Full Length Research Paper

Bioinformatics analysis of *Agouti* gene among different species

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***Agouti* gene plays an important role in the synthesis and distribution of coat color in domestic animals. In this report, a total of 71 *Agouti* gene sequences with the complete coding region (CDS) belonging to 15 species were analyzed. The result indicated that the averaged net genetic distance (Da) and the averaged nucleotide divergence (Dxy) were 0.2256 and 0.2145, respectively. Much more genetic diversity existed in *Peromyscus maniculatus* and *Lama pacos*. The genetic relationship between mammalian and *Danio rerio* was closer than that between birds and *Danio rerio*. The predicted promoter region of chicken *Agouti* gene was from 2600 bp to 3000 bp upstream of start codon.**

Key words: *Agouti* gene, genetic diversity, differentiation, chicken promoter prediction.

INTRODUCTION

Hair and feather pigmentation is mainly determined by the distribution of two kinds of melanin, eumelanin and pheomelanin. *Agouti* gene encodes agouti-signaling protein (ASIP), which acts as an antagonist or an inverse agonist of the melanocortin 1 receptor (MC1R). The ASIP is normally produced in the skin, in which it promotes the synthesis of reddish-yellow pigment by hair follicle melanocytes (Jackson et al., 2006), and interacts with MC1R and proopiomelanocortin gene at the surface of pigment-producing cells (melanocytes) and constitutes the machinery responsibility for the ability of melanocytes to switch between the production of dark brown/black (eumelanin) and light yellow/red pigment (pheomelanin) (Gantz and Fong, 2003). About the colour study, some reports found that colour change were caused by variation of *Agouti* gene. In mice, the dark dorsal and pale ventral wild-type coat color pattern was shown to be caused by the spatial ex-pression of different transcripts from a single *ASIP* gene (Vrieling et al., 1994). A mutation in exon 3 of mice *Agouti* gene resulting in a change of stop codon premature from glutamine at amino

acid position 65 ($a^{Q65term}$) could eliminates exon 4 (Kingsley et al., 2009). A homozygous 11bp deletion in exon 2 of horse *Agouti* gene was associated with recessive black coat color (Stefan et al., 2001). In goat, the 5275bp of goat A*gouti* gene was obtained (EF587236), including intron1 (87bp), exon2 (169bp), intron2 (1316bp), exon3 (65bp), intron3 (3461bp) and exon4 (177bp), and 16 SNPs were found in 4 Chinese indigenous goat breeds (LI et al., 2008). Tang et al. (2008) postulated that the T of *5700G>T* in exon4 might be associated with the black phenotype, or linked with the site controlling black coat color in goat. Tang et al. (2009) also showed that AA of 128delT in intron1 might have relationship with brown phenotype and BB might correlates with black phenotype. The research on the relationship between mutation of *Agouti* gene and coat color provided us many useful information for studying the mechanism of color formation. On the other hand, plenty of *Agouti* gene sequences from different species in Genbank have been provided and need to be investi-gated further using bioinformatics methods in order to understand its genetic diversity and differentiation within and among species, especially the differentiation between mammalian and birds. In this paper, the gene structure, genetic diversity and differentiation of *Agouti* gene within and among species were analyzed, and the

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promoter of chicken *Agouti* gene was also identified.

MATERIALS AND METHODS

Analysis of genetic diversity and differentiation

All the complete gene sequences and CDSs were obtained from NCBI and Ensembl (Table 1). All of the 71 CDSs were aligned using the Clustal W program implemented in BioEdit (version7.0.9.0). DnaSP (version 4.0) software was used to analyze the following parameters, including net genetic distance(Da) , nucleotide divergence (Dxy), haplotypes (h), haplotype diversity (Hd), average number of nucleotide differences (K), polymorphic site (S), and Nucleotide diversity (π).

Prediction of promoter of chicken *Agouti* gene

Based on the results of sequencing (Chromosome 20: 1, 554, 566-1, 581, 604), we used ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/) to find the start codon. Then the Neural Network Promoter Prediction (http://fruitfly.org:9005/seq_tools/promoter.htm), the TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html), the Promoter 2.0 (http://www.cbs.dtu.dk/services/Promoter/), Promoter Scan (http://www-bimas.cit.nih.gov/molbio/proscan/), MATCH (http://www.gene-regulation.com/pub/programs.html#match) were used to predict the promoter of chicken *Agouti* gene.

