

RESEARCH / INVESTIGACIÓN

Genomic benchmarking studies reveal variations of the polyubiquitination domain of the PSD95 protein in *Homo neanderthalensis* and other primates of the Hominidae family: Possible implications in cognitive functions?

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DOI. 10.21931/RB/2021.06.01.23

Abstract: Modern humans' unique cognitive abilities regarding Neanderthals and other primate's lineages are frequently attributed to the differences in brain size development and evolution. However, recent studies have established the critical role of genomic and genetic benchmarking in analyzing the cognitive evolution between modern humans and primates, focused mainly on searching for involved genes in neurogenesis. PSD95 protein (named PSD95p) has a key role in modulating synaptic plasticity, learning, and memory skills. Thus, the present study aimed to determine the possible variations of the PSD95 gene between modern humans, Neanderthals, and other hominid primate species using bioinformatics tools. The results showed 14 polymorphisms compared with the contemporary human PSD95 gene, of which 13 were silent mutations, and only one was a non-silent mutation at the nucleotide position 281. Despite polymorphisms found at the nucleotide sequences, the PSD95p of humans and chimpanzees are 100% identical. Likewise, the gorilla and orangutan PSD95p are 100% identical, although a 103-amino acid deletion characterizes them at the N-terminal end (1-103), suggesting that it behaves like a non-functional protein. Interestingly, the single nucleotide polymorphism (SNP) found at position 281 in the Neanderthal PSD95 gene leads to a change of the E⁹⁴ to valine V⁹⁴ in the polyubiquitination domain (PEST) and variation in the three-dimensional structure of PSD95 protein. We prompt that this structural change in the PEST domain could induce a loss of PSD95p function and, therefore, an alteration in synaptic plasticity forms such as long-term potentiation (LTP) and long-term depression (LTD). These findings open a possible hypothesis supporting the idea that humans' cognitive evolution after separating our last common ancestor with Neanderthals lineage could have been accompanied by discrete changes in the PSD95p polyubiquitination domain.

1593

Key words: Evolution, cognitive development, intelligence, memory, ARC gene, PES.

Introduction

Since our last common ancestor's divergence with Neanderthals, the early humans' evolution process, between 430,000 to 765,000 years ago¹⁻⁴, and with chimpanzees, between 4 to 7 million years ago^{5,6}, has become a subject of great controversy. One of the most relevant issues within this matter is precisely the interbreeding that humans and Neanderthals could have after migrated from Sub-Saharan Africa in two events. The first time carried out 100,000 years ago⁴ and the second time 50,000 years ago^{3,7-10}. Based on studies of fossil remains, it has been reported that the second interbreeding event allowed the introgression of around 6-9% of the Neanderthal genome into the early human genome^{4,7}. However, due to positive selection processes, a recent Asian and European genomes analysis has shown that the percentage of genetic segments introgressed from Neanderthal to human fell 1-3%^{3,7,11,12}.

The persistent Neanderthal genes in the genome of modern non-African humans are mainly those involved in phenotypic traits, directly influenced by the environment and other genes involved in both cognitive and skeletal development^{8,9}. Consequently, these genes have been linked to the expansion of modern humans throughout Europe, which subsequently led to the disappearance of Neanderthals 30,000 years ago^{8,13}. Nevertheless, it is evident that beyond a disappearance, Neanderthals were diluted in the early human genome.

On the other hand, despite the evolutionary processes in time and space between modern humans and chimpanzees,

unlike Neanderthals, it has been found that their genome is 98.7% identical^{5,6}. Despite their high identity percentage, likewise other hominids, chimpanzees also differ from modern humans in many morphological, behavioral, and cognitive characteristics⁵.

Modern humans' behavior and unique cognitive abilities concerning Neanderthals and other hominid primates have been attributed to the brain's evolution process^{5,14-18}. Several studies have suggested that the lower development of the brain's cranial capacity and organization could be the possible cause of the Neanderthal extinction^{14,18-20}. Based on these facts, it has been reported that the Neanderthal adaptive system was unable to survive the competitive environment with modern humans²⁰⁻²², therefore, emphasizing that modern humans had a more remarkable cognitive ability to develop adaptive strategies^{4,7}. However, a report described that the Neanderthals and modern humans had similar brain sizes, advocating that it is wrong to infer differentiated cognitive development between these two species based on archaeological data analysis²³. Recent studies reveal the importance of molecular biology and bioinformatics tools for the cognitive development investigation between modern humans and their common ancestors, which are focused mainly on the search of involved genes in neurogenesis, a mechanism associated with learning and memory developments^{6,18,24}. Therefore, it has been suggested that the evolutionary development of the brain is a direct cause of a more significant generation of neural

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networks, hence the rounded form that has been attributed to the modern human brain compared with the elongated shape of the Neanderthal brain²⁴.

