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In-Silico Study of Protein-Protein Interaction, Secondary Structural Analysis and Annotation of Functional Domains of Indoleamine 2,3-Dioxygenase1

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ABSTRACT

Indoleamine-2,3-dioxygenase-1 (IDO1) is the first catabolizing enzyme of tryptophan. IDO1 is extensively expressed in both immune and non-immune tissues and its activation is related to inflammation as well as severity of depressive symptoms, leading neurodegeneration and anxiety like behaviors. Moreover, IDO1 acts as novel immune checkpoint protein with regulatory pathways of immune response and may interact with several proteins. In this paper, physicochemical characterization, functional characterization, subcellular localization, secondary structural analysis and domains/motif/family of IDO1 interacting proteins were done using in silico tools such as STRING, ProtParam-Expasy, SOSUI, SOPMA and MOTIF Search. Eighteen interacting proteins were obtained by STRING. Briefly, all interacting proteins were hydrophilic in nature except AANAT. MAOA and MAOB were almost similar to each other and predicted as more stable in high range of temperature. Further, all interacting proteins showed diverse secondary structure. Higher percentage of α-helix structure was present in remaining proteins except KYNU, MAOA, IL4I1, MAOB and 4930438A08RIK. These proteins showed higher percentage of random coil. Moreover, almost equal percentage of α-helix and random coil were present in TPH1. Secondary structure β-turn was low in percentage in comparison to all other secondary structures. Taken together IDO1 interacting protein or enzyme which are involved in tryptophan catabolic process to kynurenine, serotonin synthesis, tryptophan metabolism, response to corticosterone and glucocorticoids, several other biological process. Such studies are useful to understand IDO1 dependent regulation in the context of tryptophan/kynurenine pathway and helpful in therapeutics of neurological disorders.

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Abbreviations

NMDAR: N-Methyl-D-Aspartate Receptor
α7 nAChR A7-Nicotinic Acetylcholine Receptor
NCBI: National Centre for Biotechnology Information
FASTA: Fast Alignment Sequence Test for Application
STRING: Search Tool for the Retrieval of Interacting Genes/ Proteins
SOPMA: Self-Optimized Prediction Method with Alignment
MW: Molecular Weight
EC: Extinction Coefficient
II: Instability Index
AI: Aliphatic Index
GRAVY: Grand Average of Hydropathicity

Introduction

Indoleamine 2,3-dioxygenase is a heme-containing enzyme which is physiologically expressed in a number of tissues, cells and catalyzes first and rate limiting step of the catabolism of essential amino acid tryptophan (Trp) along the kynurenine pathway (KP) [1]. Trp is an essential amino acid (AA) that is exclusively obtained from dietary intake in human. Trp and its metabolites plays an important role in several physiological processes, cell growth, maintenance and serve as neurotransmitters in signalling molecules. The Trp level in the organisms depends on food intake and several activities of Trp metabolizing pathways. Trp enter the gut and metabolized into four main pathways; KP, serotonin pathway, indolic pathway (bacterial degradation) and tryptamine pathway [2]. Moreover, 1-2% Trp metabolized into serotonin neurotransmitter and regulates gut motility, intestinal permeability and mood. IDO1 is the first-rate limiting enzyme which convert 90-95% Trp to KP metabolites which plays important role in immune tolerance, inflammation, neurotransmission and several other cellular and biological process [2]. Further, IDO1 activation is significantly associated with inflammation and the severity of depressive symptoms which leads to many neurodegenerative, depressive and anxiety like behavior [3].

Depression is associated with excessive stimulation of hypothalamic pituitary adrenal-axis (HPA-axis) which also represents route of gut-brain-crosstalk [4]. HPA-axis activation involves in the sequential release of corticotrophin-releasing factor (CRF) followed by adrenocorticotrophin hormone (ACTH) from the hypothalamus and pituitary respectively. Thereafter, ACTH stimulates the adrenal glands to synthesize glucocorticoids (GCs). Excessive stimulation of HPA-axis leads to circulating high level of GCs (i.e., cortisol and corticosterone). Moreover, GCs stimulate immune cells in the central nervous system (CNS) to produce proinflammatory cytokines which includes interferon- (IFN-ϒ) tumour necrosis factor- (TNF-α) and interleukin-6 (IL-6) leading

to the activation of IDO1 [5]. Thereafter, IDO1 activation leads to convert serotonin pathway to abnormal KP and produce end product quinolinic acid (QA) and kynurenic acid (KynA). KynA is a metabolite of the astrocytic processing and binds with glycine co-agonist site of the N-methyl-D-aspartate receptor (NMDAR). On the other hand, KynA also acts as antagonist on binding with α7-nicotinic acetylcholine receptor. Both these receptors play an important role in synaptic plasticity and cognitive processes and show neuroprotection when activated physiologically. KynA has anti-inflammatory properties and has ability to clear over activation of glutamate in the brain [6]. Similarly, QA is synthesized as a result of microglial processing. Further, QA agonist to NMDAR, specifically with NMDAR subtypes containing the NR2A and NR2B subunits, with massive calcium entry into neurons and promotes neurodegeneration or neuronal apoptosis [7].

The beneficial effect of aripiprazole on poststroke depression may be mediated through the inhibition of IDO1-dependent neurotoxic of kynurenine metabolism [8]. Moreover, inhibition or knockout IDO1 gene reduce depressive like behaviour in rats. Over the decades, several studies suggested that IDO1 may acts as novel immune checkpoint and involved in several CNS functions and related disorders [9,10]. Proteins have dynamic physiochemical connections to perform biological functions at cellular and system level. Further, protein interaction networks have ability to prevent disease and several health problems, such as help in prevention, diagnosis and treatment [11,12]. Several studies have shown that IDO1interacting proteins, their domain and families are corelated to several brain functions as well as gut related biological functions. The KP also regulates vitamin B3 and hepatic heme synthesis. Kynurenine hydroxylase (KMO) is a flavoprotein of mitochondria that uses as flavin adenine dinucleotide–dependent enzyme. Its activity may decrease in riboflavin (vitamin B2) deficiency that's why riboflavin serves as an essential determinant of the KP [13].

Several studies have shown that computational biology built many platforms and methods for predicting protein structure, protein-protein interaction, conserve domain determination, analyzing active site, protein-ligand interaction, protein functional and structural properties and several other factors about protein [14-18]. Therefore, the present study is focused on in silico analysis of IDO1 interacting proteins replace by using several computational tools which are linked to their functional, structural and physicochemical properties. In silico studies have been used for prediction of drug metabolism and interaction [19]. Such studies may add further information in the context of KP metabolites which related to neurological disorders like depression and helpful in therapeutics.

Materials and Methods

Identification of IDO1 interacting proteins

IDO1 interacting proteins were analyzed by using STRING 11.5 database (https://string-db.org/) which showed 0.900 in the highest confidence.

Sequence Retrieval

The proteins sequences and accession number were analyzed by using FASTA (https://www.ncbi.nlm.nih.gov/) from NCBI database.

Physicochemical Characterization

The physicochemical parameter of IDO1 interacting proteins such as AA, molecular weight (MW) of protein, Theoretical pI, total number of negatively charged residues (Asp + Glu), total number of positively charged residues (Arg + Lys), Extinction Coefficient (EC), Instability index (II), Aliphatic Index (AI) and Grand Average of Hydropathicity (GRAVY) from primary structure of protein were analyzed by using ProtParam (https://web.expasy.org/protparam/) server of Expasy [15,20,21].

Subcellular Location and Functional Characterization

The nature of proteins either soluble or trans membrane and sub cellular localization were analyzed by using SOSUI database (https://harrier.nagahama-i-bio.ac.jp/sosui/mobile/) [22].

Secondary Structure Prediction

The secondary structure of IDO1 interacting proteins were analysed by using SOPMA assessment database (https://npsa-prabi.ibcp.fr/NPSA/npsa_sopma.html) [18,23,24].

Functional Annotation Prediction

For identification of domain/motif search among IDO1 interacting proteins were analyzed by using motif search (https://www.genome.jp/tools/motif/). There are several computational tools are available for identification of motifs [25,26].

