Current Applied Science and Technology Vol. 22 No. 5 (September-October 2022)

Research article

In-Silico Analysis of the NT-3 (Neurotrophin-3) Protein Expressed by the NTF-3 (Neurotrophic Factor-3) Gene in Homo sapiens

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Received: 3 January 2021, Revised: 1 December 2021, Accepted: 4 January, 2022

DOI: 10.55003/cast.2022.05.22.007

Keywords

in-silico;

NT-3;

NTF-3;

Homo sapiens

Abstract

Neurotrophic Factor 3 (NTF-3) is a gene that encodes the Neurotrophin 3 (NT-3) protein. The NTF3 gene works as a neurotrophic factor that is responsible for enhancing the development of neuron function. Several disorders in the nervous system have become a discussion topic of health problems, such as a study about NTF-3 in pathophysiology. The aim of this research was to study the phylogenetic and tertiary structure of the protein expressed by the NTF-3 gene in humans. The computation method in this study uses in-silico analysis and data from GenBank with accession code M61180.1, and has a protein product with accession code AAA63231.1. The results of this study show humans are quite closely related to primate species based on phylogenetic bootstrap values. The alignment result of the sequences illustrates a high level of similarities. The NT-3 protein is composed of 257 aa (amino acids) with a weight of 29353.03 Daltons. The number of serine, arginine, and leucine contained in the protein is more than other amino acids, i.e. 24 (9.34%), 22 (8.56), and 22 (8.56%), respectively. The 1-41 and 101-251 amino acid sequences of the protein are the conserved areas of the NT-3 protein. The structure of the protein consists of a beta-sheet and coiled structure without an alpha helix. The polar region of the protein structure consists of several amino acids with polar groups that have hydrophilic properties. The result of the study can be utilized in the further study of genetic information related to the protein expressed by the NTF-3 gene in humans, and its role in pathophysiology.

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1. Introduction

Neurotrophic Factor 3 (NTF-3) comes from the neurotrophin factor family, which are a group of protein growth factors and nerve cell protectors [1-3]. There are four factors of neurotrophins, including NGF, BDNF, NT-3, and NT-4 [4]. However, NTF-3 is the only one of the four neurotrophins that can prevent the degeneration of noradrenergic neurons in the coeruleus locus [5]. It plays a role in controlling the survival and differentiation of nerve cells [6]. Moreover, it significantly increases the differentiation of nerve stem cells into cholinergic neurons [7]. It is found in the central nervous system and peripheral nerves [8], with different locations on chromosomes and exons. In humans, NTF-3 is on chromosome 12p13 exon 3 [4].

NTF-3 is the gene that encodes the NT-3 protein [8], and function as a neurotrophic factor that is responsible for enhancing the development of neuron function [9]. The NT-3 protein can increase the regeneration of damaged neurons by binding to the ERBb gene [10]. The NT-3 protein plays an important role in controlling the number of neurons and the growth of dendrites [11]. The regulation of the NT-3 protein can cause phosphorylation due to receptor activation [12]. Moreover, neurotrophin factors have two receptors that work with different affinities, namely p75NTR and tyrosine kinase [4]. The affinity strength of the p75NTR receptor is less than the Trk receptor [13]. The affinity difference shows the characteristics and uniqueness of the NT-3 protein in the human nervous system. This feature is still very rarely studied, so it needs to be studied further. The lack of information and molecular studies of the NT-3 protein can affect the existence of studies in the future about the development of knowledge of the NT-3 protein. Analyzing the NT-3 protein using in-silico analysis is a very practical because it is free to access in molecular research and can provide detailed information.

Several disorders in the nervous system have become a discussion topic in health issues, and the NTF-3 gene is studied to show its relation to pathophysiology [14]. Further analysis of the NT-3 protein is needed to add references to the development of molecular analysis related to the structure and biochemical function of the NT-3 protein. The purpose of this study is to examine the phylogenetic and tertiary structure of proteins expressed by the NTF-3 gene in humans using in-silico analysis. In-silico analysis has great potential to predict biochemical processes. It is used for determining the relationship between the structure and function of biomolecules. The results of this study can be used as additional knowledge to discuss the genetic protein expressed by the NTF-3 gene in humans, and its relation to pathophysiology.

2. Materials and Methods

This study uses in-silico analysis with computational methods. The materials used in this study are protein sequences resulting from the expression of the NTF-3 gene with access code M61180.1. The protein sequence data is from the GenBank database with accession code AAA63231.1, and is originally from the National Center for Biotechnology and Information (NCBI) website. This data was accessed online via https://www.ncbi.nlm.nih.gov/. The alignment of the NT-3 sequences is from the analysis of the BLAST results of the NT-3 protein on the NCBI website. The phylogenetic information was analyzed through the Construct/Test UPGMA Tree using MEGA X. The amino acid sequences of the NT-3 protein were analyzed using the Expasy Protparam tool to determine the properties of the protein. Meanwhile, the analysis of the tertiary structure of the protein was analyzed using Chimera and Consurf server.

2.1 Tracking the target protein

The target protein (Figure 1) used in this study is a protein expressed by the NTF-3 gene in humans, accessed on http://www.ncbi.nlm.nih.gov/. The target protein is the human NT-3 protein. It has an accession code AAA63231.1 with a length of 257 aa (amino acid). It is the expression result of the NTF-3 gene in humans with an accession code M61180.1.

