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EVOLUTION OF HYPOTHALAMUS-PITUITARY GROWTH AXIS AMONG FISH, AMPHIBIAN, BIRDS AND MAMMALS

M. MOAEEN-UD-DIN[1þ], G. BILAL[1] and James M. REECY[2]

[1] Laboratories of Animal Breeding & Genetics, Faculty of Veterinary & Animal Sciences, PMAS-Arid Agriculture University, Rawalpindi (46300, Pakistan
[2] Department of Animal Science, Iowa State University, 2255 Kildee Hall, Ames, IA

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Hypothalamus-pituitary growth axis (HP growth axis) regulates animal growth and development in pre-natal and post natal life governed by many factors. However, until recently, the evolutionary history of this axis among lineages is not understood. Aim of the present study was to understand the major events in evolution and evolutionary history and trend of HP growth axis. The diversity among *Homo sapience, Mus musculus, Rattus norvegicus, Gallus gallus, Danio rerio* and *Xenopus laevis* was determined for genes involved in HP growth axis in current study. Sequences of HP growth axis genes were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/). Nucleotide diversity using Kimura's two-parameter method; codon-based test of positive selection using the Nei-Gojobori; equality of evolutionary rate with Tajima's relative rate test and phylogenetic history using the RelTime method were estimated in MEGA6. Estimates of the coefficients of evolutionary differentiation based on nucleotides and amino acids substitution patterns of HP growth axis genes showed contrasting evolutionary patterns among the lineages. The results demonstrated that although these genes might have crucial functional roles in each of the species, however, their sequence divergence did not necessarily reflect similar molecular evolution among the species. Codon-based test of positive selection revealed that Human vs Mouse, Chicken vs Rat, Human vs Rat and Mouse vs Rat had similar and higher non synonymous substitutions ($P > 0.05$). Higher rate of non-synonymous substitutions at similar orthologs level among species indicated a similar positive selection pressure in these species. Results for relative rate test assessed with the chi-squared test showed difference on unique mutations among lineages at synonymous and non synonymous sites except Chicken vs Mouse, Human vs Mouse, Chicken vs Rat, Human vs Rat and Mouse vs Rat. This indicated that the mutagenic process that generates substitutional mutation is taking place at approximately the same

Corresponding author: Muhammad Moaeen-ud-Din, Lab of Animal Breeding & Genetics,Faculty of Veterinary & Animal Sciences,PMAS-Arid Agriculture University, Rawalpindi (46300), Pakistan, Email: drmoinawan@gmail.com,Ph: 0092-3438702053

rate at synonymous and non-synonymous sites these lineages. Moreover, despite of common ancestry, our results indicate a different divergent time among genes of these species. This is the first demonstration that variable rates of molecular evolution may be present within HP growth axis genes among different species. This difference could be of interest for comparative genomics analysis and physiological genes functions identification among tho comparative genomics, evolution rate, HP growth axis, positive selection se species whose HP growth axis is not explored.

Key words: genetic linkage map, genome length, Lily, map coverage, RIL, SRAP

INTRODUCTION

Neurocrine axis or hypothalamus-pituitary growth axis (HP growth axis) plays a major role of regulation in the evolution of animal growth and development. It consists of somatostatin (SS) and growth hormone releasing hormone(GHRH) secreted by hypothalamus, growth hormone (GH) secreted by pituitary, growth hormone receptor (GHR) and insulin-like growth factor-I (IGF-I) secreted by target organs (such as liver), as well as the path of hypothalamus-pituitary-target organs (SZCZEPANKIEWICZ *et al.* 2010). Other major genes involved in HP growth axis are growth hormone releasing hormone receptor (GHRHR), growth hormone secretagogue receptor (GHSR), Ghrelin, insulin-like growth factor binding protein-2 (IGFBP-2), insulin (INS), leptin receptor (LEPR), thyroid-stimulating hormone beta subunit (TSH-β), and pituitary-specific transcription factor-1 (PIT-1).

