Altered 3D Structure of Human Tryptophan Hydroxylase-2 Caused by Change in the amino acid 341: In Silico Analysis

Juan Pablo Castaño Casas[1] Juan Sebastián Pérez Barona[1] Flavio Edgar Cerón Betancourth[1] Doris Haydee Rosero Salazar[1]

[1] Biomedical Research Group, Department of Basic Medical Sciences, Faculty of Health, Universidad Icesi, Cali, Colombia

J Morphol Sci 2018;35:198–202.

Address for correspondence Doris Haydee Rosero Salazar, MSc, Grupo de Investigación Biomédica, Departmento de Ciencias Básicas Médicas, Facultad de la Salud, Universidad Icesi, Street 18 No. 122-135, Postal Code 760031, Cali, Colombia (e-mail: dorhaross@gmail.com).

Abstract

Introduction Neuropsychiatric syndromes have an important connection with disorders in the regulation of serotonin, with human tryptophan hydroxylase-2 being one of the related biosynthetic enzymes of this neurotransmitter. Evidence-based genetic studies suggest a possible involvement of this enzyme in neuropsychiatric disorders caused by abnormalities in the synthesis and regulation of serotonin.

Objective To analyze the structural effects of single nucleotide polymorphism (SNP) in the enzyme tryptophan hydroxylase-2 and the changes that lead to functional alterations.

Materials and Methods In this study, we performed an in silico analysis of SNPs associated with abnormal folding of the tryptophan hydroxylase-2 protein. Different programs were used to identify amino acid changes evidencing pathogenic effects and possible functional impairments.

Results A change in the amino acid 341 (lysine [L]for phenylalanine [F]) (L341F) of the protein chain affects the total enthalpy of the protein. The enthalpy turned positive due to the energy required for the amino acid to return to its original condition. The protein function is also affected negatively because of the altered structured.

Conclusion The change in the L341F leads to serious structural defects in the tryptophan hydroxylase-2. Those defects can be further related with functional instability and associated to the etiology of neuropsychiatric diseases.

Keywords
- polymorphism
- serotonin
- tryptophan hydroxylase
- SNPs
- amino acid

Introduction

Human tryptophan hydroxylase 2 (hTPH-2) is one of the two isoforms of the tryptophan hydroxylase (TPH) enzyme. One of them is found in a phosphorylated way in the digestive system (hTPH-1). The other is present in the central and the peripheral nervous system. The function of the hTPH-2 is the hydroxylation of tryptophan to 5-hydroxytrytophan, a crucial step in the biosynthesis of the neurotransmitter serotonin.[1,2] The deficit or absence of the cofactor Fe (III) affects negatively the efficiency of the production of 5-hydroxytrytophan. The hTPH-2 has three domains: the N-terminus that evidences a regulatory function; the Catalytic α-helix domain, with the function of tetramerization of tryptophan; and the C-terminus domain.[3]

Serotonin is the neurotransmitter associated in normal conditions with the regulation of grades of pain, thermonociceptive response, intake, emotional behavior, and stress response, among other functions.[4] A decrease in the serotonin production affects the muscle tissue of the vascular system. For instance, this smooth muscle tissue evidences altered contractile responses resulting on blood pressure disease due to a significant reduction of 5-hydroxytrytophan.[5]

received
November 28, 2017
accepted
September 26, 2018

DOI https://doi.org/10.1055/s-0038-1675830.
ISSN 2177-0298.

