Alzheimer disease: An interactome of many diseases

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Abstract

Alzheimer Disease (AD) is an outcome as well as source of many diseases. Alzheimer is linked with many other diseases like Diabetes type 2, cholesterolemia, hypertension and many more. But how each of these diseases affecting other is still unknown to scientific community. Signaling Pathways of one disease is interlinked with other disease. But to what extent healthy brain is affected when any signaling in human body is disturbed is the question that matters. There is a need of Pathway analysis, Protein-Protein interaction (PPI) and the conserved interactome study in AD and linked diseases. It will be helpful in finding the potent drug or vaccine target in conscious manner. In the present research the Protein-Protein interaction of all the proteins involved in Alzheimer Disease is analyzed using ViSANT and osprey tools and pathway analysis further reveals the significant genes/proteins linking AD with other diseases.

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Introduction

Alzheimer disease (AD) is the most common type of dementia; accounts for an estimated 60-80% of cases. "Dementia" describes a variety of diseases and conditions that develop when neuron in the brain dies or no longer function normally. The hallmark abnormalities are protein fragment beta-amyloid (plaques) and twisted strands of the protein tau (tangles) as well as evidence of nerve cell damage and death in the brain which are represented in Figure 1. To target any molecule we need to validate its biochemical network properly as its unconscious targeting may leads to affect other vital cell signaling (side effect). It is reported that streptozotocin induced removal of insulin receptors leads to strong risk factors.[1] There is a strong correlation between glucose utilization and memory enhancement because insulin-enhancing therapeutic agents enhance memory.[2] It is found that Insulin is involved in cholesterol biosynthesis. Insulin increases 3-Hydroxy-3-methylglutaryl-coA HMGR levels by at least 10-fold in rat hepatoma cells.[3] Impairment in insulin signaling cascade may

be implicated in the pathways through which soluble Amyloid beta (Aβ) induces Tau phosphorylation. The phosphorylation of Akt and glycogen synthase kinase-3GSK3 β upon insulin stimulation is less activated under AD leads to Aβ induced Tau hyperphosphorylation. The strong link between cholesterol and insulin is that Type II diabetes is associated with high synthesis and low absorption of cholesterol. Other important regulatory enzymes of insulin metabolism are acyl-CoA: Cholesterol acyltransferase (ACAT).[4] Oxysterols cholesterol 7 α-hydroxylase and sterol 27-hydroxylase,[5] sterol- regulatory-element-binding proteins-lc.[6] Neurosteroids plays a vital role on memory processing, elevations in cholesterol could increase the production of neurosteroids such as dehydro-epiandrosterone, δ5-androstene-3 β,17 β-diol, pregnenolone and 7 α-OH-dehydroepiandrosterone. γ-aminobutyric acid A receptors (GABAA), N-methyl-D-aspartate (NMDA) and cholinergic and sigma opioid systems are all potential targets of neurosteroids. Allopregnano-lone and pregnanolone are the most potent known modulators of GABAA receptors; neurosteroid levels were negatively correlated in one report with levels of phosphorylated tau protein and β-amyloid, two biochemical markers of AD. Allopregnanolone is 20 times more potent than benzodiazepines at potentiating GAB aergic neurotransmission. It can be synthesized in the brain from peripherally derived progesterone.[7] Cholesterol metabolites synthesized in the periphery would find it much easier to reach the brain than cholesterol itself. Long-term potentiation of NMDA receptor during Aβ production by ACAT in cholesterol esterification also impairs many negative circumstances.[8] Aβ is the mediator of tau hyperphosphorylation by impaired insulin signaling. It is observed that decreasing membrane fluidity
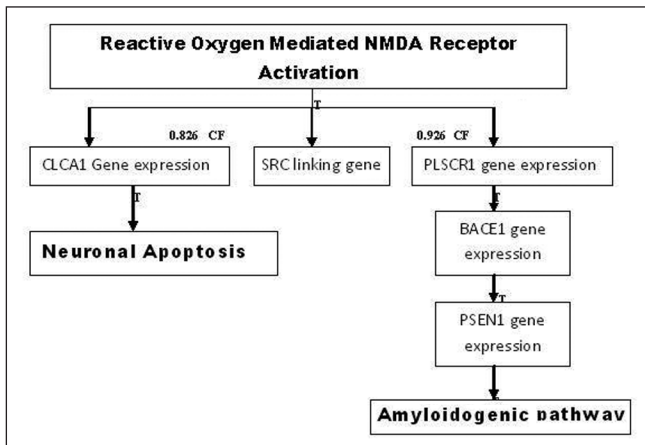
Figure 1: Role of Proto-oncogene tyrosine-protein kinase protein Src; EC = 2.7.10.2 in linking neuronal apoptosis and amyloidogenic pathway in Alzheimer disease

