Investigation of Diabetes as a Risk Factor for Development of Alzheimer's Disease

By

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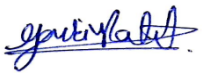

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PATIL GOURI VIJAY

"Alzheimer's is not a part of getting older"

-Stephanie Vasque

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Abbreviations

Aβ	Amyloid beta
AβPP	Amyloid β precursor protein
AD	Alzheimer's Disease
AEP	Asparagine endopeptidase
AGE	Advanced Glycation End-products
AICD	AβPP intracellular domain
APH 1	Anterior pharynx defective
ATP	Adenosine triphosphate
BDNF	Brain-derived neurotrophic factor
BSA	Bovine Serum Albumin
CaMK-II	Calcium/calmodulin-dependent kinase II
CDK 5	Cyclin dependent kinase
CML	Carboxymethyl lysine
CEL	Carboxyethyl lysine
CSF	Cerebrospinal fluid
CTF	C-terminal fragments
DAVID	Database for Annotation, Visualization and Integrated Discovery
DIA	Data Independent Acquisition
DMEM	Dulbecco's Modified Eagle's medium
EOAD	Early-onset alzheimer's disease
ERK	Extracellular signal-regulated kinase

FAD	Familial alzheimer's disease
FBS	Fetal Bovine Serum
FDR	False Determination Rate
GABA	Gamma-Aminobutyric acid
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GFAT	Glutamine fructose 6 phosphate amidotransferase
GSK3β	Glycogen synthase kinase-3 beta
HDMS	High definition mass spectrometry
HMGB1	High mobility group box protein 1
HSA	Human Serum Albumin
iNOS	Inducible Nitric oxide Synthase
IDA	Information Dependant Acquisition
IP3	inositol 1,4,5-trisphosphate
JAK	Janus activated kinase
JNK	c-Jun N-terminal kinase
LC	Liquid chromatography
LDL	Low Density Lipoprotein
MMP	Matrix metalloproteinase
MS/MS	Tandem mass spectrometry
MS	Mass Spectrometry
MS^E	MS Exponential
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa B
NFT	Neurofibrillary tangles
NMDA	N-methyl-D-aspartate
OPLS-DA	Orthogonal Projections to Latent Structures Discriminant Analysis
P38-MAPK	p38 mitogen-activated protein kinase
PCA	Principal Component Analysis
PBS	Phosphate Buffer Saline
PHF	Paired helical filaments
PKA	cAMP-dependent protein kinase
PPAR	Peroxisome proliferator-activated receptor
PRM	Parallel reaction monitoring
PSEN1	Presenilin1
PSEN2	Presenilin2
PTM	Post translational modification
PVDF	Polyvinylidene fluoride
RAGE	Receptor for Advanced Glycation End-products
RBC	Red blood cells
ROS	Reactive Oxygen Species
sAPPα	Soluble amyloid precursor protein alpha
sAPPβ	Soluble amyloid precursor protein beta
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SP	Signal peptide

SRM	Selected reaction monitoring
STAT	Signal transducers and activators of transcription
SWATH	Sequential Window Acquisition of Theoretical Masses
TACE	tumor necrosis factor α-converting enzyme
TGFβ	Transforming growth factor beta
TrkB	Tropomyosin receptor kinase B

DEDICATED TO

MY BELOVED FAMILY

AND EVERYONE WHO

SHOWED FAITH IN ME

Chapter 1

Introduction

Chapter 1 Introduction

1.1 Alzheimer's Disease

Alzheimer's disease (AD) was first discovered by Dr. Alois Alzheimer (1906) in a 51 year-old woman patient suffering from cognitive impairment and hallucinations. AD is one of the major neurodegenerative disorders at present [1]. According to the Alzheimer's Disease International (ADI) 2019 report, over 50 million people are suffering from dementia globally, and this number shall rise to 152 million by 2050 [2]. The prevalence of dementia in the age group of 65 years and above is 6-7%, with Alzheimer's accounting for 2/3 rd of these cases [3]. There are various risk factors, such as increased age, hypertension, and diabetes [4]. Advanced age, lack of education, presence of Apolipoprotein E ε4 allele and few other mutations in genes are strongly linked with AD [5-7]. People with midlife raised higher blood pressure, traumatic head injury, and elevated serum cholesterol has been found to have a higher risk of AD [8-10]. There are reports stating sex and geographical areas being a key determinant in AD development. Women, African Americans, and Caribbean Hispanics are more prone to the disease [4]. Population-based studies have shown that smoking increases the risk of AD development [11, 12]. Engagement in leisure activities and regular physical activities after the age of 65 has been found to reduce disease development [5, 13]. Diet has also been found to affect the occurrence of the disease. Wine and any alcohol in a light to moderate amount have been found to induce beneficial effects, especially in people aged 55 years or more, because of its antioxidant property [14, 15].

