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ARTICLE

# Clustering and Differentiation of glr-3 Gene Function and Its Homologous Proteins

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ABSTRACT

In order to adapt to the low temperature environment, organisms transmit excitement to the central system through the thermal sensing system, which is a classic reflex reaction. The cold receptor GLR-3 perceives cold and produces cold avoidance behavior through peripheral sensory neurons ASER. In order to further understand the gene encoding of the cold sensing glr-3 gene and the evolution of its homologous gene group function and protein function, the nucleotide sequence and amino acid sequence of the glr-3 gene and its homologous gene in 24 species were obtained and compared. By clustering with the GRIK2 gene sequence of Rana chensinensis, the bioinformatics method was used to predict and sequence analyze the change of gene, evolution rate, physical and chemical properties of protein, glycosylation sites, phosphorylation sites, secondary structure and tertiary structure of protein. The analysis results show that the glr-3 gene and its homologous gene have obvious positive selection effect. The protein prediction analysis showed that the glr-3 gene and its homologous genes encoded proteins in these 25 species were hydrophilic proteins, and the proportion of side chains of aliphatic amino acids was high. The transmembrane helix was widespread and there were more N-glycosylation sites and O-glycosylation sites. The protein phosphorylation sites encoded were serine, threonine and tyrosine phosphorylation sites. Secondary structure prediction showed that the secondary structure units of the encoded protein were α-helix, β-turn, random coil and extended chain, and the proportion of α-helix was the largest. This study provides useful information on the evolution and function of the cold sensing gene glr-3 and its homologous genes.

## 1. Introduction

As a kind of stressor, the low temperature environment can easily induce the body to produce cold stress, which directly or indirectly affects the physiological state and behavior of the animal, and even causes the death of the animal [1-3]. During the stress response, the changes of the animal body are very complicated [2,4]. Therefore, to maintain optimal function in a cold environment, animals must detect the temperature of their body and the environment, and make appropriate responses [5]. The information of environmental cold is expressed and transmitted by cold-sensitive ion channels in the peripheral sensory nerve endings of the skin. Neurons respond to cold stimuli, and the animal body will produce the corresponding cold escape mechanism [5,6]. When an animal stays in a cold stress

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environment for a long time, the neuroendocrine system will respond to the cold stimulus. When the physiological and hormone levels are balanced, the animal can adapt to this low temperature environment, and the animal body will overcome the stressor. Obtained cold adaptation [2,5,7,8].

In order to survive, organisms have evolved sophisticated heat-sensing systems to detect low temperatures and respond accordingly [9], but as of August 2019, only one cold receptor TRPM8 (transient receptor potential cation channel subfamily M) has been discovered. member 8), TRPM8 plays a central role in detecting somatosensory environmental low temperature [10], can be activated by low temperature and coolant menthol [11-13], its ability to sense cold can be fine-tuned in various species, In order to adapt well to the environmental temperature and better participate in energy metabolism [14]. The kainic acid glutamate receptor homolog GLR-3 was only identified as a cold receptor on August 29, 2019. GLR-3 senses cold in peripheral sensory neurons ASER to trigger the cold escape mechanism [15,16], its homolog GluK2 (glutamate ionotropic receptor kainate type subunit 2) can functionally replace GLR-3 in the body for cold sensation [17]. By selecting glr-3 genes and their homologs from 25 species Gene, and the protein sequence that the gene encodes. Use bioinformatics methods to conduct comparative analysis to explore whether the gene has undergone adaptive evolution among different species, and provide useful information for the glr-3 gene and its homologous genes, as well as their evolution and function.

## 2. Materials and Methods

### 2.1 Acquisition and Evolution Rate Calculation of glr-3 Gene and Its Homologous Gene Sequences in 25 Species

The gene sequences and protein sequences of 24 different species were obtained from the GenBank database of NCBI (Table 1) on the official website. In addition, the GRIK2 gene sequence of Rana dybowskii was obtained by polymerase chain reaction (PCR), and its protein sequence was obtained on emboss _ transeq. The ratio of dN / dS was calculated by pamlX-CodeML, namely, ω value, to detect the evolution rate of glr-3 gene and its homologous genes.