Polymorphisms of hTHP-2 lead to changes in the amino acids and the structure of the enzyme. These polimorphisms cause a declining serotonin outputs due non-functional enzyme activity. Neuropsychiatric diseases such as depression, schizophrenia, and autism are related with those changes.[6] Depression, for instance, is a common disease nowadays, evidenced by constant feelings of sadness, loss of interest in self-care activities and self-esteem. The rs7305115 single nucleotide polymorphism (SNP) at the TPH2 may predispose to suicide attempts in patients in the suicide and major depressive disorders (MMD) spectrum.[7] It is estimated that by 2020 depression will be the second most disabling disease in the world.[8]

Thus, it is important to focus the research on neuropsychiatric and systemic serotonin-related disorders, in order to implement therapeutic targets useful to improve treatments.[9] This in silico analysis corresponds to an initial modeling study of the reported alteration on the enzyme structure. The results of this analysis serve as a background of the SNPs associated to possible alterations in the function of the protein. The purpose of this study is to analyze the influence on the stability and alterations caused by changes in the associated SNPs of the hTHP-2 enzyme.

Materials and Methods

In this study, we applied bioinformatics techniques by using websites designed by universities and organizations for the analysis of different SNPs. The code for TPH2 is Ensembl: ENSG00000139287.

The procedures used were:

1. Protein Data Bank (PDB)
   We consulted the division of medicine found on this page to look for a protein according to its function. The analyzed enzyme had to do with the production of serotonin, so it was selected to determine the way in which an amino acid change could affect the role of the treated protein. By using the PDB, we established the protein chain, primary and secondary structures, as well as SNP. The primary structure was identified with codes; in this case, the enzyme was labeled with the code 1MLW and contained 14 SNPs on its structure. In this database, the files necessary to perform the following steps in the investigation, such as those in Fast-All (FASTA) sequence and PDB format (3D structure) were found.
2. Sorting Intolerant from Tolerant (SIFT)
   This program indicated the probability of tolerance of the amino acids changed. Likewise, the biochemical tolerance to the 3D structure of the chosen protein was determined. To use this program, we started with the FASTA file that was downloaded from the PDB. Then, the unique protein chain that constitutes the enzyme was selected from this file. After adding the sequence of the enzyme in the space provided, we selected the option of locating the position of the SNP and acquire the data for the analysis. The fragment of protein sequence with the amino acid 341 (L), was as follows: **GAGLLSSISELKHALSGHAKVKPF**
3. Polymorphism Phenotyping v2 (PolyPhen-2)
   By this software, it was demonstrated that making the change of an amino acid in the protein sequence can lead to a pathogenic protein, based on biological characteristics and protein evolution.
   To obtain the results, the information of the protein chain and the position of SNP to be treated were established. Then, the software evidenced the pathogenicity of the amino acid change in the structure of the protein.
4. Non-synonymous single nucleotide polymorphism Analyzer (nsSNPAnalyzer):
   To predict disease-associated non-synonymous single nucleotide polymorphism.
   This tool provides additional information about the SNP investigated by reading and interpreting the results in an easier way. With this software we used information of multiple sequence alignment, and the information contained in the 3D structure of the protein to make predictions through the analysis of biochemical and biological information.
   The procedure for data collection began with the use of the corresponding tryptophan hydroxylase protein FASTA sequence. Following the procedure, the amino acid change was placed in the protein sequence, and then the PDB file previously obtained was used. The protein chain to be analyzed was introduced and the steps were repeated with each of the SNPs.
5. Swiss-PDB Viewer 4.1.0

After the completion of the analysis with the described software, the 3D structure of the studied protein was evidenced by Swiss-PDB. This 3D structure was obtained through the option "file," in which the "PDB file" option was selected. Then, the structure of the protein was observed, and its protein chain for tryptophan hydroxylase was used. The sequence of ordered protein chains was observed by using the bar called *control panel*. In the control panel, the corresponding chain of interest was selected. We proceeded to activate the *Compute H-Bonds*, near the command bar *tools*, to identify the hydrogen bonds that naturally possess the protein when it has normal or abnormal changes on its structure.

For the analysis of the SNPs that were considered pathogens, we selected the amino acid of interest in the control panel, and its location on the screen was presented. First, the amino acid was located in the protein structure. Then, the amino acid change or "mutation" was performed by selecting the corresponding option and finally, by choosing the amino acid in the chain to make the exact change to the SNP.

