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cDNA Cloning, Sequence Identification and Tissue Expression Profile of Three Novel Duck Genes *MJD1*, *RHOG* and *RB11A*

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Abstract: The complete CDS sequences of three duck genes-*MJD1*, *RHOG* and *RB11A* were amplified using RT-PCR based on the sequence information of the mouse or other vertebrates. Sequence analysis of these three genes revealed that the duck *MJD1* gene encodes a protein of 352 amino acids and has high homology with the Machado-Joseph disease protein 1 homolog (MJD1) of four species-rat (85%), human (87%), mouse (86%) and chicken (76%). The duck *RHOG* gene encodes a protein of 191 amino acids and has high homology with the RhoG precursor (RHOG) of six species-pig (100%), human, mouse and rat (98%), zebrafish (79%) and chiken (81%). The duck *RB11A* gene encodes a protein of 216 amino acids and has high homology with the Ras-related protein Rab-11A (RB11A) of nine species-chicken (99%), rat, human, rabbit, pig, mouse, dog and orangutan (99%) and bovine (98%). The phylogenetic tree analysis revealed that the duck MJD1 has a closer genetic relationship with the MJD1 of human and the duck RHOG has closer genetic relationships with the RHOG of pig but the duck RB11A has a closer genetic relationship with the RB11A of chicken. The *RT-PCR* gene expression analysis indicated that the duck *MJD1*, *RHOG* and *RB11A* gene was differentially expressed in tissues including lung, pancreas, intestine, fat, heart, spleen, liver and muscle. The study established the primary foundation for further research on these three duck genes.

Key words: Duck, MJD1, RHOG, RB11A, gene expression profile, China

INTRODUCTION

Mutation of *MJD1* gene had been identified to be the major factor responsible for the Machado-Joseph Disease (MJD) which is a hereditary neurodegenerative disease with symptoms presented to be cerebellar ataxia, external ophthalmoplegia, pyramidal and extrapyramidal signs and muscle wasting (Gu *et al*., 2004; Ishikawa *et al*., 2002; Ikeda *et al*., 2001). Transgenic mice carrying pathological alleles of the MJD1 locus exhibit a mild and slowly progressive cerebellar deficit and this implied that not only human but also other animals might suffer from this disease (Cemal *et al*., 2002).

RhoG, alike to Rho GTPase is highly similar to members of the Rac subfamily; homology area includes the regions involved in effector recognition and binding. RhoG activates Rac1 through Elmo and Dock180 to control cell morphology. RhoG has also been shown to play a role in caveolar trafficking and has a novel role in signaling the neutrophil respiratory burst stimulated by G Protein-Coupled Receptor (GPCR) agonists (Prieto-Sanchez and Bustelo, 2003; Katoh and Negishi, 2003; Prieto-Sanchez *et al*., 2006; Hiramoto *et al*., 2006). RB11A regulates the recycling pathways from endosomes to the plasma membrane and to the trans-Golgi network and is also thought to function in the histamine-induced fusion of tubulovesicles containing H+, K+ATPase with the plasma membrane in gastric parietal cells and in insulin-stimulated insertion of GLUT4 in the plasma membrane of cardiomyocytes (Duman *et al*., 1999; Gromov *et al*., 1998; Bhartur *et al*., 2000; Palmieri *et al*., 2006).

Based on above described about these three genes, it is necessary to isolate these three genes from duck for they are associated with health, caveolar trafficking, neutrophil respiratory burst, fusion of tubulovesicles and other important functions. These functions are potentially related with the duck production. But until today the duck MJD1, RHOG and RB11A have not been reported yet.

In present study, there will isolate the coding sequences of duck *MJD1*, *RHOG* and *RB11A* genes based on the conserved coding sequence information of the *MJD1*, *RHOG* and *RB11A* genes from mouse and other mammals, subsequently perform some necessary sequence analysis and finally conduct the tissue expression analysis for these three genes. These will establish the primary foundation of understanding these three duck genes.