RESULTS AND DISCUSSION

Genetic diversity within and among species

The alignment of the 71 CDSs listed in Table 1 was carried out using BioEdit. The subsequent DnaSP analysis indicated that the selected region (1 to 412) of 71 sequences (Table 2) shows the polymorphic information of *Agouti* gene for each species. Different species have different genetic diversity estimates for the *Agouti* gene. Usually, more genetic diversity is more useful for nature selection. Due to the limited sequences from each species, we could only compare the species with more than two of sequences. Therefore, for the species with two and more sequences, a total of 246 segregating sites were found, which could be sorted into 30 haplotypes with the diversity of 0.9485. The average number of nucleotide differences is 82.2749 with the diversity of 0.2434. We could know from Table 2 that *Peromyscus maniculatus* and *Lama pacos* with 6 segregating sites have more haplotypes (4 and 6), higher average number of differences (1.6667 and 3.2000), and higher nucleotide diversity (0.0049 and 0.0094).

Genetic differentiation among species

Table 3 shows the polymorphic information of *Agouti* gene for each species. Different species have different genetic diversity estimates for the *Agouti* gene. The averaged net genetic distance (Da) is 0.2256 ranged from 0.0088 to 0.5289. The averaged nucleotide divergence (Dxy) is 0.2145 ranged from 0.0103 to 0.5297. The smallest Da (0.0088) and Dxy (0.0103) were all found between *Homo sapiens* and *Pan troglodytes.* The largest Da (0.5289) and Dxy (0.5297) were all found between *Gallus gallus* and *Danio rerio*.

The phylogenic tree of all species was built based on Dxy (Figure 1). We could know from Table 3 and Figure 1 that the differentiation is different among different cluster of species. The cluster 1 including *Homo sapiens*, *Callithrix pygmaea*, *Papio Anubis*, *Pan troglodytes*, and *Pongo pygmaeus* with closer relationship in Figure 1 has the smallest averaged value of Da (0.0496) and Dxy (0.0405), while the cluster 3 including *Peromyscus maniculatus* and *Rattus norvegicus* with closer relationship has the highest value of Da (0.1152) and Dxy (0.1177). The cluster 2 including *Sus scrofa, Felis catus, Lama pacos,* and *Ovis aries* has the averaged value of Da (0.1076) and Dxy (0.1102). The cluster 4 including *Gallus gallus* and *Coturnix japonica* has the averaged value of Da (0.0559) and Dxy (0.0596). From Figure 1 we also notice that *Oryctolagus cuniculus* has closer relationship with cluster 1 and make much higher Da (0.0756) and Dxy (0.0698).

The averaged Da (0.1263) and Dxy (0.1270) within mammalian are higher than that (0.0559 & 0.0596) within birds including *Gallus gallus* and *Coturnix japonica*. The averaged Da (0.4534) and Dxy (0.4544) between mammalian and *Danio rerio* is smaller than that (0.5211 and 0.5230) between birds and *Danio rerio*. Therefore it could be inferred that the relationship between mammalian and *Danio rerio* was closer than that between birds and *Danio rerio*.

The promoter prediction of chicken *Agouti* gene

The whole length of chicken *Agouti* gene (Chromosome 20: 1, 554, 566-1, 581, 604) in Ensembl is 27039 bp. We named the start codon of chicken *Agouti* gene predicted by ORF Finder at 1559614 bp as 0. Three programs of promoter prediction software were use to predict chicken *Agouti* gene (Table 4). We could know from Table 4 that 6 regions of promoter were predicted by Neural NetworkPromoter Prediction. Two and one regions of promoter were predicted by Promoter 2.0 and PromoterScan respectively. It was obvious that the region from 2600bp to 3000bp upstream of start codon might be promoter region of chicken *Agouti* gene.

In order to make sure the scope of core promoter of chicken *Agouti* gene, we used TFSEARCH and MATCH software to analyze the transcription factor of chicken *Agouti* gene. The TATA box (2647-2662 from start codon), CAAT box (NF-1, 2740-2758), and GC box (SP1, 2870-2880) were found and were listed in Figure 2. Meanwhile, many other transcription factors were also found in predicted promoter region, such as OCT-1, ATF,

Table 1. *Agouti* sequences of different species from GenBank and Ensembl.