Likewise, it has been found that the regulation of genes involved in the migration and detrital development of neurons has led to a dissimilar functional development between the neurons of humans, chimpanzees, and bonobo¹⁸. *In-silico* analysis done in this work are not enough to confirm that the polymorphism of the PSD95 can be associated with neural networks. However, the results open a window to the scientific community because they allow knowing and infer a possible cause in the cognitive development differences between Neanderthals and modern humans and other primates.

It is known that the learning process through memory lies in the organization of neurons and their interactions²⁵. Memories are stored in a neuronal circuit by synapse mechanisms and specific neurons²⁶. Synaptic plasticity or dynamic changes of neuronal connections change the neuron's chemical composition and its capacity to communicate with its neighbors. Also, that allows the generation of new functional neurons (neurogenesis), contributing even more to plasticity²⁷. The me-

chanisms involved in synaptic plasticity changes are long-term potentiation (LTP) and long-term depression (LTD), characterized by an increase and weakening of the synaptic force, respectively. Therefore, the favoring of LTP and the development of memory and learning have been associated with an increased expression of the PSD95 gene, which encodes a protein of postsynaptic density 95 (PSD95p) characteristic of an electrodense region called postsynaptic density^{28,29}.

PSD95p is a specialized scaffold protein composed of several multiple protein interaction domains that include: i) a polyubiquitination domain (PEST), ii) three PDZ domains, iii) an SH3 domain, and iv) a guanylate kinase (GK)-like domain^{30,32}. PSD-95p is the best-studied protein of the guanylate kinase-associated membrane family (MAGUK)³⁰. Within this family, there are other scaffolding proteins such as SAP102, SAP97, and PSD93³². PSD95p with PSD93p can interact in postsynaptic sites to form a multimeric scaffold for grouping associated receptors, ion channels, and signaling proteins^{30,33,34}. As shown in Figure. 1, PSD95p is the anchoring axis of a complex of postsynaptic proteins, ion channels, and NMDA receptors (N-methyl-D-aspartate) and AMPA (α -amino-3-hydroxy-5-