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MATERIALS AND METHODS

Samples collection, RNA extraction and first-strand cDNA synthesis: The tissue samples of lung, pancreas, small intestine, fat, heart, spleen, liver and muscle were derived from one 60 days old Sheldrake. Total RNA extraction and first-strand cDNA synthesis for these tissue samples were performed as the methods describe by Liu *et al.* (2004).

Isolation of coding sequences for the duck *MJD1*, *RHOG* and *RB11A* genes: The RT-PCR was performed to isolate the coding sequences for the duck *MJD1*, *RHOG* and *RB11A* gene using the cDNAs from different tissues above. The 25 μL reaction system was: 2.0 μL cDNA (100 ng μL^{-1}), 2.5 μL, 2 mM mixed dNTPs, 2.5 μL, 10×Taq DNA polymerase buffer, 2.5 μL , 25 mM $MgCl_2$, 2.0 μL, 10 μM forward primer, 2.0 μL, 10 μM reverse primer, 2.0 units of Taq DNA polymerase (1 U μL^{-1}) and 9.5 μL sterile water. The primers for duck *MJD1*, *RHOG* and *RB11A* gene isolation were designed based on the conserved coding sequences sequences information from human and mouse *MJD1* gene. These primer sequences and their annealing temperature for RT-PCR reaction were shown in Table 1. The PCR program initially started with a 94°C denaturation for 4 min followed by 35 cycles of 94, Ta, 72°C 1 min^{-1}, then 72°C extension for 10 min, finally 4°C to terminate the reaction. These PCR products for duck *MJD1*, *RHOG* and *RB11A* genes were then cloned into PMD18-T vector and sequenced.

RT-PCR for tissue expression profile analysis: RT-PCR for tissue expression profile analysis was performed as previously described elsewhere (Fehr *et al*., 2000; Daigo *et al*., 2006; Liu *et al*., 2005). The primers and annealing temperature for duck β-actin gene (EF667345) amplification were shown in Table 1. The primers of duck *MJD1*, *RHOG* and *RB11A* gene which were used to perform the RT-PCR for tissue expression profile analysis were same as the primers for isolation RT-PCR above. The PCR reactions were optimized for a number of cycles to ensure product intensity within the linear phase of amplification. The 25 μL reaction system was: 2 μL cDNA (100 ng μL^{-1}), 5 pmoles each oligonucleotide primer, 2.5 μL, 2 mmol L^{-1} mixed dNTPs, 2.5 μL 10×Taq DNA polymerase buffer, 2.5 μL, 25 mmol L^{-1} $MgCl_2$, 1.0 units of Taq DNA polymerase and finally add sterile water to volume 25 μL. The PCR program initially started with a 94°C denaturation for 4 min, followed by 25 cycles of 94, Ta, 72°C 1 min^{-1} then 72°C extension for 10 min, finally 4°C to terminate the reaction.

Table 1: PCR primers for duck MJD1, RHOG, RB11A and β-actin and annealing temperature

Gene	Primer sequence	Ta/°C
MJD1	Forward :5-ATGGAGTCCATCTTCCAC-3	-
	Reverse: 5-TTATTTTTTCCCTTCTGTTT-3	58
RHOG	Forward: 5'-ATGCAGAGCATCAAGTGC G-3'	-
	Reverse: 5'-TCACAAGAGGACGCAGGA-3'	57
RB11A	Forward: 5'-ATGGGCACCCGCGACGAC-3	-
	Reverse: 5'-TTAGATGTTCTG ACAGCACTG-3	54
β-actin	Forward :5'-AGGGCTGTGATCTCCTTCTG-3'	-
	Reverse: 5'-CATGCCATCCTCCGTCTG -3'	55

Sequence analysis: The cDNA sequence prediction was conducted using GenScan software (http://genes.mit.edu/GENSCAN.html). The protein prediction and analysis were performed using the Conserved Domain Architecture Retrieval Tool of BLAST at the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST) and the ClustalW software (http://www.ebi.ac.uk/clustalw).

RESULTS AND DISCUSSION

RT-PCR results for duck *MJD1*, *RHOG* and *RB11A* genes: Through RT-PCR with different tissue cDNAs fromlung, pancreas, small intestine, fat, heart, spleen, liver and muscle, for duck *MJD1*, *RHOG* and *RB11A* gene, the resulting PCR products were 1059, 576 and 651 bp (Fig. 1).