Species	Number	CDS sequence from GenBank	Gene from Ensembl
Homo sapiens	2	NM_001672, L37019.1	Chromosome 20: 32,848,171-32,857,150
Callithrix pygmaea	3	EF094496, EF094494, EF094495	Chromosome 10: 30,312,665-30,315,177
Papio anubis	2	EF094487, NM_001164331	
Pan troglodytes	3	EF094481, AB236870, NM_001135627	Chromosome 20: 31,314,965-31,325,056
Pongo pygmaeus	2	EF094482, AB236872	
Peromyscus maniculatus	9	GQ340879, GQ340881, GQ340883, GQ340885, GQ340887, GQ340889, GQ340891, GQ340893, GQ340895	
Rattus norvegicus	3	NM_052979, AB045590, AB045587	Chromosome 3: 145,530,367-145,536,831
Sus scrofa	8	NM_001011648, NM_001011646, NM_001011647, AY916525, AY308997, AY308996, AY308998, GQ373180	Chromosome 17: 39,717,458-39,801,515
Felis catus	2	AY237394, NM_001009190	GeneScaffold_794: 590-5,404
Lama pacos	6	FJ847231, FJ847229, FJ847230, HM768322, HM768323, HQ008273	GeneScaffold_575:110917-114835
Ovis aries	10	EU420022, EU420024, EU420026, EU420028, EU420030, EU420025, EU420027, EU420029, FJ889180, NM_001134303	
Oryctolagus cuniculus	5	FN547132, FN547134, AM748787, FN547133, NM_001122939	
Gallus gallus	11	EU370216, EU370215, EU370217.1, AB518061.1, AB518062.1, AB518063.1, AB518064, AB518066, AB518065, AB518067, NM_001115079	Chromosome 20: 1,554,566-1,581,604
Coturnix japonica	2	AB304509, AB304510	
Danio rerio	3	NM_001128801, BC162411, BC162417	Chromosome 6: 49,927,795-49,934,959

Table 2. Polymorphic information of *agouti* gene for each species.

Species	S	h	Hd	K	π
Homo sapiens	1	2	1.0000	1.0000	0.0029
Callithrix pygmaea	2	2	0.6667	1.3333	0.0039
Papio anubis	0	1	0.0000	0.0000	0.0000
Pan troglodytes	0	1	0.0000	0.0000	0.0000
Pongo pygmaeus	1	2	1.0000	1.0000	0.0029
Peromyscus maniculatus	6	4	0.6944	1.6667	0.0049
Rattus norvegicus	0	1	0. 0000	0.0000	0.0000
Sus scrofa	1	2	0.2500	0.2500	0.0007
Felis catus	0	1	0.0000	0.0000	0.0000
Lama pacos	6	6	1.0000	3.2000	0.0094
Ovis aries	0	1	0.0000	0.0000	0.0000
Oryctolagus cuniculus	0	1	0.0000	0.0000	0.0000
Gallus gallus	2	3	0.4727	0.5091	0.0015
Coturnix japonica	2	2	1.0000	2.0000	0.0059
Danio rerio	0	1	0.0000	0.0000	0.0000

Number of segregating sites, S; Number of haplotypes, h; Haplotype diversity, Hd; Average number of differences, K; Nucleotide diversity, π.
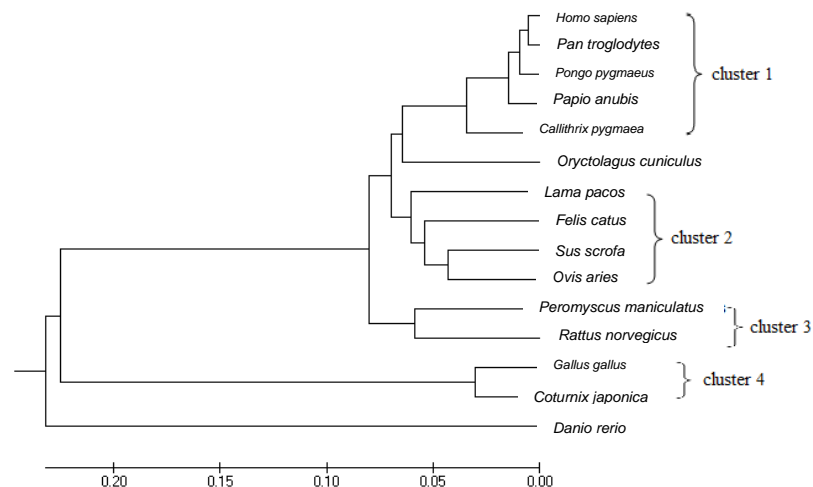
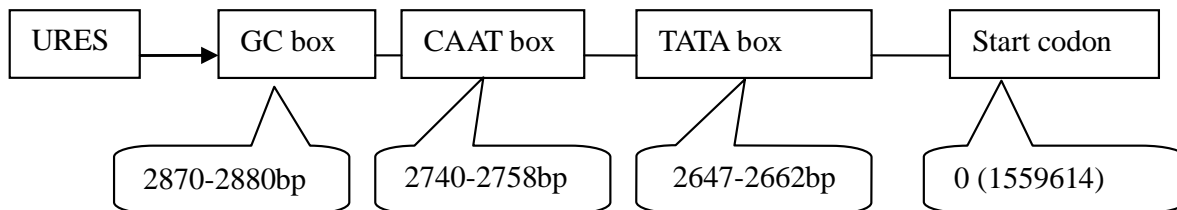**Figure 1.** Phylogenetic tree of 71 CDS among different species.

