Alleles of the Cholesterol 7 Alpha-Hydroxylase (CYP7) Gene in Pigs Selected for High or Low Plasma Total Cholesterol (44259)

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Abstract. **Crossbred pigs were selected for high (HTC) or low (LTC) plasma total cholesterol (TC). Pigs from the seventh ($n = 51$) and eighth ($n = 92$) generations were used to determine restriction fragment length polymorphisms (RFLP). Using *TaqI* restriction enzyme digestion, the frequencies of two alleles (2.8- or 5.0-kb fragments) of the cholesterol 7 alpha-hydroxylase (CYP7) gene were determined in the two populations as a potential indicator of TC concentration at 8 weeks of age. Only the 2.8-kb fragment allele was present in the 26 HTC pigs tested in Generation 7. In the LTC pigs both the 2.8- and 5.0-kb alleles were present in 12 pigs, and only the 5.0-kb allele was present in 13 pigs. The allele frequencies of the 2.8 and 5.0 fragments, respectively, were .26 and .74 in LTC pigs and 1.00 and 0 in HTC pigs. There was an association ($P < .001$) between the 5.0- and 2.8-kb CYP7 alleles, respectively, and low and high TC concentrations. In Generation 8, all HTC pigs were homozygous for the 2.8-kb allele. The 5.0 kb allele was present in all LTC pigs tested and was homozygous in 57% of LTC pigs. Mean plasma TC was 105.0 mg/dl in 30 pigs homozygous for the 2.8-kb allele in Generation 8; means for LTC pigs were 53.5 and 60.4 mg/dl in 35 pigs homozygous for the 5.0-kb allele and in 27 heterozygous pigs, respectively. High TC was associated with the presence of the 2.8-kb allele, and low TC was associated with the presence of the 5.0-kb allele in both Generations 7 and 8. We conclude that *TaqI* RFLP analysis of the CYP7 gene is a reliable indicator for TC in these swine.** [P.S.E.B.M. 1998, Vol 217]

The relatively high heritability (h2 = .25–.45; 1, 2, 3) of plasma total cholesterol (TC) in swine prompted the development of a population divergent in TC at 8 weeks of age, (4, 5) for use in experiments to study nutrition × genetic interactions. The TC was measured in pigs fed a diet with no cholesterol and <4% fat, by weight. The mean (± SD) TC in Generation 7 was 117 ± 18 and 64 ± 18 mg/dl for pigs selected for high (HTC) and low (LTC) total plasma cholesterol, respectively (6). Plasma TC concentration is regulated by multiple genetic factors that modulate cholesterol uptake, synthesis, and catabolism.

Using restriction fragment length polymorphism (RFLP) analysis with *Taq*I, Davis *et al.* (7, 8, 9) reported two alleles of the low-density lipoprotein receptor (LDLR) and two alleles of the cholesterol 7 alpha-hydroxylase (CYP7) locus (10) in swine. The LDLR is the major receptor for uptake of cholesterol-rich lipoproteins, and CYP7 is the first and rate-limiting enzyme in the conversion of cholesterol to bile acids for excretion.

The purpose of the present study was to determine the relationship between TC of pigs selected phenotypically for eight generations for high or low TC and the CYP7 genotype (heterozygous or homozygous for one or the other of two alleles of the CYP7 locus).

