription factor(s) for these *CYP* genes, because the levels of serum androgen showing inhibitory effects on these expression are almost the same and because the transcription factors selective for these genes are different from each other [33–36]. In addition, the androgen-mediated downregulation of the *CYP1A* subfamily genes would occur, at least in part, through a complex formation between the androgen receptor (AR) and the aryl hydrocarbon receptor (AhR), a positive transcription factor of these genes [37,38]. This is because an AR-AhR complex suppresses the AhR-mediated transcription of the *CYP1A* subfamily genes [39]. The androgen-mediated effects are generally considered to occur through activation of androgen receptor by binding of androgen. However, the androgen receptor-binding element(s)

have never been found in the promoter regions of the *CYP* genes. Accordingly, androgen is thought to indirectly down regulate the expression of the *CYP* genes.

The CYPs, including CYP1A1, CYP1A2, *CYP2A19*, and CYP2E1, play an important role in the metabolism of various drugs and carcinogens. For example, CYP1A1 and CYP1A2 catalyze the metabolic activations of carcinogenic aryl hydrocarbons and aromatic amines, respectively [10,40–43]. Furthermore, the activities of CYP1A1 and CYP1A2 in target tissues are one of the host factors that determine the susceptibility of experimental animals toward carcinogenic aryl hydrocarbons [40,44] and aromatic amines [8,45], respectively. Likewise, *CYP2A19*, which is homologous to human CYP2A6/2A13 [20,46], catalyzes the oxidative metabolisms of coumarin [47], nicotine [48], and carcinogenic nitrosamines [49]. CYP2E1 is responsible for the bioactivations of several toxicants, including alcohol [50], carcinogenic nitrosamines [49], and styrenes [51,52]. Furthermore, *CYP2A19* and CYP2E1 are also associated with the clearance of skatole, a major component of boar taint [53]. Thus, further studies on the androgen-associated expression of these *CYP* genes would contribute not only to our understanding of the individual differences in the susceptibilities to drugs and environmental toxicants in humans, but also help in development of meat production avoiding boar taint.

In conclusion, we demonstrate for the first time that the physiological level of serum androgen is determined by autosomal dominant inheritance and further confirm that the level of serum androgen is one of the endogenous factors that downregulate the constitutive expression of the *CYP1A1*, *CYP1A2*, *CYP2A19,* and *CYP2E1* genes in the pig liver.

Acknowledgments

We thank the Swine Management Section of the National Institute of Livestock and Grassland Science (Tsukuba, Japan) for the care of the animals and for collecting tissue samples. This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (No. 22590166, M.K.).