Results and Discussion

Identification of IDO1 Interacting Proteins

Protein- protein interactions between IDO1 interacting proteins was determined by prediction confidence score. Confidence score meant for probability of interaction between two proteins and having value varying from 0 to 1. Value closer to 1 predicts maximum probability to interact [24]. Eighteen IDO1 interacting proteins were observed with highest confidence in score 0.900 shown in (Fig. 1) by STRING. Hence, detailed about IDO1 interacting proteins namely; Kynu protein (KYNU), Tryptophan2,3-dioxygenase (TDO2), Cytochrome p450 1A1 (CYP1A1), Tryptophan5-monooxygenase 1 isoform 2 (TPH1), Kynurenine formamidase isoform 4 (AFMID), Cytochrome p450 1A2 (CYP1A2), Cytochrome P450 1B1 isoform 2 (CYP1B1), Amine oxidase (flavin-containing) A (MAOA), Aromatic-L-amino-acid decarboxylase (DDC), L-amino-acid oxidase precursor (IL4I1), Acetylserotonin O-methyltransferase (ASMT), Indolethylamine N-methyltransferase (INMT), Tryptophan hydroxylase 2 partial (TPH2), Serotonin N-acetyltransferase (AANAT), Monoamine oxidase B (MAOB), L-amino acid oxidase 1 (LAO1), Uncharacterized protein LOC73988 isoform X1 (4930438A08Rik) and GM20708. We excluded GM20708 for further analysis as it is present only in Drosophila sechellia having very little information.

Sequence Retrieval

The AA sequence of interacting proteins were taken from the NCBI database and further information of the proteins mentioned in Table 1.

Table 1: IDO1 interacting proteins retrieval

S.N.	NCBI Accession Number	Gene Name	Protein Name
1.	AAH69848.1	KYNU	Kynu protein
2.	NP_064295.2	TDO2	Tryptophan2,3-dioxygenase
3.	NP_001129531.1	CYP1A1	Cytochrome p450 1A1
4.	NP_001263301.1	TPH1	Tryptophan5-monooxygenase 1 isoform 2
5.	NP_001350089.1	AFMID	Kynurenine formamidase isoform 4
6.	NP_034123.1	CYP1A2	Cytochrome P450 1A2
7.	NP_001351818.1	CYP1B1	Cytochrome P450 1B1 isoform 2
8.	NP_776101.3	MAOA	Amine oxidase [flavin-containing] A
9.	O88533.1	DDC	Aromatic-L-amino-acid decarboxylase
10.	NP_034345.2	IL4I1	L-amino-acid oxidase precursor
11.	NP_001295417.1	ASMT	Acetylserotonin O-methyltransferase isoform 2
12.	EDK98724.1	INMT	Indolethylamine N-methyltransferase
13.	EDL21762.1	TPH2	Tryptophan hydroxylase 2 partial
14.	NP_033721.1	AANAT	Serotonin N-acetyltransferase
15.	NP_776101.3	MAOB	Monoamine oxidase B
16.	EDL30476.1	LAO1	L-amino acid oxidase 1
17.	XP_017170355.1	4930438A08RIK	Uncharacterized protein LOC73988 isoform X1
18.	-	GM20708*	-

*No information available

Physicochemical Properties

The study of characteristics of each proteins AA can be determined by how the physicochemical features of proteins are defined. ProtParam (https://web.expasy.org/protparam/) server of Expasy were utilized to defined the physicochemical properties of protein. IDO1 interacting proteins consisted a number of several AA as, KYNU (428), TDO2 (406), CYP1A1 (524), TPH1 (155), AFMID (270), CYP1A2 (513), CYP1B1 (487), MAOA (526), DDC (480), IL4I1 (630), ASMT (387), INMT (163), TPH2 (462), AANAT (205), MAOB (526), LAO1 (523), 4930438A08RIK (307), and AA Drosophila sechellia GM20708 is undefined protein. Moreover, hydrophobic AA Leucine is most abundant in remaining proteins except DDC and ASMT. DDC and ASMT proteins contains alanine as most abundant AA (Table 2). Leucine AA plays an important role in maintaining the tryptophan kynurenine ratios in the brain and inhibit lipopolysaccharide -induce depressive like behaviour inhibiting kynurenine to cross from blood to brain [27]. Leucine administration may help to reduce downstream kynurenine metabolites in brain and help in therapeutics [28].

Table 2: Amino acids composition of IDO1 interacting proteins

AA	KYNU	TDO2	CYP1A1	TPH1	AFMID	CYP1A2	CYP1B1	MAOA	DDC	IL4I1	ASMT	INMT	TPH2	AANAT	MAOB	LAO1	4930438A08RIK	GM20708*
Ala	7.0%	3.7%	4.6%	3.2%	7.4%	5.3%	9.7	6.7%	10.0%	9.0%	15.0%	3.1%	5.0%	6.8%	6.7%	7.6%	8.5%	
Arg	4.7%	6.4%	5.7%	4.5%	4.8%	4.9%	7.2	4.9%	6.5%	6.0%	11.6%	2.5%	5.8%	7.8%	4.9%	5.0%	5.5%	
Asn	3.5%	4.9%	3.4%	7.1%	3.3%	5.8%	3.3	3.2%	2.3%	3.5%	0.3%	1.2%	3.0%	1.5%	3.2%	3.1%	3.3%	
Asp	5.6%	4.7%	5.7%	8.4%	5.6%	4.5%	5.1	4.4%	4.8%	4.1%	5.4%	5.5%	5.8%	2.0%	4.4%	4.4%	7.5%	
Cys	1.9%	0.7%	1.7%	1.3%	0.7%	1.2%	1.2	1.9%	2.5%	0.6%	1.8%	1.8%	2.8%	4.9%	1.9%	1.5%	1.0%	
Gln	1.9%	5.2%	4.4%	3.2%	7.0%	4.5%	4.5	3.2%	2.7%	4.1%	2.1%	6.1%	3.9%	3.4%	3.2%	3.3%	3.9%	
Glu	7.7%	8.9%	4.8%	9.7%	5.2%	5.1%	3.7	7.6%	7.1%	7.8%	3.9%	7.4%	8.7%	7.8%	7.6%	4.8%	4.9%	
Gly	6.8%	5.9%	6.5%	2.6%	6.7%	5.5%	6.2	7.4%	6.7%	8.3%	12.7%	7.4%	5.6%	7.3%	7.4%	7.3%	5.5%	
His	4.2%	3.0%	2.7%	3.2%	3.0%	2.5%	2.9	1.9%	2.7%	4.9%	1.6%	1.2%	2.2%	4.9%	1.9%	3.1%	2.6%	
Ile	6.3%	3.9%	5.0%	5.8%	4.1%	5.7%	2.9	7.0%	5.6%	2.9%	0.5%	4.9%	3.7%	3.4%	7.0%	5.9%	4.6%	
Leu	12.1%	12.3%	10.9%	10.3%	10.0%	9.6%	10.9	8.2%	9.8%	11.6%	13.2%	11.0%	9.7%	15.1%	8.2%	10.5%	9.8%	
Lys	6.5%	6.2%	5.3%	7.7%	4.1%	6.0%	2.3	7.4%	4.2%	4.1%	0.0%	4.9%	7.4%	2.0%	7.4%	7.1%	3.3%	
Met	3.0%	3.0%	1.7%	1.9%	1.9%	1.8%	1.8	2.5%	2.9%	1.9%	2.1%	0.6%	1.3%	1.0%	2.5%	1.9%	1.3%	
Phe	4.4%	5.9%	5.9%	5.2%	4.4%	6.6%	6.6	3.2%	5.6%	3.2%	3.9%	4.3%	6.3%	6.3%	3.2%	3.4%	6.5%	
Pro	4.7%	2.5%	5.7%	3.2%	7.0%	6.4%	6.4	5.3%	4.8%	5.1%	5.7%	6.1%	4.3%	4.9%	5.3%	4.6%	5.9%	
Ser	5.8%	6.7%	7.4%	10.3%	7.0%	6.8%	8.6	4.6%	6.5%	6.0%	7.0%	10.4%	6.9%	6.8%	4.6%	8.6%	7.5%	
Thr	3.7%	3.9%	6.5%	3.9%	4.1%	5.8%	4.9	5.5%	4.0%	4.4%	4.7%	6.7%	6.1%	6.3%	5.5%	6.3%	6.5%	
Trp	1.2%	1.5%	1.3%	0.6%	1.5%	1.8%	2.1	2.3%	1.9%	1.7%	0.8%	2.5%	0.6%	1.5%	2.3%	1.1%	1.3%	

Tyr	2.6%	4.9%	3.1%	0.6%	3.3%	2.3%	2.1	4.8%	3.1%	3.2%	2.3%	6.1%	4.3%	1.5%	4.8%	4.4%	4.6%	
Val	6.3%	5.9%	7.6%	7.1%	8.9%	8.0%	7.8	8.0%	6.5%	7.5%	5.7%	6.1%	6.5%	4.9%	8.0%	6.1%	6.2%	
Pyl	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
Sec	0.0%	0.0	0.0%	0.0%	0.0%	0.0%	0.0	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	

*No information available

Further, theoretical pI (isoelectric point pI), MW, EC, II, AI and total number of atoms of the proteins are mentioned in (Table 3). Furthermore, protein pI value is important for determining the buffer system purification [29]. pI is the pH at which protein surface is covered with charge and at this point net charge of protein is zero [30]. Proteins are more stable at zero pI net value. A pI value below 7 indicates acidic in nature whereas above 7 indicates basic in nature. In this study negatively charged residues computed as aspartic acid and glutamic acid whereas positively charged residues computed as arginine and lysine. Hence, (Table 3) showed that pI value less than 7 indicates high aspartic acid and glutamic acid. Moreover, pI value of nine proteins namely; KYNU, TDO2, TPH1, AFMID, DDC, IL4L1, INMT, TPH2 and 4930438A08RIK were below 7 which means all these proteins acidic in nature and remaining eight proteins namely; CYP1A1, CYP1A2, CYP1B1, MAOA, ASMT, AANAT, MAOB and LAO1 are having pI value higher than 7, henceforth basic in nature.