Sequence analysis: The cDNA nucleotide sequence analysis for these sequenced PCR products using the BLAST software at NCBI server (http://www. ncbi. nlm. nih.gov/BLAST) revealed that these genes were not homologous to any of the known duck genes and they were then deposited into the GenBank database (Accession number: EU244433-EU244435). The sequence prediction was carried out using the GenScan software and results showed that these 1059, 576 and 651 bp cDNA sequences represented three single genes which encoded 352, 191 and 216 amino acids, respectively. The complete coding sequences of these genes and the encoded amino acids were shown in Fig. 2-4.

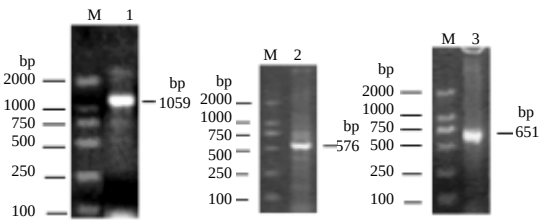

Fig. 1: RT-PCR results for duck MJD1, RHOG and RB11A. M, DL2000 DNA markers; 1, PCR product for duck *MJD1*gene from heart, spleen and liver tissues; 2, PCR product for duck *RHOG* gene from lung, pancreas and small intestine tissues; 3, PCR product for duck *RB11A* gene from lung, small intestine, heart, liver and tissues

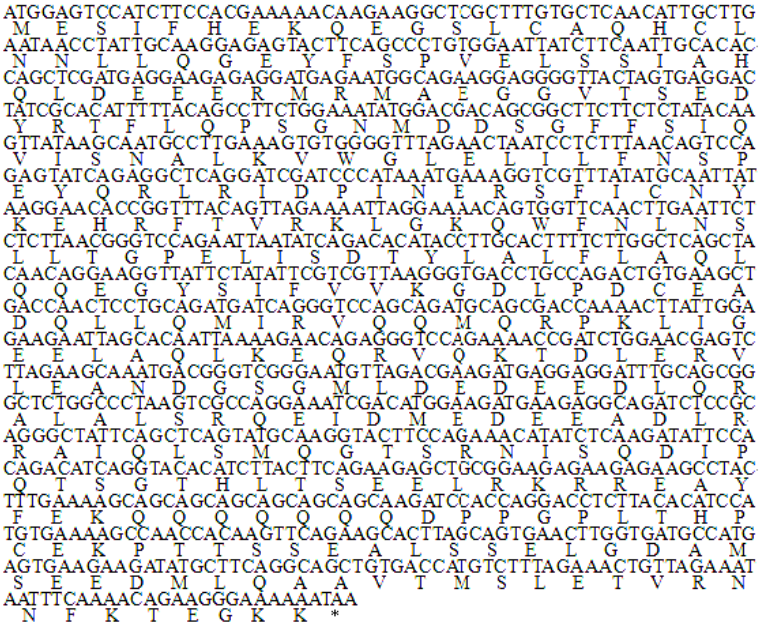

Fig. 2: The complete CDS of duck *MJD1* gene and its encoding amino acids; *indicates the stop codon

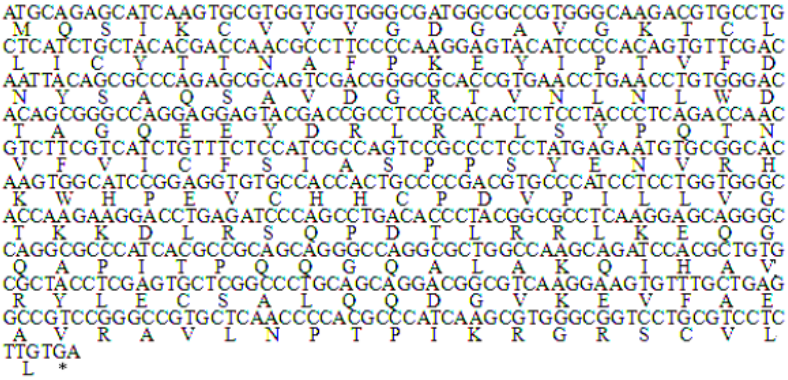

Fig. 3: The complete *CDS* of duck *RHOG* gene and its encoding amino acids; *indicates the stop codon