References

[1] D.R. Nelson, L. Koymans, T. Kamataki, J.J. Stegeman, et al., P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature, Pharmacogenetics 6 (1996) 1–42.
[2] C.J. Omiecinski, R.P. Remmel, V.P. Hosagrahara, Concise review of the cytochrome P450s and their roles in toxicology, Toxicol. Sci. 48 (1999) 151–156.
[3] F.P. Guengerich, Cytochromes P450, drugs, and diseases, Mol. Interventions 3 (2003) 194–204.
[4] A. Parkinsin, D.R. Mudra, C. Johnson, et al., 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 (2004) 193–209.
[5] M. Murray, Altered CYP expression and function in response to dietary factors: potential roles in disease pathogenesis, Curr. Drug Metab. 7 (2006) 67–81.
[6] M.J. Graham, B.G. Lake, Induction of drug metabolism: species differences and toxicological relevance, Toxicology 254 (2008) 184–191.
[7] K. Monostory, J.-M. Pascussi, L. Kóbori, et al., Hormonal regulation of CYP1A expression, Drug Metab. Rev. 41 (2009) 547–572.
[8] M. Degawa, M. Kojima, T. Hishinuma, et al., 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 (1985) 96–102.
[9] M. Degawa, C. Yamaya, Y. Hashimoto, Hepatic cytochrome P-450 isozymes induced by dietary carcinogenic aromatic amines preferentially in female mice of DBA/2 and other strains, Carcinogenesis 9 (1988) 567–571.
[10] M. Degawa, K. Hanaki, M. Sekimoto, A hepatocarcinogenic tryptophan-pyrolyzate component, Trp-P-1, decreases serum total testosterone level and induces hepatic Cyp1a2 in male mice, Cancer Sci. 97 (2006) 32–37.
[11] P. Honkakoski, A. Kojo, M.A. Lang, Regulation of the mouse liver cytochrome P450 2B subfamily by sex hormones and phenobarbital, Biochem. J. 285 (1992) 979–983.
[12] C.A. Wiwi, M. Gupte, D.J. Waxman, Sexually dimorphic P450 gene expression in liver-specific hepatocyte nuclear factor 4α-difficient mice, Mol. Endocrinol. 18 (2004) 1975–1987.
[13] M.T. Skaanild, C. Friis, Cytochrome P450 sex differences in minipigs and conventional pigs, Pharmacol. Toxicol. 85 (1999) 174–180.
[14] G. Zamaratskaia, G. Chen, K. Lundstrom, Effects of sex, weight, diet and hCG administration on levels of skatole and indole in the liver and hepatic activities of cytochrome P4502E1 and P4502A6 in pigs, Meat Sci. 72 (2006) 331–338.
[15] G. Zamaratskaia, V. Zlabek, E. Ropstad, et al., In vitro and In vivo association of porcine hepatic cytochrome P450 3A and 2C activities with testicular steroids, Reprod. Dom. Anim., in press, doi: 10.1111/j.1439-0531.2012.01986.x.
[16] M.K. Rasmussen, G. Zamaratskaia, B. Ekstrand, Gender-related differences in cytochrome P450 in porcine liver – implication for activity, expression and inhibition by testicular steroids, Reprod. Dom. Anim. 46 (2011) 616–623.
[17] M. Kojima, M. Sekimoto, M. Degawa, A novel gender-related difference in the constitutive expression of hepatic cytochrome P4501A subfamily enzymes in Meishan pigs, Biochem. Pharmacol. 75 (2008) 1076–1082.
[18] M. Kojima, M. Sekimoto, M. Degawa, Androgen-mediated down-regulation of CYP1A subfamily genes in the pig liver, J. Endocrinol. 207 (2010) 203–211.
[19] R.D. Hughes, R. Williams, Use of bioartificial and artificial liver support device, Semin. Liver Dis. 16 (1996) 435–444.
[20] B. Achour, J. Barber, A. Rostami-Hodjegan, Cytochrome P450 pig liver pie: determination of individual cytochrome P450 isoform contents in microsomes from two livers using liquid chromatography in conjunction with mass spectrometry, Drug Metab. Dispos. 39 (2011) 2130–2134.
[21] J.A. Krauser, M. Voehler, Li-H. Tseng, et al., Testosterone 1b-hydroxylation by human cytochrome P450 3A4, Eur. J. Biochem. 271 (2004) 3962–3969.
[22] H. Agematu, N. Matsumoto, Y. Fujii, et al., Hydroxylation of testosterone by bacterial cytochrome P450 using the *Escherichia coli* expression system, Biosci. Biotechnol. Biochem. 70 (2006) 307–311.
[23] W.L. Miller, R.J. Auchus, The molecular biology, biochemistry, and physiology of human steroidgenesis and its disorders, Endocrine Rev. 32 (2011) 81–151.
[24] G. Zamaratskaia, V. Zlabek, G. Chen, et al., Modulation of porcine cytochrome P450 enzymes activities by surgical castration and immunocastration, Animal 3 (2009) 1124–1132.
[25] A.K. Agrawal, B.H. Shapiro, Intrinsic signals in the sexually dimorphic circulating growth hormone profiles of the rat, Mol. Cell. Endocrinol. 173 (2001) 167–181.
[26] A. Mode, J.-A. Gustafsson, Sex and the liver – a journey through five decades, Drug Metab. Rev. 38 (2006) 197–207.
[27] D.J. Waxman, M.G. Holloway, Sex differences in the expression of hepatic drug metabolizing enzymes, Mol. Pharmacol. 76 (2009) 215–228.
[28] J.R. Arbona, D.N. Marple, R.W. Russell, et al., Secretory patterns and metabolic clearance rate of porcine growth hormone in swine selected for growth, J. Anim. Sci. 66 (1988) 3068–3072.
[29] S.L. Davis, D.L. Ohlson, J. Klindt, et al., Episodic growth hormone secretory patterns in sheep: relationship to gonadal steroid hormones, Am. J. Physiol. Endocrinol. Metab. 233 (1977) E519–E523.
[30] R. Somana, S. Visessuwan, A. Samridtong, et al., Effect of neonatal androgen treatment and orchidectomy on pituitary levels of growth hormone in the rat, J. Endocrinol. 79 (1978) 399–400.
[31] K. Link, R.M. Blizzard, W.S. Evans, et al., The effect of androgens on the pulsatile release and the twenty-four-hour mean concentration of growth hormone in peripubertal males, J. Clin. Endocrinol. Metab. 62 (1986) 159–164.
[32] P. Dubreuil, P.Y. Couture, H. Lapierre, et al., Castration and testosterone effects on endogenous and somatocrinin-induced growth hormone release in intact and castrated male pigs, Dom. Anim. Endocrinol. 6 (1989) 15–24.
[33] J.F. Shadley, K. Divakaran, K. Munson, et al., Identification and functional analysis of a novel human *CYP2E1* far upstream enhancer, Mol. Pharmacol. 71 (2007) 1630–1639.
[34] A. Nannelli, F. Rossignolo, R. Tolando, P. Rossato, et al., Effect of β-naphthoflavone on AhR-regulated genes (CYP1A1, 1A2, 1B1, 2S1, Nfr2, and GST) and antioxidant enzymes in various brain regions of pig, Toxicology 265 (2009) 69–79.
[35] J. Wang, J. Nekvindova, M. Ingelman-Sundberg, et al., IL-4-mediated transcriptional regulation of human CYP2E1 by two independent signaling pathways, Biochem. Pharmacol. 80 (2010) 1592–1600.
[36] S. Yokota, E. Higashi, T. Fukami, et al., Human CYP2A6 is regulated by nuclear factor-erythroid 2 related factor 2, Biochem. Pharmacol. 81 (2011) 289–294.
[37] O. Hankinson, The aryl hydrocarbon receptor complex, Annu. Rev. Pharmacol. Toxicol. 35 (1995) 307–340.
[38] M.S. Denison, S.R. Nagy, Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals, Annu. Rev. Pharmacol. Toxicol. 43 (2003) 309–334.
[39] N. Sanada, Y. Gotoh, R. Shimazawa, et al., Repression of activated aryl hydrocarbon receptor-induced transcriptional activation by 5α-dihydrotestosterone in human prostate cancer LNCaP and human breast cancer T47D cells, J. Pharmacol. Sci. 109 (2009) 380–387.
[40] A.H. Conney, Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes memorial lecture, Cancer Res. 42 (1982) 4875–4917.
[41] T. Shimada, Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons, Drug Metab. Pharmacokinet. 21 (2006) 257–276.
[42] Q. Ma, A.Y.H. Lu, CYP1A induction and human risk assessment: An evolving tale of in vitro and in vivo studies, Drug Metab. Dispos. 35 (2007) 1009–1016.