### 2.2 Construction of Phylogenetic Tree of glr-3 Gene and Its Homologous Genes

The ML tree was constructed by evolutionary analysis software MEGAX. ModelFinder and MrBayes in PhyloSuite-Pylogeny were used for model selection and Bayesian inference tree construction.

**Table 1.** GeneID and GenBank accession numbers of species

| Species | GenBank accession numbers | GeneID | homologous gene |
|---|---|---|---|
| *Homo sapiens* | NM_001166247 | 2898 | GRIK2 |
| *Pan troglodytes* | XM_001142208 | 462899 | GRIK2 |
| *Macaca mulatta* | XM_015136995 | 695660 | GRIK2 |
| *Canis lupus familiaris* | XM_038684247 | 481938 | GRIK2 |
| *Bos taurus* | NM_001193063 | 615226 | GRIK2 |
| *Mus musculus* | NM_001111268 | 14806 | Grik2 |
| *Rattus norvegicus* | NM_019309 | 54257 | Grik2 |
| *Gallus gallus* | XM_015284534 | 428628 | GRIK2 |
| *Xenopus tropicalis* | XM_031902289 | 100495093 | grik2 |
| *Danio rerio* | XM_021466798 | 556013 | grik2 |
| *Drosophila melanogaster* | NM_142668 | 42473 | KaiR1D |
| *Anopheles gambiae str. PEST* | XM_003437056 | 4576020 | AgaP_ AGAP000801 |
| *Caenorhabditis elegans* | NM_059616 | 172449 | glr--3 |
| *Sus scrofa* | XM_021073336 | 100516526 | GRIK2 |
| *Equus caballus* | XM_001503914 | 100066235 | GRIK2 |
| *Felis catus* | XM_019831025 | 101089440 | GRIK2 |
| *Ailuropoda melanoleuca* | XM_034670902 | 100466021 | GRIK2 |
| *Ictalurus punctatus* | XM_017479112 | 108271497 | grik2 |
| *Dermochelys coriacea* | XM_038395673 | 119853121 | GRIK2 |
| *Balaenoptera musculus* | XM_036871504 | 118905139 | GRIK2 |
| *Cygnus atratus* | XM_035561788 | 118255532 | GRIK2 |
| *Zootoca vivipara* | XM_035109324 | 118082226 | GRIK2 |
| *Artibeus jamaicensis* | XM_037135552 | 119042005 | GRIK2 |
| *Manis pentadactyla* | XM_036890423 | 118915022 | GRIK2 |

### 2.3 Prediction of glr-3 Gene, glr-3 Homologous Gene Encoding Protein Properties

ProtParam was used to predict the physicochemical properties of glr-3 gene and its homologous gene encoded protein. ProtScale was used to analyze the hydrophilicity and hydrophobicity of the encoded protein. TMHMMServerv2.0 was used to analyze the transmembrane topological structure of the encoded protein. PredictionServers was used to analyze the glycosylation sites of the encoded protein.

Use SOPMA to predict and analyze the secondary structure of the protein; use Swiss-Model. Predict the tertiary structure of proteins.

## 3. Results and Analysis

### 3.1 Phylogenetic Analysis and Evolution Rate of glr-3 Gene and Its Homologous Genes

In order to compare the phylogenetic relationships of



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glr-3 gene and its homologous genes in different species. In order to compare the phylogenetic relationship of glr-3 genes and their homologous genes in different species, phylogenetic trees were constructed for 25 species obtained. The construction methods were Maximum Likelihood (ML) method (Figure 1) and Bayesian inference method. The results show that the two phylogenetic trees are divided into two branches, the ML tree diagram shows that mammals are on the same branch, and the Bayesian inference tree diagram shows that Mus musculus and Rattus norvegicus are separated On the branch where the mammal is. The evolution rate analysis of the glr-3 genes of 25 species showed that the ω value of Mus musculus and Rattus norvegicus was 2.40, and the ω value of the remaining 12 mammals was 1.37, which is obviously compared to the other 12 mammals, Grik2 Genes make more favorable selection in Mus musculus and Rattus norvegicus; the ω value of 25 species is 1.28, which has obvious positive selection effect.