Our understanding on regulation of basic aspects of animal's physiology is continuously expanding. The importance of HP growth axis in regulating animal growth and development in pre-natal and post natal life phases is well documented and already established (SZCZEPANKIEWICZ *et al.* 2010). It has been pointed out that the functions of this axis are complex and governed by many factors such as nutrient availability and multiple interactions between the endocrine system, the genetic, and the environmental factors (ROGOL 2011). Some of the genetic factors affecting HP growth axis were described elsewhere (DATTANI and PREECE, 2004; ROGOL, 2011). HP growth axis plays very important role in production of farm animals. HP growth axis acts as a crossing point between input factors that includes genotype, sex, environmental conditions, nutrition, etc., and the apprehended output as growth rate, feed efficiency, carcass composition, egg or milk production in animals by mediating and regulating metabolism.

Although our knowledge of HP growth axis is expanded vastly until recently; there are studies on evaluation of functions and evolutionary history of various genes involved in HP growth axis (MOAEEN-UD-DIN and YANG 2009; MALIK *et al.* 2013). However, still the evolutionary history of this axis among lineages is not completely understood. This knowledge is necessary for cross species comparison in the present scenario of rapid development in the areas of chip technology for animal evaluations. Therefore, the aim of the present paper was to understand the major events in evolution and evolutionary history and trend of HP growth axis.

MATERIALS AND METHODS

GH, GHR, GHRH, GHRHR, GHSR, Ghrelin, SS, IGF-I, IGFBP-2, INS, LEPR, TSH-β, and PIT-1 genes sequences were used to investigate the level of divergence among *Homo sapience, Mus musculus, Rattus norvegicus, Gallus gallus, Danio rerio* and *Xenopus laevis* genes. Both protein and DNA coding strand sequences were investigated. No 5'- or 3'-untranslated

regions were included. In first step all known human genes sequences were retrieved from gene bank (http://www.ncbi.nlm.nih.gov/). These sequences were blasted using NCBI blast tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find out their orthologous in *Mus musculus, Rattus norvegicus, Gallus gallus, Danio rerio* and *Xenopus laevis*. The evolutionary distances were computed using the p-distance method. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm at a search level of 1. The Neighbor-joining algorithm (SAITOU and NEI, 1987) was used to generate the initial tree. The analysis involved 151 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair.

Nucleotide diversity (K) was performed using Kimura's two-parameter method (KIMURA, 1980) as implemented within Molecular Evolutionary Genetics Analysis version 6 MEGA6; (TAMURA *et al.*, 2013). Standard error estimate(s) were obtained by a bootstrap procedure (50 replicates). The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing alignment gaps and missing data were eliminated in pairwise sequence comparisons (Pairwise deletion option). The number of amino acid differences per site from mean interpopulational diversity were conducted in MEGA6 (TAMURA *et al.*, 2013).

The accumulation of sequence differences was further investigated by calculating codon-based test of positive selection. The probability of rejecting the null hypothesis of strict-neutrality ($d_N = d_S$; where d_S and d_N are the numbers of synonymous and nonsynonymous substitutions per site) in favor of the alternative hypothesis ($d_N > d_S$) was calculated. Values of *P* less than 0.05 were considered significant at the 5%. The variance of the difference was computed using the bootstrap method (50 replicates). Analyses were conducted using the modified Nei-Gojobori (assumed transition/transversion bias = 2) method (NEI and GOJOBORI, 1986) using MEGA6 (TAMURA *et al.*, 2013).

The equality of evolutionary rate among sequences of *Homo sapience, Mus musculus, Rattus norvegicus, Gallus gallus, Danio rerio* and *Xenopus laevis* were estimated in Tajima's relative rate test (TAJIMA, 1993). The χ^2 test statistic was used and P-value less than 0.05 were used to reject the null hypothesis of equal rates between lineages. Evolutionary analyses were conducted in MEGA6 (TAMURA *et al.*, 2013).