Table 3: Physicochemical properties of IDO1 interacting proteins

S.N.	Protein	No. of AA	M.W.	Theoretical pI	Total number of negatively charged residues (Asp+Glu)	Total number of positively charged residues (Arg+Lys)	Total Number of Atoms	EC	II	AI	GRAVY
1.	KYNU	428	48151.70	6.07	57	48	6798	44390	43.00 (Unstable)	97.29	-0.107
2.	TDO2	406	47756.45	6.34	55	51	6688	62925	46.84 (Unstable)	84.24	-0.471
3.	CYP1A1	524	59229.99	8.25	55	58	8348	62840	29.92 (Stable)	88.49	-0.186
4.	TPH1	155	17778.97	5.02	28	19	2486	7115	49.84 (Unstable)	86.71	-0.565
5.	AFMID	270	30132.27	5.90	29	24	4230	35535	88.07 (Unstable)	53.93	-0.242
6.	CYP1A2	513	58183.89	8.92	49	56	8214	67755	32.01 (Stable)	87.74	-0.203
7.	CYP1B1	487	54158.81	8.49	43	46	7590	70275	43.85 (Unstable)	85.93	-0.067
8.	MAOA	526	59601.88	7.90	63	65	8417	103875	38.05 (Stable)	89.13	-0.223
9.	DDC	480	53874.09	6.14	57	51	7551	72600	43.30 (Unstable)	88.85	-0.019
10.	IL4I1	630	70162.53	6.28	75	64	9827	90550	49.23 (Unstable)	87.02	-0.338
11.	ASMT	387	40772.48	9.55	36	45	5716	30285	47.67 Unstable	84.88	-0.021
12.	INMT	163	18421.74	4.60	21	12	2569	37025	50.60 Unstable	83.07	-0.279
13.	TPH2	462	53026.27	6.03	67	61	7412	47050	49.42 Unstable	76.15	-0.452
14.	AANAT	205	23068.68	7.12	20	20	3236	21595	55.98 Unstable	93.27	0.040
15.	MAOB	526	59601.88	7.90	63	65	8417	103875	38.05 Stable	89.13	-0.223
16.	LAO1	523	58051.80	9.24	48	63	8212	67770	31.44 Stable	89.52	-0.191
17.	4930438A08RIK	307	34574.95	5.17	38	27	4818	42985	41.81 Unstable	82.31	-0.216
18.	GM20708*										

Molecular weight: MW; Extinction Coefficient: EC; Instability index: II; Aliphatic Index: AI; Grand Average of Hydropathicity: GRAVY

Besides other parameters, protein stability was determined by instability index. If proteins having value lower than 40 that means the protein is recognized as stable in laboratory condition whereas, II value higher than 40 is defined as unstable. ProtParam- Expasy server result for IDO1 interacting proteins represent that only five proteins namely; CYP1A1, CYP1A2, MAOA, MAOB and LAO1 are stable and remaining twelve are unstable. Further, EC value plays an important role in protein-protein interaction and protein-ligand interaction for several drug development processing. Tryptophan, Tyrosine and Cystine AA residues in the protein sequence are responsible for EC value [16,26]. Because of these AA residues help to measured optical density of denatured protein at 276-282 nm range [31]. In this study, using ProtParam- Expasy server, EC value was measured at 280nm based on the concentration of tryptophan, tyrosine and cysteine AA residues in the protein sequences. High amount of these AA indicates high EC value. Thus,

TPH1 was showed very low EC value because of little tryptophan and tyrosine AA in comparison to other interacting proteins however cystine AA number is low in IL4I1. MAOA and MAOB still showed high EC value due to presence of tryptophan and tyrosine AA residues. Cystine AA number is high in AANAT. This result suggests that cysteine AA residues might not be responsible for EC values.

Moreover, AI value determines its balance over a broad temperature scale. AI value of protein indicates their stability in high range of temperature which means higher the AI value indicate high thermal stability of proteins or enzymes [14,32]. Our result showed that KYNU have high thermal stability. Whereas, AFMID had low thermal stability indicating AFMID contain more flexible structure as compared to other interacting proteins. GRAVY index determines the proteins solubility and positive interaction with water [30]. Greater hydrophobicity indicates the increase in positive scores. Low GRAVY value favours a good interaction between water and proteins. In this study GRAVY indicated that all IDO1 interacting proteins were hydrophilic in nature except AANAT which is hydrophobic. GRAVY value of DDC protein suggested better communication with water. In 3D conformation of proteins hydrophobic AA reside within the structure. However, due to polarity all surfaces interact with water containing hydrophilic residues i.e., asparagine, cysteine, glutamine, serine, tyrosine, threonine and tyrosine as those have ability to interact with aqueous environment [14,33].

Sub-cellular Location Determination and Functional Characterization

The SOSUI data server was used for subcellular location assessment and determination of the functional characterization of IDO1 interacting proteins. Protein present in extracellular showed secretory nature. Signal peptides play an important role in the determination of transportation of particular proteins to target location and figure out the cleavage site [24,26]. Therefore, the secretory nature of proteins can be predicted through peptide analysis. Moreover, server predicted the TDO2, TPH1, AFMID, CYP1B1, MAOA, DDC, IL4I1, ASMT, INMT, TPH2, AANAT and 4930438A08RIK as a cytoplasmic protein. Out of these cytoplasmic proteins, MAOA, IL4I1, and ASMT are secretory in nature due to the presence of signal peptide (Table 4). CYP1A1 and LAO1 are identified as inner membrane proteins and LAO1 has signal peptide. Out of all interacting proteins only CYP1A2 is identified as periplasmic protein. Further, the localization of both KNYU and MAOB are predicted unknown and signal peptide is present in MAOB whereas absent in KYNU.

Table 4: Sub-cellular localization of IDO1 interacting proteins

Proteins	Subcellular localization Site	Signal peptide
KYNU	Unknown	NO
TDO2	C (Cytoplasmic)	NO
CYP1A1	IM (inner membrane)	NO
TPH1	C (Cytoplasmic)	NO
AFMID	C (Cytoplasmic)	NO
CYP1A2	P (Periplasm)	NO
CYP1B1	C (Cytoplasmic)	NO
MAOA	C (Cytoplasmic)	YES
DDC	C (Cytoplasmic)	NO
IL4I1	C (Cytoplasmic)	YES
ASMT	C (Cytoplasmic)	YES
INMT	C (Cytoplasmic)	NO
TPH2	C (Cytoplasmic)	NO
AANAT	C (Cytoplasmic)	NO
MAOB	Unknown	YES
LAO1	IM (inner membrane)	YES
4930438A08RIK	C (Cytoplasmic)	NO
Gm20708*		

Moreover, cellular localization of proteins are very important to be involved in several biological process such as signaling, transportation and energy transduction. Further, Trp also metabolizes into indolic pathway. Around 4-6% of Trp moves with gastrointestinal tract and get metabolized into indole and indolic compounds by microbiome. These indolic compounds bind with aryl hydrocarbon receptor (AhR) for maintenance of intestinal homeostasis. Indoles may act as neuroprotective compounds. Under normal conditions, activation of AhR results in transcription of genes, CYP1A1, CYP1B1 and AhRR (Aryl-Hydrocarbon Receptor Repressor), that control AhR activity. However, dysregulation of AhR activation is correlated with inflammatory processes, including Irritable Bowel Disorder (IBD), multiple sclerosis, cardiovascular conditions, allergic responses and carcinogenesis [2]. Meanwhile, this study showed that IDO1 interacted with CYP1A1 and CYP1B1.

Further, serotonin neurotransmitter (5-HT) synthesizes in the brain by TPH2, whereas, 90% of serotonin synthesize in the gut through TPH1 [34]. AANAT, INMT ASMT enzymes play an important role in synthesis of serotonin and melatonin neurotransmitter [35]. Moreover, dysregulation in serotonin level in the brain is linked to sickness behaviour and depression [36].

Hence, in this study IDO1 interacting proteins namely; CYP1A1 (1 trans membrane), CYP1A2 (1 Trans membrane), CYP1B1 (1 trans membrane), MAOA (2 trans membrane), DDC (1 trans membrane), IL4I1 (membrane protein) and LAO1 (membrane protein) are membrane proteins. However, ASMT and MAOB are trans membrane also soluble proteins. Rest of the proteins namely; KYNU, TDO2, TPH1, AFMID, INMT, TPH2, AANAT and 4930438A08RIK was classified as a soluble protein (Table 5).