due to hypercholesterolaemia impairs memory.[9] Membrane rigidity due to Phospholipase A2 inhibition positively correlates with AD.[10] apolipoprotein E is an important cholesterol transport protein associated with low-density lipoprotein;[11] Reactive Oxygen Species (ROS) production causes oxidative stress and there are many negative circumstances going on in the brain. It is experimentally verified that oxidative stress results in an increase in the activity of beta secretase (BACE1) through activation of the PKR-eIF2α pathway, another major potent target for AD.[12] Disturbed metal metabolism in the brain leads to ROS production and the antioxidants inhibits the metals ions responsible for ROS production. Whether the antioxidants is exogenous or endogenous, all these scavenge the harmful effect of ROS.

Materials and Methods

Collection of alzheimer and alzheimer linked genes
The first thing comes in mind is the genes involved in AD and its associated diseases. We collected all the genes from GeneCards,[13] integrated, database of human genes that provides concise genomic related information, on all known and predicted human genes. By literature survey it is reported that Alzheimer patients have a high risk of having obesity,[14] cholesterolemia,[15] Diabetes type 2[16] and hypertension.[17] There are some biochemical marker which is reported in research papers. These include reduced levels of melatonin,[18] disturbed metal metabolism,[19] Beta amyloid plaques and tangles formation.[20]

On considering the above facts, we considered all the genes associated with AD, obesity, cholesterolemia, diabetes type 2, hypertension, reduced levels of melatonin, disturbed metal metabolism, beta amyloid plaques and tangles formation.

In GeneCards we selected genes using below mentioned criteria:

Gene set enrichment analysis
Molecular signature Database[21] was used to collect the training gene sets for the biological processes that are modulated in

alzheimer pathogenesis such as neuronal apoptosis, regulator of neurotransmitter release and neuronal system process. To perform candidate gene prioritization, Toppgene[22] was used. DAVID[23] tool was also used to crosscheck the results coming from Toppgene. We performed individual analysis for the candidate genes (test genes) of each diseases and gene prioritization was done with each biological processes as training set. The sorted genes list were created for each disease linked with AD.

Analysis of protein-protein interactions (PPIs) with applying gene ontology (GO) and experimental filter
The best candidate genes in accordance with Alzheimer pathogenesis were analyzed for PPIs in ViSANT[24] tool. Each network of diseases were created and checked once again with suitable biological GO[25] terms matching AD Pathogenesis. Each individual disease network was checked for any linking protein with AD and with each other. Linking of each gene in the network were carried out with significant interaction method. The whole PPIs was is a predictome with genes linking diseases like diabetes type 2, cholesterolemia, hypertension and many more with AD. Based on the training set relative importance of candidate gene in gene network was also carried out from ToppNet.[26]

Pathways and disease analysis
All the genes were checked for pathway and signaling to validate its contribution in AD pathogenesis. Again ViSANT was used. To cross check the results of ViSANT, DAVID was also used for pathway annotation so that significance of a particular gene in AD pathogenesis can be traced.

Key node and centrality analysis
On the basis of degree of candidate genes, key nodes were searched. In ViSANT, we applied from 2 to 6 value for degree of nodes and found significant master and accessory genes. For crosscheck we feed the result of ToppGene or ToppNet into ToppGenet for Prioritization of neighboring genes in PPI network.