Phylogenetic history of these genes was estimated by determining gene-specific evolutionary rates. The time tree shown was generated using the RelTime method (TAMURA *et al.*, 2012). Divergence times for all branching points in the user-supplied topology were calculated using the Maximum Likelihood method based on the Tamura-Nei model (TAMURA *et al.*, 2012). Relative times were optimized and bars around each node represent 95% confidence intervals which were computed using the method described in TAMURA *et al.* (2013). A discrete Gamma distribution was used to model evolutionary rate differences among sites. Evolutionary analyses were conducted in MEGA6 (TAMURA *et al.*, 2013).

RESULTS

The evolutionary history inferred using the Maximum Parsimony method and Minimum Evolution methods with bootstrap value of 50 replicates yielded similar trees.

Data describing sequence diversity among *Homo sapience, Mus musculus, Rattus norvegicus, Gallus gallus, Danio rerio* and *Xenopus laevis* coding regions are based on individual available

common genes in gene bank. Our study reports levels of sequence diversity derived using HP growth axis genes transcripts.

Table 1 shows that *Homo sapience, Mus musculus, Rattus norvegicus, Gallus gallus, Danio rerio* and *Xenopus laevis* genes were highly although variably related, with maximum coefficient of evolutionary difference of 0.077 (±0.032) between Mouse and Frog and minimum of -0.076 (±0.036) between human and chicken (Table 1).

Table 1. Coefficient of Evolutionary Differentiation estimates of HP growth axis orthologues

Groups	**Diversity parameter**					
	Nucleotide			**Amino acid**		
	Number of sequences	**Nucleotide positions in the final dataset**	**K (±SE)**	**Number of genes**	**Positions in the final dataset**	**p-distance**
Human VS Mouse	68	3655	0.058±0.031	66	1183	0.007±0.001
Human VS Rat	67	3655	-0.021±0.029	65	1183	0.006±0.001
Human VS Frog	64	3655	-0.037±0.030	61	1183	0.014±0.002
Human VS Zebra fish	62	3655	-0.059±0.023	60	1183	0.007±0.001
Human VS Chicken	62	3655	-0.067±0.036	60	1183	0.005±0.001
Mouse VS Rat	49	3655	0.025±0.024	51	1183	-0.007±0.000
Mouse VS Frog	46	3655	0.076±0.032	50	1183	0.011±0.001
Mouse VS Zebra Fish	44	3655	-0.018±0.034	46	1183	0.011±0.001
Mouse VS Chicken	44	3655	0.033±0.035	46	1183	0.004±0.001
Rat VS Frog	45	3655	0.001±0.030	46	1183	0.001±0.001
Rat VS Zebra Fish	43	3655	-0.026±0.025	45	1183	0.008±0.001
Rat VS Chicken	43	3655	-0.060±0.028	45	1183	-0.004±0.001
Frog VS Zebra Fish	40	3655	-0.030±0.026	41	1183	0.007±0.001
Frog VS Chicken	40	3655	-0.046±0.034	41	1183	-0.004±0.001
Zebra Fish VS Chicken	38	3655	-0.056±0.028	40	1183	0.001±0.001

The accumulation of sequence differences was further explored by calculating the number of nucleotide substitutions per synonymous site (dS) and per non-synonymous site (dN). This was performed separately using HP growth axis genes of *Homo sapience, Mus musculus, Rattus norvegicus, Gallus gallus, Danio rerio* and *Xenopus laevis* orthologues. Human vs Mouse, Chicken vs Rat, Human vs Rat and Mouse vs Rat had similar non synonymous substitutions ($P > 0.05$) whereas in case of rest of the groups; there were higher non synonymous substitutions ($P < 0.05$) (Table 2). Likewise, the non synonymous sites; synonymous sites were also different on unique mutations among lineages except Human vs Mouse, Chicken vs Rat, Human vs Rat and Mouse vs Rat.