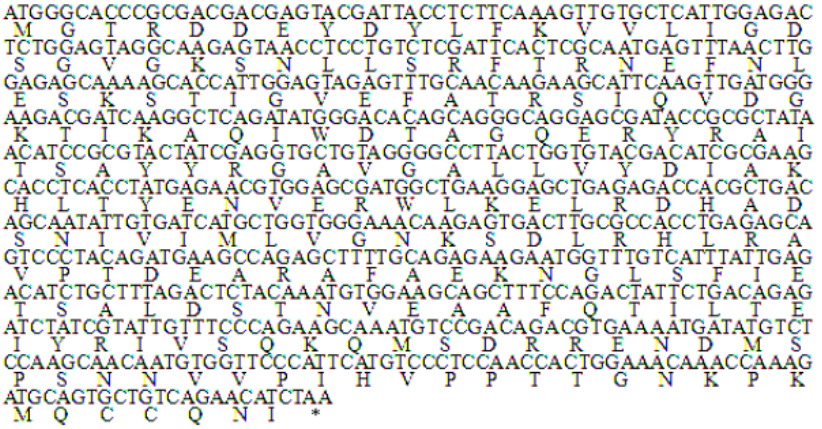

Fig. 4: The complete CDS of duck *RB11A* gene and its encoding amino acids; *indicates the stop codon

These putative protein were also blasted using the Conserved Domain Architecture Retrieval Tool of Blast at the NCBI server (http://www. ncbi.nlm.nih.gov/BLAST) and the conserved domain of duck MJD1 protein was identified as Josephin, the conserved domains of duck RHOG protein was identified as RhoG and the conserved domains of duck RB11A protein was identified as Rab11-like (Fig. 5).

Further BLAST analysis of these proteins revealed that duck MJD1 has high homology with MJD1 of four species rat (85%), human (87%), mouse (86%) and chicken (76%). The duck RHOG has high homology with RHOG of six species pig (100%), human, mouse and rat (98%), zebrafish (79%) and chiken (81%). The duck *RB11A* gene has high homology with the RB11A of nine species chicken (99%), rat, human, rabbit, pig, mouse, dog, orangutan (99%) and bovine (98%) (Fig. 6-8).

Based on the results of the alignment analyses of MJD1, RHOG and RB11A, the phylogenetic trees were constructed using the ClustalW software (http://www. ebi.ac.uk/clustalw) as shown in Fig. 9.

The phylogenetic tree analysis revealed that the duck MJD1 has closer genetic relationships with the MJD1 of human but the duck RHOG has a closer genetic relationship with the RHOG of pig and the duck RB11A has closer genetic relationships with the RB11A of chicken.

