Integrative Genomic Analysis of REM Sleep Behavior Disorder

Pratikiran Bajgain, St. Xavier's College, Maitighar, Kathmandu, Nepal & BioScience Project, Wakefield, MA, 01880

Introduction

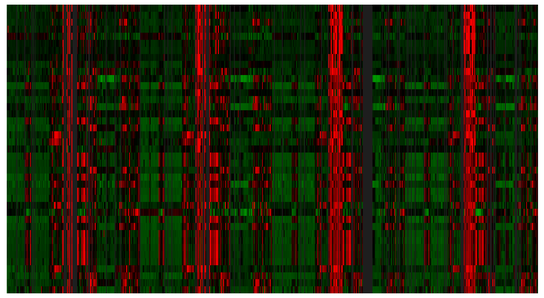
- Rapid Eye Movement Sleep behavior disorder (RBD) is a Parasomnia in which people act out their dreams; characterized by talking, yelling, punching, kicking while asleep.
- REM sleep accounts for 20%- 25% of our sleep period.
- Hypothalamus (HTH) is a sector of brain correlated with sleep/wake cycle, maintaining equilibrium, producing/releasing hormones and linking the Nervous-Endocrine System.
- In this study, integrative genomic methods are used to find genes of interest and profile gene expression in hypothalamus of four different human donors.

Methods

- Gene expression data from Hypothalamus was obtained from Allen Brain Atlas(http://www.brain-map.org) using differential search option in Hypothalamus (HTH) in contrast to Gray Matter (GM) in the donors. (H0351.1012, H0351.1015, H0351.2001, H0351.2002)
- Gene lists from four donors was compared using Venny 2.1.0 (http://bioinfogp.cnb.csic.es/tools/venny)
- Cluster analysis and Gene ontology classification were obtained from DAVID (http://david.ncifcrf.gov)
- The string database (http://string-db.org) was used to identify genes related to REM disorder on the basis of interaction evidence and experiments as the interaction source.
- NCBI (http://www.ncbi.nlm.nih.gov/gene), Genecards (http://genecard.org) and Wikipedia (http://en.wikipedia.org) were used to obtain additional info about genes and the disease

Results

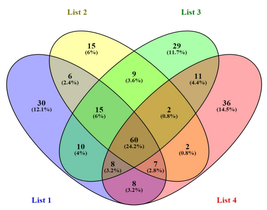
1. Gene Expression Profiling



Study subject vs. Control
Red = expression greater than control
Green = expression less than control
Black = expression equal to control

The heat map shown above gives the gene expression pattern in the hypothalamus in contrast to the gray matter. It was mainly for the four donors: H0351.1012, H0351.1015, H0351.2001, H0351.2002

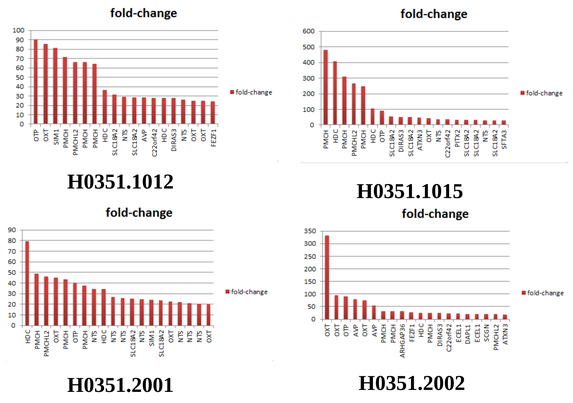
2. Common Genes



After cleaning the data obtained from Allen Brain Atlas, the data of four donors was used to draw Venn diagram. The picture shows us that there are 24.2% common genes among the 4 donors which consists of 60 genes listed at the right side of the Venn diagram.

