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The Amino Acid Composition and Diversity Analysis of the Porcine Myogenin

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Abstract: A total of 63 individuals from 10 different pig breeds were utilized to sequence the nucleotide sequence of the porcine *myogenin* gene in this experiment. The amino acid sequences of the porcine myogenin were deduced from the nucleotide sequences to study the amino acid composition and diversity of the porcine myogenin. The following conclusions can be drawn from the experiment. The porcine myogenin consists of 220 amino acids, all of the 20 conventional amino acids can be found in all detected pig breed populations. Only one nonsynonymous mutation happened in Neijiang pig breed caused the substitution of Arg131→Cys131, the myogenin has completely identical deduced amino acid sequence in all the other pig breeds. That means mutations happen in *myogenin* gene mainly are synonymous mutation and the myogenin gene is very conservative. The structure of porcine myogenin is very stable. In all the detected pig populations the values of Ka and Ks in myogenin are very small that means the porcine *myogenin* gene keeps in a nature status does not be influenced by artificial selection.

Key words: Pig, *MRFs* gene family, myogenin, amino acid composition, genetic diversity, China

INTRODUCTION

As an important candidate gene responsible for muscle fiber characteristics the *myogenin* gene is a member of the Myogenic Regulatory Factors (MRFs) family. The *MRFs* gene family is a kind of myogenic basic helix-loop-helix transcription factors plays important roles in skeletal muscle development (Weintraub, 1993; Kim *et al*., 2009). In the *MRFs* gene family, myogenin is the only gene that expresses in all of skeletal muscle cell linages (Wang and Jaenisch, 1997).

Myogenin is a major player in the initiation and maintenance of myogenic differentiation in a mouse myoblast cell line and knockdown of Rbm 24 expression by RNA interference significantly decreased myogenin expression associated with the inhibition of myogenesis. In contrast the overexpression of Rbm 24 by stable transfection of a plasmid increased myogenin expression and had a positive effect on myogenic differentiation (Jin *et al*., 2010).

The expression of myogenin begins in all myoblasts with the onset of differentiation and terminates at the end of myoblast proliferation (Soumillion *et al*., 1997; Te Pas *et al*., 2000). Several experiments have proved that because of its crucial function during myogenesis, the genetic variation in the porcine *myogenin* gene may cause changes of myoblast and muscle fibers and corresponding growth, lean meat production and meat quality traits (Knapp *et al*., 2006; Anton *et al*., 2006; Cieslak *et al*., 2000; Te Pas *et al*., 2000; Kim *et al*., 2009). The porcine *myogenin* gene located at the region of 9q21-q2.6 and consists of 3 extron and 2 intron (Soumillion *et al*., 1997). Genetic diversity is the base and core of ecosystem diversity which is the sum total of genetic information within a species and the sum of genetic variation within a population. To different breeds the genetic diversity is the difference exists between individuals at the DNA level (Wang and Li, 2002).

The evolution potential of a species is decided by the extent of genetic variation and the genetic distribution of these variations (Wang and Li, 2002). With the development of gene sequencing technology in the recent years, it is possible to research the genetic diversity from the DNA level and researches about the nucleotide variation promoted the development of phylogeny, evolutionary genetics and molecular evolution (Nei and Kumar, 2000). In this study, the complete nucleotide sequence of *myogenin* gene (~2605 bp) was sequenced in 63 individuals from 10 different pig breeds collected in China. The amino acid sequences of porcine myogenin were deduced based on the nucleotide sequence to study the amino acid composition and genetic diversity of the porcine myogenin.

MATERIALS AND METHODS

Sampling: Muscle samples of 63 individuals from 10 breeds (Eight Chinese domestic breeds, one recently

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developed breed and one introduced breed) were collected from five provinces in China (Li *et al*., 2008). One reference myogenin sequence from GenBank (GenBank accession number X89007) was used in the following analysis.

DNA amplification and sequencing: Total genomic DNA was isolated from muscle samples by standard phenol/chloroform methods. To ensure the accuracy, the complete *myogenin* gene sequence was divided into three parts. Each part was amplified separately and jointed together after sequencing. There are overlap sequences exist in each part (Li *et al*., 2008).