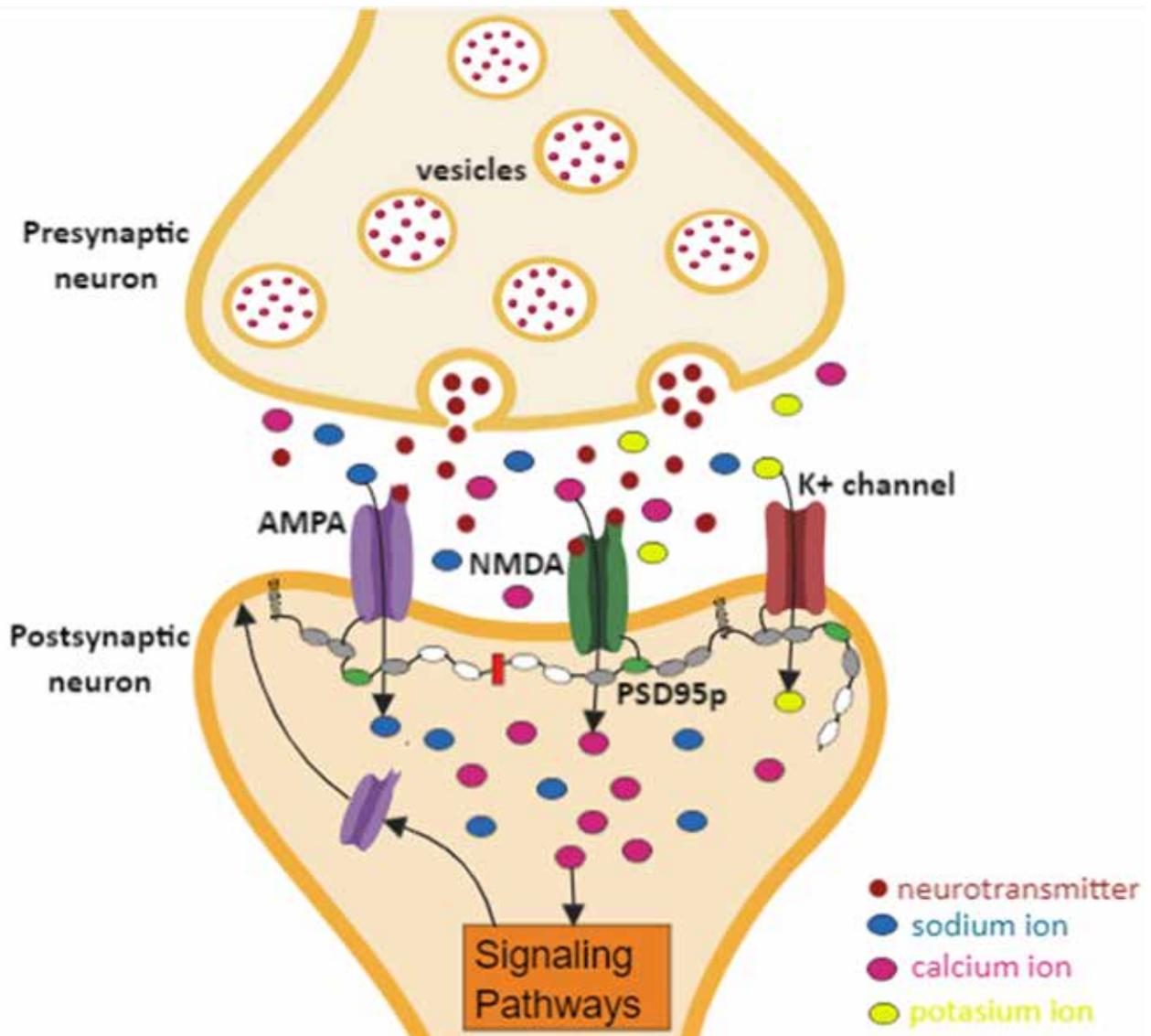


Figure 1. Interaction of PSD95p with AMPA and NMDA receptors. Generation of signaling pathways to increase the localization of AMPA receptors to the postsynaptic membrane; thus, inducing an increase of the strength of the synaptic connections between neighboring neurons.

methyl-4-acid isoxazole propionic) type. The presence of the NMDA and AMPA proteins is essential in the synaptic plasticity and cognitive functions development. However, their correct function will depend directly on the presence of PSD95p. For this reason, this study was only focused on the function of PSD95p, which plays an essential role in synaptic plasticity during LTP by allowing better localization of AMPA receptors in the synaptic membrane; thus, inducing an increase in the synaptic connections' strength between neighboring neurons³³⁻³⁷.

Experiments performed in mice have shown that mutations in the gene that encodes the PSD95 protein lead to a deficit in learning and memory abilities, abnormal social behavior, repetitive behaviors, increased stress, anxiety-related responses, and different motor alterations^{34,38}. In the case of mutations and erroneous genetic variants in the ARCp-PSD95p complex, it has been found that there may be a high susceptibility to schizophrenia, autism disorders, intellectual disabilities, epileptic mutations, and abnormal variants in intelligence^{33,39,40}. In future works, mutation analysis in the other proteins involved with cognitive development, such as ARCp, AMPA, and NMDA receptors, among others, will be elucidated.

In this research, we hypothesized that if in an evolutionary past, humans acquired specific variations in the nucleotide sequence of the PSD95 gene, then the structure and function of PSD95p, as well as the development of cognitive abilities, could have been improved compared to Neanderthals and other primates of the Hominidae family.

(Neanderthal), *Pan troglodytes* (Chimpanzee), *Pan paniscus* (Bonobo), *Gorilla gorilla* (Gorilla) and *Pongo abelii* (Orangutan). The PSD95 nucleotide (without introns) and PSD95p amino acid sequences of human, Chimpanzee, bonobo, gorilla, and orangutan were downloaded from the Ensembl Database⁴¹ and GenBank database⁴². The Neanderthal's nucleotide and amino acid sequences were downloaded from the Neanderthal Genome database⁴³, as shown in Tables 1 and 2.

Due to the present study's complexity, only one sequence was selected per Hominidae primate's species studied. However, in the case of Neanderthals, there is only one access to a sequenced genome in the Neanderthals Genome database.

In spite that this approach only uses one sequence by a specimen to make the comparison between species regarding PSD95 genes and proteins, it is useful to release scientific questions and hypothesis and to planning strong *in vivo* and *in vitro* experiments allowing to determine the relationships between variations of the polyubiquitination domain of the PSD95 protein and its possible implications in cognitive functions in Homo neanderthalensis and other primates of the Hominidae family, including modern human adequately.

Prediction of 3D protein structures

Prediction of the tertiary structure of human PSD5p was performed with Protein Homology/analogy Recognition Engine V 2.0 (PHYRE2) server⁴⁵, which also allowed us to predict the 3D structure domains have not been crystallized previously. Prediction of the PSD5p 3D structures of Neanderthal, Chim-

Organism	Scientific name	Chromosome	Orientation	ID (%)	E-value	Accession	References
Human	<i>Homo sapiens</i>	17:7196755-7197052	Reverse	100	0	U83192.1	Stathakis, Hoover, You, and Bryant (1997)
Neanderthal	<i>Homo neanderthalensis</i>	17:7033933-7063745	Reverse	99.91	0	ENSG00000132535	The Neanderthal Genome Project - May 2010
Chimpanzee	<i>Pan troglodytes</i>	17:7450950-7451247	Reverse	99.83	0	XM_16931399.2	MODEL REFSEQ.
Bonobo	<i>Pan paniscus</i>	17:7214437-7214734	Reverse	99.48	0	XM_03810105.3	MODEL REFSEQ.
Gorilla	<i>Gorilla gorilla</i>	17:7202712-7203009	Reverse	99.67	0	XM_19013126.1	MODEL REFSEQ.
Orangutan	<i>Pongo abelii</i>	17:7210380-7210677	Reverse	99.17	0	XM_24234382.1	MODEL REFSEQ.