A conformational change in the protein structure is evidenced when the SNP has a significant effect on the protein function. This is one of the determinants in the protein to be considered pathogenic. Another factor is given by the analysis through Force Field (compute energy) in the *Tools* tab. After performing the last step, the Force Field analysis demonstrated the total enthalpy of the protein and each amino acid in the chain.

Results

During evaluation and analysis of the protein 1MLW with its corresponding SNPs, it was possible to demonstrate that only one SNP caused a pathogenic effect on the protein structure. This polymorphism was classified by the four software as harmful/not tolerable. The pathogenic SNP is the amino acid 341, which had a change of amino acid L to amino acid F. Likewise, different results were found including the pathogenicity/tolerability of SNPs. The first software, SIFT, performed a chemical assessment and determined whether the protein is tolerable or not to the change. The second software, PolyPhen-2, evaluated the biological characteristics of the protein, its evolution and whether it is pathogenic or not. PolyPhen-2 labeled the SNPs as benign and possibly pathogenic. ►**Table 1** summarizes the data obtained from the software for each SNP.

PDB

1. In the PDB, we found the following SNPs:

rs201585879 change: K (lysine) - R (arginine)
rs147638867 change: V (valine) - I (isoleucine)
rs201751661 change: L (leucine) - F (phenylalanine)
rs200937558 change: R (arginine) - C (cysteine)
rs145855109 change: A (alanine) - T (threonine)
rs184973363 change: M (methionine) - L (leucine)
rs142170901 change: A (arginine) - V (valine)
rs41274348 change: T (threonine) - M (methionine)
rs139617975 change: R (arginine) - W (tryptophan)
rs147488937 change: E (glutamic acid) - K (lysine)
rs20222394 change: Q (glutamine) - R (arginine)
rs41274350 change: L (leucine) - I (isoleucine)
M.rs189455467 change: K (lysine) - N (asparagine)
rs145479597 change: R (arginine) - C (cysteine)

SIFT

The software evidenced that 9 of the 13 SNPs studied were tolerable; therefore, 4 SNPs were considered intolerable (►**Table 1**).

PolyPhen-2

After analysis of the SNPs, this software revealed that 6 of the 13 SNPs were benign. Thus, more than half of the SNPs were considered as possibly pathogenic. (►**Table 1**).

Non-synonymous Single Nucleotide Polymorphism Analyzer (nsSNPAnalyzer)

This software showed that eight SNPs were neutral, while five might be disease-causing (►**Table 1**).

Enthalpy of the 3D Structure

On the analysis of the enthalpy of the 3D structure of the protein, a change on its primary structure was observed. The effect of the SNP that generated this outcome was considered pathogenic. Likewise, the same SNP was evidenced as pathogenic in the previous analysis performed through the software applications (►**Table 1**). ►**Fig. 1A** shows the primary structure of the protein 1MLW without changes on its structure. In contrast, ►**Fig. 1B** evidences the change on the 3D structure of the chain, due to the change on the L341F, which generated an aromatic ring.

Discussion

This study evidenced an important change in the SNP 341 of the hTPH-2 enzyme that caused a pathogenic effect. This

Table 1 Analysis of single-nucleotide polymorphisms according to tolerance, pathogenic effect and enthalpy of the 3D structure of human tryptophan hydroxylase 2 (hTHP2)