[43] S.F. Zhou, B. Wang, L.P. Yang, et al., Structure, function, regulation and polymorphism and the clinical significance of human cytochrome P450 1A2, Drug Metab. Rev. 42 (2010) 268–354.

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

[45] M. Kleman, E. Overvik, G. Mason, et al., Effects of the food mutagens MeIQx and PhIP on the expression of cytochrome P450IA proteins in various tissues of male and female rats, Carcinogenesis 11 (1990) 2185–2189.

[46] M. Kojima, T. Morozumi, 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 (2004) 518–529.

[47] C.H. Yun, T. Shimada, F.P. Guengerich, Purification and characterization of human liver microsomal cytochrome P-450 2A6, Mol. Pharmacol. 40 (1991) 679–685.

[48] M. Nakajima, T. Yamamoto, K. Nunoya, T. Yokoi, et al., Role of human P4502A6 in C-oxidation of nicotine, Drug Metab. Dispos. 24 (1996) 1212–1217.

[49] H. Yamazaki, K. Inoue, M. Hashimoto, et al., Cytochrome P450 2E1 and 2A6 as major catalysts of metabolic activation of N-nitrodiakylamines and tobacco-related nitrosamine in human liver microsomes, Carcinogenesis 13 (1992) 179–1794.

[50] T.M. Badger, M.J. Ronis, H.K. Seitz, et al., Alcohol metabolism: role in toxicity and carcinogenesis, Alcohol Clin. Exp. Res. 27 (2003) 336–347.

[51] F.P. Guengerich, D.H. Kim, M. Iwasaki, Role of human cytochrome P-450IIE1 in the oxidation of many low molecular weight cancer suspects, Chem. Res. Toxicol. 4 (1991) 168–179.

[52] J. Chung, S. Shen, Z. Jiang, et al., Structure-toxicity relationship study of para-halogenated styrene analogues in CYP2E1 transgenic cells, Toxicol. Lett. 210 (2012) 353–359.

[53] G. Zamaratskaia, E.J. Squires, Biochemical, nutritional and genetic effects on boar taint in entire male pigs, Animal 3 (2009) 1508–1521.