The function of histamine receptor H4R in the brain revealed by interaction partners

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1. ABSTRACT

The histamine H_4 receptor is mainly expressed in haematopoietic cells, hence is linked to inflammatory and immune system conditions. It has been recently discovered that the receptor is expressed also in the mammalian central nervous system (CNS), but its role in the brain remains unclear. We address the potential functions of the histamine H_4 receptor in the human brain using a 'guilty by association' logic, by close examination of protein-protein functional associations networks in the human proteome.

2. INTRODUCTION

Histamine H_4 receptor (H4R), the most recently discovered member of the histamine receptors family, is described as the immune system histamine receptor (1), since it is dominantly expressed in cells of the immune system and in peripheral cells and tissues, such as blood, spleen, lung, liver and gut (2, 3). It has been related to pathological conditions as inflammation (4) and allergy (5, 6), and linked to autoimmune diseases (7, 8) and cancer (9), although its precise functional characterization is an open

Table 1. H3R predicted functional interactions

UniProt_ID	Name
RBGP1_HUMAN	Rab GTPase-activating protein 1
Q9NWP4_HUMAN	cDNA FLJ20703 fis, clone KAIA1965
TM7S4_HUMAN	Transmembrane 7 superfamily member 4
OS9_HUMAN	Protein OS-9
RBG1L_HUMAN	Rab GTPase-activating protein 1-like
GREM2_HUMAN	Gremlin-2
RFPL1_HUMAN	Ret finger protein-like 1
Q9BW99_HUMAN	Amplified in osteosarcoma, isoform CRA_d
GLRA3_HUMAN	Glycine receptor subunit alpha-3
ACHA9_HUMAN	Neuronal acetylcholine receptor subunit alpha-9
ABCG1_HUMAN	ATP-binding cassette sub-family G member 1
PASK_HUMAN	PAS domain-containing serine/threonine-protein kinase
XRCC1_HUMAN	DNA repair protein XRCC1
Q96RZ2_HUMAN	N-acetylglucosamine-1-phosphate transferase, gamma subunit, isoform CRA_b
A0PJU7_HUMAN	TSHR protein
ACHA6_HUMAN	Neuronal acetylcholine receptor subunit alpha-6
Q8WWH1_HUMAN	ATP-binding cassette transporter
TSHR_HUMAN	Thyrotropin receptor
SRGN_HUMAN	Serglycin
Q16503_HUMAN	Thyrotropin receptor-I
ERLEC_HUMAN	Endoplasmic reticulum lectin 1
ACHB2_HUMAN	Neuronal acetylcholine receptor subunit beta-2
WFDC8_HUMAN	WAP four-disulfide core domain protein 8
Q5T508_HUMAN	Leucine-rich repeat-containing G protein-coupled receptor 6
PI42B_HUMAN	Phosphatidylinositol-5-phosphate 4-kinase type-2 beta
GLRA2_HUMAN	Glycine receptor subunit alpha-2
Q8WWH2_HUMAN	ATP-binding cassette transporter
SPCS2_HUMAN	Signal peptidase complex subunit 2
ACH10_HUMAN	Neuronal acetylcholine receptor subunit alpha-10

and very active research field. It is clear that H4R can participate in diverse and complex physiological processes sometimes intertwined, this situation makes the identification and characterization of the receptor functions a hard task, which must be helped by systems biology technologies. Recently H4R has been reported to be functionally expressed on neurons (10), which broads the potential areas of function for this new histamine receptor. In this work we use the principle 'guilty by association' to establish firm hypothesis that drive new experimental approaches to unravel the role of H4R in the brain from its predicted functional partners.

3. METHODS

3.1. The Knowledgegram and the Predictogram

We used the Knowledgegram (KG) to find known functional associations for H4R in the human proteome and the Predictogram (PG) to predict new H4R functional partners. They comprise a set of methods that retrieve and combine protein-protein associations from major biological databases into a network (KG) and predict functional interactions (PG) (11).

Briefly, the KG includes the protein-protein association data from Reactome (12), Kegg (13), GO (14), FunCat (15), Intact (16), MINT (17) and HRPD (18). On the other hand the PG predicts binary protein functional associations in the human proteome (PG) by integrating different computational methods: Gene Expression COmparison (GECO) measures the correlation of gene expression profiles between protein pairs; homology

Inherited Protein-Protein Interactions (hIPPI) scores potential protein-protein interactions based on their homology to known interacting protein pairs; and Co-Occurrence Domain Analysis (CODA) (19) looks for and scores protein pairs in a given target genome (e.g. human) found as fused (Co-Occurring) domain architectures in homologues from genomes of 575 different species. The integration of different computational prediction methods in the PG brings more accurate functional associations between proteins than using each individual method. The cumulative frequency distributions were calculated for each of the prediction score datasets (GECO, hiPPI and CODA). The particular Probability Density Functions (PDF) associated with the score distributions for each of the methods was calculated in order to translate the scores into p-values. The different methods p-values for each protein-protein pair were integrated into one single score using the Fisher weighted method (20). Integrated prediction scores were benchmarked using the highest quality annotations of the human proteome in the Gene Ontology. Precision associated with the Fisher scores was calculated as the ratio of cumulative TP/TP+FP at different prediction p-values, where TP (True Positives) is the rate of hits predicted as true protein binary associations in GO, and FP (False positive) is the average rate of hits predicted from 1000 random iterations.