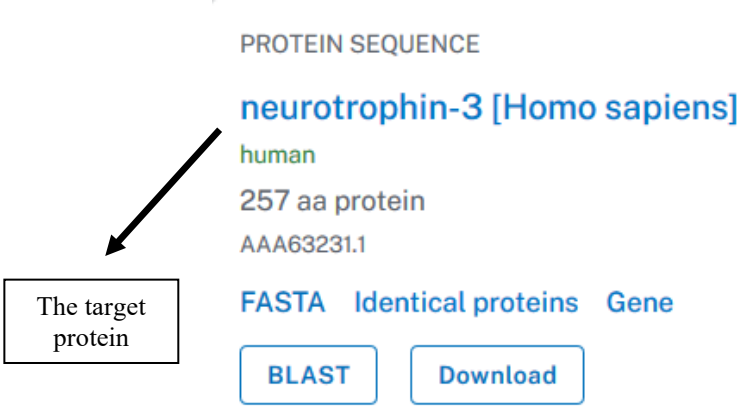


Figure 1. The target protein

2.2 Determining homologous proteins

Several protein sequences from Genbank were compared with target proteins to determine the homologous proteins. The determination of homologous proteins was analyzed using BLAST protein in the NCBI program. As a result, 100 samples were homologous with the target protein based on some parameters shown in Figure 2. This study only used the top 20 samples as comparison data. Overall, these samples had a high degree of similarity and came from several primate species. These selections were used to simplify information related to the genetic relationship level between humans and several primates based on the identity value of the NT-3 protein sequences. The percentage of the identity value reveals how close the genetic relationships are. The higher the identity value of a protein against the target protein in the BLAST protein results, the more similar it is to the target protein because it has a similar sequence of amino acids to the target protein. Therefore, this technique can be used to explain the relationship between species.

2.3 Tracing templates through analysis of the target protein domain using Phyre2 server

The database of the protein sequences was input into the workspace of Phyre2 Server to search the templates of the target protein. The templates were selected based on the homology levels of the protein in order to predict the most accurate 3D structure of the protein. The result shows d1bndb was the template with the highest level of homology (Figure 3), a confidence model of 100.0 %, and the structural coverage of about 42% of the target protein sequences (Figure 4). The result of the model structure was the closest part to the actual protein structure based on the analysis of the NT-3 tertiary structure. In addition, the analysis results of the Phyre2 Server showed that the percentage was maximum. The determiner in selecting the target model structure was the confidence and coverage values.