1.1.1 Pathophysiology of Alzheimer's Disease

AD is characterized by dementia, progressive memory loss, decline in thinking ability, mental confusion, and disorientation, ultimately leading to death. Extracellular amyloid-β (Aβ) senile plaques (SP), and intracellular neurofibrillary tangles (NFTs) are two major hallmarks of AD. SPs and NFTs majorly form in the hippocampus and cortical areas of the brain, which leads to neurodegeneration and loss of synaptic plasticity.

Aβ is formed from amyloid-β protein precursor (AβPP) upon cleavage by secretases. AβPP plays a central role in "amyloid cascade hypothesis" in AD pathology, termed by Hardy and Higgins in 1992 [16]. According to this hypothesis, Aβ forms neurotoxic Aβ plaques which causes neurodegeneration. AβPP is a type 1 transmembrane protein that is processed either by alpha-secretases (α-secretases) or by aspartyl protease β-secretase (BACE1). AβPP processing by α-secretases follows a non-amyloidogenic pathway leading to minimal accumulation of Aβ levels, whereas BACE1 processing leads to an amyloidogenic pathway resulting in enhanced accumulation of Aβ, a pathogenic hallmark of AD [17-19]. α-secretases are metalloproteases requiring zinc ion for its activity. *In vivo*, three enzymes belonging to a family of proteins termed as ADAM (a disintegrin and metalloproteinase) have shown α-secretase activity viz. ADAM 9, ADAM 10, and ADAM 17. ADAM 17 is also termed as tumor necrosis factor α-converting enzyme (TACE). In non-amyloidogenic pathway, α-secretases act at the extracellular domain of AβPP, precluding the formation of Aβ peptide. This cleavage forms a non-toxic soluble amyloid precursor protein α (sAPPα), leaving membrane-tethered C83 fragment, also called as C-terminal fragment α (CTFα). C83 is then acted upon by γ secretase at the transmembrane region of AβPP forming p3 and AβPP intracellular domain (AICD) fragments. γ secretase complex consists of presenilins (PS), nicastrin**,** anterior pharynx defective (APH 1), and presenilin enhancer (PSEN-2) [20]. sAPPα has neurotrophic properties such as modulation of synaptic plasticity, neurite outgrowth, cell survival, and protection of neurons against excitotoxicity and so on [21, 22]. sAPPα has also been found to inhibit the activity of cyclin-dependent kinase 5 (CDK 5). CDK 5 is one of the major kinases responsible for tau protein phosphorylation leading to the formation of neurotoxic neurofibrillary tangles, an important pathological characteristic in AD [23]. sAPPα has also been shown to interact and inhibit BACE1 activity leading to decreased Aβ production [24].

AβPP upon action by BACE1 (also called as memapsin 2), forms soluble amyloid precursor protein β (sAPPβ) and membrane-tethered C99, also called at C terminal fragment 99 (CTF 99). C99 is then acted by γ secretase, releasing intact Aβ peptide and AICD. There are two major forms of Aβ peptide, $A\beta_{1-40}$ and $A\beta_{1-42}$. Aggregation propensity and toxicity has been found to be more in the case of $A\beta_{1-42}$ due to its

hydrophobic nature. Released Aβ peptide then goes through various stages such as oligomers, protofibrils ultimately forming insoluble extracellular Aβ plaques. Along with Aβ plaques, Aβ oligomers have been shown to cause various neurotoxic effects [20]. Along with Aβ, sAPPβ has been shown to have neurotoxic effects. sAPPβ has been found to interact with death receptor 6 leading to axon pruning and neuronal death via activation of caspases 6 (**Figure 1.1**) [19, 25-27]. Along with BACE1, cathepsin B has shown β secretase activity [28, 29]. Aβ plaque is known to interact with a cell surface receptor for advanced glycation end products (RAGE) and induce oxidative stress, which further activates inflammatory pathways causing neuronal death [30, 31].