Analysis of key proteins linking AD to various diseases
The individual genes in the network were analyzed in H-invitational database[27] to find gene structures, alternative splicing variants, non-coding functional RNAs, protein functions, protein 3D structure, functional domains and sub-cellular localizations. Again metabolic pathways, genetic polymorphisms single-nucleotide polymorphism (SNP) indels and microsatellite repeats), relation with diseases, gene expression profiling, and molecular evolutionary features and gene families/groups. The final network were optimized so that the genes in form network has high weightage.

Results, Discussion and Conclusions

We collected 246, 107, 60, 194, 1 and 2 genes from GeneCards for AD, diabetes types 2, high cholesterol, hypertension, reduced melatonin and metal ions. based on neurotransmitter regulation, neuronal apoptosis and neuronal system processing important genes from AD, diabetes types 2, high cholesterol and hypertension whose pathways have been mapped are shown in Table 1.

Genes responsible for highcholestrome, hypertension, diabetes type 2 and alzheimers were represented in light green, grey, light blue and red in respective metanodes, whereas the remaining colors indicates as linking genes which are associated with all the four disease, among all the linked genes park 2 is common in all the four diseases were mentioned in [Table 2]. Apart from hypertension DRD2 gene also plays an important role in remaining diseases. Important genes involved in Neurotransmitter regulation are shown in Figure 2. Based on the node centrality and degree analysis, DRD2 and PARK2 is the most important gene regulating the four major diseases. As per our observation, it is clearly visible that PARK2, yellow color node is linked with all the four metanode i.e., all the four metanode has at least one yellow color node.

Similarly, DRD2 (Blue color) is linked with AD, diabetes type 2 and high cholesterol, but not with hypertension as there is no blue color node in hypertension metanode.

PARK2: The precise function of this gene is unknown; however, the encoded protein is a component of a multiprotein E3 ubiquitin ligase complex that mediates the targeting of substrate proteins for proteasomal degradation [Table 3]. Mutations in this gene are known to cause Parkinson disease and autosomal recessive juvenile Parkinson disease. Alternative splicing of this gene produces multiple transcript variants encoding distinct isoforms. Additional splice variants of this gene have been described, but currently lack transcript support.

Table 1: Key genes involved in pathways involved in neurotransmitter regulation, neuronal apoptosis and neuronal system processing