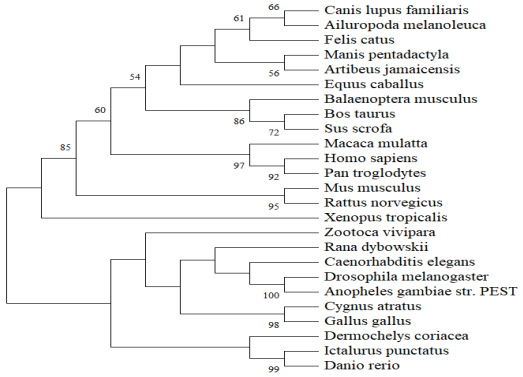


**Figure 1.** The evolutionary tree

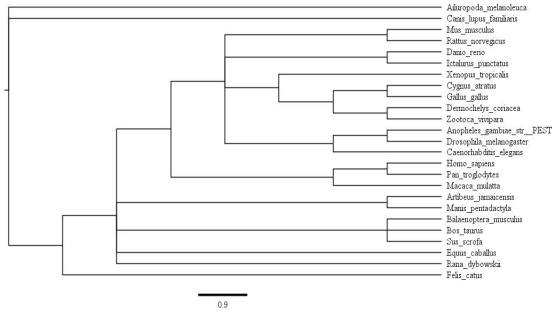


**Figure 2.** The evolutionary tree

## 3.2 Functional Protein Analysis of glr-3 Gene and Its Homologous Genes

The protein sequences encoded by glr-3 gene and its homologous genes in 25 species were obtained and analyzed. The physical and chemical properties of the encoded protein were predicted by online analysis software. The results showed that the protein sequence length was 432-915 AA, and the average protein length was 860 AA. The isoelectric point is between 6.22 and 9.75, and the average isoelectric point is 7.80. The instability index was between 36.73 and 47.75, and the instability index of coded proteins in 11 species was lower than the threshold, which was predicted to be stable proteins. The instability index of coded proteins in 14 species was higher than the threshold, which was predicted to be unstable proteins. The total average hydrophobicity was between-0.283 and-0.062, which were hydrophilic proteins. The predicted values of fat coefficient ranged from 80.53 to 97.48, and the proportion of side chains composed of aliphatic amino acids in proteins was higher, which reflected the strong thermal stability of proteins controlling these genes [18].

**Table 2.** 25 Species *glr-3* gene and its homologous gene expression protein physical and chemical properties analysis

| Species | AAs | PI | Instability index | GRAVY | Aliphatic index |
|---|---|---|---|---|---|
| *Homo sapiens* | 892 | 6.91 | 39.36 | -0.077 | 90.72 |
| *Pan troglodytes* | 908 | 8.05 | 39.99 | -0.120 | 89.23 |
| *Macaca mulatta* | 908 | 8.05 | 39.99 | -0.120 | 89.23 |
| *Canis lupus familiaris* | 908 | 8.06 | 40.10 | -0.125 | 89.23 |
| *Mus musculus* | 908 | 7.83 | 40.29 | -0.113 | 89.65 |
| *Bos taurus* | 908 | 8.05 | 40.10 | -0.126 | 89.12 |
| *Rattus norvegicus* | 908 | 8.04 | 40.56 | -0.108 | 89.65 |
| *Gallus gallus* | 908 | 7.8 | 40.47 | -0.112 | 89.12 |
| *Xenopus tropicalis* | 913 | 8.02 | 39.28 | -0.118 | 87.57 |
| *Danio rerio* | 908 | 7.29 | 40.62 | -0.138 | 88.9 |
| *Drosophila melanogaster* | 853 | 7.59 | 37.81 | -0.085 | 94.20 |
| *Anopheles gambiae str. PEST* | 888 | 6.22 | 40.8 | -0.193 | 85.56 |
| *Rana dybowskii* | 432 | 9.75 | 47.75 | -0.482 | 80.53 |
| *Caenorhabditis elegans* | 836 | 6.83 | 37.54 | -0.062 | 97.48 |
| *Sus scrofa* | 908 | 8.05 | 39.66 | -0.125 | 89.23 |
| *Equus caballus* | 908 | 8.05 | 40.10 | -0.126 | 89.23 |
| *Felis catus* | 583 | 8.00 | 36.73 | -0.091 | 93.48 |
| *Ailuropoda melanoleuca* | 908 | 8.05 | 40.10 | -0.126 | 89.23 |
| *Ictalurus punctatus* | 915 | 7.86 | 42.98 | -0.125 | 89.60 |
| *Dermochelys coriacea* | 908 | 7.80 | 40.06 | -0.111 | 89.02 |
| *Balaenoptera musculus* | 895 | 8.35 | 41.80 | -0.185 | 86.72 |
| *Cygnus atratus* | 859 | 7.20 | 39.82 | -0.160 | 87.29 |
| *Zootoca vivipara* | 733 | 8.13 | 39.27 | -0.283 | 84.58 |
| *Artibeus jamaicensis* | 908 | 8.05 | 40.10 | -0.126 | 89.23 |
| *Manis pentadactyla* | 887 | 6.94 | 39.83 | -0.162 | 87.40 |