Table 3. Nucleotide divergence and net genetic distance among species.

species	Homo sapiens	Callithrix pygmaea	Papio anubis	Pan troglodytes	Pongo pygmaeus	Peromyscus maniculatus	Rattus norvegicus	Sus scrofa	Felis catus	Lama pacos	Ovis aries	Oryctolagus cuniculus	Gallus gallus	Coturnix japonica	Danio rerio
Homo sapiens		0.0735	0.0353	0.0088	0.0206	0.1730	0.1529	0.1412	0.1353	0.1541	0.1412	0.1294	0.4589	0.4515	0.4500
Callithrix pygmaea	0.0735		0.0647	0.13658	0.09456	0.1508	0.1471	0.1412	0.1471	0.1512	0.1402	0.1412	0.4540	0.4466	0.4539
Papio anubis	0.0368	0.0667		0.0265	0.0235	0.1613	0.1441	0.1265	0.1353	0.1394	0.1294	0.1235	0.4523	0.4544	0.4618
Pan troglodytes	0.0103	0.0667	0.0265		0.0118	0.1642	0.1441	0.1324	0.1294	0.1453	0.1324	0.1206	0.4530	0.4456	0.4559
Pongo pygmaeus	0.0235	0.0623	0.0250	0.0132		0.1462	0.1412	0.1324	0.1324	0.1424	0.1265	0.1235	0.4515	0.4471	0.4559
Peromyscus maniculatus	0.1770	0.1552	0.1652	0.1667	0.1627		0.1152	0.1675	0.1672	0.1860	0.1555	0.1482	0.4652	0.4588	0.4564
Rattus norvegicus	0.1544	0.1490	0.1441	0.1441	0.1427	0.1177		0.1559	0.1588	0.1806	0.1529	0.1412	0.4354	0.4427	0.4588
Sus scrofa	0.1430	0.1435	0.1268	0.1327	0.1342	0.1703	0.1563		0.1147	0.1022	0.0853	0.1412	0.4383	0.4279	0.4463
Felis catus	0.1368	0.1490	0.1371	0.1294	0.1338	0.1696	0.1588	0.1151		0.1355	0.1000	0.1235	0.4354	0.13099	0.4559
Lama pacos	0.1603	0.1578	0.1441	0.1500	0.1485	0.1931	0.1823	0.1072	0.1402		0.1080	0.1370	0.4409	0.4404	0.4551
Ovis aries	0.1427	0.1422	0.1294	0.1324	0.1279	0.1680	0.1529	0.0857	0.1000	0.1128		0.1441	0.4501	0.4309	0.4500
Oryctolagus cuniculus	0.1309	0.1431	0.1235	0.1206	0.1250	0.1507	0.1412	0.1415	0.1235	0.1417	0.1441		0.4490	0.4515	0.4412
Gallus gallus	0.4611	0.4567	0.4537	0.4537	0.4567	0.4684	0.4479	0.4394	0.4361	0.4464	0.4508	0.4497		0.0559	0.5289
Coturnix japonica	0.4559	0.4515	0.4574	0.4485	0.4515	0.4642	0.4456	0.4313	0.4397	0.4480	0.4338	0.4544	0.0596		0.5132
Danio rerio	0.4515	0.4559	0.4618	0.4559	0.4574	0.4588	0.4588	0.4467	0.4559	0.4589	0.4500	0.4412	0.5297	0.5162	

Above diagonal is net genetic distance (Da)；below diagonal is nucleotide divergence (Dxy).

Table 4. The promoter region prediction of chicken Agouti gene.

Program	Prediction result		
	Position	Score	Likelihood
Promoter 2	3200	1.092	Highly likely prediction
http://www.cbs.dtu. dk/services/Promoter	6700	1.078	Highly likely prediction
	Start	End	Score
	8210	8160	0.99
	7945	7895	0.91
Neural NetworkPromoter Prediction	2672	2622	0.94
http://fruitfly.org:9005/seq_tools/promoter.html	2647	2597	0.90
	2377	2327	0.92
	625	575	0.98
PromoterScan	Start	End	Score
http://www-bimas.cit.nih.gov/molbio/proscan/	2872	3122	64.15

Figure 2. Predicted structure of chicken *agouti* gene promoter region.

HSF, ADR1, GATA, and CdxA named as URES in Figure 2.

Conclusion

It could be concluded that more genetic diversity of *Agouti* gene was existed among different species. *Peromyscus maniculatus and Lama pacos* have much more genetic diversity than other species. *Homo sapiens* and *Pan troglodytes* has closer relationship, and *Gallus gallus* and *Danio rerio* are with the largest genetic distance. The genetic relationship between mammalian and *Danio rerio* was closer than that between birds and *Danio rerio*. The promoter region of chicken *Agouti* gene was predicted from 2600bp to 3000bp upstream of start codon. The TATA box, CAAT box and GC box were predicted at 2647 to 2662, 2740 to 2758 and 2870 to 2880 upstream of start codon.

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