Polymerase chain reaction was performed using about 20 ng of DNA in a 25 μL reaction volume with 32 cycle (94°C for 1 min, annealing for 1 min and 72°C for 1 min). The products were purified with a gel extraction kit (Watson Biomedical Inc., Shanghai, China) and sequenced on an Applied Biosystem ABI 3100 Sequencer using the Bigdye TM. Terminator Cycle Sequencing kit (P.E. Biosystems Inc., Foster City, CA, USA). Both strands were sequenced using Forward and Reverse primer.

Nucleotide sequence data analyses: Sequences were edited and jointed together with BIOEDIT software (BIOEDIT version 5.0.9, Tom Hall. 2001, USA). Complete sequences were aligned with DNASTAR software (DNASTAR version 5.00, Steve. 2001, USA). The complete myogenin gene sequence of 10 different breeds were got and put in GenBank.

Amino acid sequence data analyses: The amino acid sequence of myogenin was deduced from the nucleotide sequence by DNASTAR software (DNASTAR version 5.00, Steve. 2001, USA). DnaSP 3.53 (DNA Sequencing Polymorphism) was utilized to analyze the amino acid composition and diversity and the variation of codon usage frequency. Areliquin (Ver 2.001, Laurent Excoffier, 2001, Switzerland) was used to analyze the population demographic parameter.

RESULTS AND DISCUSSION

Amino acid composition of myogenin in different pig breeds: The deduced amino acid sequence of myogenin in different pig breeds is shown in Table 1. From Table 1, the researchers know that in the detected 10 pig breeds, the myogenin consists of 220 amino acids. Although, all of the 20 conventional amino acids can be found in the myogenin, the content of each amino acid in the porcine myogenin is quite different. Leu is most abundant in every breed (Average content is 11.36%), the content of Glu, Pro and Ser are high as 10.00, 9.09 and 8.64%, respectively. The Met and Trp are the rare amino acids in the porcine myogenin the both content are <1% (0.45 and 0.91%, respectively).

The amino acid variation of porcine myogenin in different pig breeds: The amino acid sequences of the porcine myogenin were compared among different pig breeds to analyze the genetic variation (Table 2). Among the 10 pig breeds only one nonsynonymous mutation happened in

Table 1: The amino acid composition frequencies of the myogenin in different pig breeds (%)

Breed	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu
Ya'nan pig	6.36	2.73	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
Neijiang pig	6.36	3.18	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
Chenghua pig	6.36	2.73	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
Rongchang pig	6.36	2.73	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
Dahe pig	6.36	2.73	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
Tibetan pig	6.36	2.73	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
Wuzhishan pig	6.36	2.73	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
Meishan pig	6.36	2.73	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
Dawu pig	6.36	2.73	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
Duroc	6.36	2.73	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
X89007	6.36	2.73	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
Average	6.36	2.77	4.55	10.00	2.73	6.36	3.18	2.27	4.09	11.36
Breed	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr
Ya'nan pig	0.45	3.18	9.09	3.64	7.27	8.64	4.09	5.45	0.91	3.64
Neijiang pig	0.45	3.18	9.09	3.64	6.82	8.64	4.09	5.45	0.91	3.64
Chenghua pig	0.45	3.18	9.09	3.64	7.27	8.64	4.09	5.45	0.91	3.64
Rongchang pig	0.45	3.18	9.09	3.64	7.27	8.64	4.09	5.45	0.91	3.64
Dahe pig	0.45	3.18	9.09	3.64	7.27	8.64	4.09	5.45	0.91	3.64
Tibetan pig	0.45	3.18	9.09	3.64	7.27	8.64	4.09	5.45	0.91	3.64
Wuzhishan pig	0.45	3.18	9.09	3.64	7.27	8.64	4.09	5.45	0.91	3.64
Meishan pig	0.45	3.18	9.09	3.64	7.27	8.64	4.09	5.45	0.91	3.64
Dawu pig	0.45	3.18	9.09	3.64	7.27	8.64	4.09	5.45	0.91	3.64
Duroc	0.45	3.18	9.09	3.64	7.27	8.64	4.09	5.45	0.91	3.64
X89007	0.45	3.18	9.09	3.64	7.27	8.64	4.09	5.45	0.91	3.64
Average	0.45	3.18	9.09	3.64	7.23	8.64	4.09	5.45	0.91	3.64

Neijiang pig caused the change of Arg131→Cys131, the myogenin has completely identical deduced amino acid sequence in all the other pig breeds. That means mutations happen in porcine *myogenin* gene mainly are synonymous mutation and the myogenin is very conservative. The above result is consistent with a previous study that most of nucleotide mutations were exist at the non-coding region of *myogenin* gene (Li *et al*., 2008).

