Revealing the hidden mystery of Piezo: A phylogenetic study

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Abstract. Piezo proteins are a group of important mechanical-responsive ion channel proteins, which play an indispensable role in the normal physiological process of the organism. Therefore, Piezo proteins have attracted many researchers to develop relevant studies. In this work, an evolutionary tree of the Piezo1 gene was constructed using the gene sequences from National Center for Biotechnology Information (NCBI). Using MEGA, the ancestral gene sequence was constructed and the conserved and variable regions of Piezo1 protein were marked. According to the tree, the evolution of Piezo genes can be analysed, and then it is possible to predict the direction of gene mutation. The pathogenic mutation site to the corresponding regions was also mapped. Although there is still much work to be improved, the efforts to analyze the Piezo 1 gene from an evolutionary perspective is valuable, which might be helpful for future disease diagnosis and individualized therapy.

1. Introduction

Piezo proteins, whose function was firstly identified in 2010, are regarded as mechanical-responsive ion channels in many cells [1]. Ge et al determined the structure of the mouse Piezo1 protein by using cryo-electron microscopy, which forms a structure similar to a trimeric propeller [2]. When mechanically stimulated, the structure of a Piezo protein will be changed, followed by positively charged ions entering the cell. In many vertebrates’ organs, there are two-channel isoforms named Piezo1 and Piezo2, which contribute to a series of important physiological roles. Although much knowledge of this type of protein remains unknown, scientists have already proposed some functions of Piezo1 and Piezo2 in some human tissues, such as skin, bones, lungs, and so on [3]. Therefore, it is not surprising why so many research articles on Piezo proteins have been published in the past ten years [4-7].

Recent studies showed that Piezo channels were essential for vertebrates’ survival. For example, knocking out Piezo1 or Piezo2 will cause mice to die during their midgestation [8-10]. According to the genetic central dogma, many diseases are caused by the changes in protein structure, which is imputed to the genes. In fact, more than 25 mutations in Piezo1 have been related to human diseases, such as congenital lymphatic dysplasia. Besides, over 12 mutations in Piezo2 have been linked to arthrogryposis disorders. Therefore, it is necessary to take the gene mutation sites of Piezo proteins as the objects of researches, especially Piezo1. S.L. Alper has listed tables to show genetic diseases caused by different mutation sites, which can provide a reference for subsequent research [11]. In these tables, many diseases such as lymphatic dysplasia, arthrogryposis syndrome are listed, followed by the detailed information of the mutation sites, including the nucleotids, the corresponding domain, the functional phenotype, etc.
Herein, the Piezo gene sequences of different species from NCBI were used to build an evolutionary tree. Through the analysis of the evolutionary tree, the level of humans in the evolution of this gene can be obtained. This evolutionary tree is constructed using PhyML, a fast and accurate algorithm based on maximum likelihood (ML) [12]. Then the genes of five species were selected for horizontal comparison, and the ancestral genes of these species were constructed using MEGA [13]. Then the conserved and mutation region of Piezo1 were divided. Moreover, the mapping of human pathogenic mutation sites and these regions were established. It is believed that analyzing the Piezo gene from an evolutionary perspective is innovative. From the tree, the branch nodes of evolution can be obtained, and then the direction of gene mutation can be predicted. In addition, because human pathogenic mutation sites are manifestations of genetic polymorphisms, this connection between regions and mutation sites can be used for future diagnosis of diseases and individualized therapy.

2. Method
The sequences used in this work were collected from the NCBI database, and then they were preliminary operated in the software named Seaview. It was applied for the alignment of different sequences and construction of the evolution tree.

Next, MEGA was used to the following genetic analysis, including building the ancestral gene sequence and obtaining the conserved and variant regions.

3. Result & Discussion
Generally, an evolutionary tree is a schematic diagram of biological entities (such as species) that are connected through common offspring [14]. Compared with other manifestations, the evolutionary tree has significant advantages. For example, they are more direct as a representation of macro-evolutionary processes [15]. Therefore, it is valuable to use an evolutionary tree to consider the evolution of the same gene in different species and associated evolution with current genetic mutations.