Table 1. General characteristics of the studied Hominidae primates PSD95 genes.

Organism	Scientific name	ID (%)	E-value	Accession	References
Human	<i>Homo sapiens</i>	100	0	AAC52113.1	Stathakis, Hoover, You, and Bryant (1997)
Neanderthal	<i>Homo neanderthalensis</i>	99.87	0	ENSG00000132535	The Neanderthal Genome Project - May 2010
Chimpanzee	<i>Pan troglodytes</i>	100	0	XP_016786888.1	MODEL REFSEQ.
Bonobo	<i>Pan paniscus</i>	99.61	0	XP_03810153.1	MODEL REFSEQ.
Gorilla	<i>Gorilla gorilla</i>	100	0	XP_018868671.1	MODEL REFSEQ.
Orangutan	<i>Pongo abelii</i>	100	0	XP_024090150.1	MODEL REFSEQ.

Table 2. General characteristics of the studied Hominidae primates PSD95 proteins.

The multiple sequence alignments were performed with the MUSCLE server directly linked to the Molecular Evolutionary Genetics Analysis software, MEGA X 10.0.5⁴⁴

Materials and methods

Obtaining PSD95 sequences and multiple alignments

The primates selected for the present study were chosen based on the species belonging to the Hominidae clade, specifically: *Homo sapiens* (Human), *Homo neanderthalensis*

panzee, bonobo, gorilla, and orangutan was performed by SWISS-MODEL server⁴⁶ previous model generated for humans as a template. Finally, the models obtained were analyzed using PyMOL 2.3 software⁴⁷.

Phylogenetic trees

Two phylogenetic trees were built using nucleotide and amino acid sequences of the PSD95 gene and the PSD95p. The

evolutionary history was performed using MEGA X⁴⁸, the maximum likelihood method. Additionally, the Tamura-Nei model was used⁴⁹ for nucleotides and the model Le_Gascuel_2008⁵⁰ for amino acids. To increase the reliability of the analyzed taxa's consensus trees, a bootstrap corresponding to 2500 replicates was established⁵¹.

To decrease the construction complexity of the consensus tree that presents an optimal topology, the heuristic search method was used. For this, an initial tree was automatically obtained, applying the algorithms of Neighbor-Join and BioNJ to a matrix of distances by pairs estimated using the Maximum Composition Probability (MCL) approach, for the nucleotide sequences. The heuristic search's initial tree was obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using a JTT model. The analysis involved 6 nucleotide sequences and 6 amino acid sequences.

Results

Polymorphisms are found in the PEST domain of the PSD95 gene in Neanderthal and other primates of the Hominidae family

Despite the high percentage of identity (Table 1), the results obtained showed 14 polymorphisms along the PSD95 gene (Figure. 2A). The Neanderthal PSD95 gene is different from the human gene only by replacing one nucleotide at position 281 (A²⁸¹ by T²⁸¹). Simultaneously, the Chimpanzee is different from humans by substituting two nucleotides at positions 75 and 1503 (A⁷⁵ by C⁷⁵ and T¹⁵⁰³ by C¹⁵⁰³). The bonobo PSD95 gene is discriminated from humans by the substitution of 2 nucleotides at positions 75 and 1494 (A⁷⁵ by C⁷⁵ and T¹⁴⁹⁴ by C¹⁴⁹⁴) and by deletion of 9 nucleotides at positions 279-289 that are highly conserved in humans, Neanderthal, and Chimpanzee (5'...GAGTGMCA...3'). Gorilla and orangutan were the species that presented a lower percentage of identity with humans (99.67 and 99.17, respectively). This differentiation is related to the substitution of 5 and 8 nucleotides, respectively, and the deletion of 309 nucleotides in the 5'-end at positions 1-309 concerning the human, Neanderthal, Chimpanzee PSD95 genes.

Change of A²⁸¹ by T²⁸¹ in the Neanderthal PSD95 gene leads to alteration of Glu⁹⁴ to Val⁹⁴ in the PEST domain.