Table 5: Functional characterization of IDO1 interacting proteins

Interacting proteins	N- terminal	Trans-membrane region	C-terminal	Type	Length	Characters
KYNU	-	-	-	-	-	Soluble
TDO2	-	-	-	-	-	Soluble
CYP1A1	9	AFVSATELLLAVT VFCLGFWVVR	31	Primary	23	MEMBRANE PROTEIN which has 1 transmembrane helices
TPH1	-	-	-	-	-	Soluble
AFMID	-	-	-	-	-	Soluble
CYP1A2	9	QYISLAPELLLA TAIFCLVFWMV	27	Primary	23	MEMBRANE PROTEIN which has 1 transmembrane helices
CYP1B1	21	TTLLLLFSVLAAV HLGQWLLRQW	43	Primary	23	MEMBRANE PROTEIN which has 1 transmembrane helices
MAOA	10 499	TGHMFDVVVIG GGISGLAAAKLL PGLLKITGFSTS VALLCFVLYK	32 520	Secondary Primary	33 22	MEMBRANE PROTEIN which has 1 transmembrane helices
DDC	87	PAMLADMLCGAIGC IGFSWAASP	109	Primary	23	MEMBRANE PROTEIN which has 1 transmembrane helices
IL4I1	1	MAGLALRLVLAATL LGLAGSLDW	22	Primary	22	Membrane protein
ASMT	1	MHRGRSASARQERDFRALMDL AHGFMASQVLFAGCA	36	-	36	Soluble
INMT	-	-	-	-	-	Soluble
TPH2	-	-	-	-	-	Soluble
AANAT	-	-	-	-	-	Soluble
MAOB	1	MSNKSDVIVVGGGIS GMAAAKLLHD	25		25	Soluble
LAO1	1	SGILVWGILLCVSS CLALYENLV	29	Primary	29	Membrane protein
4930438A08RIK	-	-	-	-		Soluble
Gm20708*						

Secondary Structure Prediction

Secondary structure elements play an important role in protein folding [24]. It contains helix, turn, coli and strand, and have relationship with protein structure, function and engagement. In this study SOPMA server showed the percentage of all secondary structures in (Table 6) and (Fig. 2-18). However, the higher percentage of α-helix structure present in proteins namely; TDO2, CYP1A1, AFMID, CYP1A2, CYP1B1, DDC, ASMT, INMT, TPH2 and LAO1 in comparison to other secondary structures, except KYNU, MAOA, IL4I1, AANAT, MAOB and 4930438A08RIK. These exception proteins had high percentage of random coil. Further, TPH1 showed equal percentage of α-helix and random coil. Alanine, glutamic acid and leucine play a significant role in high helix structure formation [14]. The high percentage of coil might be responsible just because of flexible glycine and hydrophobic proline AA. The proline has special property of creating kinks in polypeptide chains and thus results in coiling [33].

Table 6: Secondary structure predictions showing content of different conformations

Proteins	α - helix	Extended strand	β - turn	Random coil
KYNU	158 (36.92%)	79 (18.46%)	30 (7.01%)	161 (37.62%)
TDO2	250 (61.58%)	16 (3.94%)	14 (3.45%)	126 (31.03%)
CYP1A1	249 (47.52%)	56 (10.69%)	28 (5.34%)	191 (36.45%)
TPH1	66 (42.58%)	20 (12.90%)	3 (1.94%)	66 (42.58%)
AFMID	112 (41.48%)	40 (14.81%)	15 (5.56%)	103 (38.15%)
CYP1A2	244 (47.56%)	54 (10.53%)	23 (4.48%)	192 (37.43%)
CYP1B1	237 (48.67%)	56 (11.50%)	26 (5.34%)	168 (34.50%)
MAOA	196 (37.26%)	92 (17.49%)	39 (7.41%)	199 (37.83%)
DDC	227 (47.29%)	69 (14.37%)	32 (6.67%)	152 (31.67%)
IL4I1	249 (39.52%)	95 (15.08%)	31 (4.92%)	255 (40.48%)
ASMT	204 (52.71%)	43 (11.11%)	24 (6.20%)	116 (29.97%)
INMT	79 (48.47%)	15 (9.20%)	8 (4.91%)	61 (37.42%)
TPH2	225 (48.70%)	50 (10.82%)	15 (3.25%)	172 (37.23%)
AANAT	58 (28.29%)	35 (17.07%)	8 (3.90%)	104 (50.73%)
MAOB	196 (37.26%)	92 (17.49%)	39 (7.41%)	199 (37.83%)
LAO1	228 (43.59%)	83 (15.87%)	26 (4.97%)	186 (35.56%)
4930438A08RIK	116 (37.79%)	53 (17.26%)	12 (3.91%)	126 (41.04%)
GM20708*				

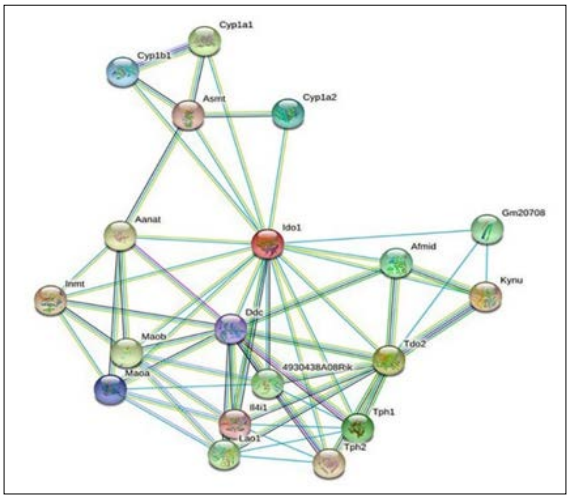

Figure 1: Protein- protein interaction networks between IDO1 and other proteins using STRING