SNPs	SIFT	PolyPhen-2	nsSNPAnalyzer	Enthalpy of the 3D structure
SNP 124 M-L	Tolerable	Benign 0.001	Neutral	12,296.042
SNP 142 R-C	Not tolerable	Possibly pathogen 1000	Disease	11,821.518
SNP 146 K-R	Tolerable	Possibly pathogen 0.471	Neutral	12,442.093
SNP 163 K-N	Tolerable	Benign 0.189	Neutral	12,441.610
SNP 177 V-I	Tolerable	Benign 0.015	Neutral	12,213.241
SNP 207 R-W	Tolerable	Possibly pathogen 0.999	Disease	12,033.135
SNP 224 L-I	Tolerable	Benign	Disease	12,030.536
SNP 274 L-I	Tolerable	Benign 0.375	Neutral	12,046.070
SNP 300 A-T	Not tolerable	Possibly pathogen 0.925	Neutral	12,020.028
SNP 304 A-V	Tolerable	Benign	Neutral	12,271.819
SNP 310 T-M	Not tolerable	Possibly pathogen 0.996	Disease	11,446.873
SNP 341 L-F	Not tolerable	Possibly pathogen 0.999	Disease	14,452.046
SNP 362 Q-R	Tolerable	Possibly pathogen 0.990	Neutral	12,400.587

Abbreviations: A, alanine; C, cysteine; F, phenylalanine; I, isoleucine; K, lysine; L, leucine; M, methionine; nsSNPAnalyzer, non-synonymous single nucleotide polymorphism analyzer; PolyPhen-2, polymorphism phenotyping v2; Q, glutamine; R, arginine; SIFT, sorting intolerant from tolerant; SNP, single-nucleotide polymorphism; T, threonine; V, valine; W, tryptophan.

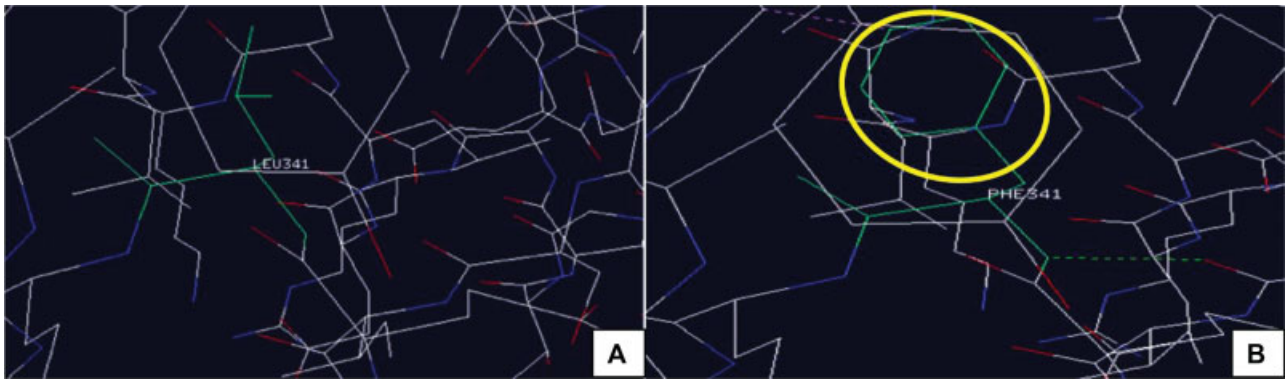

Fig. 1 (**A**) Normal protein. (**B**) Changes in the protein structure caused by mutation. The evident conformational change is the formation of an aromatic ring (circle).

negative impact resulted in the presence of an abnormal aromatic ring in the structure of the protein (►**Fig. 1B**). This structural disorder affects the polarity of the protein, causing loss of the hydrogen bonds required for the stability so, leading to a functional alteration.

The changes on the other amino acids of the protein sequence mostly did not affect it negatively; however, they caused minimal variations on the enthalpy. These outcomes can be associated with abnormal expression in determined diseases that cause functional defects. For instance, defects related with the decrease in the serotonin synthesis that carries out symptoms evidenced in neurological diseases.

The change in a specific amino acid of the chain of this protein affects the serotonin levels. This condition might lead to neuropsychiatric disorders such as depression, schizophrenia, Parkinson's disease, aggressiveness, suicidal behavior, attention deficit or hyperactivity, and autism.[8] In neuropsychiatric degenerative diseases, such as Parkinson's disease, according to Ostrosky-Solis (2000),[10] the lack or deficiency in the synthesis of the serotonin is associated with depressive and anxiety disorders in these patients.