Go terms used	Key genes involved in pathways involved in neurotransmitter regulation, neuronal apoptosis and neuronal system processing
Neurotransmitter regulation_AD	GRIN1, GRIA1, PARK2, NOS1, STX1A, DRD2, GAD2, PSEN1
Neurotransmitter regulation_diabetes type 2	MAPK10, ALDH2, ACHE, CCL2, NOS2, PARK2, AGT, DRD2, PTS, SULT1A3, MAOB, ADCY1, GSK3B, RET, SOD2, SOD1, HTR2A, ATP1A2, CACNA1A, HTT, MPZ, PRL, TNF, TGFB1, ADRA2A, CNR1, GNB3, ACE
Neurotransmitter regulation_high cholesterol	EDN1, LRP5, DNTT, CS, NPPA, ADORA2A, NOS3, DHCR7, NPC2, MAP2K1, INS, PLTP, VNN1, ACE, B2M, REN, ABCG5, APOC3, LRP6, LIPOC, CDCD1, AGTR1, ATK1
Neurotransmitter regulation_hypertension	AVP, ADCY1, ADRB2, MAOA, ADRA2A, DRD5, CHRNA7, VAMP8, AGTR2, DRD4, CDC42, AR, BDNF, DRD2, KCNMA1, RET, TH, MAPK10, CHRNB1, DRD1, CACNA1C, DRD3, SLC18A3, CACNA1A
Neurotransmitter regulation_reduced melatonin	PER2
Neurotransmitter regulation_metal ions	IL8
Apoptosis regulation_AD	BCL2L1, XIAP, MAPK3, NOS2, HTT, EPHA4, FASLG, BIRC3, TP73, DLG4
Apoptosis regulation_DT2	GAD1, DRD2, GPX1, IL1R1, IL18, PPARGC1A, IL6, PTGS2, NOS2, APC, LRP6, GPX1, PRL, ACHE, KNG1, AKT1, SOD2, HTT, BMP2, NOS1, NRP1, CVTSB, TNFRSF1B, TNFRSF1A, IGF1R
Apoptosis regulation_high cholesterol	NPPA, NPC1, CS, NPC1, LRP5, APOB, NPC2, NOS3, AGT, NPPA, NR3C2, EDN1, ADORA2A, ACE, APOC3, DNTT, ABCG8, SP1, VNN1, CTNNB1, CGA, MAP2K1, B2M, DHCR7, CDKN1B, INS, PLTP
Apoptosis regulation_hypertension	SLC6A4, IL6, TH, IGF1, NFKB1, BIRC5, LRP6, NGF, NOS2, DRD2, ADORA2, SOD2, APC, MAPK8, NEDD4, ITPR1, MAPK14
Apoptosis regulation_melatonin	PER2
Apoptosis regulation_metal ions	IL8
Neuronal system processing_AD	PDE4D, ADORA1, SLC17A7, NPY1R, NEFM, CHRNA3, CACNA1C, CNR1, CHRM1, ITPR1, DRD3, CHRNB2, GABRG2, EDNRB, STX1A, DNM1, GRIN3A, GRIN3B, NEFL, CAMK2A, TACR1, HRH1, NR3C1, CACNA1S
Neuronal system processing_DT2	AKT1, IL4, PRKCB, ACHE, ADRB2, TGFBR2, TNF, IGF1, CNR1, PARK2, ADRA2A, GNB3, PDE5A, ATP1A2, AVPR2, MAOB, TGFBR2, ADRA2C, RET, NEUROD1, CACNA1A, ADCY1, ADRA2B, MYH9, CCL2
Neuronal system processing_hyper cholesterol	DHCR7, PLTP, VNN1, DNTT, EDN1, B2M, LRP5, AGT, NOS3, CDKN1BAPOB, LIPC, LEP, INS, ACE, ABCG8, CS, SCARB1, APOC3, MYC
Neuronal system processing_HT	F2R, SSTR1, CHRM2, TH, PDE4D, NPYR2, DRD5, CCR5, AVPR1A, NPYR1, AVPR1B, CNR1, EDNRA, HTR1A, PTGER3, CALM1, P2RX3, ADORA1, HTR2B, P2RX3, BDKRB1, ADRA1A, ADRA2A, AGTR1
Neuronal system processing_melatonin	PER2
Neuronal system processing_metal ions	IL8