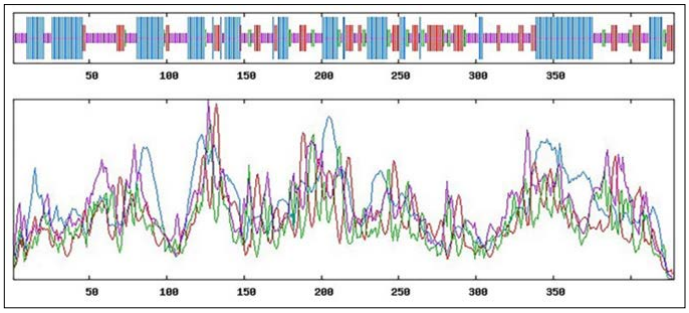

Figure 2: Secondary structure of KYNU

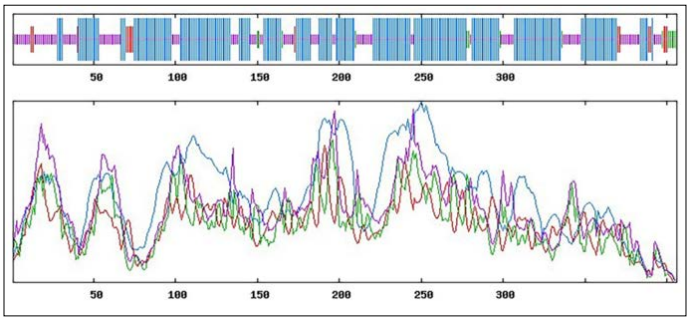

Figure 3: Secondary structure of TDO2

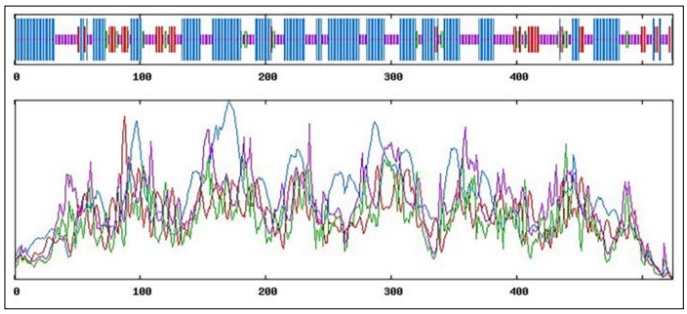

Figure 4: Secondary structure of CYP1A1

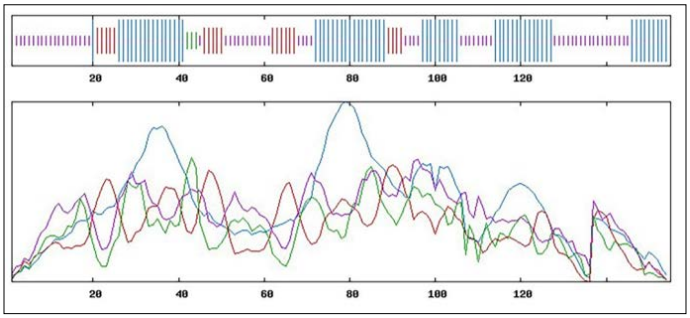

Figure 5: Secondary structure of TPH1

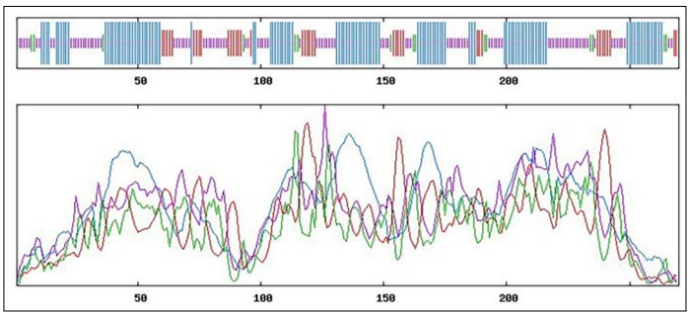

Figure 6: Secondary structure of AFMID

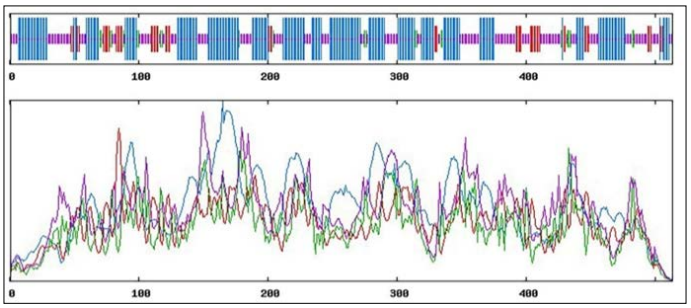

Figure 7: Secondary structure of CYP1A2

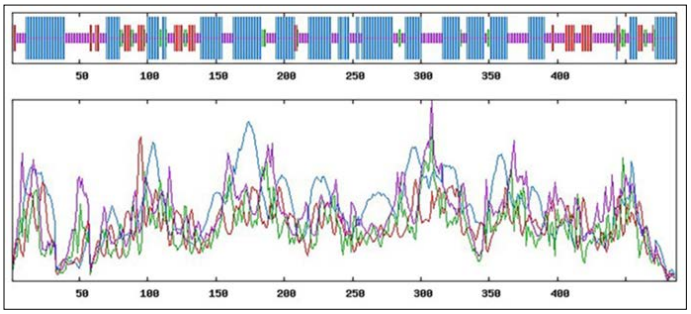

Figure 8: Secondary structure of CYP1B1

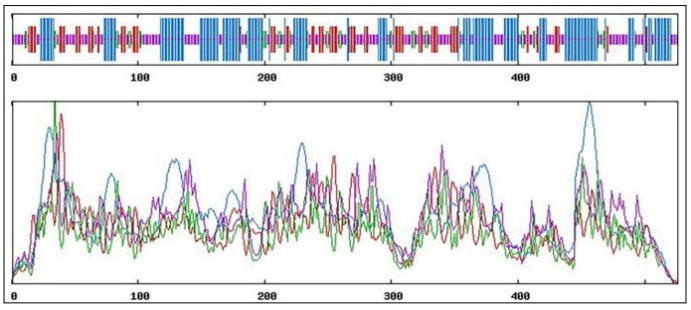

Figure 9: Secondary structure of MAOA

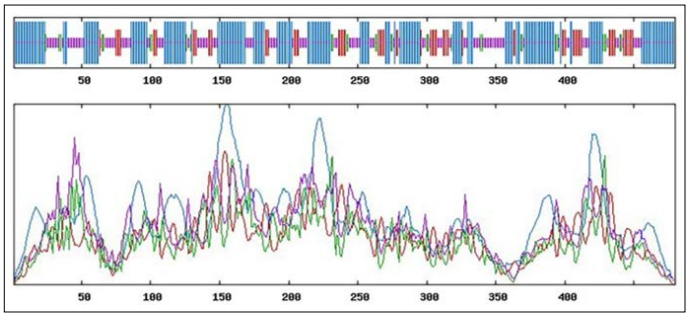

Figure 10: Secondary structure of DDC

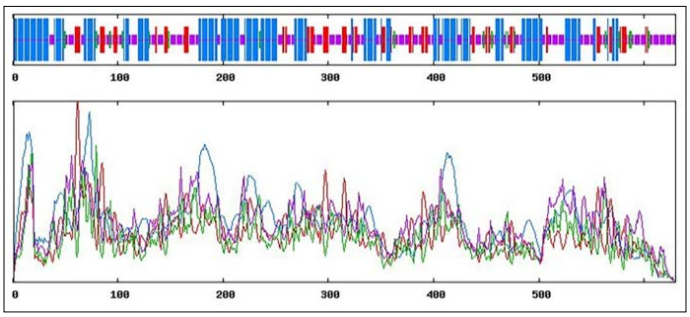

Figure 11: Secondary structure of IL4I1

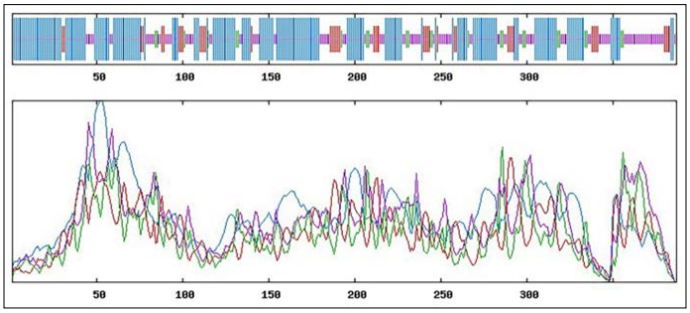

Figure 12: Secondary structure of ASMT

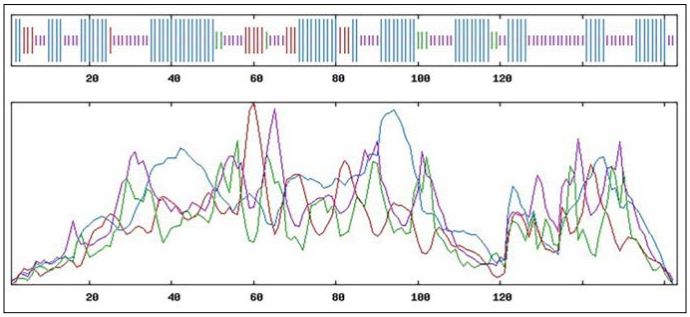

Figure 13: Secondary structure of INMT

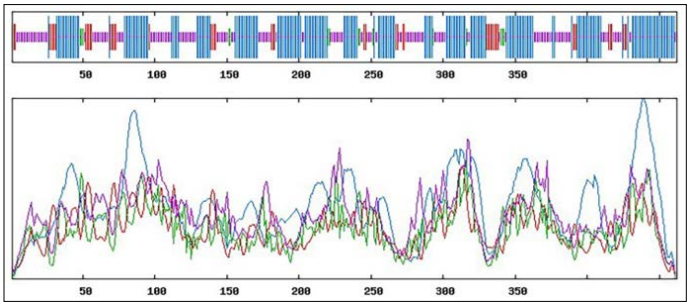

Figure 14: Secondary structure of TPH2

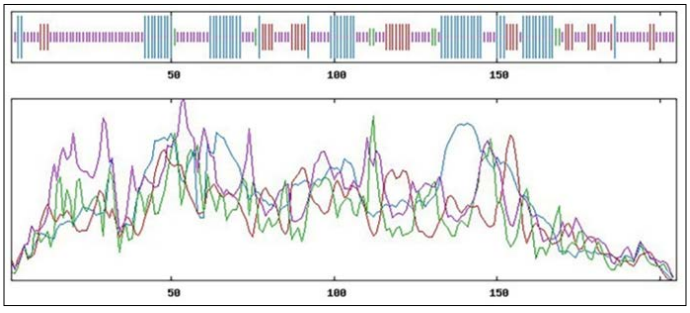

Figure 15: Secondary structure of AANAT

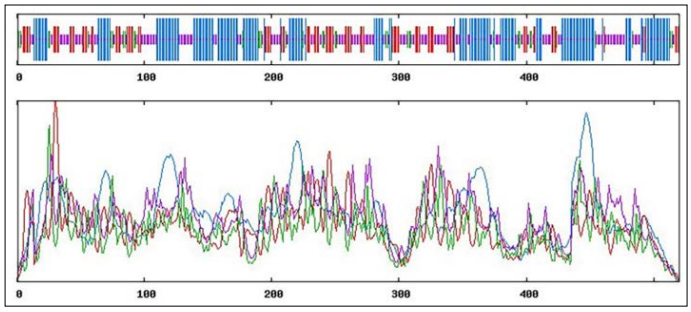

Figure 16: Secondary structure of MAOB

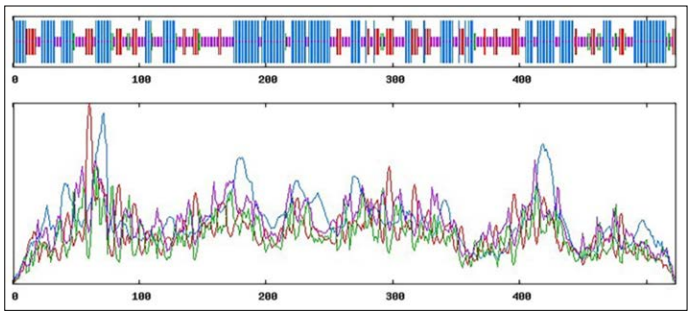

Figure 17: Secondary structure of LAO1

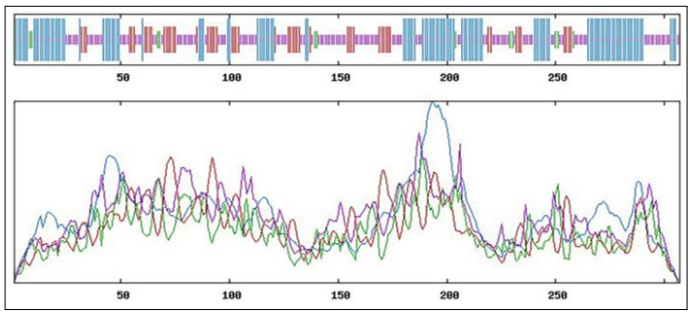

Figure 18: Secondary structure of 4930438A08RIK

The secondary structure β-turn contains low percentage in comparison to all other secondary structures. However, β-turn contains weak bonds that's why it may not favour the secondary structure and helps in folding process. The random coil is more flexible and dynamic folded chain region than other secondary structures [32].

Functional Annotation

The conserve domain/motifs serve as a catalytic site which plays an important role in manifestation of several biological functions. Annotated collection of protein domains/families classify as motifs descriptor is called PROSITE. It is either PROSITE patterns (ProPat) or PROSITE profiles (ProPro) or Pfam, received from several homologous sequences. ProPat describes qualitative motifs whereas ProPro describes quantitative protein domain demonstration and Pfam describe family level of classification [25,26]. In this study IDO1 domains and family was determined by using motif search enlisted in (Table 7-9).

Table 7: PROSITE PATTERN of IDO1 interacting proteins

Proteins	Found Motif	AA Position	Description
KYNU	-	-	-
TDO2	-	-	-
CYP1A1	CYTOCHROME_P450	454..463	Cytochrome P450 cysteine heme-iron ligand signature
TPH1	-	-	-
AFMID	-	-	-
CYP1A2	CYTOCHROME_P450	449..458	Cytochrome P450 cysteine heme-iron ligand signature.
CYP1B1	CYTOCHROME_P450	463..472	Cytochrome P450 cysteine heme-iron ligand signature
MAOA	-	-	-
DDC	DDC_GAD_HDC_YDC	296..317	DDC / GAD / HDC / TyrDC pyridoxal-phosphate attachment site.
IL4I1	-	-	-
ASMT	-	-	-
INMT	NNMT_PNMT_TEMT	60..76	NNMT/PNMT/TEMT family of methyltransferases signature.
