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Bioinformatics analysis of the presence of Ca^{2+} channels of *Acanthamoeba castellanii*

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Abstract

The free-living amoeba (FLA) belonging to the genus *Acanthamoeba* is the most widespread protozoan in the environment, found in natural and man-made environments. *Acanthamoeba* is an opportunistic protist capable of causing granulomatous amebic encephalitis (GAE), a fatal disease of the central nervous system (CNS), and *Acanthamoeba* keratitis (AK), a painful, progressive, and sight-threatening infection. A single drug that can simultaneously eliminate both trophozoite and cystic forms of the parasite is currently unavailable. Bioinformatics research has aimed to reveal the presence of new therapeutic targets and pathways in the treatment of *Acanthamoeba*.

Calcium (Ca^{2+}) channels regulate many vital functions within cells. Ca^{2+} influx mediates the regulation of physiological signaling pathways in parasites. Calcium channels in *Acanthamoeba* allow the intracellular Ca^{2+} stores to be refilled following intracellular Ca^{2+} release. It is also well known that the role of calcium in the activation of some anti-parasitic drugs is very important. The search for protein sequence homology between two-pore calcium channel protein 1 (TPC-1), TPC-2, and calmodulin was done by searching the *Acanthamoeba* Neff strain protein databases at NCBI and amoebadb.org by using BLAST*p* search. The BLAST*p* alignment option was selected to show similarities between the proteins of both species.

In conclusion, it may be an option for a narrow treatment approach for GAE and AK caused by *Acanthamoeba* species; therefore, the discovery of calcium channels (TPC-1, TPC-2, and Calmodulin) in *Acanthamoeba* could prove to be a potential therapeutic target in the future.

Keywords: Bioinformatics tools, Acanthamoeba castellanii, Ca^{2+} channels, new therapeutic targets

Introduction

Acanthamoeba is a genus of free-living amoebae that generally inhabit soil, freshwater, and other habitats. This parasite has two evolutionary forms: a metabolically active trophozoite and a dormant, stress-resistant cyst form [1]. *Acanthamoeba* is frequently isolated in media such as contact lens solutions. This opportunistic amoeba can cause various clinical symptoms such as granulomatous amoebic encephalitis (GAE), a central nervous system disease, *Acanthamoeba* keratitis (AK), a sight-threatening eye disease, and *Acanthamoeba* pneumonia and skin lesions, especially in people with suppressed immune systems [2, 3].

Acanthamoeba species are known to have resistant cyst structures that can resist traditional antimicrobial treatments. Additionally, these cysts are resistant to adverse environmental conditions and anti-microbial agents, making it difficult for the patient to be treated [4]. There is no fully effective drug against *Acanthamoeba* infections. The most commonly used drugs in the treatment of AK include combination drugs such as polyhexamethylene biguanide or chlorhexidine digluconate and propamidine isethionate or hexamidine [5]. Combination drugs such as azoles, liposomal amphotericin B, trimethoprim-sulfamethoxazole, rifampicin, meropenem, linezolid, moxifloxacin, and alkylphosphocholine compounds are used for the treatment of GAE infections. There is no definitive treatment protocol recommended for GAE yet [3]. *Acanthamoeba* infections are often misdiagnosed or diagnosed late due to their nonspecific symptoms and similarities to other ocular or central nervous system conditions. Delayed diagnosis may lead to the progression of infection and worsening prognosis [6].

Calcium (Ca^{2+}) channels play a crucial role in regulating various cellular functions, including signal transduction, muscle contraction, neurotransmitter release, and gene expression [7]. In parasites, such as *Acanthamoeba* and other protozoa, Ca^{2+} influx through specific channels mediates the regulation of physiological signaling pathways essential for their survival, replication, and virulence [8].

Ca^{2+} signaling regulates the exocytosis of secretory vesicles containing virulence factors, enzymes, and other molecules necessary for parasite survival and pathogenesis. Ca^{2+} influx triggers the fusion of secretory vesicles with the plasma membrane, facilitating the release of their contents [9]. Understanding the role of Ca^{2+} channels and signaling pathways in parasites is crucial for identifying potential targets for therapeutic intervention. Calcium channels in *Acanthamoeba* allow the intracellular Ca^{2+} stores to be refilled following intracellular Ca^{2+} release. It is also well known that the role of calcium in activating some anti-parasitic drugs is very important [8, 10].