Interestingly, as is shown in Figure 2B, the alignments of amino acid sequences showed that, from the 14 polymorphisms shown above, 13 correspond to silent mutations. Only one mutation is a non-silent type, which was found in the Neanderthal PSD95 gene. This single nucleotide polymorphism (SNP), based on substitution of adenine by thymine at position 281, led to a change of the glutamate residue at position 94 (E94R) by a valine (V94R) in the Neanderthal PSD95 protein. Similarly, the deletion of the 9 nucleotides in bonobo led to a modification of 3 amino acids (E94R-L95R-Q96R), and the deletion of 309 nucleotides in gorilla and orangutan led to a deletion of 103 amino acids at the N-terminal end of the protein (position 1-103). On the other hand, the results indicate that, despite the polymorphisms found in the nucleotide sequences, the PSD95p protein from humans and chimpanzees are 100% identical. Likewise, gorilla and orangutan proteins do not show specific amino acid changes. An important fact is that overall substitutions and deletions from species studied were found in the PEST domain of PSD95p, as shown in Figure 2C.

The evolutionary history based on the PSD95p protein indicates that modern humans have a greater kinship with chimpanzees than with Neanderthals.

Using the maximum likelihood method, the evolutionary lineage concerning the PSD95 gene shows us the three clades' formation; the first one groups includes human, Neanderthal, and gorilla. In this clade, we can observe that humans have a greater kinship with Neanderthals than with gorillas (Figure. 3A). The second clade includes Chimpanzee and bonobo, and the third one only consists of the orangutan.

Regarding the PSD95p protein, three clades are obtained (Figure. 3B). However, because the polymorphisms found were non-silent mutations, except for Neanderthal, differences were observed in the first two clades described above for the phylogenetic tree regarding the species' PSD95 genes studied. Thus, a first clade was observed for humans and chimpanzees, a second clade for Neanderthals, bonobo, and gorilla, where Neanderthal has a greater kinship with bonobo. Finally, no differences were observed in the third clade formed by the orangutan.

Discussion

Proteins can fold in a specific way depending on the amino acids that constitute them until forming three-dimensional structures. Consequently, the function of a protein is directly related to its structure. Based on the results based on PSD95p sequences, we have shown that chimpanzees have a higher percentage of identity with modern humans. However, studies have shown that the cognitive differences between these two species are not governed solely by structural changes in gene products as proteins and gene expression levels are involved in the different cognitive processes.

On the other hand, due to the SNP found in the Neanderthal PSD95 gene, a change of glutamate © by valine (V) is generated, leading to a modification in the 3D conformational of the protein, as shown in Figure. 4A-B. Thus, we believe that the function of the PSD95 protein from Neanderthal was possibly affected, with a concomitant reduction in cognitive function compared with modern humans.

Likewise, as a consequence of the SNP found in the Neanderthal PSD95 gene, a change of glutamate © by valine (V) is generated, leading to a modification in the 3D conformational of the protein and therefore in its function (Figure. 4).

According to previous studies, a change of the glutamate amino acid within the PEST domain may lose its function. The synaptic strength depends on the AMPA and NMDA type receptors, regulated in part by the PSD95 protein, which seems depleted^{52,53}. Then, based on the conservation of the E94R-L95R-Q96R motif (Figure. 5), it could be thought that the modification in the E94R residue in Neanderthal could have induced a loss of function of the PSD95p protein. Likewise, the signal peptide's de-palmitoylation at the N-terminal end of the protein can weaken synaptic strength and lead to an LTD. This sequence of about 24 amino acids, found upstream of the PEST domain when undergoing a palmitoylation process in cysteine, serine, or threonine residues, allows PSD95p to be coupled postsynaptic membrane for subsequent anchoring of receptors and other signaling and adhesion proteins^{36,54,55}. Therefore, based on the mentioned above, it could be inferred that the deletion of the N-terminal end of 103 amino acids in the gorilla and orangutan protein may be, among other elements, a probable cause that they have less cognitive development than humans. Since PSD95p doesn't have a palmitoylation