The bias of codon usage frequency of porcine myogenin: Relative Synonymous Codon Usage (RSCU) was used in this study to measure the bias of codon usage frequency of porcine myogenin (Table 3). From Table 3 researchers know that in the porcine myogenin, the CUG has the highest usage frequency which is used for 17 times with the frequency of 4.08%. The second is GAG which is used for 16 times and the frequency is 1.45%. Generally speaking, the bias of codon usage frequency of the porcine myogenin is very serious, the usage frequency of each codon is unbalance. For example, within the 6 synonymous codon for Leu (UUA, UUG, CUU, CUC, CUA and CUG) only CUG is used for 17 times (4.08%) and CUC is used for 8 times (1.92%) the other four synonymous codons are not used. As to the starting and terminal of the amino acid transition of the porcine myogenin, AUG is the special starting codon and UGA is the only terminal codon.

The synonymous and nonsynonymous substitution of porcine myogenin among pig breeds: The synonymous (or silent) and nonsynonymous substitution of the porcine myogenin among different pig breeds was analyzed and shown in Table 4. Among the 10 pig breeds, only in Neijiang pig the total number of silent sites (SilentPos, S) is 165.67 and the total number of nonsilent sites (NSynPos, N) is 494.33 and in all the other 9 pig breeds, the S is 166.17 and N is 493.83. There is very small

Table 2: The amino acid sequence of the *myogenin* gene

MELYETSPYF	YQEPHFYDGE	NYLPVHLQGF	EPPGYERTEL	SLSPEARVPL
EDKGLGTPEH	CPGQCLPWAC	KVCKRKSVSV	DRRRAATLRE	KRRLKKVNEA
FEALKRSTLL	NPNQRLPKVE	ILRSAIQYIE	R LQALLSSLN	QEERDLRYRG
GGGPQPGVPS	ECSSHSASCS	PEWGSALEFG	PNPGDHLLTA	DPTDAHNLHS
LTSIVDSITV	EDVAVAFPDE			

*: Thick line with highlighted mark is the variation exists in Neijiang pig

Table 3: Codon usage frequence of the myogenin in pigs

Codon	Frequency	Codon	Frequency	Codon	Frequency	Codon	Frequency
UUU (F)	2.0 (0.67)	UCU (S)	1.0 (0.320)	UAU (Y)	2.0 (0.500)	UGU (C)	2.0 (0.66)
UUC (F)	4.0 (1.33)	UCC (S)	7.0 (2.210)	UAC (Y)	6.0 (1.500)	UGC (C)	4.1 (1.34)
UUA (L)	0.0 (0.00)	UCA (S)	0.0 (0.000)	UAA (*)	0.0 (0.000)	UGA (*)	1.0 (1.00)
UUG (L)	0.0 (0.00)	UCG (S)	0.0 (0.000)	UAG (*)	0.0 (0.000)	UGG (W)	2.0 (1.00)
CUU (L)	0.0 (0.00)	CCU (P)	2.0 (0.400)	CAU (H)	1.0 (0.290)	CGU (R)	1.0 (0.38)
CUC (L)	8.0 (1.92)	CCC (P)	9.0 (1.800)	CAC (H)	6.0 (1.710)	CGC (R)	3.9 (1.47)
CUA (L)	0.0 (0.00)	CCA (P)	7.0 (1.400)	CAA (Q)	0.0 (0.000)	CGA (R)	3.0 (1.13)
CUG (L)	17.0 (4.08)	CCG (P)	2.0 (0.400)	CAG (Q)	8.0 (2.000)	CGG (R)	4.0 (1.51)
AUU (I)	0.0 (0.00)	ACU (T)	1.0 (0.440)	AAU (N)	2.0 (0.570)	AGU (S)	5.0 (1.58)
AUC (I)	5.0 (3.00)	ACC (T)	3.0 (1.330)	AAC (N)	5.0 (1.430)	AGC (S)	6.0 (1.89)
AUA (I)	0.0 (0.00)	ACA (T)	4.0 (1.780)	AAA (K)	0.0 (0.000)	AGA (R)	0.0 (0.00)
AUG (M)	1.0 (1.00)	ACG (T)	1.0 (0.440)	AAG (K)	9.0 (2.000)	AGG (R)	4.0 (1.51)
GUU (V)	0.0 (0.00)	GCU (A)	2.0 (0.570)	GAU (D)	4.9 (0.980)	GGU (G)	0.2 (0.05)
GUC (V)	1.0 (0.33)	GCC (A)	10.0 (2.86)	GAC (D)	5.1 (1.020)	GGC (G)	6.8 (1.95)
GUA (V)	0.0 (0.00)	GCA (A)	2.0 (0.570)	GAA (E)	6.0 (0.550)	GGA (G)	0.0 (0.00)
GUG (V)	11.0 (3.67)	GCG (A)	0.0 (0.000)	GAG (E)	16.0 (1.45)	GGG (G)	7.0 (2.00)