3.2. Mapping functions on networks

All network analysis and visualization were done using Cytoscape (21). Mapping of the Gene Ontology (GO) categories statistically overrepresented in our networks were done with the BiNGO plugin (22) within Cytoscape, searching in the Biological Process and GO Full ontologies with the hypergeometric statistical test and a significance level of 0,05.

4. RESULTS

4.1. Lack of knowledge about H4R relationships

When using H4R as a bait to check the current knowledge about proteins associated with the receptor, we found virtually no output, that is there are no H4R interactors described and compiled in the major databases yet.

4.2. H3R and H4R predicted functional associations

Our PG analysis with the histamine H_3 receptor (H3R) yields 29 statistically significant interactions (p-val $\leq$ 0,014; 80% precision; Table 1). There are no homology inherited protein-protein interactions, but now we have domain fusion a gene co-expression signals in our functional relationships prediction. The overrepresented GO categories show the H3R function in neurotransmission. We found 188 statistically significant (p-val $\leq$ 0,014; 80% precision) functional interactions with H4R in the human proteome (Table 2). As with H3R there are no predicted physical interactions between the proteins in this highly reliable set and H4R, we have mainly predictions from gene co-expression signals and domain fusion. The Gene Ontology categories statistically overrepresented in this set of highly confiable H4R interactors, give us an overall picture of the cellular function H4R is involved in (Figure 1).