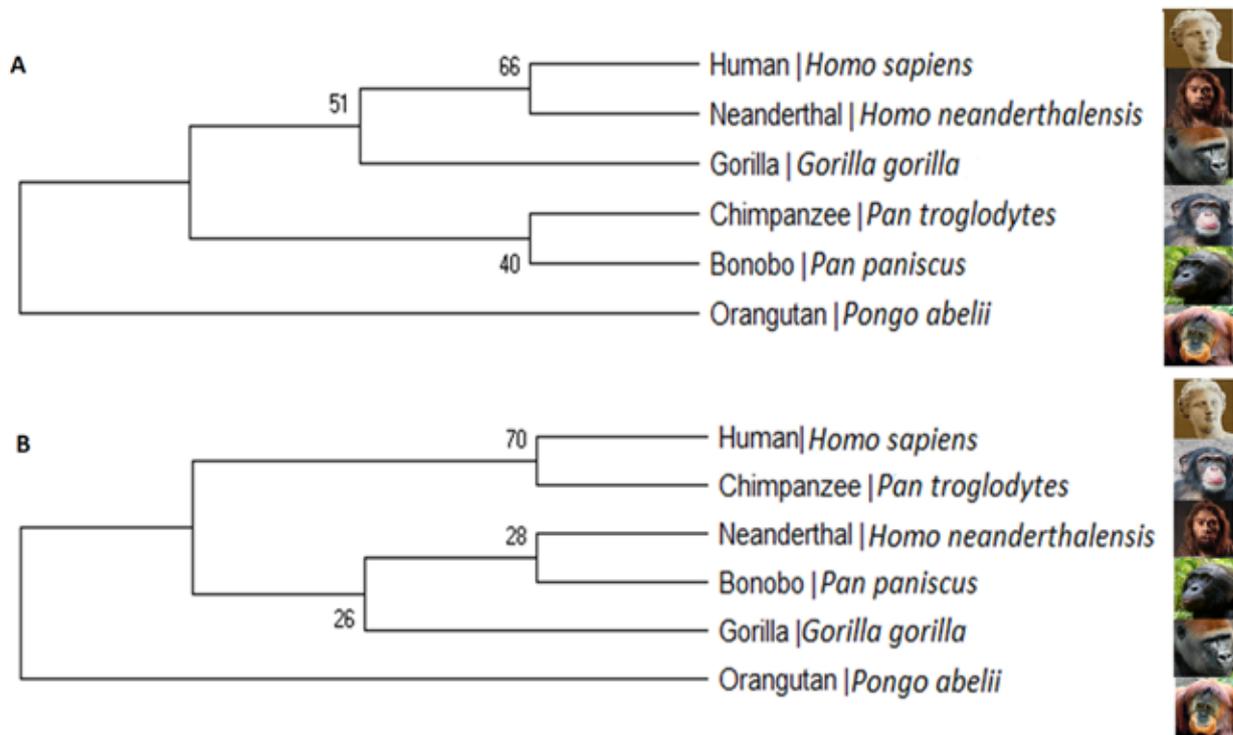


Figure 3. Evolutionary history of the Hominidae primate's family concerning the PSD95 gene and its encoded protein. (A) The genes' evolutionary history was inferred using the Maximum Likelihood method and the Tamura-Nei model. (B) The evolutionary history concerning the proteins was inferred by using the Maximum Likelihood method and Le_Gascuel_2008 model. The bootstrap consensus tree inferred from 2500 replicates both for genes and proteins represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (2500 replicates) are shown next to the branches.

pathologies, such as depression, post-traumatic stress disorder, and stroke are generated⁶². Therefore, it is suggested that proteolysis by the proteasome of human PSD95p induced by polyubiquitination could act as a regulatory mechanism in the disengagement of AMPA receptors to decrease the flow of Ca²⁺ ions dependent on NMDA receptors (Figure. 6), favoring in this way the modulation of the synaptic force between neighboring neurons and so the increase of cognitive capacity.

Conclusions

In conclusion, these findings support the idea that the cognitive evolution of modern humans after the separation of our last common ancestor with Neanderthals and other hominid primates could have been accompanied by discrete changes in the domain of polyubiquitination of PSD95p, which has a crucial role in the modulation of synaptic plasticity and cognitive development. However, it should be considered that PSD95p is not the only protein involved in synaptic plasticity processes. Therefore, to verify our claims, it is necessary to perform *in vitro* and *in vivo* experiments to evaluate that the change of glutamate by valine at position 94 in the PEST domain could lead to the loss of the PSD95p functions and concomitant deterioration of cognitive development. The *in-silico* findings presented in this work are only the beginning and could open a window for future robust investigations.

Acknowledgment

We thank the excellent opportunity to study at the prestigious Universidad Regional Amazónica Ikiam. The authors

thank A. Naranjo, for your grammatical support.