Calcium channels in human cells play an essential role in various physiological processes, and their modulation can affect drug action in various ways. Understanding the existence of calcium channels of *Acanthamoeba* in this study, their different roles, and their participation in various physiological processes is crucial for elucidating the mechanisms of drug action and developing therapeutic strategies targeting calcium signaling pathways. This study aims to analyze *Acanthamoeba* with bioinformatics methods using software and online databases containing analysis to show evidence of the presence of calcium channel proteins and receptor homologs.

Methods

Bioinformatics Sequence Data

Amino acid and nucleotide sequence data of the *Acanthamoeba castellanii* Neff strain were accessed using NCBI (https://www.ncbi.nlm.nih.gov/), Amoeba DB (https://amoebadb.org/amoeba/app), and UniProtKB (https://www.uniprot.org/) databases. NCBI and UniProtKB databases also found human two-pore calcium channel protein 1 (TPC-1), TPC-2, and calmodulin sequence data [11, 12].

Homologous Structures and Predicting Ligand Binding Dynamics

The search for protein sequence homology between two-pore calcium channel protein 1 (TPC-1), TPC-2, and calmodulin was done by searching the *Acanthamoeba* Neff strain protein databases at NCBI and amoebadb.org by using BLASTp search. The BLASTp and UniProtKB alignment option was selected to show similarities between the proteins of both species [12, 13].

FASTA sequences of proteins from the *Acanthamoeba* castellanii Neff strain and human TPC1, TPC2, and calmodulin were submitted to a BLASTp search. After submitting the sequences, the BLASTp search aligns them with sequences in the database to identify regions of similarity. The UniProtKB align was the selected option to visualize and analyses the similarities between the proteins of both species. Finally, we compared the downloaded data (identities, similarities, and E-values) for human and *Acanthamoeba* proteins using pairwise alignment tools such as EMBL EMBOSS mapper [14].

After comparing the amino acid sequence of *Acanthamoeba* and the human calcium channel calmodulin protein with BLASTp, the 3D structure and ligand binding