Table 2. H4R predicted functional interactions

UniProt ID	Name
A1ATR_HUMAN	Putative alpha-1-antitrypsin-related protein (Serpin A2)
ACHB4_HUMAN	Neuronal acetylcholine receptor subunit beta-4
APC2_HUMAN	Adenomatous polyposis coli protein 2 (Adenomatous polyposis coli protein-like) (APC-like)
APR2_HUMAN	Apoptosis-related protein 2 (APR-2)
ASCL2_HUMAN	Achaete-scute homolog 2 (ASH-2) (hASH2) (Mash2) (Class A basic helix-loop-helix protein 45) (bHLHa45)
AT2B3_HUMAN	Plasma membrane calcium-transporting ATPase 3 (PMCA3) (EC 3.6.3.8) (Plasma membrane calcium ATPase isoform 3) (Plasma membrane calcium pump isoform 3)
ATF7_HUMAN	Cyclic AMP-dependent transcription factor ATF-7 (cAMP-dependent transcription factor ATF-7) (Activating transcription factor 7) (Transcription factor ATF-A)
ATS20_HUMAN	A disintegrin and metalloproteinase with thrombospondin motifs 20 (ADAMTS-20) (ADAM-TS 20) (ADAM-TS20) (EC 3.4.24.-)
ATX3L_HUMAN	Putative ataxin-3-like protein (EC 3.1.2.15) (Machado-Joseph disease protein 1-like)
AUXI_HUMAN	Putative tyrosine-protein phosphatase auxilin (EC 3.1.3.48) (DnaJ homolog subfamily C member 6)
B3GT1_HUMAN	Beta-1,3-galactosyltransferase 1 (Beta-1,3-GalTase 1) (Beta3Gal-T1) (Beta3GalT1) (EC 2.4.1.-) (UDP-galactose:beta-N-acetyl-glucosamine-beta-1,3-galactosyltransferase 1) (UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase-I)
B3GT5_HUMAN	Beta-1,3-galactosyltransferase 5 (Beta-1,3-GalTase 5) (Beta3Gal-T5) (Beta3GalT5) (b3Gal-T5) (EC 2.4.1.-) (Beta-3-Gx-T5) (UDP-galactose:beta-N-acetylglucosamine beta-1,3-galactosyltransferase 5) (UDP-Gal:beta-GlcNAc beta-1,3-galactosyltransferase 5)
BPEC1_HUMAN	Putative BPES syndrome breakpoint region protein (BPES candidate 1)
BTC_HUMAN	Probetacellulin [Cleaved into: Betacellulin (BTC)]
C144A_HUMAN	Coiled-coil domain-containing protein 144A
CBPC3_HUMAN	Cytosolic carboxypeptidase 3 (EC 3.4.17.-) (ATP/GTP-binding protein-like 3)
CDX4_HUMAN	Homeobox protein CDX-4 (Caudal-type homeobox protein 4)
CI031_HUMAN	Putative uncharacterized protein C9orf31 (Protein MOST-2)
CJ079_HUMAN	WD repeat-containing protein C10orf79
CLCA3_HUMAN	Calcium-activated chloride channel regulator family member 3 (Calcium-activated chloride channel family member 3) (hCLCA3)
CN143_HUMAN	EF-hand domain-containing protein C14orf143
CO034_HUMAN	Uncharacterized protein C15orf34
CP7A1_HUMAN	Cholesterol 7-alpha-monooxygenase (EC 1.14.13.17) (Cytochrome P450 7A1) (CYPVII) (Cholesterol 7-alpha-hydroxylase)
CRFR2_HUMAN	Corticotropin-releasing factor receptor 2 (CRF-R-2) (CRFR-2) (CRF-R2) (Corticotropin-releasing hormone receptor 2) (CRH-R-2) (CRH-R2)
CRGA_HUMAN	Gamma-crystallin A (Gamma-A-crystallin) (Gamma-crystallin 5)
CRIS1_HUMAN	Cysteine-rich secretory protein 1 (CRISP-1) (Acidic epididymal glycoprotein homolog) (AEG-like protein) (ARP)
CXY02_HUMAN	Putative uncharacterized protein encoded by NCRNA00105
DCAKD_HUMAN	Dephospho-CoA kinase domain-containing protein
DEC1_HUMAN	Deleted in esophageal cancer 1 (Candidate tumor suppressor CTS9)
DSCR4_HUMAN	Down syndrome critical region protein 4 (Down syndrome critical region protein B)
E15L2_HUMAN	Putative epidermal growth factor receptor pathway substrate 15-like protein 2
ELOA2_HUMAN	RNA polymerase II transcription factor SIII subunit A2 (Elongin-A2) (EloA2) (Transcription elongation factor B polypeptide 3B)
EXOG_HUMAN	Nuclease EXOG, mitochondrial (EC 3.1.30.-) (Endonuclease G-like 1) (Endo G-like 1)