Competing Interests

Authors declare there is no competing of interest

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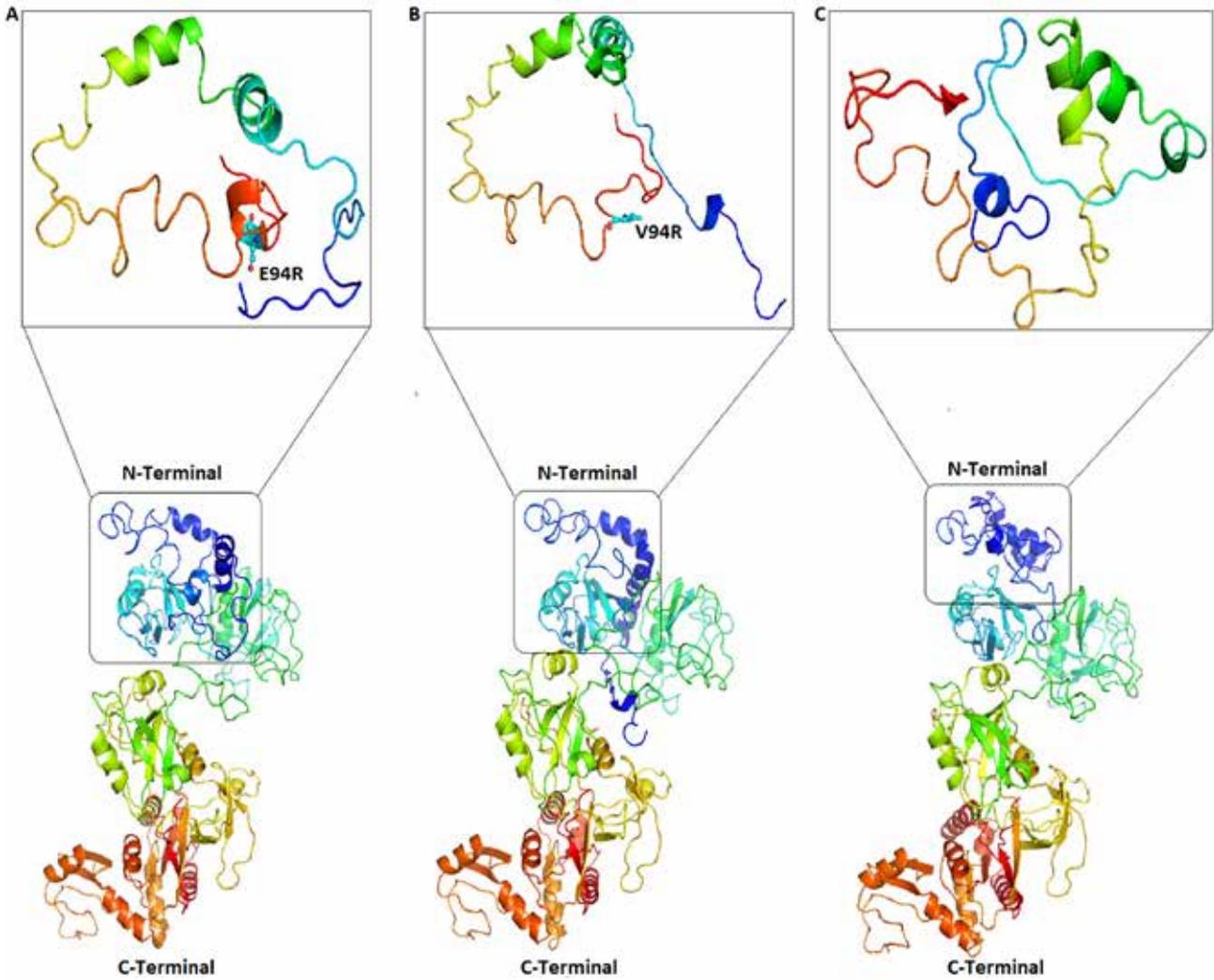


Figure 4. Conformational changes in the PSD95p three-dimensional structure. (A) Complete 3D structure prediction of human and chimpanzee proteins and the 3D structure of its N-terminus end are shown, indicating the glutamic amino acid E94 in balls and sticks figures. (B) Prediction of the 3D structure of the Neanderthal PSD95p shows that its N-terminal end differs from human protein. (C) The bonobo protein 3D structure is indicated, and, as well as the Neanderthal's, the N-terminal end diverges from human PSD95p.

	Q	K	Y	R	Q	D	E	D	T	P	P	L	E	H	S	P	A	H	L	P	N	Q	A	N	S	P	P	V	I	N	T	D	T	L	E	A	P	G	V	E	L	Q	V	N	G	I	E	G	E	M	E	V	I											
Human	Q	K	Y	R	Q	D	E	D	T	P	P	L	E	H	S	P	A	H	L	P	N	Q	A	N	S	P	P	V	I	N	T	D	T	L	E	A	P	G	V	E	L	Q	V	N	G	I	E	G	E	M	E	V	I											
Elephant	Q	K	Y	R	Q	D	E	D	T	P	P	L	E	H	S	P	A	H	-	L	P	N	Q	A	S	P	P	V	I	N	T	D	T	L	E	A	P	G	V	E	L	Q	V	N	G	I	E	G	E	M	E	V	I											
Rabbit	Q	K	Y	R	Q	D	E	D	T	P	P	L	E	H	S	P	A	H	-	L	P	N	Q	A	N	S	P	P	V	I	N	T	D	T	L	E	A	P	G	V	E	L	Q	V	N	G	I	E	G	E	M	E	V	I										
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Koala	K	K	Y	R	Q	D	E	D	T	P	P	L	E	H	S	P	A	H	-	L	P	N	Q	A	N	S	P	P	V	I	N	T	D	T	L	E	A	P	G	V	-	-	-	V	N	G	I	E	G	E	M	E	V	I										
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Ostrich	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E	T	I	P	S	T	I	L	P	-	Q	A	N	P	P	F	I	L	I	N	S	D	T	L	I	T	S	T	V	-	-	V	N	G	I	D	A	D	Y	F	V	I

Figure 5. Conserved motifs in the PEST domain of the N-terminal end of diverse macro vertebrate proteins. The red color indicates fully conserved motifs; the gray color indicates partially conserved motifs and, the yellow color indicates the non-conserved amino acids.