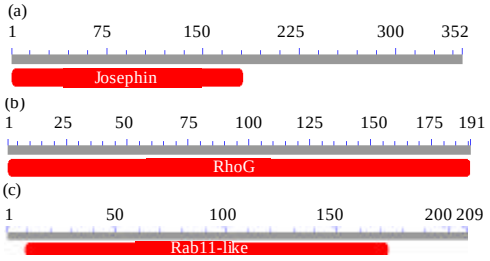

Fig. 5: The putative domains of the protein encoded duck *MJD1*, *RHOG* and *RB11A* genes; a) Josephin domain of duck *MJD1* gene; b) RhoG domain of duck *RHOG* gene; c) Rab11-like domain of duck *RB11A* gene

```
Duck        MESIFHEKQEGSLCAQHCLNNLLQGEYFSPVELSSIAHQLDEEERMRMAEGGVTSEDYRT
Human       MESIFHEKQEGSLCAQHCLNNLLQGEYFSPVELSSIAHQLDEEERMRMAEGGVTSEDYRT
Rat         MESIFHEKQEGSLCAQHCLNNLLQGEYFSPVELSSIAHQLDEEERLRMAEGGVTSEDYRT
Mouse       MESIFHEKQEGSLCAQHCLNNLLQGEYFSPVELSSIAHQLDEEERLRMAEGGVTSEDYRT
Chicken     MESIFHERQEGSLCAQHCLNNLLQGEYFSPVELSSIAQQLDEEERMRMAEGGVSSEEYRT
            *******:******************************:*******:*******:**:***

Duck        FLQ-PSGNMDDSGFFSIQVISNALKVWGLELILFNSPEYQRLRIDPINERSFICNYKEHR
Human       FLQQPSGNMDDSGFFSIQVISNALKVWGLELILFNSPEYQRLRIDPINERSFICNYKEHW
Rat         FLQQPSGNMDDSGFFSIQVISNALKVWGLELILFNSPEYQRLRIDPINERSFICNYKEHW
Mouse       FLQQPSGNMDDSGFFSIQVISNALKVWGLELILFNSPEYQRLRIDPINERSFICNYKEHW
Chicken     FLQQPSVNMDDSGFFSIQVISNALKVWGLELILFNSPEYQRLGIDPINEKSFICNYKEHW
            *** ** ********************************** ******:*********

Duck        FTVRKLGKQWFNLNSLLTGPELISDTYLALFLAQLQQEGYSIFVVKGDLPDCEADQLLQM
Human       FTVRKLGKQWFNLNSLLTGPELISDTYLALFLAQLQQEGYSIFVVKGDLPDCEADQLLQM
Rat         FTVRKLGKQWFNLNSLLTGPELISDTYLALFLAQLQQEGYSIFVVKGDLPDCEADQLLQM
Mouse       FTVRKLGKQWFNLNSLLTGPELISDTYLALFLAQLQQEGYSIFVVKGDLPDCEADQLLQM
Chicken     FTVRKLGKQWFNLNSLLMGPELISDTYLALFLAQLQQEGYSIFVVKGDLPDCEADQLLQM
            ***************** ****************************************

Duck        IRVQQMQRPKLIGEELAQLKEQRVQKTDLERVLEAND---GSGMLDEDEEDLQRALALSR
Human       IRVQQMHRPKLIGEELAQLKEQRVHKTDLERMLEAND---GSGMLDEDEEDLQRALALSR
Rat         IKVQQMHRPKLIGEELAHLKEQSALKADLERVLEAAD---GPGMFDDDEDDLQRALAMSR
Mouse       IKVQQMHRPKLIGEELAHLKEQSALKADLERVLEAAD---GSGIFDEDEDDLQRALAISR
Chicken     IRVQQVQRPKLIGEETAQSRDQRLPRSDVDQAIEVSHPFDGTGMLDEDEENFQRALALSR
            *:***::******** *: ::*   ::*::: :*. .   *.*::*:**:::*****:**

Duck        QEIDMEDEEADLRRAIQLSMQGTSR---------NISQDIPQTSGTHLTSEELRKRREAY
Human       QEIDMEDEEADLRRAIQLSMQGSSR---------NISQDMTQTSGTNLTSEELRKRREAY
Rat         QEIDMEDEEADLRRAIQLSMQGSSR---------GMCEDSPQTSSTDLSSEELRKRREAY
Mouse       QEIDMEDEEADLRRAIQLSMQGSSR---------SMCENSPQTSSPDLSSEELRRRREAY
Chicken     QEIDMEDEEADLRRAIQLSMQGSRQSEFSNSLPQNASQPPHTSQTDSLSSEDLRRRRQAY
            **********************: :         . .:    :.   *:**:**:**:**

Duck        FEKQQQQQQQ--------------------DPPGPLTHPCEKPTTSSEALSSELG-DAMS
Human       FEKQQQKQQQQQQQQQQQQQQQQQQQQQQRDLSGQSSHPCERPATSSGALGSDLG-KACS
Rat         FEKQQHQQQE--------------------ADRPGYLSYPCERPTTSSGGLRSNQAGNAMS
Mouse       FEKQQQQQQE--------------------VDRPGPLSYPRERPTTSSGGRRSDQGGDAVS
Chicken     FEKQQQQLQQ----------------------QDLTLNLHDKPTINSSTLEADPG-GDMS
            *****:: *:                      .      ::*: .*    :: .    *

Duck        EEDMLQAAVTMSLETVRNNFKTEGKK---
Human       PFIMFATFTLYLTYELHVIFALHYSSFPL
Rat         EEDVLRATVTVSLETAKDSLKAERKK---
Mouse       EEDMLRAAVTMSLETAKDNLKAERKK---
Chicken     EEDMLQAAMNMSLESARNHLSTEEKK---