TPH2	BH4_AAA_HYDROXYL_1	286..297	Biopterin-dependent aromatic amino acid hydroxylases signature.
AANAT	-	-	-
MAOB	-	-	
LAO1	-	-	-
4930438A08RIK	-	-	-
GM20708*			

Table 8: PROSITE PROFILE of IDO1 interacting proteins

Proteins	Found Motif	AA Position	Description
KYNU	-	-	-
TDO2	-	-	-
CYP1A1	-	-	-
TPH1	ACT BH4_AAA_HYDROXYL_2	22..97 96..155	ACT domain profile Biopterin-dependent aromatic amino acid hydroxylase family profile
AFMID	-	-	-
CYP1A2	-	-	-
CYP1B1	-	-	-
MAOA	-	-	-
DDC	-	-	-
IL4I1	-	-	-
ASMT	SAM_OMT_II PROKAR_LIPOPROTEIN	19..356 1..35	SAM-dependent O-methyltransferase class II-type profile Prokaryotic membrane lipoprotein lipid attachment site profile
INMT	SAM_MT_NNMT_PNMT_TEMT	9..163	SAM-dependent methyltransferase NNMT/PNMT/TEMT-type profile.
TPH2	BH4_AAA_HYDROXYL_2 ATC	111..457 28..103	BH4_AAA_HYDROXYL_2 ACT domain profile

AANAT	GNAT	33..194	Gcn5-related N-acetyltransferase (GNAT) domain profile
MAOB	-	-	
LAO1	-	-	-
4930438A08RIK	-	-	-
GM20708*			

Table 9: Functional domains using pfam

Proteins	Pfam	AA Position	Description	Number
KYNU	Aminotran_5	117-381	Aminotransferase class-V	1
TDO2	Trp_dioxygenase,	27-372	Tryptophan 2,3-dioxygenase	1
CYP1A1	p450,	44-497	Cytochrome P450	1
TPH1	Biopterin_H, ACT ACT_4 RRM_7	109-138 22-72 21-92 5-60	Biopterin-dependent aromatic amino acid hydroxylase ACT domain ACT domain RNA recognition motif	4
AFMID	BD-FAE, Abhydrolase_3 COesterase, Hydrolase_4 LIDHydrolase Abhydrolase_6 FSH1 Peptidase_S9 Lipase_3	74-260 90..247 50-130 132..201 133-208 89..198 158-264 136..258 133..171	BD-FAE alpha/beta hydrolase fold Carboxylesterase family Serine aminopeptidase, S33 Lipid-droplet associated hydrolase Alpha/beta hydrolase family Serine hydrolase (FSH1) Prolyl oligopeptidase family Lipase (class 3)	9
CYP1A2	p450, Sec8_exocyst, COG7,	41..500 249..280 248..298	Cytochrome P450 Sec8 exocyst complex component specific domain Golgi complex component 7 (COG7)	4
CYP1B1	p450	51..473	Cytochrome P450	1
MAOA	Amino_oxidase NAD_binding_ DAO Pyr_redox_2 Thi4 FAD_binding_2 HI0933_like Pyr_redox FAD_binding_3 FAD_oxidored Pyr_redox_3 Lycopene_cycl AlaDh_PNT_C GIDA, NAD_binding_9, Trp_halogenase,	23..460 18..83 15..58 15..47 14..52 15..52 14..49 16..51 15..46 15..52 17..45 15..48 15..54 15..44 17..63 15..45	Flavin containing amine oxidoreductase 8 NAD(P)-binding Rossmann-like domain FAD dependent oxidoreductase Pyridine nucleotide-disulphide oxidoreductase Thi4 family FAD binding domain HI0933-like protein Pyridine nucleotide-disulphide oxidoreductase FAD binding domain FAD dependent oxidoreductase Pyridine nucleotide-disulphide oxidoreductase Lycopene cyclase protein Alanine dehydrogenase/PNT, C-terminal domain Glucose inhibited division protein A FAD-NAD(P)-binding Tryptophan halogenase	16
DDC	Pyridoxal_deC, Beta_elim_lyase, Aminotran_1_2, Autoind_bind,	35..414 104..358 175..280 293..371	Pyridoxal-dependent decarboxylase conserved domain Beta-eliminating lyase Aminotransferase class I and II Autoinducer binding domain	4