Table 2. Synonymous and non-synonymous substitution rates of HP growth axis

Species 1	Species 2	Number of genes	Probability	dN - dS
Zebrafish	Chicken	38	*0.000	5.835
Zebrafish	Human	62	*0.000	8.765
Chicken	Human	62	*0.047	1.691
Zebrafish	Mouse	44	*0.000	8.937
Chicken	Mouse	44	*0.034	1.846
Human	Mouse	68	1.000	-3.633
Zebrafish	Rat	43	*0.000	5.959
Chicken	Rat	43	0.305	0.510
Human	Rat	67	1.000	-0.646
Mouse	Rat	49	1.000	-0.023
Zebrafish	Frog	40	*0.000	9.920
Chicken	Frog	40	*0.000	7.596
Human	Frog	64	*0.000	7.196
Mouse	Frog	46	*0.000	6.306
Rat	Frog	45	*0.000	6.173

*Significant ($P < 0.05$); Nucleotide positions in the final dataset = 11563

Molecular clock hypothesis was tested, which assumes an approximately constant rate of evolution between different lineages. Specifically, rate constancy was tested between the lineages that arose following divergence using relative rate test described by (TAJIMA, 1993) that makes no assumptions regarding patterns of nucleotide substitution. It facilitated the calculation of the number of unique substitutions that have accumulated in *Homo sapience, Mus musculus, Rattus norvegicus, Gallus gallus, Danio rerio* and *Xenopus laevis* lineages by comparison with the outgroups (Table 3). The outgroups were defined based on evolutionary tree inferred using the Minimum Evolution method (Figure 1b). The relative rate test was separately applied to third codon positions and to the first and second codon positions. The relative rate did not differed at third-position and first & second codon positions sites for Chicken and Mouse, Human and Mouse, Chicken and Rat, Human and Rat and Mouse and Rat ($P < 0.05$).

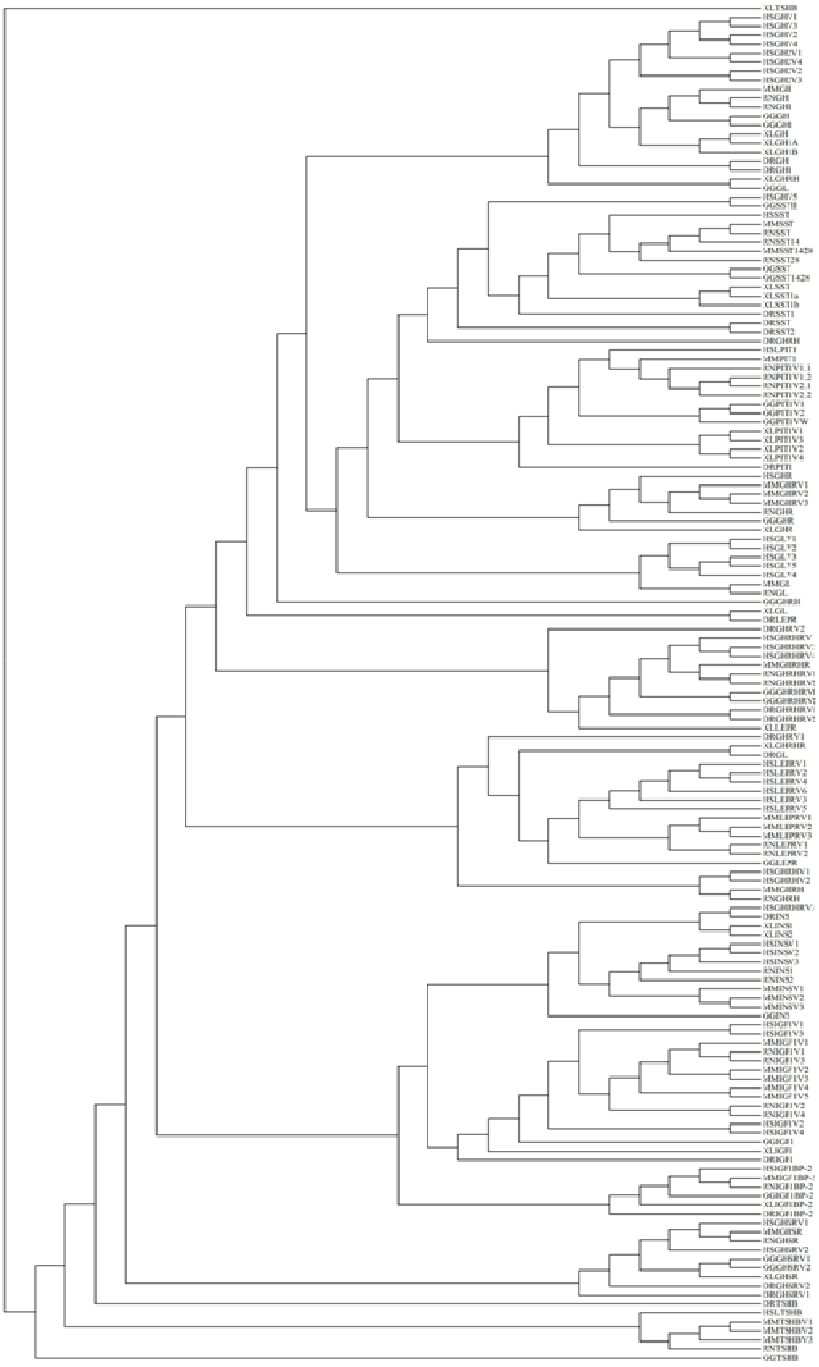
Divergent time of species was documented by (KUMAR and HEDGES, 1998) that indicated the evolution of Human, Rat, Mouse, Galliformes (Birds), Lissamphibia (Amphibian/Frog) and Actinopterygii (Fish/Zebra fish) as 5.5 ± 0.2, 40.7 ± 0.9, 40.7 ± 0.9, 112 ± 11.7, 360 ± 14.7, and 450 ± 35.5 Million Years Ago (MYA), respectively based on large sets of molecular data with