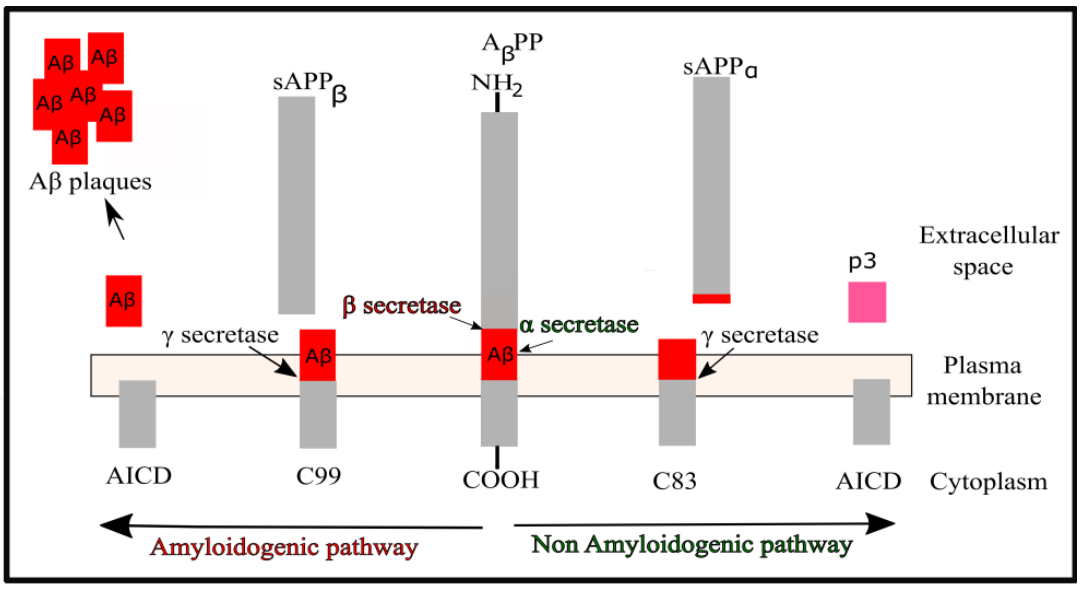

Figure 1. 1: AβPP processing and its cleaved products

Amyloid-β protein precursor can be processed by amyloidogenic and non-amyloidogenic pathways. Processing by α-secretases leads to the formation of sAPPα and p3 fragments, which are non-toxic, whereas BACE1 processing leads to the formation of neurotoxic sAPPβ and Aβ. Aβ further aggregates to form plaques, which cause neuroinflammation and oxidative stress causing neurodegeneration (figure adapted and modified from [32, 33])

Along with Aβ plaques, NFTs are essential to AD pathology. NFTs consist of hyperphosphorylated tau protein [34, 35]. Human tau gene is localised on chromosome 17. Tau is a protein belonging to a group of proteins termed as microtubule-associated protein (MAP). Tau undergoes multiple splicing and exists in six isoforms in the human brain [36]. Tau interacts with neuronal microtubules

through its tubulin binding domains and stabilizes neuronal microtubules, which is crucial for maintaining neuronal cytoskeleton and function. The neuronal cytoskeleton has three parts, microtubules, microfilaments and intermediate filaments. Microtubules plays an important role in maintaining neuronal cytoskeleton. Several proteins help microtubules in holding neuronal structure such as microtubule-associated proteins MAP1, MAP2, and tau with tau majorly localised in axons [37]. Binding of tau protein to microtubules is maintained by an equilibrium between phosphorylation and dephosphorylation mediated by kinases and phosphatases, respectively. Tau has an N-terminal projection followed by a proline rich region, microtubule binding region (MTBR) and a C- terminal region. N-terminus is rich in acidic proteins and C-terminal has basic amino acids. A basal level of phosphorylation is important for tau to carry its function, but it loses its function in a hyperphosphorylated state. The longest tau isoform (441 amino acids long) has 80 serine and threonine sites at which probable phosphorylation can take place [38]. The majority of these sites lie in the MTBR and C-terminus. Multiple kinases regulate tau phosphorylation, such as glycogen synthase kinase-3 beta (GSK-3β), cyclin dependent kinase-5 (CDK 5), cAMP-dependent protein kinase (PKA), extracellular signal-regulated kinases 2 (ERK 2), and calcium/calmodulin-dependent kinase II (CaMK-II) [39]. GSK-3β is the major kinase involved in tau phosphorylation being able to phosphorylate at Ser^{199}, Thr^{231}, Ser^{396}, Ser^{400}, Ser^{404}, and Ser^{413} residues. Phosphorylation by GSK-3β at Thr^{231} makes conformational changes, making tau more accessible for other kinases [38]. Cytosolic phosphorylated tau interferes in the binding of normal tau and MAP1 and MAP2. Protein phosphatases, PP1, PP-2A, PP-2B, PP-2C dephosphorylate tau, with PP-2A being down regulated along with decreased activity in AD brains, thus emphasizing its importance in AD pathology [40, 41]. Upon hyperphosphorylation, tau loses its binding capacity to neuronal microtubules and it is released as a monomer, which further through tau-tau interactions forms oligomers. Further aggregation leads to the formation of insoluble paired helical filaments (PHFs), which ultimately forms neurofibrillary tangles (NFTs) which causes neurodegeneration and decreased neuronal functions [42, 43] (**Figure 1.2**). Along with phosphorylation, tau undergoes several other post-translational modifications (PTMs) such as acetylation, glycation, ubiquitination, nitration, sumoylation, and so on, which have been found to cause loss of tau function