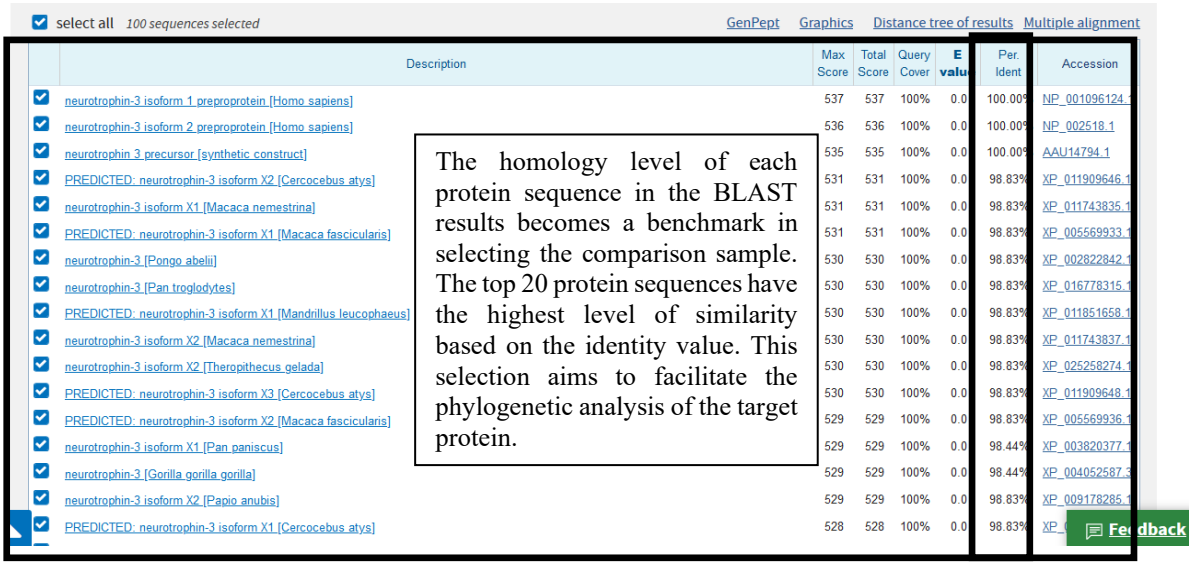


Figure 2. The result of BLASTP

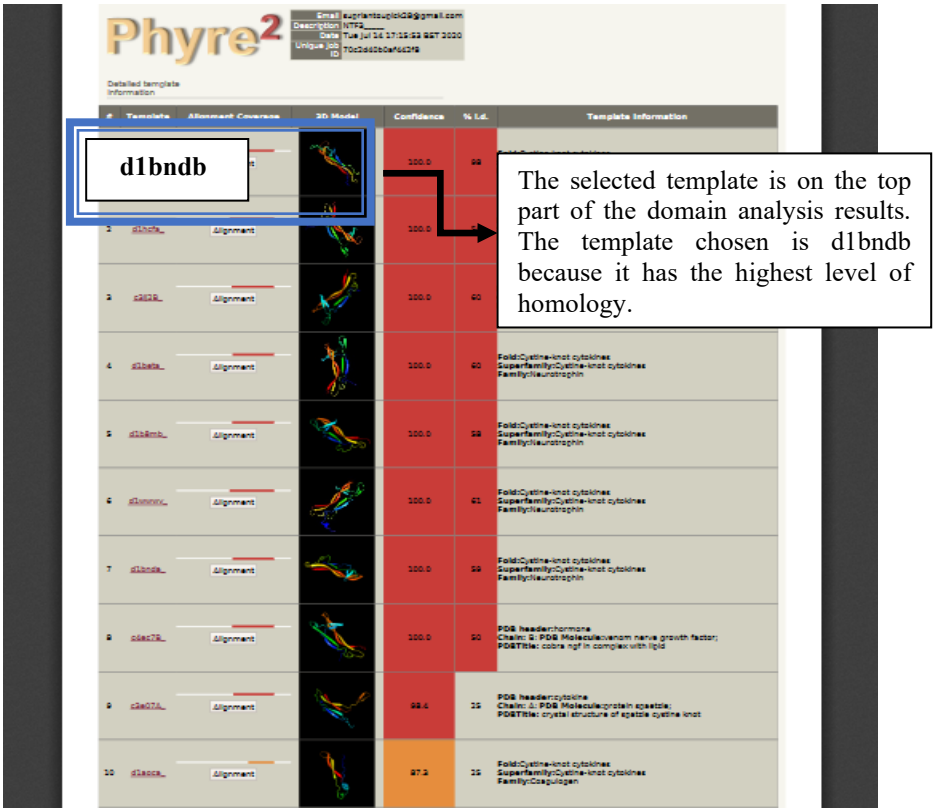


Figure 3. Tracing template of the protein

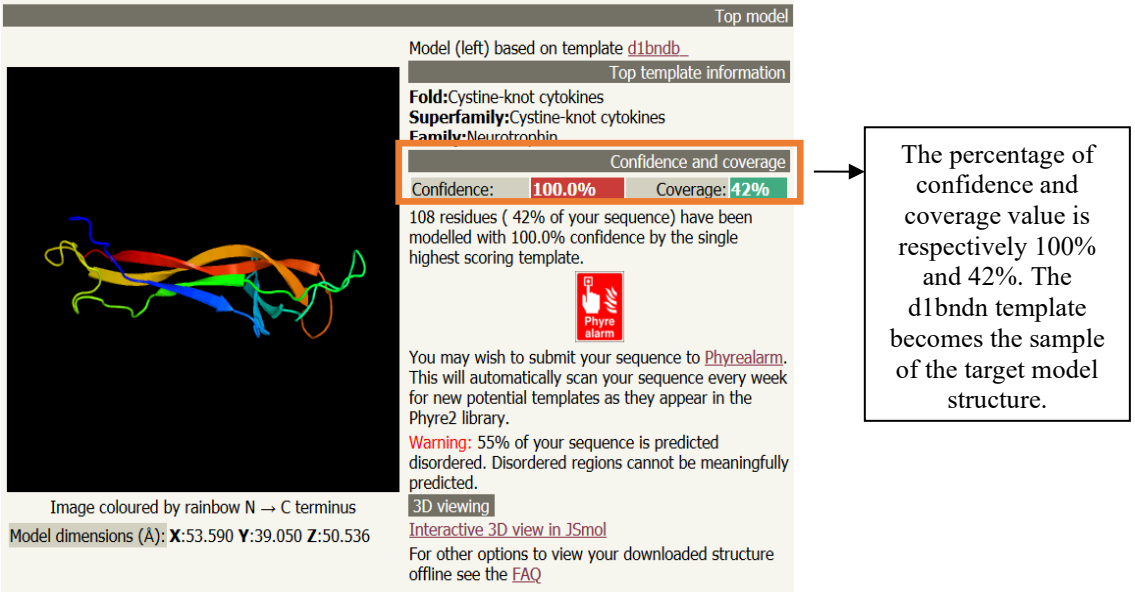


Figure 4. The accuracy of the model structure of the d1bndn template

2.4 The analysis of the tertiary structure of the target protein

The Consurf Server is used for analyzing the tertiary structure of the target protein, which can be accessed on https://consurf.tau.ac.il/ [15]. The analysis results showed the quality of residues and the atom that constructed the tertiary structure of the target protein. These include the conserved area of the sequences, the structure types, the residues descriptions, and the atom types.

3. Results and Discussion

3.1 The alignment of sequences and phylogenetic

The study of the genetic variation of the target protein (NT-3) in humans and other primates indicated a close relationship. Based on the alignment results of the target sequence and several primate species, it is clear that there is a difference between the first amino acids and the ninetieth amino acids, from the total alignment length of 348 aa. This difference explicates that around 258 amino acids are similar to amino acids from the 90th to 258th amino acids, implying that humans are closely related to several other primate species (Figure 5). The alignment results based on the Blast result have the appropriate data accuracy after the target sequence is aligned using ClustalW in the Bioedit version 7.2.5 program. The similarity level starts from the 90th residues to the 340th residues indicates that humans and several primate species have a nearly high level of similarity, which can be seen by looking at the similarity of the sequence composition of the target protein (Figure 6).