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Material and Methods

A total of 143 crossbed pigs from the seventh (n = 51) and eighth (n = 92) generation of selection for HTC or LTC (4, 5) were studied. All pigs were weaned at 4 weeks of age to a standard commercial grower diet (18% protein, 3% fat, 0% cholesterol) composed mainly of corn, soybean meal, other plant by-products, and supplemented with mineral and vitamin premixes. Blood was sampled from the anterior vena cava of each pig at 8 weeks of age into tubes containing EDTA as an anticoagulant and centrifuged at 3000 × g for 10 min at 5°C. Plasma was removed and used for determination of TC by an enzymatic assay with cholesterol esterase and cholesterol oxidase to yield hydrogen peroxide, which was reacted with p-hydroxybenzoate and 4-aminoantipyrine and measured spectrophotometrically at a wavelength of 500 nm (11), using an automated analyzer (Ciba-Corning 550 Express Chemistry Analyzer, Ciba-Corning Diagnostics, Oberlin, OH). The white buffy coat containing leukocytes at the interface of plasma and erythrocytes was removed for RFLP analysis of the CYP7 gene locus and the LDLR gene locus using *Taq*I restriction enzyme. The DNA was isolated from leukocytes, and digested overnight. Digested DNA samples underwent agarose gel electrophoresis and were transferred to nylon membranes. Prehybridization, hybridization, and washing conditions were as described by Davis *et al.* (7, 8, 9). The probe for CYP7 was a 2.0-kb EcoRI fragment excised from λ7∝6 clone that contains 95% of the rat CYP7 cDNA (12). The probe for LDLR was a 2.2-kb EcoRI fragment excised from λrLDL2-1a that contains a full-length 4.7-kb cDNA encoding the rat LDLR (13). Twenty-five LTC and 26 HTC pigs from Generation 7 and 30 HTC and 62 LTC pigs from Generation 8 were tested for TC concentration and CYP7 genotype. The animal care and experimental protocols were approved by the Institutional Animal Care and Use Committee, Baylor College of Medicine (Houston, TX).

Blood plasma TC data were analyzed using one-way analysis of variance (14) and Student's t test for selected pairs of data sets. Statistical comparisons of CYP7 allele frequencies with plasma TC concentrations were performed by chi-square analysis.

Results

Generation 7. The association between plasma TC concentrations of HTC and LTC pigs, at 8 weeks of age, and genotype (2.8- and 5.0-kb fragments) is summarized in Figure 1. The mean plasma TC of each group is in Table I. Observed frequencies of the 2.8- and 5.0-kb alleles of the CYP7 locus were 1.00 and 0, respectively, in HTC pigs and .26 and .74 in LTC pigs. The expected equal frequency was not supported by chi-square analysis (P < 0.01). Mean (± SD) plasma TC concentration was 115.6 ± 11.1 mg/dl in HTC pigs, all of which were homozygous for the 2.8-kb CYP7 fragment; mean plasma TC concentration of LTC pigs was 63.9 ± 8.2 mg/dl and tended to be lower (not statistically significant, P > 0.05) in pigs homozygous for the 5.0-kb fragment (59.2 ± 11.1 mg/dl) than in heterozygous pigs (68.5 ± 5.3 mg/dl). The data suggest the homozygous presence of the 2.8-kb CYP7 fragment is associated with higher concentrations of plasma TC, whereas the heterozygous or homozygous presence of the 5.0-kb CYP7 fragment is associated with lower plasma TC.

The *Taq*I RFLP analysis of both the CYP7 gene and the LDLR gene was performed on 42 pigs for which plasma TC

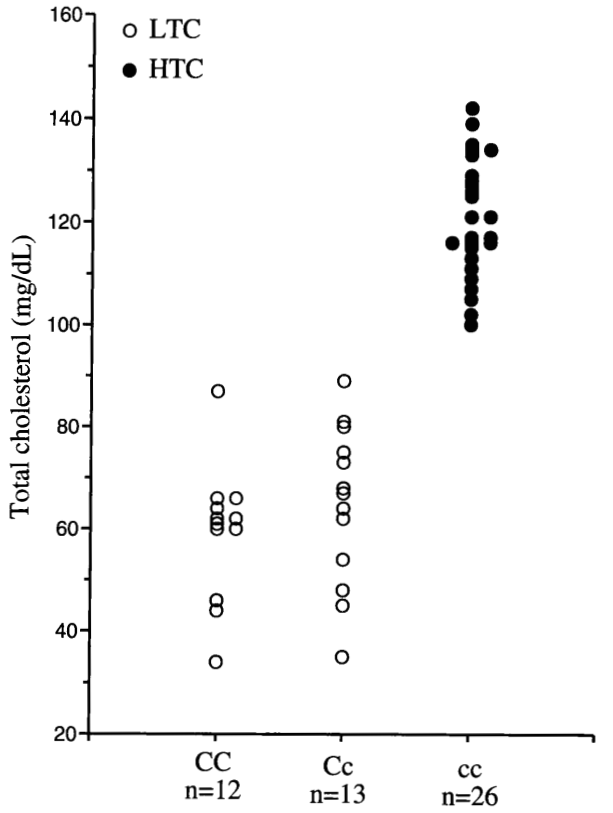

Figure 1. Association between plasma total cholesterol (TC) concentration and cholesterol 7α-hydroxylase genotype in pigs selected for seven generations for high or low TC at 8 weeks of age. C = 5.0-kb allele, c = 2.8-kb allele based on *Taq*I restriction fragment length polymorphism analysis.