*AAs: number of amino acids; PI: isoelectric point; GRAVY: Grand average of hydropathicity

Membrane proteins play an important role in biological activity, including cell communication, ion transport, transport, signal transduction, and functions as a "sensory organ" of cells. Transmembrane proteins are usually divided into three regions, which are distributed on both sides of the membrane. The hydrophilic part and the hydrophobic part that cross the membrane and form a stable helical structure exist [19,20]. Prediction and analysis of transmembrane regions of encoded proteins (Table 3), except for Rana dybowskii, there are transmembrane spirals in 24 species. Homo sapiens, Pan troglodytes, Macaca mulatta and other 14 species have 3 transmembrane spirals and their positions are the same.

**Table 3.** *glr-3* gene and its homologous gene expression protein in 25 species

| Species | Number of transmembrane spirals | Position | | |
|---|---|---|---|---|
| | | transmembrane region | extra membrane | intramembrane |
| *Homo sapiens* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583~638<br>845~892 |
| *Pan troglodytes* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583-638 845-908 |
| *Macaca mulatta* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583-638 845-908 |
| *Canis lupus familiaris* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583-638 845-908 |
| *Mus musculus* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583-638 845-908 |
| *Bos taurus* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583-638 845-908 |
| *Rattus norvegicus* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583-638 845-908 |
| *Gallus gallus* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583-638 845-908 |
| *Xenopus tropicalis* | 3 | 568~587<br>644~666<br>827~849 | 1~567<br>667~826 | 588~643<br>850~913 |
| *Danio rerio* | 4 | 13~32<br>563~582<br>639~661<br>822~844 | 33~562<br>662~821 | 1~12<br>583~638<br>845~908 |
| *Drosophila melanogaster* | 3 | 548~567<br>622~644<br>814~836 | 1~547<br>645~813 | 568~621<br>837~853 |
| *Anopheles gambiae str. PEST* | 3 | 516~535<br>592~614<br>784~806 | 1~515<br>615~783 | 536~591<br>807~888 |
| *Equus caballus* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583~638<br>845~908 |
| *Sus scrofa* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583~638<br>845~908 |
| *Felis catus* | 1 | 563~582 | 1~562 | 583 |
| *Rana dybowskii* | 0 | / | 1~432 | / |
| *Ailuropoda melanoleuca* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583~638<br>845~908 |
| *Ictalurus punctatus* | 3 | 570~589<br>646~668<br>829~851 | 1~569<br>669~828 | 590~645<br>852~915 |
| *Dermochelys coriacea* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583~638<br>845~908 |
| *Balaenoptera musculus* | 2 | 624~646<br>807~829 | 1~623<br>830~895 | 647~806 |
| *Cygnus atratus* | 3 | 514~533<br>590~612<br>773~795 | 1~513<br>613~772 | 534~589<br>796~859 |
| *Zootoca vivipara* | 1 | 638~660 | 1~637 | 661~733 |
| *Artibeus jamaicensis* | 3 | 563~582<br>639~661<br>822~844 | 1~562<br>662~821 | 583~638<br>845~908 |
| *Manis pentadactyla* | 3 | 542~561<br>618~640<br>801~823 | 1~541<br>641~800 | 562~617<br>824~887 |
| *Caenorhabditis elegans* | 3 | 525~544<br>601~623<br>781~803 | 1~524<br>624~780 | 545~600<br>804~836 |