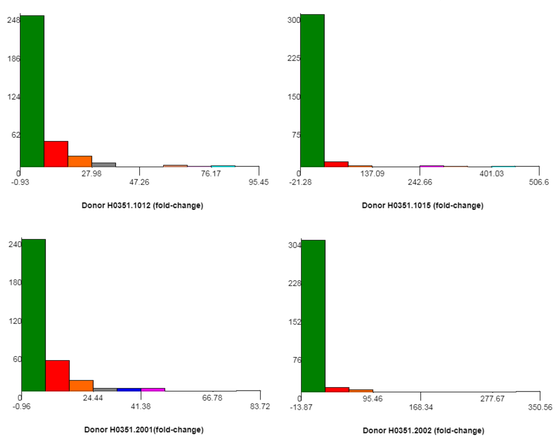
BAIAP3
C22orf42
CALB2
CALCR
CARTPT
CENPVP1
CFC1
CHODL
CITED1
CRNDE
CXorf57
CXorf59
DAPL1
DDC
DHRS2
DIRAS3
ECE2
ECEL1
EPCAM
FAM183A
FAM70A
FEZF1
FNDC9
GABRQ
GNG8
GPRIN2
HCRT
HDC
HTR2C
IGSF1
IL13RA2
IRX3
ISL1
KLHL1
LOC389458
LOXL1
MUC3B
NTS
OTP
OXT
PMCH
PMCHL2
PTGER3
QPCT
QRFPR
SAMD11
SCN7A
SCN9A
SERPINA3
SFTA3
SIM1
SLC18A2
STEAP1B
TEX26
TH
TRH
VAT1L
VWA5A
WNT4
ZCCHC12

3. Top 20 Genes with High Expression Values



The Charts show the graphs for the twenty highest expressing genes for four respective donors in relationship with fold change.
X-axis: Gene names
Y-axis: Fold change values

4. Data Distribution (Statistics and Variance)



stats	Donor1	Donor2	Donor3	Donor4
count	116.000000	116.000000	116.000000	116.000000
mean	10.785509	21.473103	8.921879	11.714629
std	15.716671	62.154523	10.737052	31.467306
min	3.886000	5.116000	3.286000	4.407000
25%	4.582500	6.264250	4.166000	5.138500
50%	5.772500	8.332000	5.309500	6.189000
75%	8.536000	12.037750	8.426500	9.051000
max	90.629000	480.211000	79.481000	332.340000
variance				
Donor2	247.013755			
Donor2	3861.184739			
Donor3	115.284295			
Donor4	990.191326			

- The frequency of genes (y-axis) is heavily skewed for genes with a smaller fold change (x-axis). The mean fold change for each donor ranges from 8.92 to 21.473. From the stats the mean of donor 1, 3, 4 are comparable in comparison to 2. The standard deviation varies a lot i.e., from 10.73 to 62.15

5. Genes Of Interest

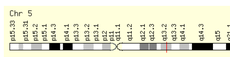
Current Gene List: List_1
Current Background: Homo sapiens
7195 DAVID IDs

55 record(s) Download File

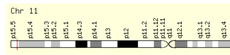
HTR2C	5-hydroxytryptamine receptor 2C(HTR2C)	Related Genes	Homo sapiens
BIOCARTA	G-Protein Signaling Through Tubby Proteins.		
GOTERM_BP_DIRECT	behavioral fear response, cGMP biosynthetic process, cellular calcium ion homeostasis, adenylate cyclase-inhibiting G-protein coupled receptor signaling pathway, phospholipase C-activating G-protein coupled receptor signaling pathway, phospholipase C-activating serotonin receptor signaling pathway, serotonin receptor signaling pathway, chemical synaptic transmission, locomotory behavior, feeding behavior, positive regulation of phosphatidylinositol biosynthetic process, regulation of neurological system process, regulation of appetite, response to drug, regulation of corticotropin-releasing hormone secretion, positive regulation of fat cell differentiation, release of sequestered calcium ion into cytosol, positive regulation of exocytic calcium ion concentration involved in phospholipase C-activating G-protein coupled signaling pathway, positive regulation of ERK1 and ERK2 cascade.		
GOTERM_CC_DIRECT	plasma membrane, integral component of plasma membrane, dendrite.		
GOTERM_MF_DIRECT	Gq/11-coupled serotonin receptor activity, G-protein coupled serotonin receptor activity, drug binding, neurotransmitter receptor activity, serotonin binding, 1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine binding.		
INTERPRO	G protein-coupled receptor, rhodopsin-like, 5-Hydroxytryptamine 2C receptor, 5-Hydroxytryptamine receptor family, GPCR, rhodopsin-like_7TM.		
KEGG_PATHWAY	Calcium signaling pathway, Neuroactive ligand-receptor interaction, Gap junction, Serotonergic synapse, Inflammatory mediator regulation of TRP channels.		
SMART	SM01381.		
UP_KEYWORDS	Alternative splicing, Behavior, Cell membrane, Complete proteome, Disulfide bond, G-protein coupled receptor, Glycoprotein, Membrane, Polymorphism, Receptor, Reference proteome, RNA editing, Signal, Transducer, Transmembrane, Transmembrane helix.		
UP_SEQ_FEATURE	chain:5-hydroxytryptamine receptor 2C, disulfide bond, glycosylation site:N-linked (GlcNAc...), mutagenesis site, sequence variant, short sequence motif:PDZ-binding, topological domain:Cytoplasmic, topological domain:Extracellular, transmembrane region,		