AD = Alzheimer disease, HT = Hypertension

Table 2: Neurotransmitter regulation: Linking genes

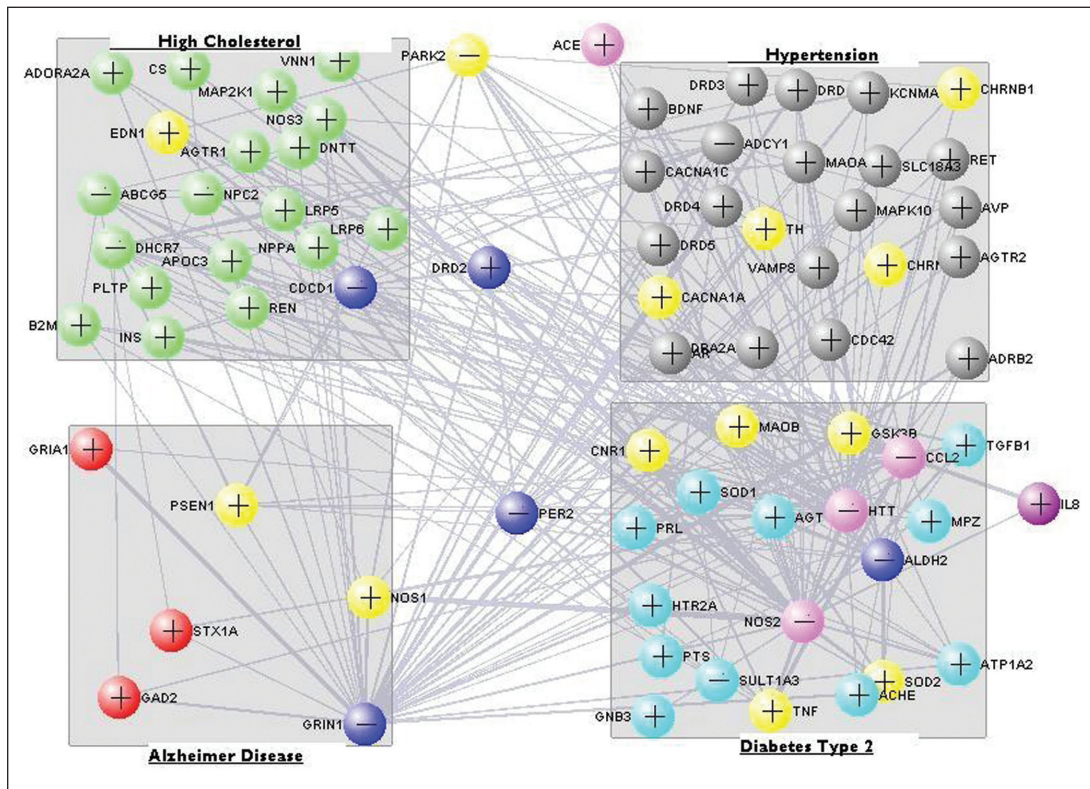| Linking genes	Alzheimer disease	Diabetes type 2	High cholesterol	Hypertension
DRD2	GRIN1	ALDH2	CDCD1	No linking with DRD2
PARK2	PSEN1, NOS1	TNF, SOD2, GSK3B, MAOB, CNR1	EDN1	CHRM, TH, CACNA1A, CHRNB1

Figure 2: Key genes linking four mentioned diseases in neurotransmitter regulation as one of the main factor in Alzheimer disease

Table 3: PARK2 and DRD2 Transcript and Protein ID

Protein ID: HIP000042030-Symbol: DRD2		

1. D(2) dopamine receptor isoform long

2. D(2) dopamine receptor; dopamine D2 receptor

3. Dopamine D2 receptor; fragment

H-InvDB IDs		
Gene cluster IDs	Transcript IDs	Protein IDs (isoforms)
HIX0010135	HIT000039132, HIT000195302, HIT000195377, HIT000321639, HIT000321672	HIP000042030
HIX0010135	HIT000072676	HIP000042031
HIX0010135	HIT000215985	HIP000042032
HIX0010135	HIT000329767	HIP000172344

Protein ID: HIP000042029-Symbol: DRD2		

1. D(2) dopamine receptor isoform short

H-InvDB IDs		
Gene cluster IDs	Transcript IDs	Protein IDs (isoforms)
HIX0010135	HIT000216352	HIP000042029
		HIP000357187

Protein ID: HIP000065822-Symbol: PARK2		

1. E3 ubiquitin-protein ligase parkin isoform 1

2. Parkin; fragment

H-InvDB IDs		
Gene cluster IDs	Transcript IDs	Protein IDs (isoforms)
HIX0023029	HIT000425387	HIP000065822
HIX0023029	HIT000058197	HIP000065821
HIX0023029	HIT000383670	HIP000174103
HIX0023029	HIT000395424	HIP000177897

H-InvDB = H-invitational database

DRD2: This gene encodes the D2 subtype of the dopamine receptor. This G-protein coupled receptor inhibits adenylyl cyclase activity [Table 3]. A missense mutation in this gene causes myoclonus dystonia; other mutations have been associated with schizophrenia. Alternative splicing of this gene results in two transcript variants encoding different isoforms. A third variant has been described, but it has not been determined whether this form is normal or due to aberrant splicing.