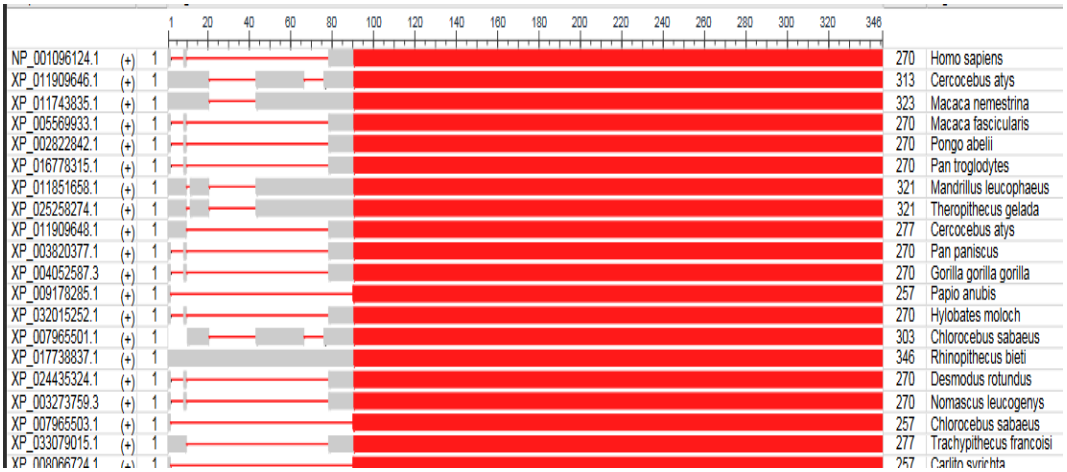


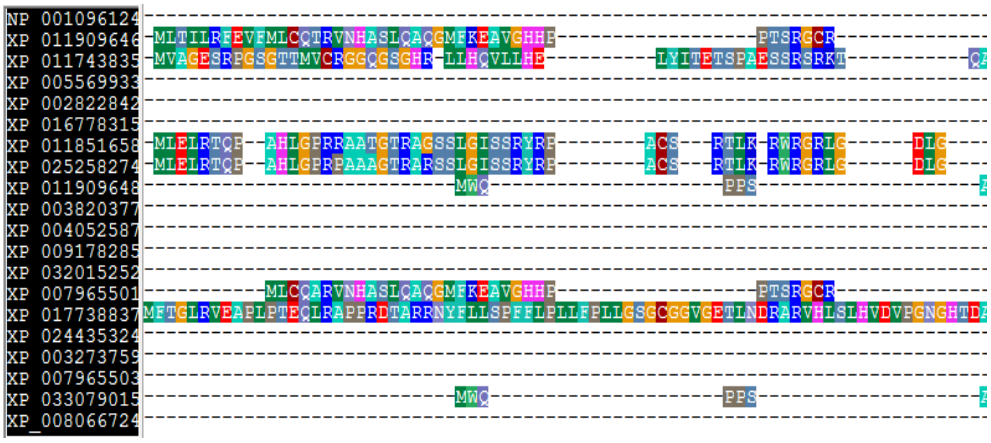
Figure 5. The sequence alignment based on the BLAST result

The Bioedit analysis results illustrated the data similarity to the nucleotide sequences obtained from the BLAST alignment results, which meant that the relationships between humans and primates are pretty close in terms of the homology of the NT-3 protein.

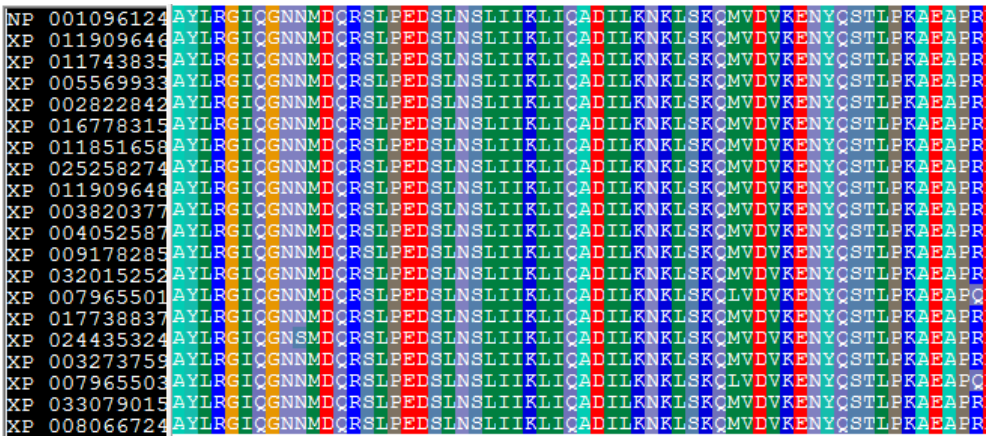
Phylogenetics is the study of the relationships between organisms based on their genetics, tracing evolutionary relationships, and the life history of a species [16]. The phylogenetic analysis and the alignment of the target sequence used data from the BLAST results of the target protein by paying attention to the similarity of the samples indicated in the BLAST results based on the database from GenBank. Several factors, such as genetic drift and natural selection, influenced the differences of genes among species [17]. The higher the value of genetic distance, the further the kinship is, and the lower the value of genetic distance, the closer the kinship is [18]. Molecular phylogenetics is the study of the evolutionary relationship between organisms using molecular data in the form of nucleotide or amino acid sequences [19].

The development of molecular techniques such as PCR (polymerase chain reaction) and DNA or protein sequencing in molecular biology has proceeded at a fast rate. The use of DNA or protein sequences in phylogenetic research has been carried out at all taxonomic levels, including the ethnic, genus, and species levels. Sequence analysis has proximity genetic relationships identified by their occupation of adjacent branches on a phylogenetic tree [20]. Phylogenetic trees are branching diagrams that help us to understand the biological evolutionary relationships between species [21]. The results of a phylogenetic tree can show the relationship between humans and some primates. It uses the NT-3 protein as the determiner. A strong categorization of the bootstrap value should be more than 85% for the data to be reliable. Reliable bootstrap value indicates a moderately high level of trust in the branch of a node formed by the protein [22].