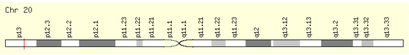
CARTPT	CART prepropeptide(CARTPT)	Related Genes	Homo sapiens
GOTERM_BP_DIRECT	activation of MAPKK activity, cellular glucose homeostasis, signal transduction, G-protein coupled receptor signaling pathway, neuropeptide signaling pathway, cell-cell signaling, chemical synaptic transmission, adult feeding behavior, cellular response to starvation, negative regulation of appetite, positive regulation of epinephrine secretion, circadian regulation of gene expression, negative regulation of osteoclast differentiation, positive regulation of blood pressure, negative regulation of bone resorption, regulation of insulin secretion, positive regulation of transmission of nerve impulse, negative regulation of glucagon secretion, somatostatin secretion.		
GOTERM_CC_DIRECT	extracellular space, secretory granule.		
GOTERM_MF_DIRECT	neuropeptide hormone activity, protein binding.		
INTERPRO	CART satiety factor.		
OMIM_DISEASE	Obesity, susceptibility to.		
UP_KEYWORDS	3D-structure, Cleavage on pair of basic residues, Complete proteome, Direct protein sequencing, Disulfide bond, Neuropeptide, Neurotransmitter, Obesity, Phosphoprotein, Polymorphism, Reference proteome, Secreted, Signal.		
UP_SEQ_FEATURE	chain:Cocaine- and amphetamine-regulated transcript protein, disulfide bond, peptide:CART(1-39), peptide:CART(42-89), sequence variant, signal peptide, strand, turn,		



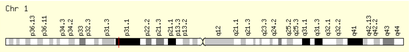
TH	tyrosine hydroxylase(TH)	Related Genes	Homo sapiens
GOTERM_BP_DIRECT	response to hypoxia, synaptic transmission, dopaminergic, response to amphetamine, heart morphogenesis, dopamine biosynthetic process from tyrosine, fatty acid metabolic process, sphingolipid metabolic process, heart development, visual perception, sensory perception of sound, learning, memory, mating behavior, locomotory behavior, regulation of heart contraction, aromatic amino acid family metabolic process, response to water deprivation, response to light stimulus, response to herbicide, response to salt stress, anatomical structure morphogenesis, organ morphogenesis, response to zinc ion, multicellular organism aging, response to activity, adrenergic neurotransmitter loading into synaptic vesicle, glycoside metabolic process, phthalate metabolic process, cerebral cortex development, response to nutrient levels, response to estradiol, response to lipopolysaccharide, sphingolipid catabolic process, social behavior, cellular response to drug, response to isolation stress, response to immobilization stress, neurotransmitter biosynthetic process, terpene metabolic process, dopamine biosynthetic process, epinephrine biosynthetic process, catecholamine biosynthetic process, eye photoreceptor cell development, circadian sleep/wake cycle, eating behavior, response to peptide hormone, pigmentation, response to ethanol, response to ether, response to hyperthermia, embryonic camera-type eye morphogenesis, response to corticosterone, response to electrical stimulus, ethylketazocin metabolic process, oxidation-reduction process, cellular response to manganese ion, cellular response to nicotine, cellular response to glucose stimulus, cellular response to growth factor stimulus.		
GOTERM_CC_DIRECT	nucleus, cytoplasm, mitochondrion, smooth endoplasmic reticulum, cytosol, synaptic vesicle, cytoplasmic side of plasma membrane, dendrite, cytoplasmic vesicle, melanosome membrane, neuron projection, terminal bouton, perikaryon.		
GOTERM_MF_DIRECT	tyrosine 3-monooxygenase activity, iron ion binding, protein binding, ferrous iron binding, ferric iron binding, amino acid binding, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced pteridine as one donor, and incorporation of one atom of oxygen, oxygen binding, enzyme binding, protein domain specific binding, tetrahydrobiopterin binding, dopamine binding.		
INTERPRO	Aromatic amino acid hydroxylase, Tyrosine 3-monooxygenase, Aromatic amino acid hydroxylase, iron/copper binding site, Tyrosine 3-monooxygenase-like, Aromatic amino acid hydroxylase, C-terminal, Tyrosine hydroxylase, conserved site.		
KEGG_PATHWAY	Tyrosine metabolism, Metabolic pathways, Dopaminergic synapse, Prolactin signaling pathway, Parkinson's disease, Cocaine addiction, Amphetamine addiction, Alcoholism.		
OMIM_DISEASE	Segawa syndrome, recessive.		
PIR_SUPERFAMILY	tyrosine 3-monooxygenase.		
UP_KEYWORDS	3D-structure, Alternative splicing, Catecholamine biosynthesis, Complete proteome, Disease mutation, Dystonia, Iron, Metal-binding, Monooxygenase, Neurotransmitter biosynthesis, Oxidoreductase, Parkinson disease, Parkinsonism, Phosphoprotein, Polymorphism, Proteomics identification, Reference proteome.		
UP_SEQ_FEATURE	chain:Tyrosine 3-monooxygenase, compositionally biased region:Poly-Ala, metal ion-binding site:Iron, modified residue, sequence conflict, sequence variant, splice variant,		