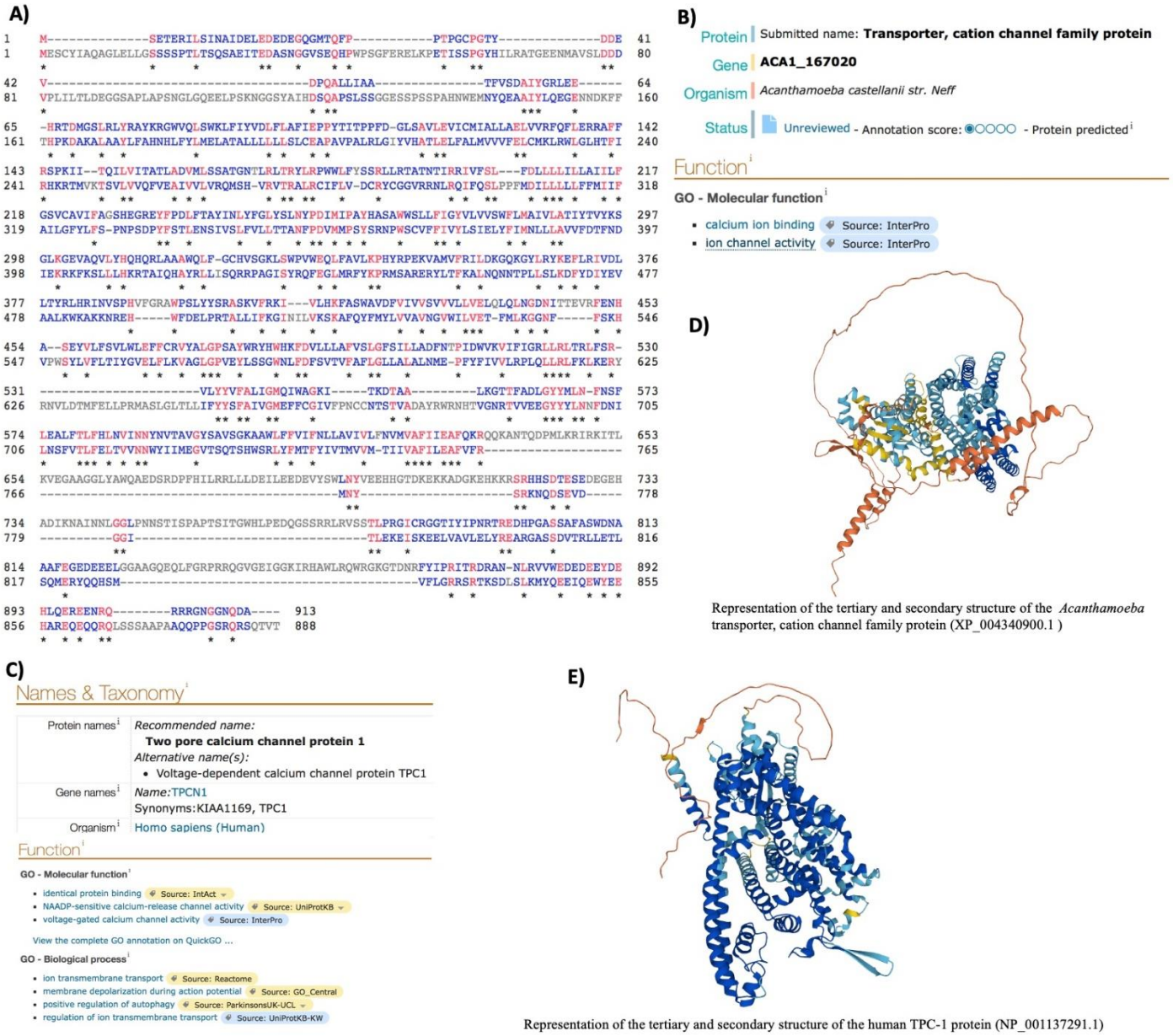

Figure 1. *A) Shows amino acid sequence homology of human two-pore channel1 (TPC-1) with the Acanthamoeba transporter, cation channel family protein accession number (XP_004340900.1). B-C) Molecular and biological functional properties of Acanthamoeba transporter cation channel family protein and Human TPC-1 protein. D-E) The amino acid sequence of the Acanthamoeba transporter, cation channel protein, and Human TPC-1 was demonstrated by the SWISS-MODEL.*

homology were established. SWISS-MODEL and Phyre2 database was used to create structural modeling/3D docking space of the calmodulin channel of *Acanthamoeba* [15, 16].

Results and Discussion

The transporter, cation channel family protein of the *Acanthamoeba castellanii* (strain ATCC 30010 / Neff) isolate was downloaded from UniProt and the amoeba database. *Acanthamoeba castellanii* cation channel family protein accession number was XP_004340900.1, and the sequence length consists of 913 amino acids. The general feature of human Two-pore channel protein 1 (TPC1) amino acid sequence homology is the intracellular channel defined as a non-selective Ca^{2+} permeable channel activated by NAADP (nicotinic acid adenine dinucleotide phosphate). The human TPC1 channel protein accession number was NP_001137291.1 and the sequence length consists of 816 amino acids. The length, identity, similarity, and gap of the amino acid sequence between *Acanthamoeba castellanii* (XP_004340900.1) cation channel protein and Human two-pore channel protein 1 are 422, 24.9%, 46.0%, and 3.8%, respectively **(Figure 1).**

Besides the general features of human TPC1 function, TPC2 is a voltage-dependent calcium channel protein and a highly selective Na^+ channel that is directly activated by PI (3,5) P2 (phosphatidylinositol 3,5-bisphosphate). The accession number of TPC-2 was NP_620714.2, and the sequence length was 752 amino acids. The length, identity, similarity, and gap of the amino acid sequence between *Acanthamoeba castellanii* (XP_004340900.1) cation channel protein and TPC-2 were 304, 28.9%, 49.7%, and 6.9%, respectively **(Figure 2)**

The general function of human calmodulin is to serve as part of the calcium signal transduction pathway, mediating the control of numerous enzymes, ion channels, aquaporins, and other proteins through calcium binding. The amino acid sequence homology results of human calmodulin accession number (AAD45181.1) with *Acanthamoeba* putative calmodulin accession number (ELR14060) showed 85.906% identity **(Figure 3)**. *Acanthamoeba* and human calmodulin sequence length consists of 149 amino acids. Additionally, their similarity was 94.6%, and the scoring value was 669.

The transporter cation protein of *Acanthamoeba*, TPC1, TPC2, and *Acanthamoeba* putative calmodulin has attributes that include calcium channel activity and functions of calcium ion hemostasis. The protein homologies of human calcium ion channels and calmodulin blockers were bioinformatically revealed, and their effects on *Acanthamoeba* were evaluated [17]. In previous studies, sequence identity and 3D modeling using the existence of *Acanthamoeba castellanii* was demonstrated by amino acids such as Na^+_v - K^{+1}_v channels and cytochrome P450 3A4. Additionally, human-like L-type and two-pore Ca^{2+} channels have been shown to be present in *Acanthamoeba* [18].