common origin. However, despite of common ancestry, our results indicated a different divergent time among HP growth axis genes of *Homo sapience, Mus musculus, Rattus norvegicus, Gallus gallus, Danio rerio* and *Xenopus laevis* as indicated in Table 4.

Table 3. Results from the Tajima's test for evolutionary rates among lineages

Group 1	Group 1	Out-Group	Synonymous					Non Synonymous				
			Identical sites in 3 sequences	Divergent sites in 3 sequences	Unique differences in Sequence A	Unique differences in Sequence B	Unique differences in Sequence C	Identical sites in 3 sequences	Divergent sites 3 sequences	Unique differences in Sequence A	Unique differences in Sequence B	Unique differences in Sequence C
Zebrafish(A)	Chicken(B)	Frog (C)	1039	829	886	653	915	2050	1497	1835	1265	1988
Zebrafish(A)	Human(B)	Frog (C)	913	917	930	794	818	1871	1721	1956	1480	1701
Chicken (A)	Human (B)	Frog (C)	1224	751	661	744	1054	2644	1400	1254	1366	2213
Zebrafish(A)	Mouse (B)	Frog (C)	923	918	931	773	790	1840	1680	1985	1475	1669
Chicken (A)	Mouse (B)	Frog (C)	*1226	770	661	719	1020	*2643	1411	1250	1324	2169
Human (A)	Mouse (B)	Frog (C)	*1624	402	361	347	1914	*3506	707	645	607	3833
Zebrafish(A)	Rat (B)	Frog (C)	887	886	914	738	750	1818	1607	1909	1384	1616
Chicken (A)	Rat (B)	Frog (C)	*1187	730	647	691	973	*2576	1345	1208	1269	2061
Human (A)	Rat (B)	Frog (C)	*1599	356	339	315	1877	*3455	615	580	535	3785
Mouse (A)	Rat (B)	Frog (C)	*1756	167	165	153	2216	*3763	346	245	254	4302
Zebrafish(A)	Frog (B)	Human(C)	913	917	930	818	794	1871	1721	1956	1701	1480
Chicken (A)	Frog (B)	Human(C)	1224	751	661	1054	744	2644	1400	1254	2213	1366
Human (A)	Frog (B)	Chicken (C)	1224	751	744	1054	661	2644	1400	1366	2213	1254
Mouse (A)	Frog (B)	Chicken (C)	1226	770	719	1020	661	2643	1411	1324	2169	1250
Rat (A)	Frog (B)	Chicken (C)	1187	730	691	973	647	2576	1345	1269	2061	1208