Table I. Association Between *Taq*I RFLP Genotypes of the CYP7 Locus and Mean Plasma Total Cholesterol Concentration in Pigs Selected for High or Low Plasma Total Cholesterol

Phenotype	n	CYP7 Genotype[a]	Plasma TC, mg/dL[b]
HTC[c]	26	2.8/2.8	115.6 ± 11.1[e]
	0	5.0/2.8	—
	0	5.0/5.0	—
LTC[d]	0	2.8/2.8	—
	13	5.0/2.8	68.5 ± 5.3[f]
	12	5.0/5.0	59.2 ± 11.1[f]

[a] 5.0 = 5.0 kb allele or C in Figures 1 and 2; 2.8 = 2.8 kb allele or c in Figures 1 and 2.
[b] Plasma total cholesterol: mean ± standard deviation.
[c] High plasma total cholesterol—selected line; seventh generation
[d] Low plasma total cholesterol—selected line; seventh generation
[e,f] Means with different superscript letters differ (P < 0.01).

values at 56 d of age were available (9). These data are summarized in Table II. The allele frequencies of the 2.1-kb and 0.5-kb fragments were 0.66 and 0.34, respectively, in LTC pigs, and 0.3 and 0.7, respectively in HTC pigs. The expected equal frequencies of the two alleles of the LDLR locus were not supported by chi-square analysis ($P < 0.01$). Three of the nine possible combinations of alleles involving the CYP7 and LDLR gene loci, based on *Taq*I RFLP analysis (Table II), were not represented in the population sampled. The data from the six genotypes that were represented suggest that the plasma TC concentration at 56 d of age of these phenotypically selected swine populations is influenced to a greater extent by the CYP7 genotype than by the LDLR genotype.

Generation 8. The association between plasma TC concentrations of HTC and LTC pigs at 8 weeks of age from Generation 8 and the CYP7 genotype (2.8- and 5.0-kb fragments) is summarized in Figure 2, and the mean plasma TC of each group is in Table III. The expected equal frequencies of the 2.8- and 5.0-kb alleles of the CYP7 locus were not supported by chi-square analysis ($P < 0.05$). The association observed in Generation 7 between plasma TC concentration and CYP7 genotype in the HTC and LTC pigs selected phenotypically for high or low plasma TC was corroborated in Generation 8. Mean plasma TC was 104.0 mg/dl for HTC pigs homozygous for the 2.8-kb fragment of the CYP7 gene ($n = 30$), 60.3 mg/dl for LTC pigs homozygous for the 5.0-kb fragment of the CYP7 gene ($n = 35$), and 66.7 mg/dl for LTC pigs heterozygous for the two alleles of the CYP7 gene ($n = 27$).