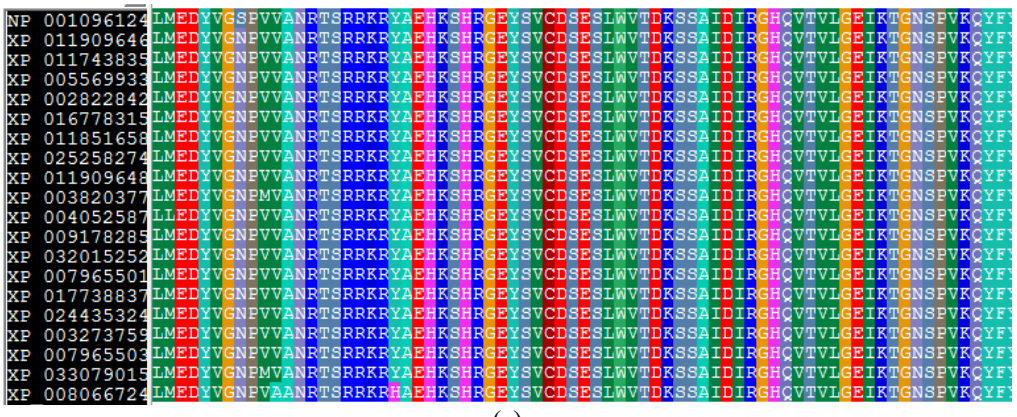
The results showed that the phylogenetic analysis between humans and several other primates had a significant bootstrap value, which meant the confidence level of the branches or nodes was very accurate according to the operation of the Construct/Test UPGMA Tree using MEGA X (Figure 7).



(a)



(b)



(c)

Figure 6. The sequence alignment based on ClustalW

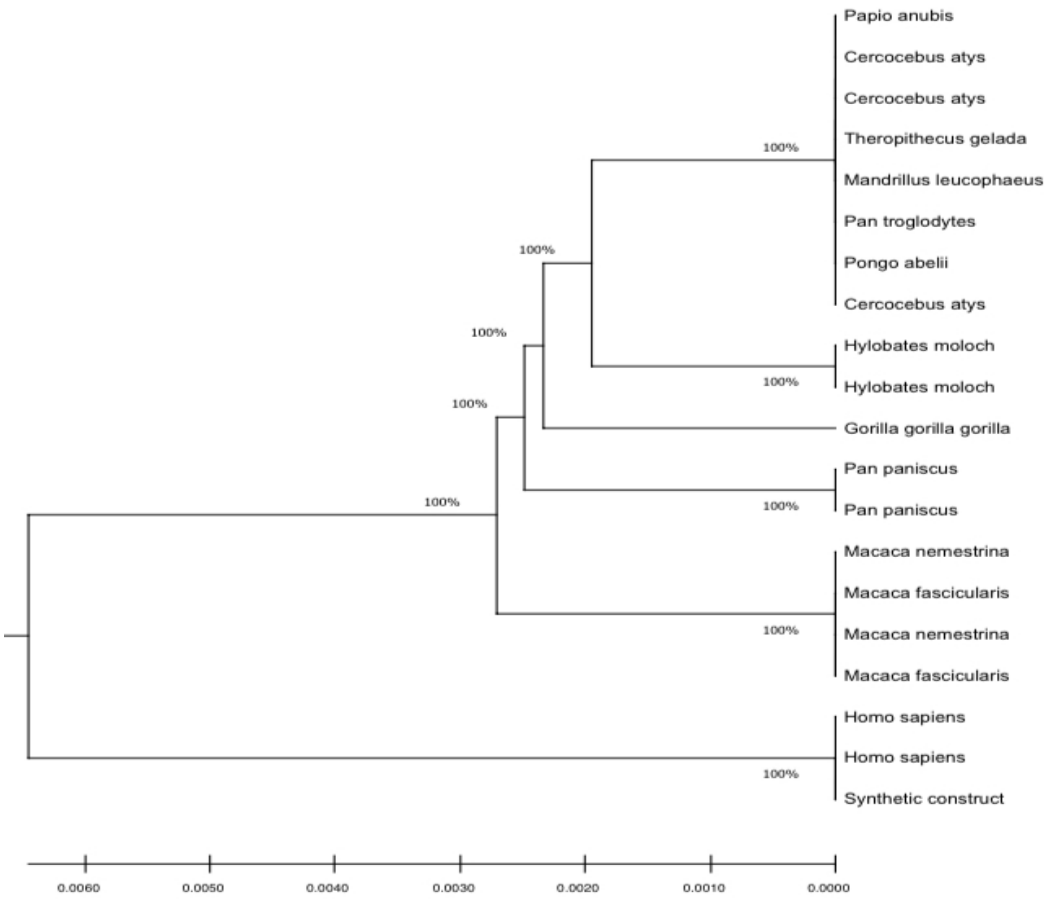


Figure 7. Diagram of the Construct/Test UPGMA tree

3.2 The composition of protein expressed by target genes

The protein composition was analyzed using Bioedit version 7.2.5. The analysis results showed the variations in the number of each type of amino acid building block of protein expressed by the target gene. Overall, this protein consists of 257 amino acids with a molecular weight of 29151.03 Daltons (see Table 1). Amino acids have a role in regulating various processes related to gene expression, including the modulation function of proteins that mediate the translation of messenger RNA (mRNA) [23]. Based on the analysis of the target protein composition, there were some amino acids with different frequencies, the amino acids serine, arginine, and leucine occurred in greater numbers of residues than other amino acids, presenting as 24 (9,3%), 22 (8,6%), and 22 (8,6%), respectively. Furthermore, based on the obtained data, 155 amino acids were in the group of nonessential amino acids, while the other 102 amino acids were in the group of essential amino acids. The human body cannot produce essential amino acids. However, the human body can produce nonessential amino acids. Essential and nonessential amino acids formed from atoms of carbon, hydrogen, nitrogen, oxygen, and sulfur atoms. Amino acids play an important role in the human body [24-26].