Based on Neuronal apoptosis, we found hunting in (HTT) protein is linked with all the four diseases with a good degree and centrality [Figure 3]. HTT:HTT is a disease gene linked to Huntington's disease, a neurodegenerative disorder characterized by loss of striatal neurons. Genes which are representing the linking genes of neuronal apoptosis and neural

processing were mentioned in Tables 4 and 6 respectively. This is thought to be caused by an expanded, unstable trinucleotide repeat in the HTT gene, which translates as a polyglutamine repeat in the protein product. A fairly broad range in the number of trinucleotide repeats has been identified in normal controls and repeat numbers in excess of 40 have been described as pathological. The HTT locus is large, spanning 180 kb and consisting of 67 exons. The HTT gene is widely expressed and is required for normal development. It is expressed as 2 alternatively polyadenylated forms displaying different relative abundance in various fetal and adult tissues. The larger transcript is approximately 13.7 kb and is expressed predominantly in adult and fetal brain, whereas the smaller transcript of approximately 10.3 kb is more widely expressed [Table 5]. The genetic defect leading to Huntington's disease may not necessarily eliminate transcription, but may confer a

Table 4: Neuronal apoptosis: Linking genes

Linking genes	Alzheimer disease	Diabetes type 2	High cholesterol	Hypertension
HTT	TP73, DLG4, FASLG, MAPK3	ACHE, NRP1, IL18, BMP2, PRL, PTGS2, GAD1, ARGC1A, IGF1R, AKT1, NOS1	NPC1, CGA, ACE, INS, B2M, NR3C2, EDN1, APOC3, APOB, SP1, NOS3, CDKN1B, DNTT	NGF, MAPKB, NFKB1, SL6A4, TH, ITPR1, IGF1, BIRC5, MAPK14

Table 5: Protein ID: HIP000035610-Symbol: HTT

1. HTT

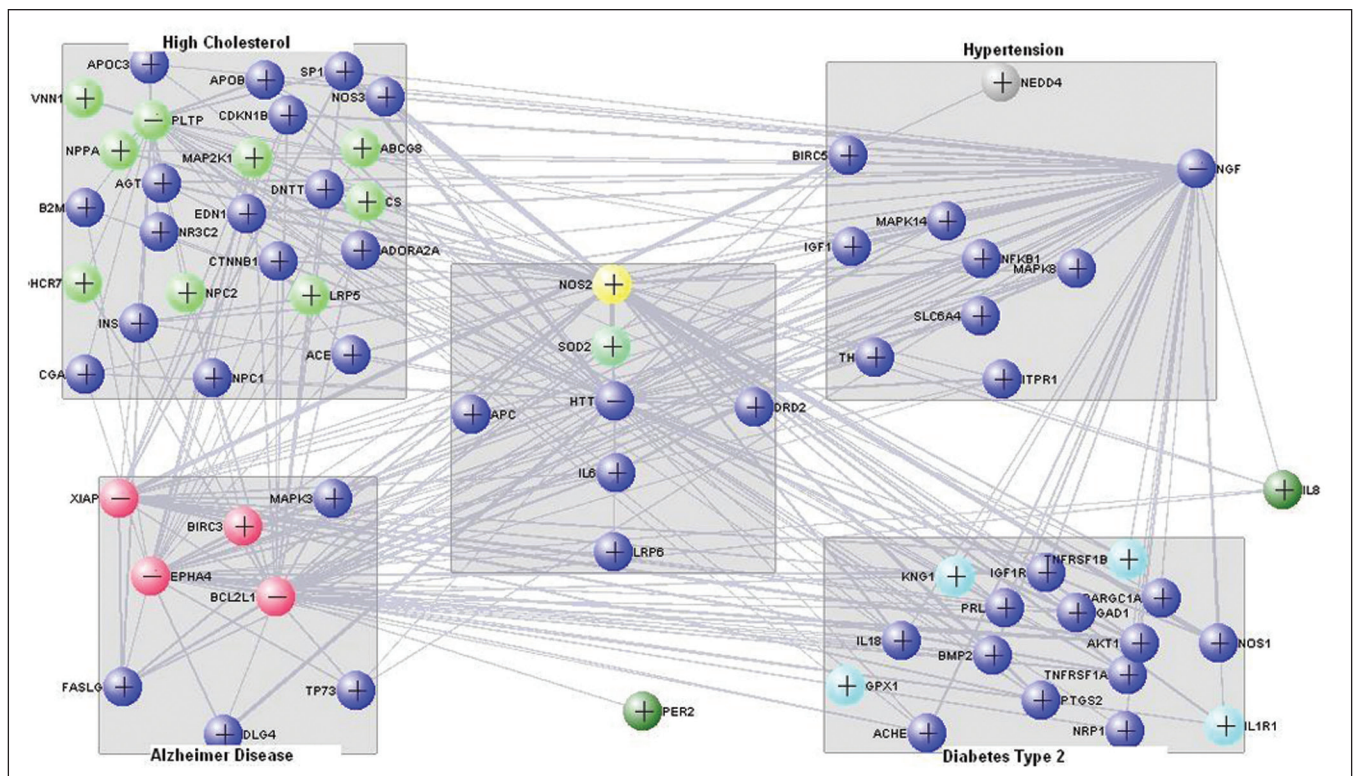|	H-InvDB IDs		Protein accession numbers
Gene cluster IDs	Transcript IDs	Protein IDs (isoforms)	
HIX0004042	HIT000058482	HIP000035610	
HIX0004042	HIT000192172	HIP000035609	Reference sequence: NP_002102, GI: 90903231