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Fig. 6: The alignment of the protein encoded by duck MJD1 with the selected MJD1 proteins from other species

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Duck_Pig            MQSIKCVVVGDGAVGKTCLLICYTTNAFPKEYIPTVFDNYSAQSAVDGRTVNLNLWDTAG
Human_Mouse_Rat     MQSIKCVVVGDGAVGKTCLLICYTTNAFPKEYIPTVFDNYSAQSAVDGRTVNLNLWDTAG
Chicken             MQTIKCVVVGDGAVGKTCLLISYTTNAFPEEYIPTVFDNYSAQMTVDGRTVSLNLWDTAG
Zebrafish           MQSIKCVVVGDGAVGKTCLLISYTTGAFPKEYIPTVFDNYSSQVSVDNRTVSLNLWDTAG
                    **:******************.***.***:***********:* :**.***.********

Duck_Pig            QEEYDRLRTLSYPQTNVFVICFSIASPPSYENVRHKWHPEVCHHCPDVPILLVGTKKDLR
Human_Mouse_Rat     QEEYDRLRTLSYPQTNVFVICFSIASPPSYENVRHKWHPEVCHHCPDVPILLVGTKKDLR
Chicken             QEEYDRLRTLSYPQTNVFVICFSIGSPSSYANVRHKWHPEVSHHCPNVPILLVGTKRDLR
Zebrafish           QEEYDRLRTLSYPQTNVFIICFSISSPPSYENIKHKWHPEVTHHCPSVPILLVGTKSDLR
                    ******************:*****.**.** *::******* ****.********* ***

Duck_Pig            SQPDTLRRLKEQGQAPITPQQGQALAKQIHAVRYLECSALQQDGVKEVFAEAVRAVLNPT
Human_Mouse_Rat     AQPDTLRRLKEQGQAPITPQQGQALAKQIHAVRYLECSALQQDGVKEVFAEAVRAVLNPT
Chicken             NDLETVKKLKEQSLAPTTPQQGTSLAKQIGAVKYLECSALNQEGVREVFAEAVRAVLYPV
Zebrafish           NDADVLKKLKEQNQAPITTQQGQALARQIHAVKYRECSALSQDGIKDVFADAVRAYLSPQ
                     : :.:::****. ** *.*** :**:** **:* *****.*:*:::***:**** * *

Duck_Pig            PIKRGRSCVLL
Human_Mouse_Rat     PIKRGRSCILL
Chicken             TKKNTRKCVLL
Zebrafish           PVANKKPCILL
                    .  . : *:**
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Fig. 7: The alignment of the protein encoded by duck *ROHG* gene with the selected ROHG proteins from other species

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Rat_Human_Rabbit_Pig_Mouse_Dog_Orangutan MGTRDDEYDYLFKVVLIGDSGVGKSNLLSRFTRNEFNLESKSTIGVEFAT
Bovine                                  MGTRDDEYDYLFKVVLIGDSGVGKSNLLSRFTRNEFNLESKSTIGVEFAT
Duck                                    MGTRDDEYDYLFKVVLIGDSGVGKSNLLSRFTRNEFNLESKSTIGVEFAT
Chicken                                 MGNRDDEYDYLFKVVLIGDSGVGKSNLLSRFTRNEFNLESKSTIGVEFAT
                                        **.**************************************************

Rat_Human_Rabbit_Pig_Mouse_Dog_Orangutan RSIQVDGKTIKAQIWDTAGQERYRAITSAYYRGAVGALLVYDIAKHLTYE
Bovine                                  RSIQVDGKTIKAQIWDTAGQERYRAITSAYYRGAVGALLVYDIAKHLTYE
Duck                                    RSIQVDGKTIKAQIWDTAGQERYRAITSAYYRGAVGALLVYDIAKHLTYE
Chicken                                 RSIQVDGKTIKAQIWDTAGQERYRAITSAYYRGAVGALLVYDIAKHLTYE
                                        **************************************************

Rat_Human_Rabbit_Pig_Mouse_Dog_Orangutan NVERWLKELRDHADSNIVIMLVGNKSDLRHLRAVPTDEARAFAEKNGLSF
Bovine                                  NVERWLKELRDHADSNIVIMLVGNKSDLRHLRAVPTDEARAFAEKNGLSF
Duck                                    NVERWLKELRDHADSNIVIMLVGNKSDLRHLRAVPTDEARAFAEKNGLSF
Chicken                                 NVERWLKELRDHADSNIVIMLVGNKSDLRHLRAVPTDEARAFAEKNGLSF
                                        **************************************************

Rat_Human_Rabbit_Pig_Mouse_Dog_Orangutan IETSALDSTNVEAAFQTILTEIYRIVSQKQMSDRRENDMSPSNNVVPIHV