F120A_HUMAN	Constitutive coactivator of PPAR-gamma-like protein 1 (Oxidative stress-associated Src activator) (Protein FAM120A)
FGF8_HUMAN	Fibroblast growth factor 8 (FGF-8) (Heparin-binding growth factor 8) (HBGF-8) (Androgen-induced growth factor) (AIGF)
FOXN1_HUMAN	Forkhead box protein N1 (Winged-helix transcription factor nude)
G6PC2_HUMAN	Glucose-6-phosphatase 2 (G-6-Pase 2) (G6Pase 2) (EC 3.1.3.9) (Islet-specific glucose-6-phosphatase catalytic subunit-related protein)
GLRA3_HUMAN	Glycine receptor subunit alpha-3
GOG6A_HUMAN	Golgin subfamily A member 6A (Golgin linked to PML) (Golgin-like protein)
GON1_HUMAN	Progonadoliberin-1 (Progonadoliberin I) [Cleaved into: Gonadoliberin-1 (Gonadoliberin I) (Luteinizing hormone-releasing hormone I) (LH-RH I) (Gonadotropin-releasing hormone I) (GnRH-I) (Luliberin I) (Gonadorelin); GnRH-associated peptide 1 (GnRH-associated peptide I)]
GPR3_HUMAN	G-protein coupled receptor 3 (ACCA orphan receptor)
GREM2_HUMAN	Gremlin-2 (Cysteine knot superfamily 1, BMP antagonist 2) (Protein related to DAN and cerberus) (DAN domain family member 3)
GRIP1_HUMAN	Glutamate receptor-interacting protein 1 (GRIP-1)
GSC2_HUMAN	Homeobox protein goosecoid-2 (GSC-2) (Homeobox protein goosecoid-like) (GSC-L)
HXD12_HUMAN	Homeobox protein Hox-D12 (Homeobox protein Hox-4H)
ICA69_HUMAN	Islet cell autoantigen 1 (69 kDa islet cell autoantigen) (ICA69) (Islet cell autoantigen p69) (ICAp69) (p69)
IFNA5_HUMAN	Interferon alpha-5 (IFN-alpha-5) (Interferon alpha-G) (LeIF G) (Interferon alpha-61)
IFNA6_HUMAN	Interferon alpha-6 (IFN-alpha-6) (Interferon alpha-K) (LeIF K) (Interferon alpha-54)
IMPG2_HUMAN	Interphotoreceptor matrix proteoglycan 2 (Sialoprotein associated with cones and rods proteoglycan) (Spacrcan) (Interphotoreceptor matrix proteoglycan of 200 kDa) (IPM 200)
INSRR_HUMAN	Insulin receptor-related protein (IRR) (EC 2.7.10.1) (IR-related receptor) [Cleaved into: Insulin receptor-related protein alpha chain; Insulin receptor-related protein beta chain]
KI3X1_HUMAN	Putative killer cell immunoglobulin-like receptor-like protein KIR3DX1 (Leukocyte receptor cluster member 12)
LBX1_HUMAN	Transcription factor LBX1 (Ladybird homeobox protein homolog 1)
LRAT_HUMAN	Lecithin retinol acyltransferase (EC 2.3.1.135) (Phosphatidylcholine--retinol O-acyltransferase)
LRIT1_HUMAN	Leucine-rich repeat, immunoglobulin-like domain and transmembrane domain-containing protein 1 (Leucine-rich repeat-containing protein 21) (Photoreceptor-associated LRR superfamily protein) (Retina-specific protein PAL)
LRTM1_HUMAN	Leucine-rich repeat and transmembrane domain-containing protein 1
MAGB1_HUMAN	Melanoma-associated antigen B1 (MAGE-B1 antigen) (MAGE-XP antigen) (DSS-AHC critical interval MAGE superfamily 10) (DAM10) (Cancer/testis antigen 3.1) (CT3.1)
MAGB3_HUMAN	Melanoma-associated antigen B3 (MAGE-B3 antigen)
MC5R_HUMAN	Melanocortin receptor 5 (MC5-R) (MC-2)
MED14_HUMAN	Mediator of RNA polymerase II transcription subunit 14 (Mediator complex subunit 14) (Cofactor required for Sp1 transcriptional activation subunit 2) (CRSP complex subunit 2) (Transcriptional coactivator CRSP150) (Vitamin D3 receptor-interacting protein complex 150 kDa component) (DRIP150) (Thyroid hormone receptor-associated protein complex 170 kDa component) (Trap170) (Activator-recruited cofactor 150 kDa component) (ARC150) (RGR1 homolog) (hRGR1)
MF2L2_HUMAN	Probable guanine nucleotide exchange factor MCF2L2 (MCF2-transforming sequence-like protein 2) (Dbs-related Rho family guanine