Glycosylation is one of the methods of protein post-translational modification. It plays an important role in changing the conformation and stability of proteins. It participates in many processes of protein transcription and translation, immune response and transportation. Mutations in glycosylation sites may change gene function and play a key role [21]. Analysis of glycosylation sites of the encoded proteins of glr-3 gene and its homologous genes (Table 4) shows that there are more glycosylation sites in 25 species, and N-glycosylation sites are more than O -Glycosylation site [22]. Rana dybowskii has the most O-glycosylation sites at 19; Rana dybowskii and Caenorhabditis elegans have 0 and 2 N-glycosylation sites, and the remaining 23 Species N-glycosylation sites are between 4-7.

**Table 4.** Analysis of glycosylation sites of *glr-3* genes and their homologous genes in 25 species

| Species | Number of O-glycosylation | Number of N-glycosylation | Position of N-glycosylation |
|---|---|---|---|
| *Homo sapiens* | 4 | 6 | 67, 73, 275, 378, 423, 546 |
| *Pan troglodytes* | 3 | 6 | 67, 73, 275, 378, 423, 546 |
| *Macaca mulatta* | 3 | 6 | 67, 73, 275, 378, 423, 546 |



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| Species | Number of O-glycosylation | Number of N-glycosylation | Position of N-glycosylation |
|---|---|---|---|
| *Canis lupus familiaris* | 3 | 6 | 67, 73, 275, 378, 423, 546 |
| *Mus musculus* | 4 | 6 | 67, 73, 275, 378, 423, 546 |
| *Bos taurus* | 3 | 6 | 67, 73, 275, 378, 423, 546 |
| *Rattus norvegicus* | 4 | 6 | 67, 73, 275, 378, 423, 546 |
| *Gallus gallus* | 2 | 6 | 67, 73, 275, 412, 423, 546 |
| *Xenopus tropicalis* | 5 | 7 | 72, 78 ,280, 383, 417, 428, 551 |
| *Danio rerio* | 7 | 7 | 67, 73, 275, 378, 412, 423, 546 |
| *Drosophila melanogaster* | 8 | 4 | 262, 293, 389, 397 |
| *Anopheles gambiae str. PEST* | 12 | 4 | 229, 359, 365, 383 |
| *Rana dybowskii* | 19 | 0 | / |
| *Sus scrofa* | 3 | 6 | 67, 73, 275, 378, 423, 546 |
| *Equus caballus* | 3 | 6 | 67, 73, 275, 378, 423, 546 |
| *Felis catus* | 2 | 4 | 67, 73, 275, 423 |
| *Ailuropoda melanoleuca* | 3 | 6 | 67, 73, 275, 378, 423, 546 |
| *Ictalurus punctatus* | 6 | 6 | 74, 80, 385, 430, 437, 553 |
| *Dermochelys coriacea* | 2 | 6 | 67, 73, 275, 378, 423, 546 |
| *Balaenoptera musculus* | 7 | 5 | 67, 73, 275, 378, 423 |
| *Cygnus atratus* | 2 | 7 | 18, 24, 226, 363, 374, 381, 497 |
| *Zootoca vivipara* | 3 | 6 | 70, 76, 278, 381, 426, 714 |
| *Artibeus jamaicensis* | 3 | 6 | 67, 73, 275, 378, 423, 546 |
| *Manis pentadactyla* | 4 | 7 | 46, 52, 254, 357, 391, 402, 525 |
| *Caenorhabditis elegans* | 5 | 2 | 257, 356 |

Protein phosphorylation is one of the common post-translational modifications of proteins in biology. It is an important mechanism in the regulation of signal transduction in cells and participates in cell transduction and maintenance of protein spatial stability. Protein phosphorylation mainly includes serine, threonine and tyrosine phosphorylation [18,23]. As shown in Figure 3, 25 species glr-3 gene and its homologous gene coding proteins contain 3 phosphorylation sites, serine, threonine and tyrosine phosphorylation sites, serine phosphorylation sites are the most, tyrosine phosphorylation sites are the least. It is predicted that the recognition and binding of these encoded proteins with receptor signals are related.