Bovine                                  IETSALDYTNVEAAFQTILTEIYRIVSQKQMSDRRENDMSPSNNVVPIHV
Duck                                    IETSALDSTNVEAAFQTILTEIYRIVSQKQMSDRRENDMSPSNNVVPIHV
Chicken                                 IETSALDSTNVEAAFQTILTEIYRIVSQKQMSDRRENDMSPSNNVVPIHV
                                        ******* ****************************************

Rat_Human_Rabbit_Pig_Mouse_Dog_Orangutan PPTTENKPKVQCCQNI
Bovine                                  PPTTENKPKVQCCQNI
Duck                                    PPTTGNKPKMQCCQNI
Chicken                                 PPTTENKPKMQCCQNI
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Fig. 8: The alignment of the protein encoded by duck *RB11A* gene with the selected RB11A proteins from other species

Tissue expression profile: Tissue expression profile analysis was carried out and results revealed that duck *MJD1* gene was moderately expressed in heart, spleen and liver and weekly expressed in muscle, hardly expressed in lung, pancreas, intestine and fat. The duck *RHOG* gene was highly expressed in lung, pancreas, intestine, fat, heart, spleen, muscle and hardly expressed in liver. The duck *RB11A* gene was highly expressed in lung, intestine, heart and liver, weakly in fat and hardly expressed in pancreas, spleen and muscle (Fig. 10).

Comparative genomics is the analysis and comparison of genomes from different species. Researchers have learned a great deal about the function of human genes by examining their counterparts in simpler model organisms such as the mouse and some results has revealed that virtually all (99%) of the protein-coding genes in humans align with homologs in mouse and over 80% are clear 1:1 orthologs (Hardison, 2003). This extensive conservation in protein-coding regions implied that this conservation of protein-coding sequences may be expected in different mammals such as including ducks, dogs, cats, rabbits, monkeys and apes. This provides us a useful method to isolate the functional regions of different genes for ducks based on the conserve sequence information of the mouse, human or other vertebrates and predict what those functions are?

In this experiment, the complete coding sequences of the duck *MJD1*, *RHOG* and *RB11A* genes were isolated based on the conserved coding sequence information of the *MJD1*, *RHO* and *RB11A* genes from mouse and other vertebrates. Sequence identification further validated that comparative genomics method is one useful tool to isolate

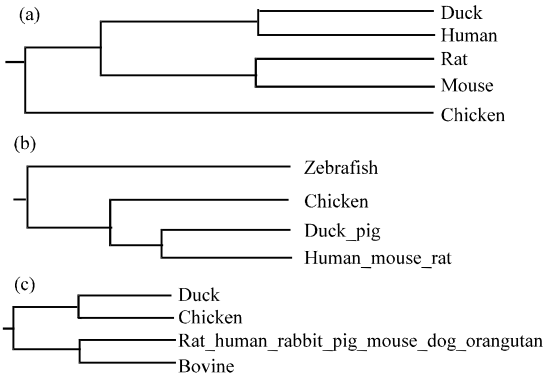

Fig. 9: The phylogenetic trees for selected MJD1, RHOG and RB11A proteins; a) phylogenetic tree analysis for selected MJD1 proteins; b) phylogenetic tree analysis for selected RHOG proteins; c) phylogenetic tree analysis for selected RB11A proteins

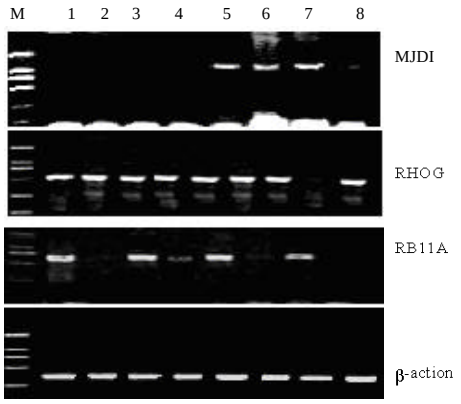

Fig. 10: Tissue expression distribution of duck *MJD1*, *RHOG* and *RB11A* gene. M, DL2000 markers; 1, lung; 2. pancreas; 3. intestine; 4. fat; 5. heart; 6. spleen; 7. liver; 8. muscle. The marker weights and PCR product sizes were same as Fig. 1