OXT	oxytocin/neurophysin I preproprotein(OXT)	Related Genes	Homo sapiens
GOTERM_BP_DIRECT	response to amphetamine, regulation of heart rate, maternal aggressive behavior, signal transduction, positive regulation of cytosolic calcium ion concentration, heart development, female pregnancy, memory, grooming behavior, response to sucrose, positive regulation of norepinephrine secretion, response to activity, sleep, response to food, positive regulation of prostaglandin secretion, response to estradiol, response to retinoic acid, response to progesterone, response to prostaglandin F, social behavior, negative regulation of urine volume, positive regulation of renal sodium excretion, response to cocaine, hyperosmotic salinity response, maternal behavior, sperm ejaculation, eating behavior, drinking behavior, response to peptide hormone, response to ether, negative regulation of blood pressure, positive regulation of blood pressure, positive regulation of ossification, positive regulation of female receptivity, positive regulation of synaptic transmission, response to glucocorticoid, response to cAMP, response to electrical stimulus, regulation of sensory perception of pain, positive regulation of synapse assembly, male mating behavior, positive regulation of penile erection, positive regulation of hindgut contraction, negative regulation of gastric acid secretion, positive regulation of uterine smooth muscle contraction.		
GOTERM_CC_DIRECT	extracellular region, extracellular space, secretory granule, terminal bouton.		
GOTERM_MF_DIRECT	neurohypophysial hormone activity, oxytocin receptor binding.		
INTERPRO	Neurohypophysial hormone, Neurohypophysial hormone, conserved site.		
KEGG_PATHWAY	Oxytocin signaling pathway.		
PIR_SUPERFAMILY	nonapeptide hormone precursor.		
SMART	NH.		
UP_KEYWORDS	Amidation, Cleavage on pair of basic residues, Complete proteome, Direct protein sequencing, Disulfide bond, Hormone, Pharmaceutical, Reference proteome, Secreted, Signal.		
UP_SEQ_FEATURE	chain:Neurophysin 1, disulfide bond, modified residue, peptide:Oxytocin, sequence conflict, signal peptide,		