IL4I1	Amino_oxidase, NAD_binding_8, DAO, AlaDh_PNT_C, FAD_binding_2, HI0933_like, Pyr_redox, FAD_oxidored, Thi4, FAD_binding_3, GIDA, MCRA, Lycopene_cycl, Pyr_redox_3, UDPG_MGDP_dh_N, Trp_halogenase, FMO-like, NAD_binding_9, 3HCDH_N, GalKase_gal_bdg, Shikimate_DH, ApbA,	68..502 63..117 60..95 49..91 61..97 60..97 60..96 61..97 60..96 60..91 60..94 60..130 61..104 62..104 60..91 60..90 59..103 62..101 60..92 458..492 57..87 61..90	Flavin containing amine oxidoreductase NAD(P)-binding Rossmann-like domain FAD dependent oxidoreductase Alanine dehydrogenase/PNT, C-terminal domain FAD binding domain HI0933-like protein Pyridine nucleotide-disulphide oxidoreductase FAD dependent oxidoreductase Thi4 family FAD binding domain Glucose inhibited division protein A MCRA family Lycopene cyclase protein Pyridine nucleotide-disulphide oxidoreductase UDP-glucose/GDP-mannose dehydrogenase family, NAD binding domain Tryptophan halogenase Flavin-binding monooxygenase-like FAD-NAD(P)-binding 3-hydroxyacyl-CoA dehydrogenase, NAD binding domain Galactokinase galactose-binding signature Shikimate / quinate 5-dehydrogenase Ketopantoate reductase PanE/ApbA	22
ASMT	Methyltransf_2 Dimerisation2 Methyltransf_25 Methyltransf_11 Methyltransf_23, Methyltransf_12, Methyltransf_31, MTS	123..338 18..105 189..288 191..291 183..338 191..289 188..296 180..217	O-methyltransferase domain Dimerisation domain Methyltransferase domain Methyltransferase domain Methyltransferase domain Methyltransferase domain Methyltransferase domain Methyltransferase small domain	8
INMT	NNMT_PNMT_TEMT,	1..122	NNMT/PNMT/TEMT family	1
TPH2	Biopterin_H, ACT,	124..453 28..77	Biopterin-dependent aromatic amino acid hydroxylase ACT domain	2
AANAT	Acetyltransf_1, Acetyltransf_10, Acetyltransf_7, Acetyltransf_9,	45..171 155.177 114..172 99..172	Acetyltransferase (GNAT) family Acetyltransferase (GNAT) domain Acetyltransferase (GNAT) domain Acetyltransferase (GNAT) domain	4
MAOB	Amino_oxidase, NAD_binding_8, DAO, FAD_binding_2, FAD_oxidored, Pyr_redox_2, Thi4, FAD_binding_3, Pyr_redox, Pyr_redox_3, HI0933_like, AlaDh_PNT_C, Lycopene_cycl, GIDA, MCRA,	14..451 9..75 6..64 6..43 6..42 5..44 4..42 5..37 7..42 8..39 6..40 4..49 6..39 6..35 6..73	Flavin containing amine oxidoreductase NAD(P)-binding Rossmann-like domain FAD dependent oxidoreductase FAD binding domain FAD dependent oxidoreductase Pyridine nucleotide-disulphide oxidoreductase Thi4 family FAD binding domain Pyridine nucleotide-disulphide oxidoreductase Pyridine nucleotide-disulphide oxidoreductase HI0933-like protein Alanine dehydrogenase/PNT, C-terminal domain Lycopene cyclase protein Glucose inhibited division protein A MCRA family	15

LAO1	Amino_oxidase, NAD_binding_8, Pyr_redox_2, DAO, FAD_binding_2 Pyr_redox, HI0933_like, FAD_oxidored, AlaDh_PNT_C, FAD_binding_3, FMO-like, Thi4, MCRA, Lycopene_cycl, GIDA, Pyr_redox_3, Trp_halogenase, NAD_binding_7, Shikimate_DH, UDPG_MGDP_dh_N, IlvN, ApbA, 3HCDH_N, HcgC, NAD_binding_9	67..508 62..115 58..93 59..93 60..94 59..93 59..96 60..96 59..91 59..89 58..105 59..94 58..117 60..92 60..91 61..91 59..89 57..89 57..87 59..90 57..86 60..91 60..91 57..92 61..102	Flavin containing amine oxidoreductase NAD(P)-binding Rossmann-like domain Pyridine nucleotide-disulphide oxidoreductase FAD dependent oxidoreductase FAD binding domain Pyridine nucleotide-disulphide oxidoreductase HI0933-like protein FAD dependent oxidoreductase Alanine dehydrogenase/PNT, C-terminal domain FAD binding domain Flavin-binding monooxygenase-like Thi4 family MCRA family Lycopene cyclase protein Glucose inhibited division protein A Pyridine nucleotide-disulphide oxidoreductase Tryptophan halogenase Putative NAD(P)-binding Shikimate / quinate 5-dehydrogenase UDP-glucose/GDP-mannose dehydrogenase family, NAD binding domain Acetohydroxy acid isomeroreductase, NADPH-binding domain Ketopantoate reductase PanE/ApbA 3-hydroxyacyl-CoA dehydrogenase, NAD binding domain FeGP cofactor biosynthesis protein, methyltransferase HcgC FAD-NAD(P)-binding	25
4930438A08RIK	Amino_oxidase,	36..283	Flavin containing amine oxidoreductase	1
Gm20708*				

Motif search data provides functional motifs of interacting proteins [30]. In this study six IDO1 interacting proteins namely; CYP1A1, CYP1A2, CYP1B1, DDC, INMT and TPH2 showed only ProPat. Further, five interacting proteins of IDO1 namely; TPH1, ASMT, INMT, TPH2 and AANAT showed only ProPro and two proteins namely; INMT and TPH2 showed both ProPat and ProPro. However, remaining KYNU, TDO2, AFMID, MAOA, IL4I1, MAOB, LAO1 and 4930438A08RIK did not show either ProPat or ProPro.

ProPat containing IDO1 interacting proteins namely; CYP1A1, CYP1A2, and CYP1B1 showed cytochrome P450 cysteine heme-iron ligand signature domain, DDC showed pyridoxal-phosphate attachment site domain, INMT showed family of methyltransferases signature domain and TPH2 showed biopterin-dependent aromatic amino acid hydroxylases signature. Similarly, ProPro contains protein; ASMT showed SAM-dependent O-methyltransferase class II-type profile domain and prokaryotic membrane lipoprotein lipid attachment site profile, INMT showed SAM-dependent methyltransferase NNMT/PNMT/TEMT-type profile and AANAT showed Gcn5-related N-acetyltransferase domain profile. TPH1 and TPH2 have shown biopterin-dependent aromatic amino acid hydroxylases signature domain from both ProPat and ProPro and ACT domain profile from ProPro only. Moreover, berberine which is a potential antidepressant improves depressive symptoms in mice by targeting tryptophan metabolising enzyme IDO1 and TPH [37].

IDO1 interacting proteins and their family and domain may involve in several biological functions. Briefly, CYPs (cytochrome p450) play an important role in the xenobiotics and toxins metabolism. AhR get directly activated by dietary molecules and xenobiotics. Also, many AhR ligands are processed and inactivated by cytochrome p450 family proteins [36]. The active form of vitamin B6 (pyridoxine or pyridoxal) is known as pyridoxal phosphate (PLP). PLP is a useful catalyst, working as a coenzyme and helps in several reactions, which includes deamination decarboxylation and transamination. Alteration in PLP levels in the brain may be responsible for several neurological dysfunctions [38]. A number of pyridoxal-dependent decarboxylases share similar regions of sequence, mostly lysine residue helping in the attachment site for the PLP group. These enzymes known as: Glutamate decarboxylase (GAD) helps in catalyses of glutamate decarboxylation into neurotransmitter GABA. IDO1 is the rate limiting enzyme of KYN and tetrahydrobiopterin (BH4). Inhibiting the function of BH4 might be a new direction of chronic pain treatment [39].

Moreover, INMT showed several cytoplasmic methyltransferases which includes nicotinamide N-methyltransferase (NNMT), phenylethanolamine N-methyltransferase (PNMT) and thioether S-methyltransferase (TEMT). Moreover, NNMT catalyses the N-methylation of nicotinamide and other pyridines to form pyridinium ions which is needed for the biotransformation of many drugs and xenobiotic compounds [40]. Further, PNMT is responsible for catalysing the biosynthesis of catecholamine, resulting in conversion of noradrenaline to adrenalin whereas TEMT catalyses the methylation of dimethyl sulphide into trimethylsulphonium. Moreover, IDO1 may be involved in aforementioned processes as showed interaction with INMT. Lastly, all IDO1 interacting proteins showed Pfam (Table 9).

Pfam result showed proteins namely; KYNU, TDO2, CYP1A1, CYP1B1, INMT and 4930438A08RIK showed only one Pfam family, TPH2 showed two, CYP1A2 showed three, TPH1, DDC, and AANAT showed four, ASMT showed eight, AFMID showed nine, MAOB showed fifteen, MAOA showed sixteen, IL4I1 showed twenty-two and finally LAO1 contains twenty-five Pfam family or domains. Further, Serine hydroxylase participating in all pathophysiological processes in mammals, including neurotransmission, pain sensation, inflammation, oxidative stress [41]. Taken together, this study might help to understand IDO1 dependent regulation in the context of tryptophan/kynurenine pathway and helpful in therapeutics of neurological disorders.

Conclusions

Present study provides new insight on in silico study of IDO1 interacting proteins using several bio-computational tools to evaluate their physiochemical properties, secondary structure and several other brain functions. Further, IDO1 interacted with TPH2 and TPH1 proteins, which is responsible for the regulation of serotonin neurotransmitter both in the brain and gut respectively. Majority of IDO1 interacting proteins having high α-helix structure and all proteins showed low β-turn secondary structure. Moreover, IDO1 may acts as therapeutic target for treatment of several psychological health issues including depression, anxiety and gut related disorders etc.