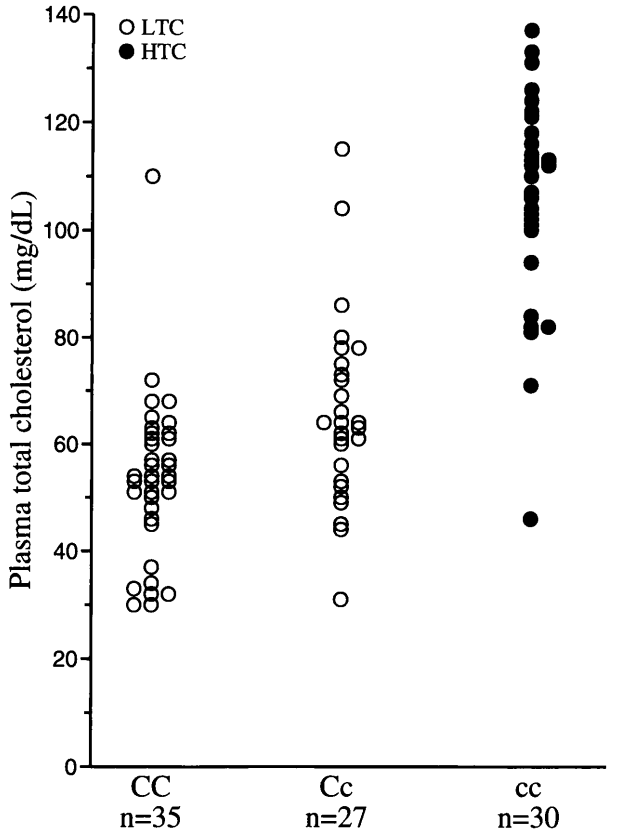

Figure 2. Association between plasma total cholesterol (TC) concentration and cholesterol 7α-hydroxylase genotype in pigs selected for eight generations for high or low TC at 8 weeks of age. C = 5.0-kb allele, c = 2.8-kb allele based on *Taq*I restriction fragment length polymorphism analysis.

Discussion

Body cholesterol homeostasis is maintained by a balance of accretion from dietary intake plus endogenous synthesis of cholesterol, and excretion by bile acid synthesis from cholesterol plus fecal sterol excretion (15). Multiple genetic factors are involved in the regulation of the plasma cholesterol level. Cholesterol uptake is primarily regulated by LDLR, synthesis is primarily regulated by the enzyme, 3-hydroxy-3-methylglutaryl-CoA reductase; and degradation is primarily regulated by CYP7, the rate limiting en-

Table II. Relationship of *Taq*I RFLP Genotypes of the CYP7 and LDLR Loci to Plasma Total Cholesterol Concentration in Pigs Selected for High or Low Plasma Total Cholesterol

Phenotype	Genotype		*n*	Plasma TC, mg/dL[c]
	CYP7[a]	LDLR[b]		
HTC[d]	2.8/2.8	0.5/0.5	8	122.1 ± 11[f]
	2.8/2.8	0.5/2.1	14	118.5 ± 10.8[f]
	2.8/2.8	2.1/2.1	0	—
LTC[e]	2.8/5.0	0.5/0.5	1	79.0
	2.8/5.0	0.5/2.1	7	74.7 ± 7.4[g]
	2.8/5.0	2.1/2.1	0	—
LTC[e]	5.0/5.0	0.5/0.5	0	—
	5.0/5.0	0.5/2.1	3	48.7 ± 13.2[h]
	5.0/5.0	2.1/2.1	9	62.9 ± 10.4[g,h]

[a] 5.0 = 5.0 kb allele or C in Figures 1 and 2; 2.8 = 2.8 kb allele or c in Figures 1 and 2.
[b] 2.1 = 2.1 kb allele; 0.5 = 0.5 kb allele.
[c] Plasma total cholesterol: mean ± standard deviation.
[d] High plasma total cholesterol—selected line; seventh generation
[e] Low plasma total cholesterol—selected line; seventh generation
[f,g,h] Means with different superscript letters differ ($P < 0.05$)

Table III. Association between *Taq*I RFLP genotypes of the CYP7 locus and mean plasma total cholesterol concentration in the eighth generation of pigs selected for high or low plasma total cholesterol.

Phenotype	*n*	CYP7 Genotype[a]	Plasma TC, mg/dL[b]
HTC[c]	30	2.8/2.8	104.0 ± 22.0[e]
	0	5.0/2.8	—
	0	5.0/5.0	—
LTC[d]	0	2.8/2.8	—
	27	5.0/2.8	66.7 ± 21.6[f]
	35	5.0/5.0	60.3 ± 21.7[f]

[a] 5.0 = 5.0 kb allele or C in Figures 1 and 2; 2.8 = 2.8 kb allele or c in Figures 1 and 2.
[b] Plasma total cholesterol: mean ± standard deviation.
[c] High plasma total cholesterol—selected line.
[d] Low plasma total cholesterol—selected line.
[e,f] Means with different superscript letters differ ($P < 0.01$).

zyme in conversion of cholesterol to bile acids. Because CYP7 is the central enzyme of cholesterol catabolism, CYP7 is thought to play an important role in cholesterol homeostasis. Rats are resistant to diet-induced hypercholesterolemia because feeding cholesterol to these animals induces a rapid increase in CYP7 activity (16). In comparison, hamster CYP7 does not respond to cholesterol feeding, and hamsters are much more susceptible to diet-induced hypercholesterolemia (17). Poorman *et al.* reported elevated CYP7 expression in a strain of hypercholesterolemia-resistant rabbits (18).