	nucleotide exchange factor)
MIA2_HUMAN	Melanoma inhibitory activity protein 2
MORC1_HUMAN	MORC family CW-type zinc finger protein 1 (Cancer/testis antigen 33) (CT33)
MOST1_HUMAN	Protein MOST-1
MOS_HUMAN	Proto-oncogene serine/threonine-protein kinase mos (EC 2.7.11.1) (Proto-oncogene c-Mos) (Oocyte maturation factor mos)
MTLR_HUMAN	Motilin receptor (G-protein coupled receptor 38)
MTMR1_HUMAN	Myotubularin-related protein 1 (EC 3.1.3.-)
MTR1B_HUMAN	Melatonin receptor type 1B (Mel1b receptor) (Mel-1B-R)
NACA2_HUMAN	Nascent polypeptide-associated complex subunit alpha-2 (Nascent polypeptide-associated complex subunit alpha-like) (NAC-alpha-like) (Alpha-NAC-like) (Hom s 2.01)
NGN2_HUMAN	Neurogenin-2 (NGN-2) (Protein atonal homolog 4) (Class A basic helix-loop-helix protein 8) (bHLHa8)
NMBR_HUMAN	Neuromedin-B receptor (NMB-R) (Neuromedin-B-preferring bombesin receptor)
NOX3_HUMAN	NADPH oxidase 3 (EC 1.6.3.-) (gp91phox homolog 3) (GP91-3) (Mitogenic oxidase 2) (MOX-2)
NPAS3_HUMAN	Neuronal PAS domain-containing protein 3 (Neuronal PAS3) (Member of PAS protein 6) (Basic-helix-loop-helix-PAS protein MOP6) (PAS domain-containing protein 6) (Class E basic helix-loop-helix protein 12) (bHLHe12)
NR2C1_HUMAN	Nuclear receptor subfamily 2 group C member 1 (Orphan nuclear receptor TR2) (Testicular receptor 2)
O10H2_HUMAN	Olfactory receptor 10H2 (Olfactory receptor OR19-23)
OR1E1_HUMAN	Olfactory receptor 1E1 (Olfactory receptor OR17-18) (Olfactory receptor-like protein HGMP07I) (Olfactory receptor 17-2/17-32) (OR17-2) (OR17-32) (Olfactory receptor 13-66) (OR13-66) (Olfactory receptor 5-85) (OR5-85) (Olfactory receptor 1E5) (Olfactory receptor 1E6)
OR2C1_HUMAN	Olfactory receptor 2C1 (Olfactory receptor OR16-1) (Olfactory receptor OR16-2) (OLFmf3) (Olfactory receptor 2C2)
OR6A2_HUMAN	Olfactory receptor 6A2 (Olfactory receptor OR11-83) (Olfactory receptor 11-55) (OR11-55) (hP2 olfactory receptor) (Olfactory receptor 6A1)
P12L2_HUMAN	POM121-like protein 2
PAR4_HUMAN	Proteinase-activated receptor 4 (PAR-4) (Thrombin receptor-like 3) (Coagulation factor II receptor-like 3)
PBOV1_HUMAN	Prostate and breast cancer overexpressed gene 1 protein (Protein UROC28) (UC28)
PBPL2_HUMAN	Platelet basic protein-like 2 (DNA-binding protein amplifying expression of surfactant protein B) (DNA-binding protein SPBPBP)
PCDA2_HUMAN	Protocadherin alpha-2 (PCDH-alpha-2)
PDE6C_HUMAN	Cone cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha' (EC 3.1.4.35) (cGMP phosphodiesterase 6C)
PLA2R_HUMAN	Secretory phospholipase A2 receptor (PLA2-R) (PLA2R) (180 kDa secretory phospholipase A2 receptor) (M-type receptor) (C-type lectin domain family 13 member C) [Cleaved into: Soluble secretory phospholipase A2 receptor (Soluble PLA2-R) (Soluble PLA2R)]
PPARD_HUMAN	Peroxisome proliferator-activated receptor delta (PPAR-delta) (Peroxisome proliferator-activated receptor beta) (PPAR-beta) (Nuclear receptor subfamily 1 group C member 2) (Nuclear hormone receptor 1) (NUC1) (NUCI)
PPY2_HUMAN	Putative pancreatic polypeptide 2
PSG1_HUMAN	Pregnancy-specific beta-1-glycoprotein 1 (Pregnancy-specific glycoprotein 1) (PS-beta-G-1) (PSBG-1) (Pregnancy-specific beta-1 glycoprotein C/D) (PS-beta-C/D) (Fetal liver non-specific cross-reactive antigen 1/2) (FL-NCA-1/2) (PSG95) (CD66 antigen-like family member F) (CD antigen CD66f)
PTN22_HUMAN	Tyrosine-protein phosphatase non-receptor type 22 (EC 3.1.3.48) (Hematopoietic cell protein-tyrosine phosphatase 70Z-PEP) (Lymphoid phosphatase) (LyP)
PURG_HUMAN	Purine-rich element-binding protein gamma
RB40A_HUMAN	Ras-related protein Rab-40A (SOCS box-containing protein RAR2A) (Protein Rar-2)
RFPL1_HUMAN	Ret finger protein-like 1 (RING finger protein 78)
RHG06_HUMAN	Rho GTPase-activating protein 6 (Rho-type GTPase-activating protein 6) (Rho-type GTPase-activating protein RhoGAPX-1)
RL3R1_HUMAN	Relaxin-3 receptor 1 (RLN3 receptor 1) (Relaxin family peptide receptor 3) (Somatostatin- and angiotensin-like peptide receptor) (G protein-coupled receptor SALPR) (G-protein coupled receptor GPCR135)
RNLS_HUMAN	Renalase (EC 1.4.-.-) (Monoamine oxidase-C) (MAO-C)
S26A1_HUMAN	Sulfate anion transporter 1 (SAT-1) (Solute carrier family 26 member 1)
SIGL8_HUMAN	Sialic acid-binding Ig-like lectin 8 (Siglec-8) (Sialoadhesin family member 2) (SAF-2) (CDw329) (CD antigen CD329)
SMG64_HUMAN	Putative smooth muscle cell-expressed and macrophage conditioned medium-induced protein 64 (Smag-64)
SNAT_HUMAN	Serotonin N-acetyltransferase (Serotonin acetylase) (EC 2.3.1.87) (Aralkylamine N-acetyltransferase) (AA-NAT)
SPAT1_HUMAN	Spermatogenesis-associated protein 1 (Sperm-specific protein SP-2)
SPCS2_HUMAN	Signal peptidase complex subunit 2 (EC 3.4.-.-) (Microsomal signal peptidase 25 kDa subunit) (SPase 25 kDa subunit)
SPTC3_HUMAN	Serine palmitoyltransferase 3 (EC 2.3.1.50) (Serine-palmitoyl-CoA transferase 3) (SPT 3) (Long chain base biosynthesis protein 3) (LCB 3) (Long chain base biosynthesis protein 2b) (LCB2b)
SRGN_HUMAN	Serglycin (Secretory granule proteoglycan core protein) (Platelet proteoglycan core protein) (P.PG) (Hematopoetic proteoglycan core protein)
T2R16_HUMAN	Taste receptor type 2 member 16 (T2R16)
TAAR2_HUMAN	Trace amine-associated receptor 2 (Trace amine receptor 2) (TaR-2) (G-protein coupled receptor 58)
THOC5_HUMAN	THO complex subunit 5 homolog (Functional spliceosome-associated protein 79) (fSAP79) (NF2/meningioma region protein pK1.3) (Placental protein 39.2) (PP39.2) (hTREX90)
TIGD6_HUMAN	Tigger transposable element-derived protein 6
TM7S4_HUMAN	Transmembrane 7 superfamily member 4 (Dendritic cell-specific transmembrane protein) (DC-STAMP) (IL-4-induced protein) (FIND)
TNF15_HUMAN	Tumor necrosis factor ligand superfamily member 15 (Vascular endothelial cell growth inhibitor) (TNF ligand-related molecule 1) [Cleaved into: Tumor necrosis factor ligand superfamily member 15, membrane form; Tumor necrosis factor ligand superfamily member 15, secreted form]
TRPC5_HUMAN	Short transient receptor potential channel 5 (TrpC5) (Transient receptor protein 5) (TRP-5) (hTRP-5) (hTRP5)
TT39A_HUMAN	Tetratricopeptide repeat protein 39A (TPR repeat protein 39A) (Differentially expressed in MCF7 with estradiol protein 6) (DEME-6)
WFDC8_HUMAN	WAP four-disulfide core domain protein 8 (Putative protease inhibitor WAP8)
WNT1_HUMAN	Proto-oncogene Wnt-1 (Proto-oncogene Int-1 homolog)
YA001_HUMAN	Uncharacterized protein FLJ14100
YE014_HUMAN	Putative uncharacterized protein PRO0255
YK016_HUMAN	Putative uncharacterized protein MGC13053
ZN132_HUMAN	Zinc finger protein 132
ZN157_HUMAN	Zinc finger protein 157 (Zinc finger protein HZF22)
ZN442_HUMAN	Zinc finger protein 442
ZN549_HUMAN	Zinc finger protein 549
ZN613_HUMAN	Zinc finger protein 613