This work mainly concentrates on the Piezo1 genes. Therefore, the Piezo1 genes of the five species were selected. In order to construct ancestral nodes and display the evolution process more accurately, the Piezo2 genes of mammals were also introduced. Interestingly, some studies have already pointed out that there is no Piezo1 or Piezo2 gene in Drosophila, but only one gene is called Piezo [16]. The evolutionary tree result is shown in Fig. 1. First of all, the confidence of most nodes is above 75, indicating that the construction of this evolutionary tree is reliable. In addition, Piezo gene of Drosophila shares the same ancestral node with the genes of other species, which indicates that Drosophila is at a more primitive level in the evolution of this gene. The Piezo2 genes of mammal and the Piezo1 genes of other species (bird, fish, etc.) have the same ancestral node. This is one piece of the evidence which shows that the original Piezo gene first mutated into two different groups (Piezo1 and Piezo2) in the ancestors, and then the ancestors evolved into different species. In other words, the differentiation of Piezo genes was earlier than the evolution of species. Hence, the Piezo1 and Piezo2 genes of different species are unique. It can be seen from Figure 1 that fish is at a relatively primitive level in the evolutionary process. Moreover, the divergence between mammal and bird, both of which are at a more modern level in the evolution, is the least.
Next, considering that the results of using software to translate genes into amino acids are not accurate enough, five amino acid sequences from different species in NCBI were selected and MEGA was used to construct Piezo1 amino acid sequences of their common ancestor. Amino acid sequences of the five species were compared with the amino acid sequences of their ancestors, followed by marking the conserved and variant regions of Piezo1 protein, which are shown in Fig. 2.

Conserved regions may represent more essential functions, while variable regions represent the diversity of biological evolution. It can be easily seen that Piezo1 protein has two regions with abundant conserved sites, which are located in the middle part and the last part, respectively. In fact, this corresponds to the two domains of the Piezo1 protein named Piezo RRas bgd and PIEZO.

Table 1. Mapping of pathogenic sites and conserved/variant regions.

| Mutation | Location       | Disease     | Effect on Channel      |
|----------|----------------|-------------|------------------------|
| R1358P   | conserved region| DHS         | Slowed inactivation    |
| E1630X   | variant region  | GLD         | Reduced expression     |
| A2020T   | conserved region| DHS         | Slowed inactivation    |
| G2029R   | variant region  | GLD         | Reduced expression     |
| T2127M   | variant region  | DHS         | Slowed inactivation    |
| E2496ELE | conserved region| DHS/HA      | Slowed inactivation    |
| H702Y    | variant region  | CAP         | Unknown                |
| L2023V   | conserved region| DHS         | Slowed inact           |
| R2488Q   | conserved region| DHS         | Incr RBC current       |
| Y1763X   | variant region  | CAP         | Unknown                |

(Abbreviations: CAP, colorectal adenomatous polyposis; DHS, dehydrated hereditary stomatocytosis; GLD, generalized lymphatic dysplasia; HA, hemolytic anemia)

Mutations sites in Piezo1 and Piezo2 genes are responsible for various hereditary human diseases. So far, autosomal recessive congenital lymphatic dysplasia, autosomal dominant hemolytic anemia, hereditary xerocytosis, dehydrated hereditary stomatocytosis, etc. have been proved to be related to the loss or mutation of the Piezo1 gene [11].

Therefore, several pathogenic mutation sites in the published articles were finally selected and their mapping with the conserved/variant regions was established in Fig. 2 [3],[11]. The results are shown in Table 1. Unfortunately, the mapping does not show regular information. From Table 1, the probability
of pathogenic mutations at conserved/variant sites seems to be random. This may be due to insufficient data. In the future, this mapping will be established based on larger data, hoping to determine that whether more mutations will occur in the conserved or in the variant regions of the Piezo1 protein, which will contribute to determining the certain value of the significance of mutations in different regions.

4. Conclusion
Piezo proteins, as a group of mechanical-responsive ion channel proteins, play a variety of important roles in organisms, including sensing stress changes. In particular, it has been proved that the knockout of Piezo1 or Piezo2 genes can cause the death of mice during the fetal period. In this work, the evolutionary tree of the Piezo1 gene was constructed and analyzed with the gene sequences from NCBI. The ancestral gene sequence was constructed, and the conserved and variable regions of Piezo1 protein were marked using MEGA. Besides, the pathogenic mutation site to the corresponding regions was also mapped. Through exploring the development of the Piezo1 gene from the perspective of biological evolution, it can be seen from the evolutionary tree that the formation of Piezo1 and Piezo2 was earlier than the formation of different species, indicating that the Piezo gene is a relatively original gene. This can lay a foundation for subsequent prediction of gene mutation and evolutionary direction. In addition, the efforts to map disease-causing mutation sites and gene conserved/variant regions can reflect the importance of gene conserved regions for the survival of organisms as well as genetic polymorphisms. This might be helpful for future disease diagnosis and individualized therapy.

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