Table 1. The characteristics of the target protein using Expasy Protparam tool

Amino Acid	Number	Mol (%)
Ala (A)	13	5.1 %
Arg (R)	22	8.6 %
Asn (N)	12	4.7 %
Asp (D)	13	5.1 %
Cys (C)	6	2.3 %
Gln (Q)	12	4.7 %
Glu (E)	15	5.8 %
Gly (G)	13	5.1 %
His (H)	4	1.6 %
Ile (I)	15	5.8 %
Leu (L)	22	8.6%
Lys (K)	18	7.0 %
Met (M)	5	1.6 %
Phe (F)	4	1.6 %
Pro (P)	14	5.4 %
Ser (S)	24	9.3 %
Thr (T)	13	5.1 %
Trp (W)	4	1.6 %
Tyr (Y)	11	4.3 %
Val (V)	17	6.6 %
Pyl (O)	0	0 %
Sec (U)	0	0 %
Length	257 Amino Acid	
Molecular Weight	29354.56 Daltons	
Total number of negatively charged residues (Asp + Glu): 28		
Total number of positively charged residues (Arg + Lys): 40		

The analysis results of the target protein sequence using the Protparam tool explained that 4132 atoms comprised the target protein. These atoms and their numbers of atoms were: carbon (1287 atoms), hydrogen (2071 atoms), nitrogen (377 atoms), oxygen (386 atoms), and sulfur (11 atoms), and the molecular formula of the target protein was $C_{1287}H_{2071}N_{377}O_{386}S_{11}$. The instability index of the target protein was 53.63%, meaning that the stability of the target protein was only 46.37%. The protein stability index shows that the target protein is unstable because the percentage of instability exceeds the expected stability. The instability index of a protein should be less than 40%, and the obtained result in our work clearly exceeds this value [27]. The aliphatic index of the target protein was 80,39%, with average of hydropathicity of -0.608. Aliphatic amino acids are amino acids that contain aliphatic side chains of functional groups. These amino acids are composed of non-polar and hydrophobic amino acids. The aliphatic index of a protein indicates the relative volume occupied by amino acids such as alanine, valine, isoleucine, and leucine, which have aliphatic side chains in their structure. The aliphatic index is a positive factor that can increase the thermostability of a globular protein [28, 29]. Hydropathic GRAVY (Grand Average of Hydropathicity) is a parameter that determines the hydrophobic nature of a protein. If the hydropathic index value of an amino acid is higher, the more hydrophobic the amino acid is [30]. The analysis results showed that the GRAVY value of the target protein indicated that the NT-3 protein in humans was hydrophilic. This property will affect the protein folding process in forming

a tertiary structure because the hydrophilic properties of the protein can complicate the forming of a decent tertiary structure.

3.3 Conserved region

Based on the analysis results of the structure of the target protein model, there are three categories of amino acid residues such as the conserved region, the average region, and the variable region (Figure 8). Each residue is represented based on the characteristic colors of the target protein structure. Figure 8a is a visualization of a model of the target protein. Figure 8b shows the distribution of amino acid residues within the conserved region (purple). Figure 8c represents the distribution of amino acid residues of the variable region (green) and Figure 8d indicates the distribution of amino acid residues in the average region (white).

The three-dimensional structure of the NT-3 protein in humans was constructed using the Consurf Server, and in particular the conserved areas of amino acid residues were determined. The conserved region explains the evolutionary relationship between species. The conserved regions of the amino acid residue indicate that the residue has an important role and can be one of the active sites of proteins in carrying out its functions [31].

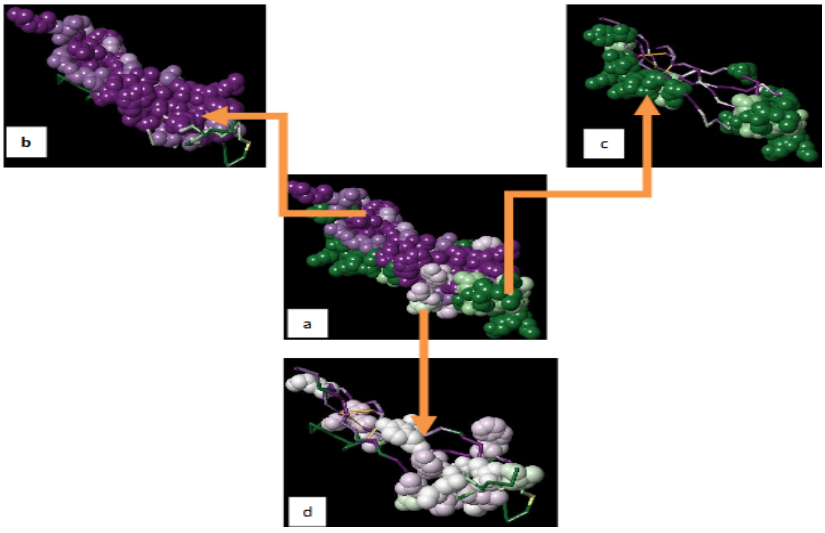


Figure 8. The visualization of model (a), the conserved region (b), the variable region (c), and the average region (d)

Based on the sequence analysis, several amino acids belonging to the conserved region have an essential role in the NT-3 protein regulation in humans. The black squares in Figure 9 show the amino acids belonging to the conserved region. The amino acid sequences of 1-41 and 101-251 are groups of amino acids included in the conserved region of the NT-3 protein in humans. Meanwhile, the amino acid sequences of 42-100 of the target protein are categorized in the variable region (Figure 9). Furthermore, this indicates that the region is not a conserved region, so it is unlikely that this area will affect the active side of NT-3 protein.