ZN749_HUMAN	Zinc finger protein 749
ZN771_HUMAN	Zinc finger protein 771 (Mesenchymal stem cell protein DSC43)
ZNF41_HUMAN	Zinc finger protein 41
ZNF80_HUMAN	Zinc finger protein 80 (ZNFpT17)
Q9P136_HUMAN	HCG1745369 (PRO3073)
Q5Q0U2_HUMAN	Fucosyltransferase 9 (EC 2.4.1.152) (Fucosyltransferase 9 (Alpha (1,3) fucosyltransferase), isoform CRA_a) (cDNA, FLJ95882, Homo sapiens fucosyltransferase 9 (alpha (1,3) fucosyltransferase) (FUT9), mRNA)
Q3SYA7_HUMAN	MAGEC3 protein (Melanoma antigen family C, 3)
Q9P159_HUMAN	HCG2016213 (PRO2613)
Q9NX68_HUMAN	CDNA FLJ20413 fis, clone KAT02170 (HCG1742967)
Q9H3B4_HUMAN	HCG2014696 (PRO1454)
Q9UI57_HUMAN	HCG40042 (PRO0514)
Q9P145_HUMAN	HCG1818458 (PRO2832)
Q9P1D0_HUMAN	HCG1786590 (PRO2533)
Q9NW32_HUMAN	HCG1770745, isoform CRA_a (cDNA FLJ10346 fis, clone NT2RM2001004) (cDNA FLJ14128 fis, clone MAMMA1002590)
Q9P1C4_HUMAN	HCG1788068 (PRO2796)
Q9BY25_HUMAN	HCG2007431 (IL-1beta-regulated neutrophil survival protein)
Q9NXD8_HUMAN	CDNA FLJ20306 fis, clone HEP06881 (Putative uncharacterized protein FLJ20306)
A4D0T8_HUMAN	G protein-coupled receptor 85 (cDNA, FLJ92962, Homo sapiens G protein-coupled receptor 85 (GPR85), mRNA)
Q96SH0_HUMAN	Transcription factor AP-2 alpha (Activating enhancer binding protein 2 alpha) (Transcription factor AP-2 alpha (Activating enhancer binding protein 2 alpha), isoform CRA_a)
A1L4Q0_HUMAN	Diacylglycerol kinase, epsilon 64kDa (cDNA FLJ75840, highly similar to Homo sapiens diacylglycerol kinase, epsilon 64kDa (DGKE), mRNA)
Q86YB4_HUMAN	PKD2L2 protein (Polycystic kidney disease 2-like 2, isoform CRA_a)
Q9P156_HUMAN	HCG2022592 (PRO2706)
Q9NYS9_HUMAN	Docking protein 1-like protein (HCG2016923) (Fragment)
Q9HD52_HUMAN	HCG1778511 (Uncharacterized gastric protein ZA52P)
Q9UFQ2_HUMAN	Putative uncharacterized protein DKFZp434K1235 (Fragment)
Q5VVL7_HUMAN	Dihydrolipoamide branched chain transacylase E2
Q9P1F9_HUMAN	PRO1942
Q9BRW5_HUMAN	SP2 protein
Q9H5H6_HUMAN	cDNA: FLJ23429 fis, clone HRC10578
Q9NZY3_HUMAN	HSPC052
Q9P166_HUMAN	PRO2435
Q5JYH9_HUMAN	Novel protein (Fragment)
Q9NYJ6_HUMAN	Ncaml
Q9P1K0_HUMAN	PRO1257
O95132_HUMAN	SOX-28 protein (Fragment)
Q68CQ5_HUMAN	Putative uncharacterized protein DKFZp781N1372
Q5G1L5_HUMAN	N-terminally extended type 3 canonical transient receptor potential channel
Q9NWP0_HUMAN	cDNA FLJ20712 fis, clone HUV01027
Q9P146_HUMAN	PRO2831
Q9P1E0_HUMAN	PRO2239
Q13528_HUMAN	Stem cell factor
Q6PIS3_HUMAN	XYLB protein (Fragment)
Q00849_HUMAN	ORF protein
Q4G0S9_HUMAN	ATP10A protein
Q9UHV1_HUMAN	PRO1386
Q9UHU9_HUMAN	PRO1510
Q9NWJ7_HUMAN	CDNA FLJ20802 fis, clone ADSU01223
Q9UI73_HUMAN	PRO0246
Q9P181_HUMAN	PRO2176
Q9BVE2_HUMAN	CDC2L5 protein
Q68D53_HUMAN	Putative uncharacterized protein DKFZp686K0887
Q5JTM4_HUMAN	Regulatory factor X, 3 (Influences HLA class II expression) (Fragment)
Q5JR97_HUMAN	Patched homolog 2 (Drosophila) (Fragment)
Q9H380_HUMAN	PRO2946
Q9H399_HUMAN	PRO2532
Q9UHT8_HUMAN	PRO1770
Q8IXE5_HUMAN	Seven transmembrane helix receptor
Q6I955_HUMAN	3β-HSD Φ1 protein
Q548W9_HUMAN	Tripartite motif protein TRIM14 alpha
Q9P155_HUMAN	PRO2710
Q9H353_HUMAN	PRO2259
Q9P194_HUMAN	PRO1728
Q9HAA9_HUMAN	cDNA FLJ11867 fis, clone HEMBA1006976, weakly similar to H.sapiens Gal-beta(1-3/1-4)GlcNAc alpha-2.3-sialyltransferase
Q9UI77_HUMAN	PRO0214
Q14DF6_HUMAN	PPEF2 protein
Q9H6Z8_HUMAN	CDNA: FLJ21625 fis, clone COL08015
Q53HV3_HUMAN	Lysyl oxidase-like 2 variant (Fragment)
MSI1H_HUMAN	RNA-binding protein Musashi-1