In this paper, we showed that *Taq*I polymorphisms of CYP7 were associated with the plasma TC concentration in pigs selected for HTC or LTC. The higher plasma TC ($P < 0.01$) in pigs homozygous for the 2.8-kg fragment than in those homozygous for the 5.0-kg fragment of the CYP7 gene was shown in data from Generation 7 of selection for high or low plasma TC concentration and confirmed in Generation 8.

Plasma TC is the sum of several genetic and environmental variables. The failure to broaden the difference between HTC and LTC lines of swine beyond Generation 4 of selection (4) was probably partly the result of the continued persistence of individuals heterozygous for the CYP7 gene in succeeding generations of selection. We did not know the CYP7 genotypes until Generation 7. Further work is required to determine how these polymorphisms affect CYP7 expression and activity.

The analysis of lipoprotein total cholesterol profile by fast performance liquid chromatography showed that elevated TC levels in HTC pigs was accounted for by the increased LDL cholesterol (data not shown). In humans, the most common cause of isolated increase in total or LDL cholesterol is polygenic hypercholesterolemia (19). Therefore, other genetic factors, in addition to the CYP7 gene, may be involved in the regulation of plasma TC level in our animals, as in humans. Endogenous synthesis of cholesterol does not appear to be different between HTC and LTC pigs (20). Therefore, it is suggested that differences in plasma TC concentrations between HTC and LTC pigs result from the differences in cholesterol degradation or cholesterol uptake, which is mainly regulated by LDLR.

The LDLR is one of several major factors in cholesterol homeostasis. Cholesterol is transported in plasma as a component of lipoproteins of varying particle sizes (i.e., very low density lipoprotein, LDL, and high density lipoprotein). Apolipoprotein B-100, present on the surface of LDL particles, allows these particles to bind to LDLR in liver and other tissues. Cholesterol is then transferred to intracellular compartments for use in biosynthetic and structural functions, or for synthesis of bile acids, to be excreted (21, 22). The LDLR gene locus has been shown by RFLP analysis to have two alleles (0.5- and 2.1-kb fragments) in swine (9). Therefore, it would be predicted that plasma TC concentration would be influenced by the LDLR genotype of these swine. However, our data suggest that in these phenotypically selected swine populations, plasma TC concentration at 8 weeks of age is influenced more by the CYP7 genotype than by the LDLR genotype, implying an important role of cholesterol degradation in controlling cholesterol homeostasis in these swine. In swine, the type 5 apolipoprotein B allele (Lpb5) also is associated with hypercholesterolemia and atherosclerosis (23). This hypercholesterolemia is not associated with a defective liver LDLR; rather, the LDL apolipoprotein is defective in its binding to a normal LDL receptor, resulting in a reduced rate of catabolism of LDL in these hypercholesterolemic swine (24). The hypercholesterolemia and atherosclerosis associated with the Lpb5 homozygous state are not limited to this gene alone; many other genes apparently contribute to the atherogenic process in that swine population (25).

High plasma TC concentration at 8 weeks of age was found to be associated with homozygosity for one allele (2.8 kb), whereas low plasma TC was associated with homozygosity of the other allele (5.0 kb) of the CYP7 gene in pigs selected for 7 or 8 generations for high or low plasma TC. This observation suggests that RFLP analysis of the CYP7 gene, using *Taq*I restriction enzyme, is a reliable indicator for plasma TC. Blood can be sampled at birth for RFLP analysis. Although plasma TC concentrations at 56 d of age are predictive of the concentrations later in life (4, 26), meaningful plasma TC values are not available until after weaning (8 weeks of age or older), as in the case of the pigs in the current experiments.

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