Other systems, besides the nervous system, can be affected by the defects in the serotonin synthesis. In the immune system, for example, evidence has been found that the synthesis and transport of serotonin can be performed by T-lymphocytes. Researches performed in psychiatric patients have shown a modification in the serotonergic system of the T-lymphocytes cells when depression occurs.[11]

Our literature search evidenced very few articles that matched specifically the change in the amino acid 341 of the structure of the hTPH-2, and/or the adverse effects caused by the exchange of an amino acid, such as the one analyzed in this study.[11] Therefore, further research is needed to bring forth improvements in the treatment for neuropsychiatric diseases.

Considering that a large percentage of people in the world suffer from depression and other neuropsychiatric diseases, this study attempts to explore one of the multiple possible causes of disorders in the synthesis of serotonin. This objective opens a new window for research on the treatment of hTPH-2 enzyme deficiency and its effects on patients.

Conclusion

While the causes of neurological and neuropsychiatric disorders are multiple, it is very likely that polymorphisms in the different proteins involved in neurophysiological processes have a significant influence on the development of these disorders. It is suitable for future works to find a similar abnormal conformational tendency in other enzymes of the nervous system. This allows researchers to establish a comparable association, similar to the observed in hTPH-2 and its association with amino acids changes.

Author Contributions

This work was a coordinated effort of the four authors; we all participated in the analysis and draft of the manuscript. No activities breakdown was presented.

Conflicts of Interest

None to declare.

Acknowledgments

The authors want to acknowledge to Dr. Andrés Castillo and Dr. Roy Romero for their motivation and significant contributions to this work.

References

1 Wang L, Erlandsen H, Haavik J, Knappskog PM, Stevens RC. Three-dimensional structure of human tryptophan hydroxylase and its implications for the biosynthesis of the neurotransmitters serotonin and melatonin. Biochemistry 2002;41(42): 12569–12574
2 Richard DM, Dawes MA, Mathias CW, Acheson A, Hill-Kapturczak N, Dougherty DM. L-Tryptophan: Basic Metabolic Functions, Behavioral Research and Therapeutic Indications. Int J Tryptophan Res 2009;2:45–60
3 Martínez A, Knappskog PM, Haavik J. A structural approach into human tryptophan hydroxylase and its implications for the regulation of serotonin biosynthesis. Curr Med Chem 2001;8(09):1077–1091
4 Santibáñez L, Ortiz R, Guzmán O, et al. Expresión de la triptófano hidroxilasa en tallo cerebral de ratas con restricción alimenticia. BIOLÓGICAS; 2008
5 Watts SW, Morrison SF, Davis RP, Barman SM. Serotonin and blood pressure regulation. Pharmacol Rev 2012;64(02):359–388

6 Zhang X, Gainetdinov RR, Beaulieu JM, et al. Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. Neuron 2005;45(01):11–16
7 Zhang Y, Zhang C, Yuan G, et al. Effect of tryptophan hydroxylase-2 rs7305115 SNP on suicide attempts risk in major depression. Behav Brain Funct 2010;6:49
8 Acosta-Hernández M, Mancilla-Percino T, Correa-Basurto J, et al. Depresión en la infancia y adolescencia: enfermedad de nuestro tiempo. Archivos Neurociencias 2011;18(01):20–25
9 Bardin L. The complex role of serotonin and 5-HT receptors in chronic pain. Behav Pharmacol 2011;22(5-6):390–404
10 Ostrosky-Solis F. Características neuropsicológicas de la enfermedad de Parkinson. Rev Neurol 2000;30(08):788–796
11 Urbina M, Lavie R, Resler G, Campos J, Mata S, Lima L. Síntesis de serotonina y presencia de hidroxilasa de triptófano en linfocitos de pacientes con depresión mayor tratados con fluoxetina y ácido fólico. Arch Venez Farmacol Ter 2008; 27:114–120