Competing Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Statement

Not applicable

References

1. Greco FA, Bournique A, Coletti A, Custodi C, Dolciami D (2016) Docking studies and molecular dynamic simulations reveal different features of IDO1 structure. Mol Inform 35: 449-459.
2. Wyatt M, Greathouse KL (2021) Targeting dietary and microbial tryptophan-indole metabolism as therapeutic approaches to colon cancer. Nutrients 13: 1189.
3. Liu W, Sheng H, Xu Y, Liu Y, Lu J (2013) Swimming exercise ameliorates depression-like behavior in chronically stressed rats: relevant to proinflammatory cytokines and IDO activation. Behav Brain Res 242: 110-116.
4. Mohajeri MH, La Fata G, Steinert RE, Weber P (2018) Relationship between the gut microbiome and brain function. Nutr Rev 76: 481-496.
5. Liberman AC, Budziñski ML, Sokn C, Gobbini RP, Steininger A, et al. (2018) Regulatory and mechanistic actions of glucocorticoids on T and inflammatory cells. Front Endocrinol (Lausanne) 9: 235.
6. Mithaiwala MN, Santana-Coelho D, Porter GA, O'Connor JC (2021) Neuroinflammation and the kynurenine pathway in CNS disease: Molecular mechanisms and therapeutic implications. Cells 10: 1548.
7. Kadriu B, Farmer CA, Yuan P, Park LT, Deng ZD (2021) The kynurenine pathway and bipolar disorder: intersection of the monoaminergic and glutamatergic systems and immune response. Mol Psychiatry 26: 4085-4095.
8. Koo YS, Kim H, Park JH, Kim MJ, Shin YI (2018) Indoleamine 2,3-dioxygenase-dependent neurotoxic kynurenine metabolism contributes to poststroke depression induced in mice by ischemic stroke along with spatial restraint stress. Oxid Med Cell Longev 30: 2413841.
9. Kim H, Chen L, Lim G, Sung B, Wang S (2012) Brain indoleamine 2,3-dioxygenase contributes to the comorbidity of pain and depression. J Clin Invest 122: 2940-2954.
10. Mammoli A, Coletti A, Ballarotto M, Riccio A, Carotti A (2020) New insights from crystallographic data: diversity of structural motifs and molecular recognition properties between groups of IDO1 structures. ChemMedChem 15: 891-899.
11. Ideker T, Sharan R (2008) Protein networks in disease. Genome Res 18: 644-652.
12. Gonzalez MW, Kann MG (2012) Chapter 4: Protein interactions and disease. PLoS Comput Biol 8: e1002819.
13. Badawy AA (2017) kynurenine pathway of tryptophan metabolism: Regulatory and functional aspects. Int J Tryptophan Res 10: 1178646917691938.
14. Sanjaya RE, Putri KDA, Kurniati A Rohman A, Puspaningsih NNT (2021) In silico characterization of the GH5-cellulase family from uncultured microorganisms: physicochemical and structural studies. J Genet Eng Biotechnol 19: 143.
15. Bharat Siva Varma P, Adimulam YB, Kodukula S (2015) In silico functional annotation of a hypothetical protein from Staphylococcus aureus. J Infect Public Health 8: 526-532.
16. Bidkar A, Thakur N, Bolshette JD, Gogoi R (2014) In-silico structural and functional analysis of hypothetical proteins of leptospira interrogans. Biochem Pharmacol 3: 2167-0501.
17. da S Vieira D, Polveiro RC, Butler TJ, Hackett TA, Braga CP (2021) An in silico, structural, and biological analysis of lactoferrin of different mammals. Int J Biol Macromol 187: 119-126.
18. Saikat ASM, Uddin M, Ahmad T, Mahmud S, Imran MAS (2021) Structural and functional elucidation of if-3 protein of chloroflexus aurantiacus involved in protein biosynthesis: An In-silico approach. Biomed Res Int 2021: 1-10.
19. Tan BH, Pan Y, Dong AN, Ong CE (2017) In vitro and in silico approaches to study cytochrome P450-mediated interactions. J Pharm Pharm Sci 20: 319-328.
20. Katalani C, Nematzadeh G, Ahmadian G, Amani J, Kiani G (2020) In silico design and in vitro analysis of a recombinant trivalent fusion protein candidate vaccine targeting virulence factor of Clostridium perfringens. Int J Biol Macromol 146: 1015-1023.
21. Saikat ASM, Uddin ME, Ahmad T, Mahmud S, Imran MAS (2021) Structural and functional elucidation of IF-3 protein of chloroflexus aurantiacus involved in protein biosynthesis: An in-silico approach. Biomed Res Int 9050026.
22. Kurrey K, Paramanik V (2018) Identification and physiochemical analysis of ERK interacting proteins using bio-computational tools. World J Neurosci 8: 303-313.
23. Roly ZY, Islam MM, Reza MA (2014) A comparative in silico characterization of functional and physicochemical properties of 3FTx (three finger toxin) proteins from four venomous snakes. Bioinformation 10: 281-287.
24. Kumari P, Kumar M, Sehgal N, Aggarwal N (2022) In silico analysis of kiss2, expression studies and protein-protein interaction with gonadotropin-releasing hormone 2 (GnRH2) and luteinizing hormone beta (LHβ) in Heteropneustes fossilis. J Biomol Struct Dyn 40: 4543-4557.
25. Kurrey K, Paramanik V (2021) Identification of secondary structure of extracellular signal regulated kinase (ERK) interacting proteins and their domain: An insilico study. World J Neurosci 11: 67-89.
26. Prabhu D, Rajamanikandan S, Anusha SB, Chowdary MS, Veerapandiyan M (2020) In silico functional annotation and characterization of hypothetical proteins from serratia marcescens FGI94. Biol Bull Russ Acad Sci 47: 319-331.
27. Walker AK, Wing EE, Banks WA, Dantzer R (2019) Leucine competes with kynurenine for blood-to-brain transport and prevents lipopolysaccharide-induced depression-like behavior

in mice. Mol Psychiatry 24: 1523-1532.
28. Remus JL, Dantzer R (2016) Inflammation models of depression in rodents: Relevance to psychotropic drug discovery. Int J Neuropsychopharmacol 19: pyw028.
29. Kantardjieff KA, Rupp B (2004) Protein isoelectric point as a predictor for increased crystallization screening efficiency. Bioinformatics 20: 2162-2168.
30. Dutta B, Banerjee A, Chakraborty P, Bandopadhyay R (2018) In silico studies on bacterial xylanase enzyme: Structural and functional insight. J Genet Eng Biotechnol 16: 749-756.
31. Dutta B, Deska J, Bandopadhyay R, Shamekh S (2021) In silico characterization of bacterial chitinase: Illuminating its relationship with archaeal and eukaryotic cousins. J Genet Eng Biotechnol 25: 19
32. Kaur A, Pati PK, Pati AM, Nagpal AK (2020) Physico-chemical characterization and topological analysis of pathogenesis-related proteins from Arabidopsis thaliana and Oryza sativa using in-silico approaches. PLoS One 15: e0239836.
33. Vidhya VG (2018) An in-silico analysis of physicochemical characterization and protein-protein interaction network analysis of human anti-apoptotic proteins. Asian J. Pharm 19: 146-155.
34. Agus A, Planchais J, Sokol H (2018) Gut microbiota regulation of tryptophan metabolism in health and disease. Cell Host Microbe 23: 716-724.
35. Mondanelli G, Volpi C (2021) The double life of serotonin metabolites: in the mood for joining neuronal and immune systems. Curr Opin Immunol 70: 1-6.
36. Ge PY, Qu SY, Ni SJ, Yao ZY, Qi YY (2023) Berberine ameliorates depression-like behavior in CUMS mice by activating TPH1 and inhibiting IDO1-associated with tryptophan metabolism. Phytother Res 37: 342-357.
37. Wichers MC, Maes M (2004) The role of indoleamine 2,3-dioxygenase (IDO) in the pathophysiology of interferon-alpha-induced depression. J Psychiatry Neurosci 29: 11-17.
38. Modoux M, Rolhion N, Mani S, Sokol H (2021) Tryptophan metabolism as a pharmacological target. Trends Pharmacol Sci 42: 60-73.
39. Clayton PT (2020) B6-responsive disorders: a model of vitamin dependency. J Inherit Metab Dis 29: 317-326.
40. Aksoy S, Szumlanski CL, Weinshilboum RM (1994) Human liver nicotinamide N-methyltransferase. cDNA cloning, expression, and biochemical characterization. J Biol Chem 269: 14835-14840.
41. Long JZ, Cravatt BF (2011) The metabolic serine hydrolases and their functions in mammalian physiology and disease. Chem Rev 111: 6022-6063.