H-InvDB = H-invitational database, HTT = Huntingtin

Figure 3: Key genes linking four mentioned diseases in neuronal apoptosis as one of another main factor in Alzheimer disease

new property on the mRNA or alter the function of the protein. One candidate is the HTT-associated protein-1, highly expressed in the brain, which has increased affinity for HTT protein with expanded polyglutamine repeats. This gene contains an upstream open reading frame in the 5' UTR that inhibits expression of the HTT gene product through translational repression. Similarly, based on neuronal processing, we found ADORA1 protein is linked with all the four diseases with a good degree and centrality [Figure 4]. ADORA1: The protein encoded by this gene is an adenosine receptor that belongs to the G-protein coupled receptor 1 family. There are three types of adenosine receptors, each with a specific pattern of ligand binding and tissue distribution and together they regulate a diverse set of physiologic functions. The type A1 receptors inhibit adenylyl cyclase and play a role in the fertilization process. Animal studies also suggest a role for A1 receptors in

kidney function and ethanol intoxication. Transcript variants with alternative splicing in the 5' untranslated region UTR have been found for this gene. The transcript and protein IDs were given in Table 7.

Conclusion

As per insilico analysis; HTT, PARK2, ADORA1 and DRD2 genes aid in linking AD, diabetes type 2, high cholesterol and hypertension. These genes must be considered for treatment as these genes have a high degree of connection and good centrality. Apart from this, there are many secondary genes are there for the same, but HTT, PARK2, ADORA1 and DRD2 are the master genes in functionally linked network. NOS2 is an also important for linking these four dreadful diseases.

Table 6: Neuronal processing: Linking genes

Linking genes	Alzheimer disease	Diabetes type 2	High cholesterol	Hypertension
ADORA1	DNRB, GABRG2, CACNA1S, GRIN3B, NEF, CHRNB2, DRD3, STX1A, GRIN3A, NPY1R, TACR1, HRH1, TACR1, CAMK2A, DNM1, CACNA1C	IGF1, ADRA2C, CACNA1A, ACHE, AVPR2, ADRA2B, AKT1, GNB3, MYH9, ADRB2, NEUROD1	INS, NOS3, SCARB1, ACE, EDN1, B2M, LIPC, VNN1	HTR1A, ADRA1A, HTR2B, CCR5, EDNRA, CALM1, BDKRB1, PTGER3, F2R, P2RX3, AGTR, SSTR1, DRD5, AVPR1B, AVPR1A, CHRM2