4.3. H3R and H4R interactors in the human brain

From the 29 H3R functional interactors predicted, 8 correspond to unknown proteins or unreviewed entries in UniProt (23). 11 out of the 21 characterized H3R predicted interactors are expressed in brain, offering a clear overview of the H3R role in the CNS.
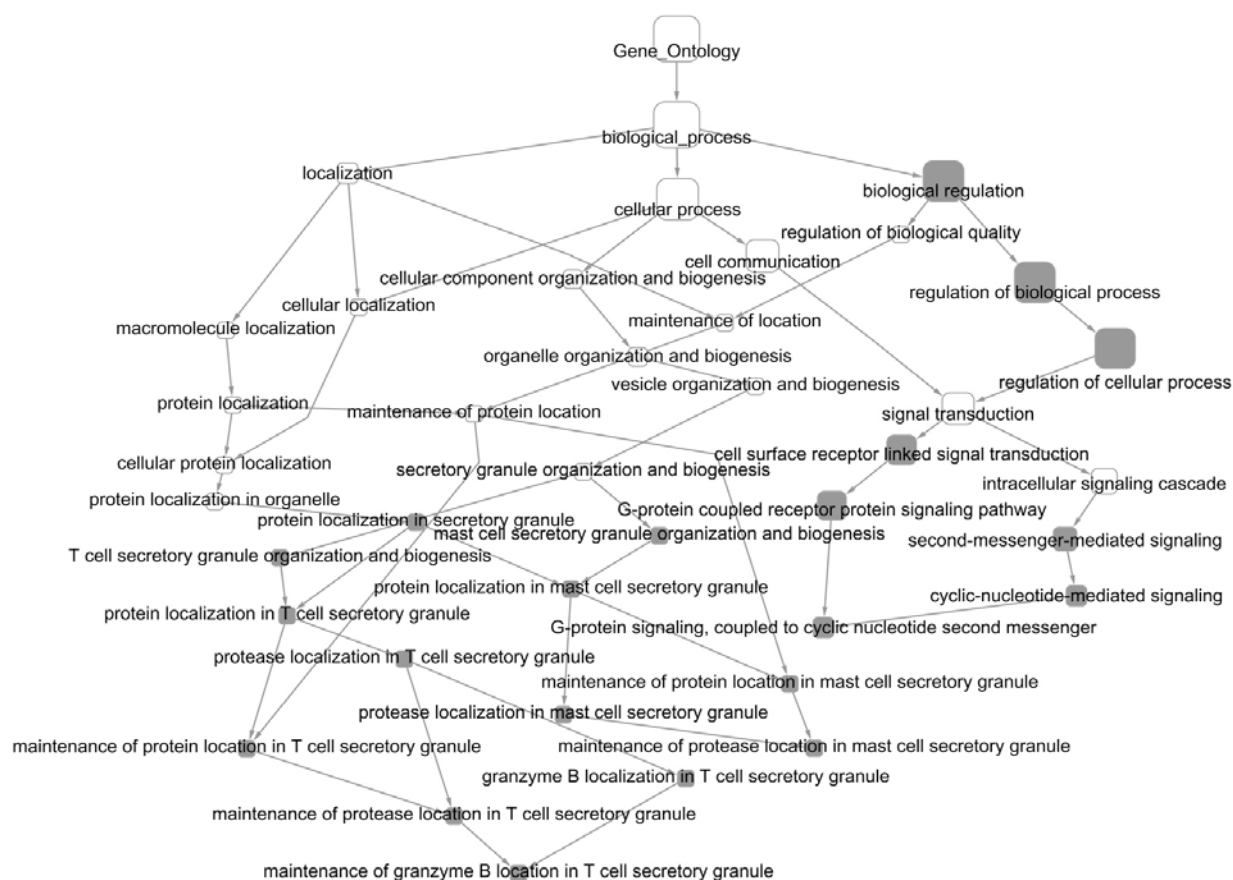
Figure 1. Functions network of the H4R predicted interactors. Nodes are labelled by their GO category. Grey nodes highlight statistically overrepresented functions in the set of H4R interactors. The size of the nodes is proportional to the number of genes in the set which are annotated to that node.

We found 15 proteins predicted to have a functional relationship with H4R and expressed exclusively or mainly in the brain (Table 3). We obtain a strong co-expression signal between each interactor and H4R and no inherited physical protein-protein interaction. Most of them are proteins of unknown specific function or involved in elemental neuron biochemistry and development, but some of the proteins predicted to be co-expressed with H4R in the CNS play clear roles in important brain functions, they are highlighted in bold typeface in Table 3.

5. DISCUSSION

H3R is a well known pre-synaptic autoreceptor controlling histaminergic neuron activity (24) and a heteroreceptor regulating the release of important neurotransmitters, such as acetylcholine (25) and serotonine (26). We can see the involvement of the receptor in these functions from the analysis of the predicted interactions using the Predictogram. We also see its implications in neuronal diseases (27) such as epilepsy through gene co-expression and domain fusion signals with the Neuronal acetylcholine receptor subunit beta-2 (UniProt ID ACHB2_HUMAN); Alzheimer's disease through gene co-expression signal with Rab GTPase-activating protein 1-like (UniProt ID RBG1L_HUMAN). This indicates that we

can obtain functional information for a protein from the analysis of its predicted functional interactors.

Our results do not include predicted protein-protein interactions (PPI) between H3R and proteins expressed by histaminergic neurons, Therefore we can not point to the role of H3R in the regulation of histamine release based on predictions. Coverage limitations are not only a shortcoming of the Predictogram methods, but it is a feature to any other experimental method able to obtain reliable PPI information, e.g. yeast two-hybrid experiments do not perform well on membrane-associated proteins and transient interactions tend to be under-reported (28). Besides, the data showing H3R as an autoreceptor in the regulation of histamine release is not even clear from the protein-protein associations described in the major databases using the Knowledgegram. We have also searched H3R protein associations with STRING (29) and iHOP (30); both powerful algorithms which uses text mining of literature, without clear outcomes in this direction (data not shown).

We address in this work the potential roles for H4R in the human brain from its predicted functional interactions in the human proteome. Its clear that the

Table 3. H4R interactors in the brain. Brain proteins co-expressed with H4R. ID, Name and Function as shown in the UniProt database