*equal evolutionary rates among lineages



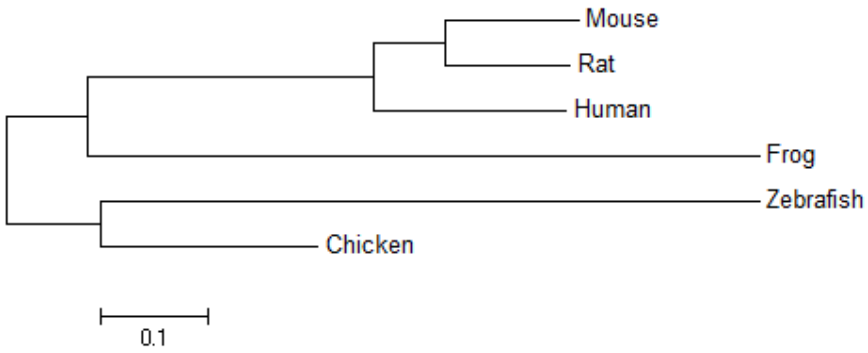


Figure 1. a) Evolutionary relationships of HP growth axis genes (linearized; inferred using the Neighbor-Joining method) b) Evolutionary relationships of species derived from all genes of HP growth axis (inferred using the Minimum Evolution method)

Table 4. A molecular timescale for vertebrate evolution of HP growth axis genes

Era	Geological period	Evolved	Group		Genes	Evolved
Palaeozoic	Ordovician	450 ± 35.5	Actinopterygii		GH	345.68
					GHR	416.24Ω
					GHRH	361.79
					GHRHR	485.63§
					GHSR	209.24
					Ghrelin	275.14
					SST	279.66©
					IGF-I	342
					IGFBP2	36.66
					INS	368.22
					LEPR	300.03
					TSH-β	257.14
					PIT-1	364.42
	Devonian	360 ± 14.7	Lissamphibia		GH	152.43≠
					GHR	381.33
					GHRH	361.79
					GHRHR	485.63
					GHSR	217.23
					Ghrelin	435.85
					SST	43.15¶
					IGF-I	342
					IGFBP2	29.60
					INS	273.75 (50.44)¥
					LEPR	561.68
					TSH-β	257.14

Mesozoic	Cretaceous	112 ± 11.7	Galliformes/Anseriformes		PIT-1	364.42∑
					GH	152.95*
					GHR	214.17*
					GHRH	336.26
					GHRHR	274.74
					GHSR	217.23(6.58)
					Ghrelin	121.72
					SST	122.00¿
					IGF-I	342
					IGFBP2	21.96
					INS	109.66
					LEPR	101.03
					TSH-β	257.14
Cenozoic	Tertiary	40.7 ± 0.9	Mouse		PIT-1	359.83√
					GH	10.44
					GHR	28.83∞
					GHRH	121.07
					GHRHR	77.05
					GHSR	29.92
					Ghrelin	84.06
					SST	11.51
					IGF-I	60.71Ħ
					IGFBP2	1.34
					INS	16.48
					LEPR	45.68
					TSH-β	95.12
	Tertiary	40.7 ± 0.9	Rat		PIT-1	22.32
					GH	10.44
					GHR	67.13
					GHRH	121.07
					GHRHR	77.05
					GHSR	29.92
					Ghrelin	84.06
					SST	25.50Æ
					IGF-I	60.71đ
					IGFBP2	1.34
					INS	39.68±
					LEPR	45.68
					TSH-β	95.12
		5.5 ± 0.2	Chimp /Human		PIT-1	22.32
					GH	10.43**
					GHR	166.92
					GHRH	173.45
					GHRHR	77.05β
					GHSR	29.92 (6.58)