*: All frequencies are averages over all taxa. Relative synonymous codon usage is given in parantheses following the codon frequency

Table 4: Synonymous and nonsynonymous substitution caused by nucleotide substitution among pig breeds

Seq1	Seq2	SilentDif	SilentPos	Ks	NSynDif	NSynPos	Ka
CHH	DaHe	2.00	166.17	0.0121	0.00	493.83	0.0000
CHH	DaWu	2.00	166.17	0.0121	0.00	493.83	0.0000
CHH	Duroc	2.00	166.17	0.0121	0.00	493.83	0.0000
CHH	NJ	2.00	165.92	0.0122	1.00	494.08	0.0020
CHH	RC	2.00	166.17	0.0121	0.00	493.83	0.0000
CHH	MS	2.00	166.17	0.0121	0.00	493.83	0.0000
CHH	WZS	2.00	166.17	0.0121	0.00	493.83	0.0000
CHH	Yanan	2.00	166.17	0.0121	0.00	493.83	0.0000
CHH	Tebetan	1.00	166.17	0.0060	0.00	493.83	0.0000
CHH	X89007	2.00	166.17	0.0121	0.00	493.83	0.0000

Table 4: Continue

Seq1	Seq2	SilentDif	SilentPos	Ks	NSynDif	NSynPos	Ka
DaHe	DaWu	0.00	166.17	0.0000	0.00	493.83	0.0000
DaHe	Duroc	0.00	166.17	0.0000	0.00	493.83	0.0000
DaHe	NJ	0.00	165.92	0.0000	1.00	494.08	0.0020
DaHe	RC	0.00	166.17	0.0000	0.00	493.83	0.0000
DaHe	MS	0.00	166.17	0.0000	0.00	493.83	0.0000
DaHe	WZS	0.00	166.17	0.0000	0.00	493.83	0.0000
DaHe	Yanan	0.00	166.17	0.0000	0.00	493.83	0.0000
DaHe	Tebetan	1.00	166.17	0.0060	0.00	493.83	0.0000
DaHe	X89007	0.00	166.17	0.0000	0.00	493.83	0.0000
DaWu	Duroc	0.00	166.17	0.0000	0.00	493.83	0.0000
DaWu	NJ	0.00	165.92	0.0000	1.00	494.08	0.0020
DaWu	RC	0.00	166.17	0.0000	0.00	493.83	0.0000
DaWu	MS	0.00	166.17	0.0000	0.00	493.83	0.0000
DaWu	WZS	0.00	166.17	0.0000	0.00	493.83	0.0000
DaWu	Yanan	0.00	166.17	0.0000	0.00	493.83	0.0000
DaWu	Tebetan	1.00	166.17	0.0060	0.00	493.83	0.0000
DaWu	X89007	0.00	166.17	0.0000	0.00	493.83	0.0000
Duroc	NJ	0.00	165.92	0.0000	1.00	494.08	0.0020
Duroc	RC	0.00	166.17	0.0000	0.00	493.83	0.0000
Duroc	MS	0.00	166.17	0.0000	0.00	493.83	0.0000
Duroc	WZS	0.00	166.17	0.0000	0.00	493.83	0.0000
Duroc	Yanan	0.00	166.17	0.0000	0.00	493.83	0.0000
Duroc	Tebetan	1.00	166.17	0.0060	0.00	493.83	0.0000
Duroc	X89007	0.00	166.17	0.0000	0.00	493.83	0.0000
NJ	RC	0.00	165.92	0.0000	1.00	494.08	0.0020
NJ	MS	0.00	165.92	0.0000	1.00	494.08	0.0020
NJ	WZS	0.00	165.92	0.0000	1.00	494.08	0.0020
NJ	Yanan	0.00	165.92	0.0000	1.00	494.08	0.0020
NJ	Tebetan	1.00	165.92	0.0061	1.00	494.08	0.0020
NJ	X89007	0.00	165.92	0.0000	1.00	494.08	0.0020
RC	MS	0.00	166.17	0.0000	0.00	493.83	0.0000
RC	WZS	0.00	166.17	0.0000	0.00	493.83	0.0000
RC	Yanan	0.00	166.17	0.0000	0.00	493.83	0.0000
RC	Tebetan	1.00	166.17	0.0060	0.00	493.83	0.0000
RC	X89007	0.00	166.17	0.0000	0.00	493.83	0.0000
MS	WZS	0.00	166.17	0.0000	0.00	493.83	0.0000
MS	Yanan	0.00	166.17	0.0000	0.00	493.83	0.0000
MS	Tebetan	1.00	166.17	0.0060	0.00	493.83	0.0000
MS	X89007	0.00	166.17	0.0000	0.00	493.83	0.0000
WZS	Yanan	0.00	166.17	0.0000	0.00	493.83	0.0000
WZS	Tebetan	1.00	166.17	0.0060	0.00	493.83	0.0000
WZS	X89007	0.00	166.17	0.0000	0.00	493.83	0.0000
Yanan_	Tebetan	1.00	166.17	0.0060	0.00	493.83	0.0000
Yanan	X89007	0.00	166.17	0.0000	0.00	493.83	0.0000
Tebetan	X89007	1.00	166.17	0.0060	0.00	493.83	0.0000