Polypeptide chains form irregular folding along one-dimensional direction by hydrogen bonds. These fragments form the secondary structural units of proteins. The common three secondary structural units are α helix, β folding, irregular curl and β rotation [24,25]. SOPMA was used to predict the secondary structure of proteins. The secondary structure units of the encoded proteins were α-helix, random coil, extended chain and β-turn, and the proportion showed a decreasing trend.

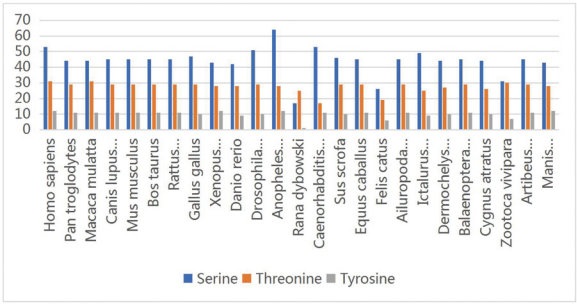


**Figure 3.** Predictive analysis of phosphorylation modification sites of glr-3 genes and their homologous genes in 25 species

**Table 5.** Prediction of secondary structure of *glr-3* gene and its homologous gene expression protein in 25 species

| Species | Alpha helix (%) | Beta turn (%) | Random coil (%) | Extended strand (%) |
|---|---|---|---|---|
| *Homo sapiens* | 41.37 | 5.27 | 35.87 | 17.49 |
| *Pan troglodytes* | 41.08 | 5.62 | 37 | 16.3 |
| *Macaca mulatta* | 41.08 | 5.62 | 37 | 16.3 |
| *Canis lupus familiaris* | 42.62 | 5.51 | 35.9 | 15.97 |
| *Mus musculus* | 42.29 | 5.62 | 36.01 | 16.8 |
| *Bos taurus* | 41.3 | 5.95 | 37 | 15.75 |
| *Rattus norvegicus* | 41.74 | 5.51 | 36.67 | 16.08 |
| *Gallus gallus* | 42.84 | 5.18 | 35.79 | 16.19 |
| *Xenopus tropicalis* | 40.64 | 5.7 | 36.69 | 16.98 |
| *Danio rerio* | 43.39 | 5.4 | 35.57 | 15.64 |
| *Drosophila melanogaster* | 42.02 | 5.48 | 35.48 | 17.02 |
| *Anopheles gambiae str. PEST* | 40.99 | 5.41 | 37.95 | 15.65 |
| *Rana dybowskii* | 34.49 | 11.81 | 33.1 | 20.6 |
| *Sus scrofa* | 40.75 | 5.73 | 37.11 | 16.41 |
| *Equus caballus* | 42.29 | 5.51 | 35.90 | 16.30 |
| *Felis catus* | 37.39 | 4.80 | 38.25 | 19.55 |
| *Ailuropoda melanoleuca* | 42.29 | 5.51 | 35.90 | 16.30 |
| *Ictalurus punctatus* | 43.17 | 5.46 | 35.74 | 15.63 |
| *Dermochelys coriacea* | 40.97 | 5.62 | 37.11 | 16.30 |
| *Balaenoptera musculus* | 41.01 | 5.25 | 37.21 | 16.54 |
| *Cygnus atratus* | 42.14 | 6.29 | 35.86 | 15.72 |
| *Zootoca vivipara* | 36.43 | 5.46 | 39.15 | 18.96 |
| *Artibeus jamaicensis* | 42.90 | 5.51 | 35.90 | 16.30 |
| *Manis pentadactyla* | 40.81 | 5.75 | 36.64 | 16.80 |
| *Caenorhabditis elegans* | 42.11 | 4.9 | 34.33 | 18.66 |


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Using Swiss-Model to predict the tertiary structure of proteins (Figure 4), the prediction results show that the tertiary structure of 8 mammals, including gorillas (Pan troglodytes), macaques (Macaca mulatta), and dogs (Canis lupus familiaris) are similar. The tertiary structure of human (Homo sapiens), zebrafish (Danio rerio) and Chinese pangolin (Manis pentadactyla) are similar.