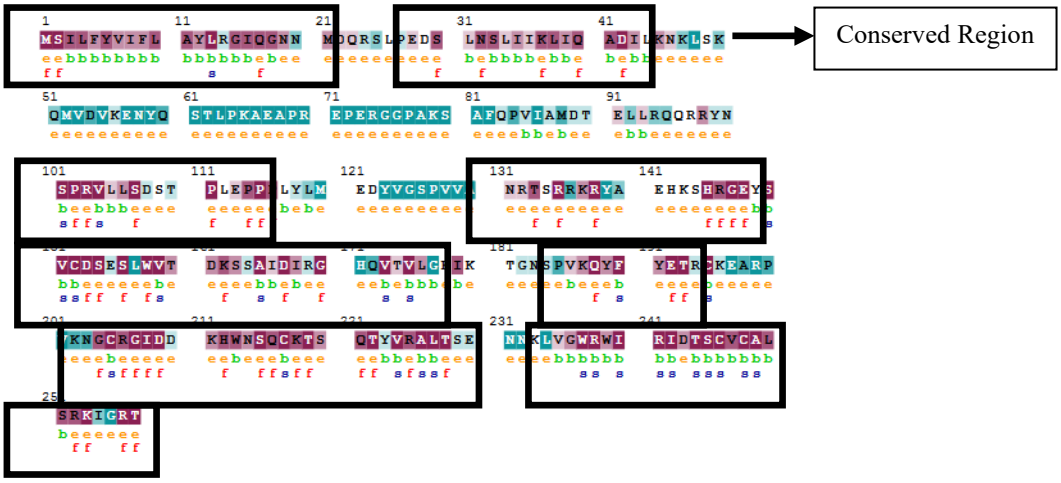


Figure 9. The analysis of conserved region of the target sequences

3.4 The tertiary structure of target protein

Tertiary structure of protein has several forms depending on the type of amino acid and its bonds. In general, proteins can form tertiary structures such as beta-strand, alpha-helix, and coil. Based on the results, the obtained model can create the beta-strand structure and coil structure without creating the alpha-helix forms (Figure 10).

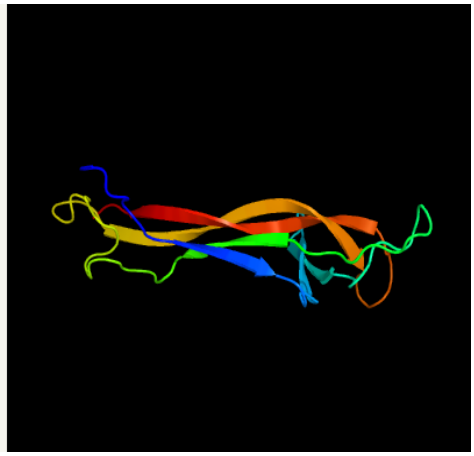
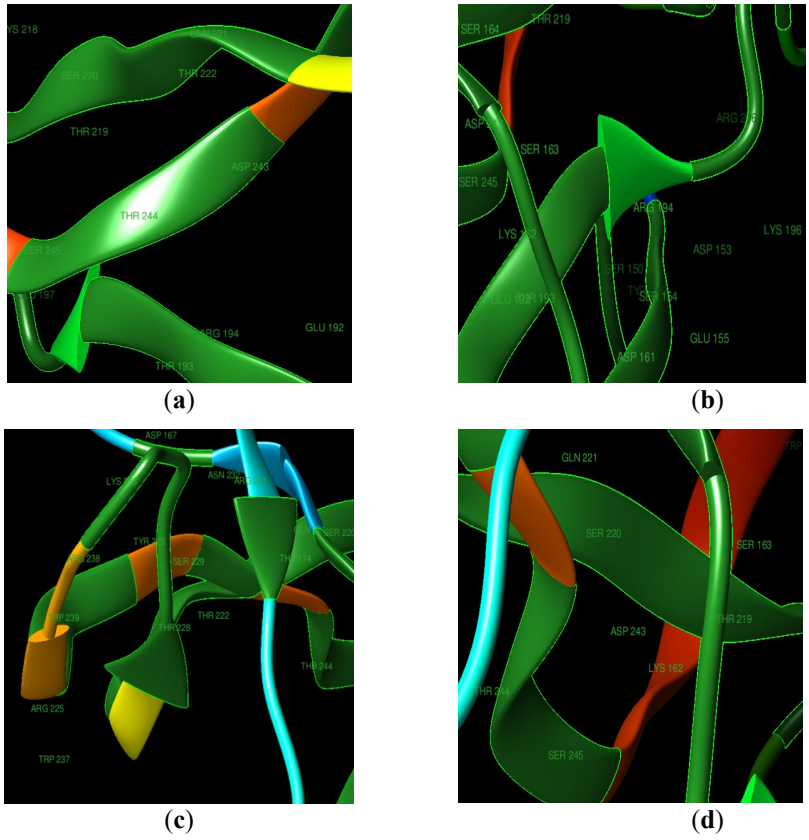


Figure 10. The tertiary structure of the target protein

The polar region of the tertiary structure of the target protein was created using the Chimera program. The analysis results indicate that there are some regions classified in the polar region with various amino acids. The polar group consists of 10 amino acids, two negatively charged—aspartic and glutamic acid, three positively charged—arginine, lysine, and histidine, and five uncharged—asparagine, glutamine, serine, threonine, and tyrosine [32]. Based on the analysis result, some parts of the protein structure are polar, and those parts are quite soluble in water because they create an

ionized region. Hydrophilic or water-soluble amino acids have ionized or polar side chains. At neutral pH, arginine and lysine are positively charged, whereas aspartic acid and glutamic acid are negatively charged, existing as aspartate and glutamate. These four amino acids are the main contributors to the overall load of proteins [33]. The polar structure in the target protein plays an essential role as it is the main contributor to the overall of the protein charge that affects the properties and biochemical functions of the protein (Figure 11).