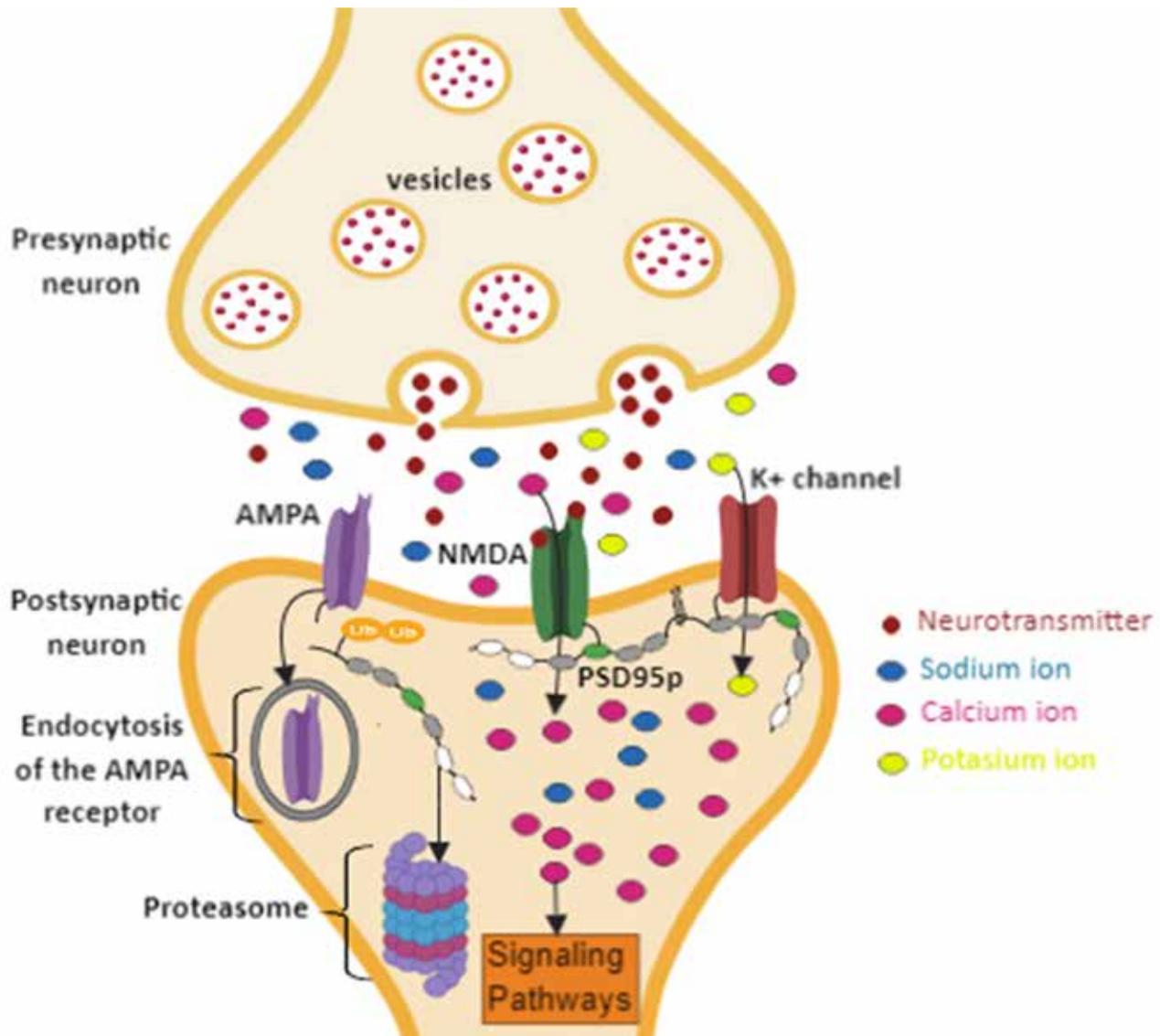


Figure 6. Regulation of synaptic strength by polyubiquitination of PSD95p. The polyubiquitination at the N-terminal end of PSD95p and subsequent proteolysis by the proteasome induces the delocalization and endocytosis of the AMPA receptors. This mechanism will decrease intracellular Ca^{2+} concentrations, modulating the synaptic connection between neurons that follow different activity profiles.

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Received: 17 november 2020

Accepted: 15 january 2021