Interestingly, various types of proteins are found in all species, such as the voltage-gated ion channel superfamily. The existence of these channels suggests that they may have extremely ancient origins, possibly dating back to the last common ancestor of all life forms [19]. Studying ion channel function on *Acanthamoeba*, application of ion channel blockers, and use of bioinformatics and computational tools provide truly valuable insight. Combining these methods may

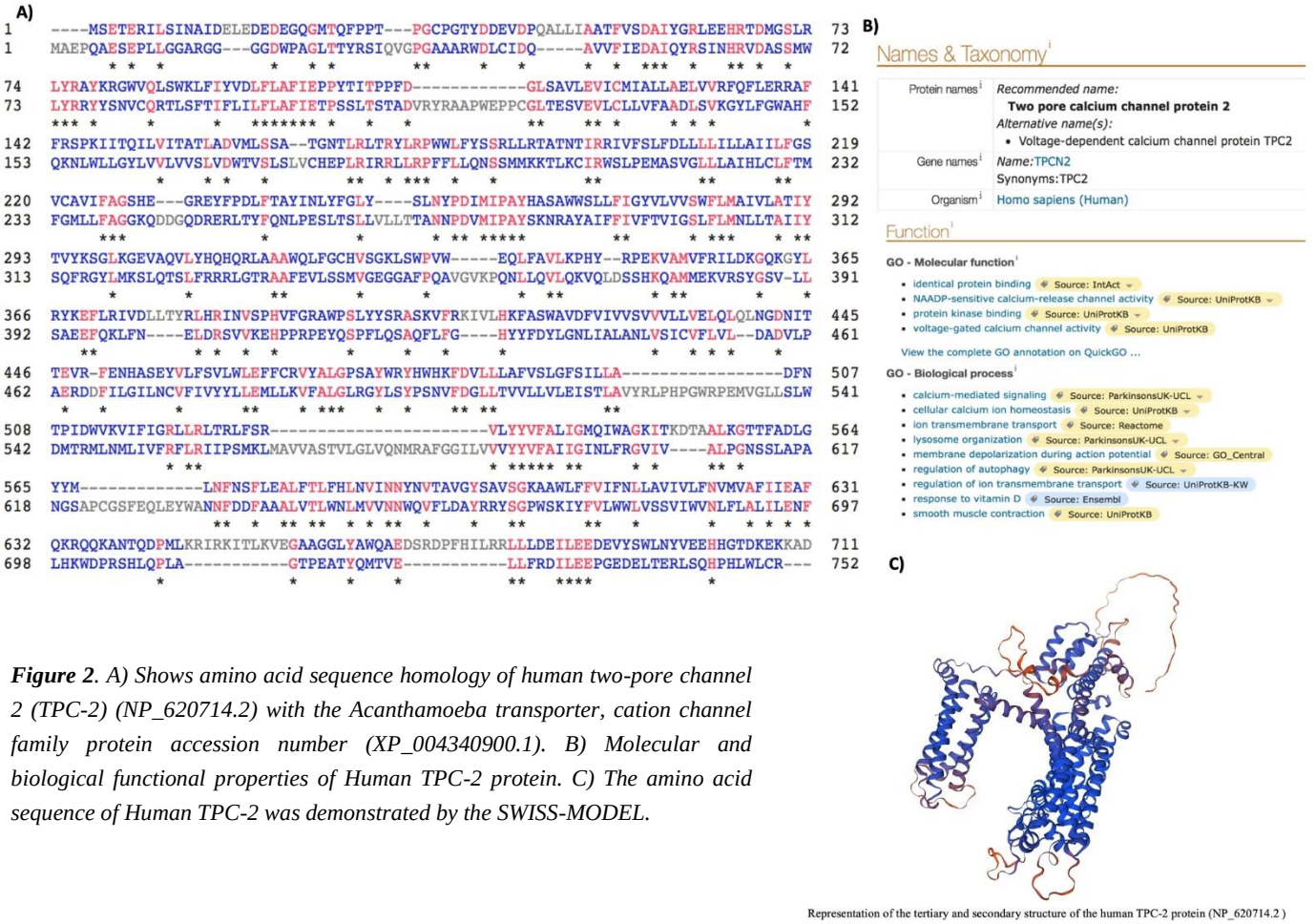

***Figure 2**. A) Shows amino acid sequence homology of human two-pore channel 2 (TPC-2) (NP_620714.2) with the Acanthamoeba transporter, cation channel family protein accession number (XP_004340900.1). B) Molecular and biological functional properties of Human TPC-2 protein. C) The amino acid sequence of Human TPC-2 was demonstrated by the SWISS-MODEL.*