Ghrelin	84.06£
SST	25.59
IGF-I	105.96þ
IGFBP2	10
INS	39.68
LEPR	101.03
TSH-β	95.12
PIT-1	50.97

£ V1 and V2 evolved 84.06 MYA but V3-5 evolved recently; § V2 274.74MYA; B V2. 3, V4 and 194.37MYA; Ω both diverged at same time; ∞ V1 and V3 at same time; ≠ A and B diverged 121.79 MYA

** 2V1, 2V4 and and V1-4 diverged at this time and evolved parallel while 2V2 and 2V3 evolved 394.57MYA; ¥ 1 and 2 diverged ; ± 2 diveged 16.48 MYA; ∑ V1,3 and V2,4 diverged 14.85 MYA; √ V1 and V2 diverged 162 MYA while V2 and W 14.85MYA; © 1 emerged 122.00 MYA; ¿ sst1 emerged 43.15 MYA; ¶ sst1a, b emerged 43.15 MYA; Æ sst emerged 11.51 MYA; þV2,4 137.97; đ V2, 4 11.95 MYA; Ħ V4, 5 11.95 MYA

DISSCUSSION

Relatively higher values for error coefficient of evolutionary difference could be because of smaller data sets used in this study as demonstrated by (KIJAS *et al.*, 2006). As data sets increased there could be cut for selection bias and therefore, chances for a robust estimation of sequence diversity could be improved. The results of such low divergence rates among species for HP growth axis genes have practical implications for researchers. The most immediate is the possibility of cross-species application of HD SNP Chip for analysis in species where it is not available. The previous studies are an evidence for this claim. For example, human cDNA arrays were successfully used to recognize transcripts with different expression in bovine tissue for orthologues genes with divergence between 5% and 15% (ADJAYE *et al.*, 2004). Again human cDNA arrays have also been used to generate reproducible results from opossum cell lines successfully with estimated pair-wise sequence diversity of 24% (WANG *et al.*, 2004). Search of transcript abundance therefore could facilitate use of known species microarray resources probably with more success. This high level of likeness could also provide the prospect to exploit genomes of species that are already sequenced for large-scale resequencing of genomes of species that are not yet sequenced in the search for SNPs.

Difference on unique mutations among lineages on non synonymous sites and synonymous sites were similar to the recent published data by (ZHANG and BROUGHTON, 2013) where rate of non synonymous substitutions is greater than synonymous in mammals and fishes nuclear and mitochondrial DNA. In general, observed dS were lower than dN. This higher rate of non-synonymous substitutions among species indicates a similar positive selection pressure in these species. As the value greater than 1 of the ratio of non-synonymous to synonymous substitutions is an indicator of occurrence of positive selection (HANADA *et al.*, 2007).

No difference in relative rate at third-position and first & second codon positions sites indicated that the mutagenic process, that generates substitutional mutation, is taking place at approximately the same rate at silent sites and non-synonymous sites in these species. This has been observed previously using primate and rodent lineages. In primate and rodent lineages rate variation was restricted to non-degenerate sites. Variation in mutation rates across mammalian lineages was uncovered previously using protein-coding sequences (LERCHER *et al.*, 2001; GIBBS *et*

al., 2004), the noncoding portion of the genome (SMITH *et al.*, 2002) and the mitochondrial genome (GISSI *et al.,* 2000).