Table 7: Protein ID: HIP000038364-Symbol: ADORA1

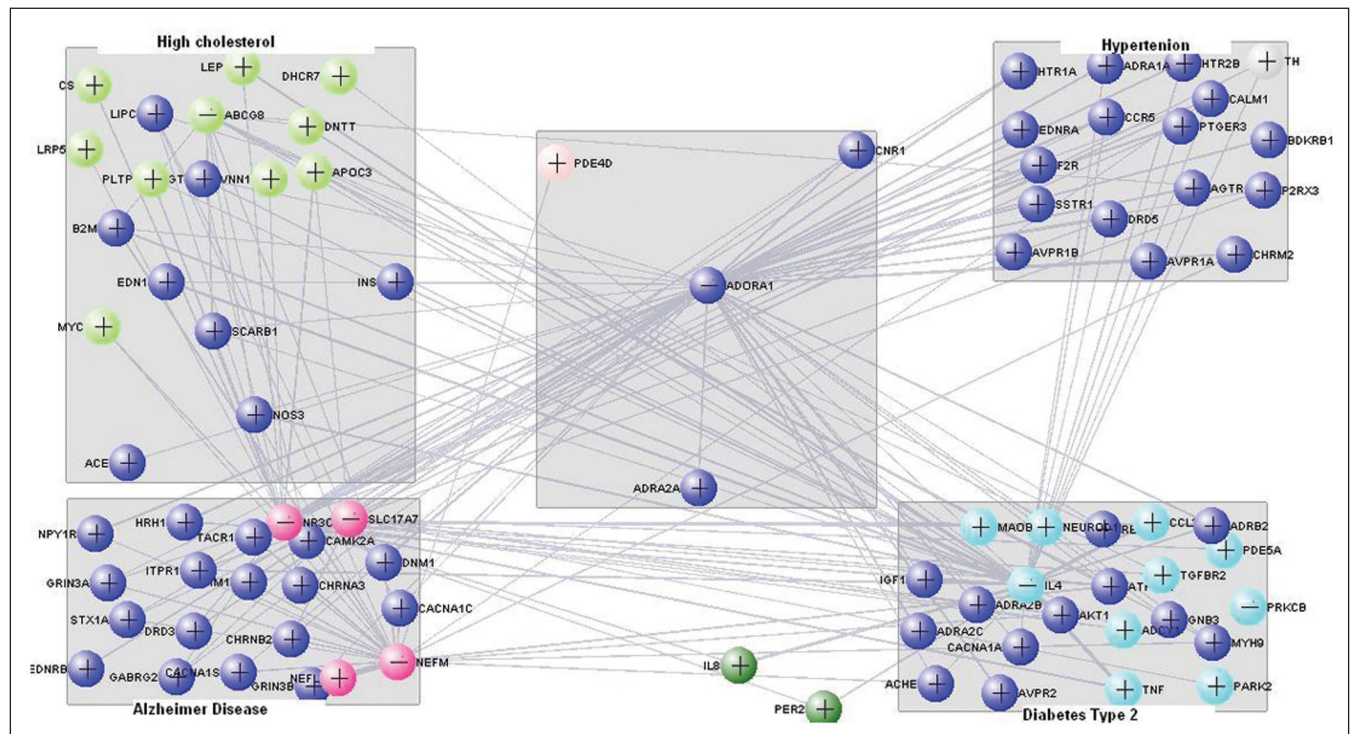| 1. Similar to adenosine receptor A1			
H-InvDB IDs			**Protein accession numbers**
Gene cluster IDs	**Transcript IDs**	**Protein IDs (isoforms)**	
HIX0001486	HIT000019915	HIP000038364	

H-InvDB = H-invitational database

Figure 4: Key genes linking four mentioned diseases in neuronal processing as one of another main factor in Alzheimer disease

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