SilentDif: The total number of synonymous differences; SilentPos: The total number of silent sites; Ks: The number of synonymous substitutions per synonymous site; NSynDif: The total number of nonsynonymous differences; NsynPos: The total number of nonsilent sites; Ka: The number of nonsynonymous substitutions per nonsynonymous site; CHH: Chenghua pig; DaHe: DaHe pig; DaWu: Dawu pig; NJ: Neijiang pig; RC: Rongchang pig; MS: Meishan pig; WZS: Wuzhishan pig; Yanan: Ya'nan pig; Tebetan: Tibetan pig

difference exists among pig breeds for the total number of synonymous differences (SilentDif) and the range is between 0~2.0. The total number of nonsynonymous differences (NSynDif) is 0 between all pig breeds, except for Neijiang pig which is 1. In accordance with the results, the number of synonymous substitutions per synonymous site (Ks) is small among different pig breeds, the range is between 0~0.0122 and the number of nonsynonymous substitutions per nonsynonymous site (Ka) is 0 among different pig breeds which means no nonsynonymous substitution. Only between Neijiang and other pig breeds, the Ka is 0.002.

CONCLUSION

Based on the complete nucleotide sequence of the porcine myogenin gene (~2605 bp), the deduced amino acid sequence of the porcine myogenin consists of 220 amino acids. All of the 20 conventional amino acids can be found in the myogenin with quite different content. The myogenin is very conservative, among the 10 pig breeds only one nonsynonymous mutation happened in Neijiang pig caused the substitution of Arg131→Cys131 and only between Neijiang pig and other pig breeds, the number of nonsynonymous substitutions per nonsynonymous site (Ka) is 0.002. The Ka is 0 among the other pig breeds which means no nonsynonymous substitution. The mutations happen in porcine *myogenin* gene mainly are synonymous mutation and the genetic effect of the Arg131→Cys131 mutation is need to do more research. The conservation of the myogenin is decided by its important function. Based on the Relative Synonymous Codon Usage (RSCU), the codon usage in the porcine myogenin is biased. The CUG has the highest usage frequency.

In the 10 detected pig breed populations in this study, both the value of the number of nonsynonymous substitutions per nonsynonymous site (Ka) and the number of synonymous substitutions per synonymous site (Ks) are very small and very similar. The possible reason is that there was no artificial selection pressure had been used to the myogenin in these populations and the populations keep in natural condition. The results can relatively reflect the true genetic background of the sampling pig breeds.

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