Until recently, this study provided first report to indicate that rate variability exists among *Homo sapience, Mus musculus, Rattus norvegicus, Gallus gallus, Danio rerio* and *Xenopus laevis*. It was not possible within this analysis to identify the factor(s) responsible for the observed rate differences; however, metabolic rate, body size, generation time, sequence context and the role of recombination have all previously been identified for their contribution (GISSI *et al.*, 2000; ELLEGREN *et al.*, 2003; COOPER *et al.,* 2004; HODGKINSON and EYRE-WALKER, 2011).

Evolutionary history and trend of the HP growth axis among taxas of current study is variable and complicated. There are evidences of parallel evolution within species for genes of HP growth axis i.e. GH and INS in human, and between species i.e. GH, GHRH and GHRHR genes in mouse and rat; GHRH and GHRHR genes in fish and frog, and GH in frog and chicken, (Table 4; Figure 1a). However, parallel evolution is common either within mammalian taxas or within non mammalian species. But, there is no eveidence of parallel evolution between mammalian and non mammalian taxas. There are also examples of spontaneous evolution of genes such as IGFBP2, GHSR, SST and INS in both mouse and rat. Likewise there are examples of sudden evolution in non mammalian species i.e. INS in fish and frog (Table 4). Examples of divergent evolution are also there i.e. GH and GHR.

This is the first demonstration that suggests that variable rates of molecular evolution may be present within HP growth axis genes among different species. This difference could be useful in comparative genomics analysis and physiological genes functions identification among unknown species.

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EVOOLUCIJA RASTA HIPOTALAMUS - PITUITARI OSOVINE RIBE, AMFIBIJE, PTICA I ŽIVOTINJA

M. MOAEEN-UD-DIN[1þ], G. BILAL[1] i James M. REECY[2]

[1]Laboratorije za oplemenjivnje i genetiku fakulteta za Veterinu i nauku o životinjama, PMAS-Arid poljoprivredni Univerzitet, Rawalpindi (46300), Pakistan
[2] Odeljenje animalne nauke, Ajova Državni univerzitet, Ames, SAD

Izvod

Rast HP – osovine reguliše rast životinja i razvoj u prenatalnom i post natalnom životu, kojim upravljaju brojni faktori. Međutim, do skoro istorija evolucije osovine među rodovima još nije razjašnjena. Diverzitet između *Homo sapience, Mus musculus, Rattus norvegicus, Gallus gallus, Danio rerio i Xenopus laevis* je determinisan ispitivanjima gena uključenih u kontrolu rasta osovine HP. Sekvence gena rasta osovine HP su dobijene iz baze sekvenci - NCBI (http://www.ncbi.nlm.nih.gov/. Diverzitet nukleotida je određivan dvoparmetarskom metodom Kimura-e : kodon – zasnovan test selekcije korišćenjem kodon – zavisnim testom pozitivne selekcije Nei-Gojobori; istovetnost evolucione brzine je određivanjem Tajima's testom, a relativne brzine i filogenetska istorija su determinisani u MEGA6. Procena koeficijenta evolucione diferencijacije zasnovana na substituciji nukleotida i način substitucije aminokiselina kod gena rasta osovine HP je pokazala kontrastni način evolucije među rodovima. Rezultati su pokazali da, iako ti geni mogu da imaju ključnu funkcionalnu ulogu u svakoj od tih vrsta, njihova divergentnost sekvenci ne odražava obavezno sličnu molekularnu evoluciju među vrstama. Detaljni rezultati ukazuju da mutageni procesi koji generišu substitucione mutacije učestvuju u apromaksitivno istoj brzini u sinonimnim i nesinonimnim mestima tih rodova. Šta više, uprkos zajedničkom pretku utvrđeno je različito vreme divergencije među genima tih rodova. Ovo je prva demostracija da različite brzine molekularne evolucije mogu biti prisutne unutar gena koji kontrolišu brzinu rasta osovine HP. Ove razlike mogu da budu od interesa za komparativne genomske analize vrsta i fiziološke funkcije gena kod kojih nije dovoljno istražena osovina rasta HP.

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