The analysis results using the Chimera program showed the presence of several amino acid residues, namely arginine, histidine, lysine, serine, glutamine, tyrosine, asparagine, threonine, aspartic acid, and glutamic acid. These amino acid residues have polar properties. A number of the polar amino acid residues obtained have different properties and characters; for example, aspartic and glutamic acids have negative loads and more carboxyl groups than arginine, lysine, and histidine acids which have positive loads and more amine groups. In addition, some amino acids such as asparagine, glutamine, threonine, and tyrosine have no loads on the R group (the side chain). However, these amino acids play an essential role in hydrogen bond formations in protein molecules. These amino acids have polarity and are hydrophilic, meaning that they dissolve in water. Figure 11 (a, b, c, d) shows the surface of the target protein structure that has polarity due to its polar amino acids. Each Figure shows the number of polar amino acids that make up the target protein. The distribution of polar amino acid residues in Figures 11 a, b, c and d varies and has different characters depending on the type of amino acid and its load.



(**a**) (**b**)

(**c**) (**d**)

Figure 11. The polar region of the structure of the target protein (a, b, c and d)

Hydrophobic amino acids have aliphatic side chains, which are insoluble or only slightly soluble in water. The side chains of alanine, valine, leucine, isoleucine, and methionine are all composed of hydrocarbons, except for the sulfur atom in methionine, and they are all nonpolar. Phenylalanine, tyrosine, and tryptophan have large aromatic side groups. Hydrophobic molecules avoid water and combine into droplets of oil or waxes [33]. Variations in the type of amino acids that make up the target protein model have varying values and amounts, consisting of protein, polar, nonpolar, backbone polar, backbone nonpolar, side-chain polar, or side-chain nonpolar types (Table 2).

Table 2. The description of the residues

The Assessment Type	Z-Score AVE	Z-Score RMS	Total
Protein	0.41	1.09	278
Polar	0.57	1.18	111
Nonpolar	0.31	1.03	167
BB-Polar	0.63	1.14	98
BB-Nonpolar	0.31	1.08	104
SC-Polar	0.11	1.43	13
SC-Nonpolar	0.31	0.95	63

Hydrophobic amino acid interactions are an entropic effect that results from the disruption of the very dynamic hydrogen bonds between water molecules by nonpolar solutes that form clathrate structures around nonpolar molecules. This formed structure is more orderly than the free water molecules that arrange themselves to interact as much as possible with themselves, and thus result in a higher entropic state that causes the nonpolar molecules to clump together and reduce the exposed surface area of the protein [34]. In-silico analysis of the surface area of the structure of the target protein model shows a breakdown of the surface structures, not including water, based on the chemical type (Table 3). Besides, this analysis provides a way to explain the model structure folding process based on the shapes and bond types between atoms.

Analysis of the surface area of the calculated structure, not including water, based on the chemical type reveals that the chemical types of the highest number are CH_2, CH_3, and O. The source of an amino group in a protein is usually nitrogen from organic matter. Theoretically, nitrogen in organic matter exists as amino acid groups in protein [35]; however, the analysis of the NT-3 tertiary structure describing the amino groups of the model does not reveal nitrogen. The numbers of carbon, hydrogen, and oxygen atoms are greater than the number of other types of atoms. The NTF-3 gene is spread all over the central nerve system and the peripheral nerves and influences the chemical types of the protein structure. The NT-3 protein has a large amount of aliphatic side chains (non-aromatic hydrocarbons), and can influence the variation of chemical type in the target protein. The major and characteristic aliphatic amino acids feature the CH_2, CH_3, O, OH, S, and H_2N chemical types. The chemical types of the protein structure can determine the characteristics of the protein in detail [36].

4. Conclusions

The results of this study indicated that humans have close genetic relationships with several primate species. This was based on phylogenetic bootstrap values and the analysis of the protein sequence alignments had significant levels of similarity. The target protein was composed of 257 aa with a weight of 29353.03 Daltons. Amino acids of the highest percentage value were serine, arginine,

Table 3. The statistical surface area of the calculated structure, not including water, based on the chemical type

No.	Chemical Type	Surface Area
1	C	288 (3.7%)
2	CH^1	448 (5.8)
3	CH^2	1683 (21.8%)
4	CH^3	967 (12.8%)
5	CR^{15}	153 (2.0%)
6	CR^{16}	510 (6.6%)
7	CR^5	15 (0.2%)
8	CR^{56}	19 (0.2%)
9	CR^6	43 (0.6%)
10	N	-
11	NC^1	72 (0.9%)
12	NC^2	524 (6.8%)
13	NC^3	475 (6.2%)
14	NH^1	127 (1.6%)
15	NH^2	249 (3.2%)
16	NR^{15}	81 (1.1%)
17	NRD^5	85 (1.1%)
18	O	1022 (13.2%)
19	OC	495 (6.4%)
20	OH^1	463 (6.0%)
21	S	-
	Total	7715 (100.0%)

and leucine. Amino acid sequences 1-41 and 101-251 were conserved areas. The target protein structure consisted of a beta-sheet and a coil structure. The protein structure had a hydrophilic polar group. The surface of the tertiary structure of the target protein consisted of 7715 chemical types and was largely composed of CH_2, CH_3, and O. The results can be used as the basis for further study of genetic information on proteins expressed by NTF-3 in humans and its relationship to pathophysiology.

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