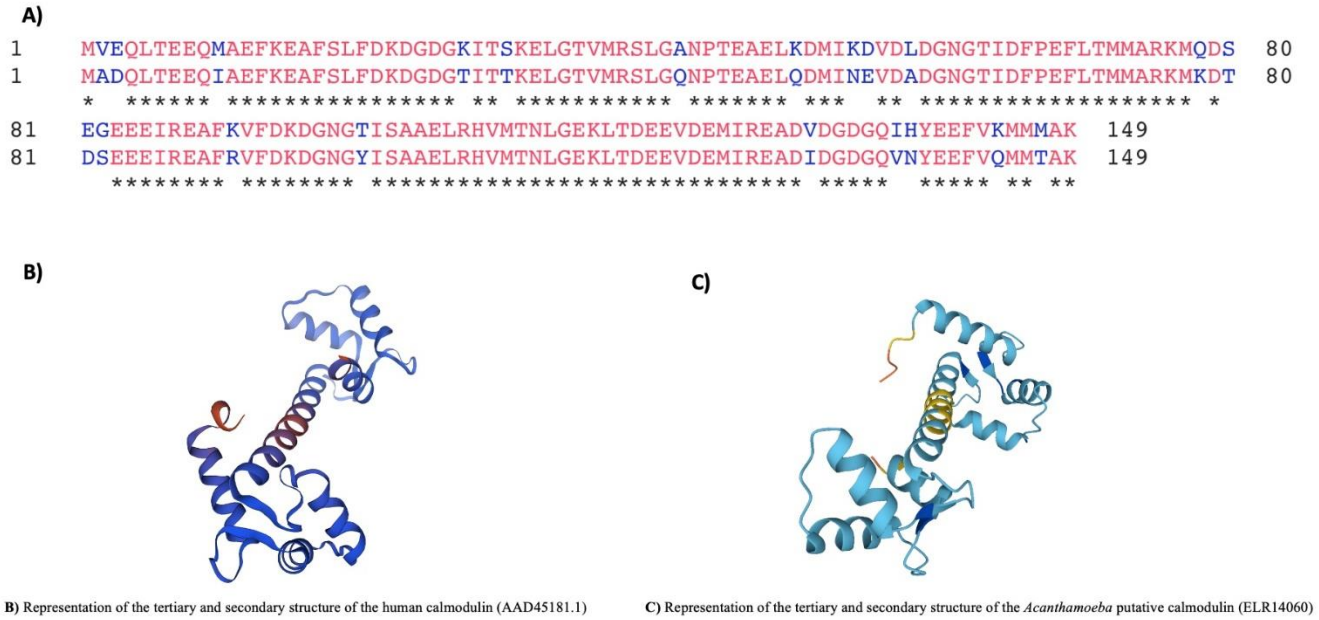

Figure 3. *A) Shows amino acid sequence homology of homology of human calmodulin (AAD45181.1) with Acanthamoeba putative calmodulin accession (ELR14060). B-C) The amino acid FASTA sequence of human calmodulin and Acanthamoeba putative calmodulin was used to build models by SWISS-MODEL.*

help us understand the functionality, evolution, and importance of ion channels found in *Acanthamoeba* [10, 18].

Administration of ion channel blockers may alter or inhibit the activity of specific ion channels in *Acanthamoeba*, helping us understand the functions and effects of these channels. In this way, we can determine the roles of specific ion channels in biological processes and understand the evolutionary adaptations of these processes in *Acanthamoeba*.

Using bioinformatics and computational tools allows us to understand the structure, function, and evolutionary origin of ion channels more deeply by analyzing the genetic data of *Acanthamoeba*. Examining genomic data allows us to determine the evolutionary relationships of specific ion channel genes and compare these channels with their counterparts in other organisms [20, 21].

In conclusion, studying the ion channel function of *Acanthamoeba* can help us understand the evolutionary history of ion channels, obtaining valuable information about the physiology and adaptation of this organism. It may be an option for a narrow treatment approach for GAE and AK caused by *Acanthamoeba* spp. therefore, the discovery of calcium channels (TPC-1, TPC-2, and Calmodulin) on *Acanthamoeba* could prove to be a potential therapeutic target in the future.

Ethical Declarations

The current study has no study with human and human participants. The study is not subject to ethics committee approval.

Conflict of Interest Statement

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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Author Contributions

All of the authors declare that they have all participated in the design, execution and analysis of the paper and that they have approved the final version.

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