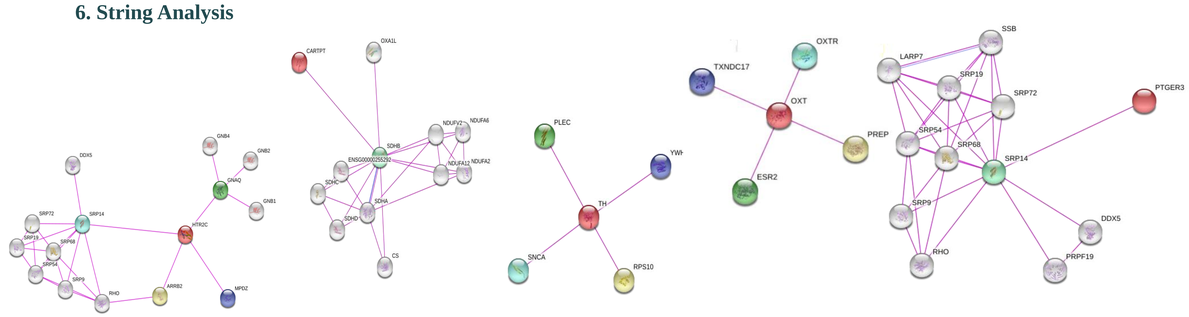


PTGER3	prostaglandin E receptor 3(PTGER3)	Related Genes	Homo sapiens
BIOCARTA	Eicosanoid Metabolism.		
GOTERM_BP_DIRECT	inflammatory response, G-protein coupled receptor signaling pathway, adenylate cyclase-activating G-protein coupled receptor signaling pathway, phospholipase C-activating G-protein coupled receptor signaling pathway, positive regulation of cytosolic calcium ion concentration, cell death, intestine smooth muscle contraction, positive regulation of fever generation, negative regulation of gastric acid secretion.		
GOTERM_CC_DIRECT	nuclear envelope, plasma membrane, integral component of plasma membrane, integral component of membrane.		
GOTERM_MF_DIRECT	prostaglandin E receptor activity.		
INTERPRO	Prostanoid EP3 receptor, G-protein-coupled receptor, rhodopsin-like, Prostaglandin DP receptor, Prostanoid EP3 receptor, type 2, Prostanoid receptor, GPCR, rhodopsin-like_7TM.		
KEGG_PATHWAY	Calcium signaling pathway, cAMP signaling pathway, Neuroactive ligand-receptor interaction, Regulation of lipolysis in adipocytes, Pathways in cancer.		
UP_KEYWORDS	Alternative splicing, Cell membrane, Complete proteome, G-protein coupled receptor, Glycoprotein, Lipoprotein, Membrane, Palmitate, Polymorphism, Receptor, Reference proteome, Transducer, Transmembrane, Transmembrane helix.		
UP_SEQ_FEATURE	chain:Prostaglandin E2 receptor EP3 subtype, glycosylation site:N-linked (GlcNAc...), sequence conflict, sequence variant, splice variant, topological domain:Cytoplasmic, topological domain:Extracellular, transmembrane region,		



The Gene Ontology analysis of the filtered common gene list was used to identify genes of interest that directly or indirectly relate to RBD: HT2RC, CARTPT, OXT, TH, PTGER3. The genomic location of the respective genes are shown below each of them.

6. String Analysis



HTR2C	CARTPT	TH	OXT	PTGER3
• Serotonin Receptor • Significant association with mental illness and behavioral disorder.	• Circadian regulation of gene expression, signal transduction. • Mutation leads to obesity	• Circadian sleep/wake cycle. • Synaptic transmission, learning and memory	• Calcium and hormone signaling. • Response to activity, sleep , food etc.	• G- protein/ Calcium signaling.

Conclusion

- RBD may occur in association with neurodegenerative and neuro developmental conditions like Parkinson's and Schizophrenia,
- Its cause is associated with alcohol, sedative- hypnotic withdrawal or serotonin reuptake inhibitor so, further research must be carried out withHTR2C.
- ARRB2 gene associated with HTR2C is somewhat related with AMPH.
- The Network Analysis suggests the gene to be associated with REM/Sleep behavior and Circadian rhythm are responsible in hormonal regulation and signaling.
- RBD causes higher risk of Narcolepsy, Periodic limb movement disorder so, regular monitoring of neurologic symptoms (tremor, Parkinson's like symptoms) should be done to prevent further complication.
- The genes related to neurological disorders may also contribute or underlie.
- Further research is needed to underlie common pathways.