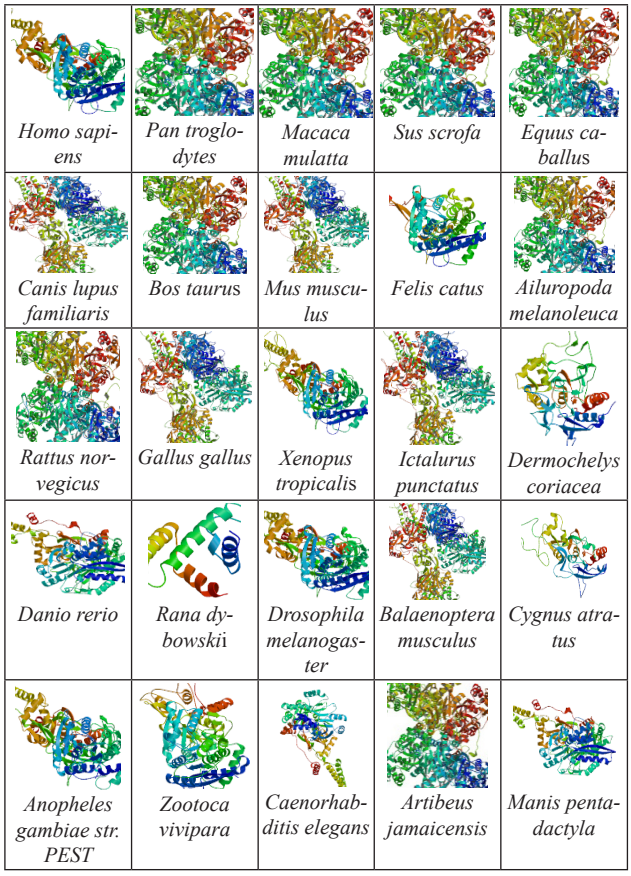


**Figure 4.** Prediction of tertiary structure of *glr-3* gene and its homologous gene expression protein in 25 species

## 4. Discussion

The regulation of temperature by organisms plays an important role in the normal conduct of life activities. In response to cold stimuli, organisms undergo the process of escape, adaptation, selection and evolution of genes and functional proteins.

The evolutionary selection of glr-3 gene in different species reflects the gain and loss of this gene [26]. The construction of ML tree and Bayesian tree reflects the phylogeny of glr-3 gene and its homologous genes. The results of the rate show that ω>1 obviously has an obvious positive selection effect, which may be related to the stress of the organism's nervous system to cold stimulation [10].

The analysis results show that the glr-3 gene and its homologous gene encoding protein of the research species are all hydrophilic proteins, and the side chain composed of aliphatic amino acids accounts for a higher proportion, indicating that the protein controlling this type of gene has strong thermal stability [18]; The encoded protein has obvious glycosylation sites, which is predicted to enhance the stability of the protein by changing the spatial structure of the protein [27,28]; the encoded proteins all contain 3 phosphorylation sites, serine, threonine and tyrosine The phosphorylation sites of amino acids, the most serine phosphorylation sites in the sequence, the least tyrosine phosphorylation sites, it is speculated that this type of protein is widely involved in cell transcription and regulation, signal recognition [29]; secondary structural unit of the encoded protein There are α-helices, random coils, extended strands and β-turns. α-helixes account for the largest proportion, maintaining the stability of the protein spatial structure. The secondary structure of the protein is also related to the coding region of the mRNA sequence, and the coding protein tends to It is encoded by the stem region of mRNA [30]. The purpose of this study is to explore the variation of glr-3 genes and their homologous genes in different species. The evolutionary rate and functional analysis of the encoded protein have certain research significance.

## 5. Conclusions

Through this study, we have reached the following conclusions: glr-3 gene and its homologous genes have obvious positive selection effects; through protein prediction analysis, it is shown that the glr-3 genes and their homologous genes of these 25 species all encode proteins It is a hydrophilic protein with a high proportion of side chains composed of aliphatic amino acids, transmembrane helixes are common, and there are more N-glycosylation sites and O-glycosylation sites, and the encoded protein phosphorylation sites There are phosphorylation sites for serine, threonine and tyrosine; the secondary structure prediction shows that the secondary structure unit of the encoded protein has α-helix, β-turn, random coil and extended chain, of which α-helix accounts for the proportion Both are the largest. This study provides useful information on the evolution and function of the glr-3 gene and its homologous genes.

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