the unknown genes especially the conserved coding region of genes for ducks or other vertebrates. From the results there can see that duck MJD1, RHOG and RB11A are highly homologous with MJD1, RHOG and RB11A of mouse or other mammals and they also have common conserved structural domains. This implied duck MJD1, RHOG and RB11A will have similar functions as MJD1, RHOG and RB11A of mouse or other vertebrates. Researchers also find duck MJD1, RHOG and RB11A do not show complete identity to those of mouse or other mammals. This implied that duck MJD1, RHOG and RB11A will have some differences in functions with those of mouse or other vertebrates. This is deserved to study further. From the alignment analyses for MJD1, RHOG and RB11A proteins, it can be seen that MJD1 protein showed more diversity in different species. Machado-Joseph disease had been well known to be caused by (CAG) n repeat numbers in the coding region of human *MJD1* gene and there is a negative correlation between the age of onset and CAG repeat numbers. From the alignment analyses of duck MJD1 proteins with the MJD1 proteins of human and other species, it can be easily found human MJD1 has nineteen more continuous glutarnine than the MJD1 proteins of duck and other species. The corresponding codon of glutarnine is just the CAG. This indicated that human *MJD1* gene has nineteen more CAG repeats in the coding region than duck *MJD1* gene and other *MJD1* genes. The more CAG repeats may be the cause of human Machado-Joseph disease.

The phylogenetic tree analysis revealed that the duck proteins-MJD1, RHOG and RB11A have closer genetic relationships with different other species, respectively. These implied that different gene has different evolutional model although, these genes are in one individual or in one species but there still could find these duck proteins have closer relationships with those of human, mouse and other vertebrates. This supported the methods used in this experiment to isolate the duck encoding regions based on the conserved encoding region information of mouse and other vertebrates. Researchers also found that duck MJD1 have closer genetic relationship with the human MJD1. This implied that researchers can use duck as the model animal to study this gene of human. Similarly, there can use duck as the model animal to research the duck *RHOG* gene.

In the experiment, researchers not only isolated the complete coding sequence of duck *MJD1*, *RHOG* and *RB11A* gene but also performed the sequence analysis and tissue expression profile analysis. From the tissue expression analysis it can be seen that these genes were obviously differentially expressed in different tissues. The suitable explanation for this is that at the same time the biological activities of these three genes were presented diversely in different tissues.

CONCLUSION

In the study, researchers first isolated encoding regions of the duck *MJD1*, *RHOG* and *RB11A* genes, performed necessary sequence analysis and tissue expression profile analysis for these three duck genes. This established the primary foundation for further research on these duck genes.

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