ID	Name	Function
TRPC5_HUMAN	Short transient receptor potential channel 5	Thought to form a receptor-activated non-selective calcium permeant cation channel. Probably is operated by a phosphatidylinositol second messenger system activated by receptor tyrosine kinases or G protein-coupled receptors. Has also been shown to be calcium-selective (by similarity). May also be activated by intracellular calcium store depletion.
ICA69_HUMAN	Islet cell autoantigen 1	May play a role in neurotransmitter secretion
AT2B3_HUMAN	Plasma membrane calcium-transporting ATPase 3	his magnesium-dependent enzyme catalyzes the hydrolysis of ATP coupled with the transport of calcium out of the cell.
GLRA3_HUMAN	**Glycine receptor subunit alpha-3**	**The glycine receptor is a neurotransmitter-gated ion channel. Binding of glycine to its receptor increases the chloride conductance and thus produces hyperpolarization (inhibition of neuronal firing).**
MC5R_HUMAN	**Melanocortin receptor 5**	**Receptor for MSH (alpha, beta and gamma) and ACTH. The activity of this receptor is mediated by G proteins which activate adenylate cyclase. This receptor is a possible mediator of the immunomodulation properties of melanocortins**
NPAS3_HUMAN	Neuronal PAS domain-containing protein 3	May play a broad role in neurogenesis. May control regulatory pathways relevant to schizophrenia and to psychotic illness (by similarity).
PCDA2_HUMAN	Protocadherin alpha-2	Potential calcium-dependent cell-adhesion protein. May be involved in the establishment and maintenance of specific neuronal connections in the brain.
ZNF41_HUMAN	Zinc finger protein 41	May be involved in transcriptional regulation.v
SNAT_HUMAN	**Serotonin N-acetyltransferase**	**Catalyzes the N-acetylation of serotonin into N- acetylserotonin. Controls the night/day rhythm of melatonin production in the pineal gland. Has a relative affinity for hydroxylated versus non-hydroxylated arylalkylamines.**
GPR3_HUMAN	G protein-coupled receptor 3	Orphan receptor with constitutive G(s) signalin activity that acti vate cyclic AMP. Has a potential role in modulating a number of brain functions, including behavioral responses to stress (by similarity), amyloid-beta peptide generation in neurons and neurite outgrowth (by similarity). Maintains also meiotic arrest in oocytes (by similarity).
RL3R1_HUMAN	**Relaxin-3 receptor 1**	**Receptor for RNL3/relaxin-3. Binding of the ligand inhibit cAMP accumulation.**
GRIP1_HUMAN	Glutamate receptor-interacting protein 1	May play a role as a localized scaffold for the assembly of a multiprotein signaling complex and as mediator of the trafficking of its binding partners at specific subcellular location in neurons.
MTR1B_HUMAN	**Melatonin receptor type 1B**	**High affinity receptor for melatonin. Likely to mediates the reproductive and circadian actions of melatonin. The activity of this receptor is mediated by pertussis toxin sensitive G proteins that inhibit adenylate cyclase activity.**
MF2L2_HUMAN	Dbs-related Rho family guanine nucleotide exchange factor	Probably functions as a guanine nucleotide exchange factor (by similarity).
APC2_HUMAN	Adenomatous polyposis coli protein 2	Promotes rapid degradation of CTNNB1 and may function as a tumor suppressor. May function in Wnt signaling.

receptor is functionally expressed in the mammalian brain (10) but the receptor's role in the brain has not been established yet. Connelly et al. suggest H4R can complement histamine H1 receptor (H1R) in the modulation of the circadian cycle (10), since H4R is much more sensitive sensors for histamine than H1R, H4R could act as the preferred histamine sensor during sleep when the histamine concentration is low. In this sense, we find a gene co-expression signal between H4R and the melatonin receptor type 1B (UniProt ID MTR1B_HUMAN) and with the enzyme serotonin N-acetyltransferase (Uniprot ID SNAT_HUMAN), both involved in the control of the night/day rhythm and circadian actions of melatonin.

Activation of H4R hyperpolarize cortical neurons (10), we find the subunit α of the glycine receptor (UniProt ID GLRA3_HUMAN) co-expressed with H4R which also inhibits neuronal firing, the glycine receptor can participate in the mechanism of this inhibitory response by H4R.

Relaxin-3 receptor 1 (UniProt ID RL3R1_HUMAN) is also co-expressed with H4R. This G protein-coupled receptor is present in the hypothalamic paraventricular nucleus, an area involved in the regulation of energy balance. Relaxin-3 is a peptide hormone belonging to the insulin superfamily that may act as signal to coordinate appetite, thyroid function and reproductive status (31). The potential coupling of this relaxin receptor and H4R functions, would imply an overlapping between H3R and H4R role in the brain, since loss of H3R

function in knockout mice is associated with hyperphagia, obesity and increased insulin and leptin levels (32).

H4R could be involved in Alzheimer's disease. It is co-expressed with G protein–coupled receptor 3, an orphan receptor acting as a modulator of amyloid-β production. Overexpression of this GPCR stimulates amyloid-β production, while genetic ablation of the receptor prevented amyloid-β accumulation in an Alzheimer's disease mouse model (33). It is interesting to note that clobenpropit, a H3R antagonist with potential therapeutic application in conditions with memory deficits, like Alzheimer's disease (34) and clozapine, a H3R antagonist and antipsychotic drug with potential use in dementia (35) bind to H4R in the nanomolar range.

5.1. Concluding remarks

Histamine receptor 4, the newest member incorporated to the histamine receptors family may have an important role to play in the histaminergic system. From its predicted functional associations we obtained data supporting the probable H4R roles previously proposed, such as neuronal firing inhibition and its participation in circadian cycle modulation. Our data show that H4R and H3R could be involved together in processes, like sleep and homeostatic regulation, and allow us to hypothesize H4R participation in thyroid function and appetite coordination, as well as the

histamine H$_4$ and glycine receptors in the mechanism of neuronal firing inhibition.

In summary, this work adds relevant information to the characterization of this G protein-coupled receptor, that can direct further research about its involvement in brain function and diseases.

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Abbreviations: H4R: Histamine H_4 receptor; H3R: Histamine H_3 receptor; H1R: Histamine H_1 receptor; PG: Predictogram; KG: Knowledgegram; PDF: Probability Density Functions; TP: True Positive; FP: False Positive; PPI: Protein-Protein Interactions

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