[44]. Tau has 12 glycation sites, with 7 belonging to MTBR. Glycation has been found to stabilize aggregated tau [45].

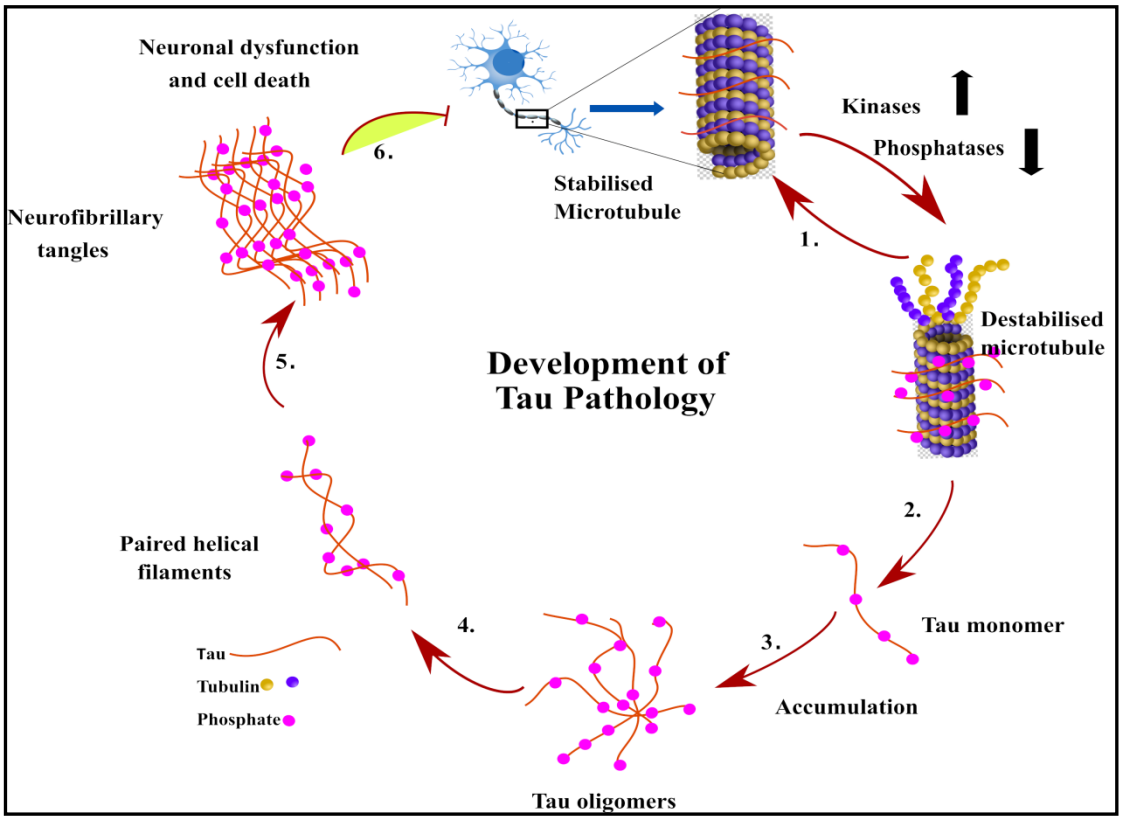

Figure 1. 2: Tau hyperphosphorylation.

Hyperphosphorylation of tau protein due to increased kinase activity cause its destabilization and accumulation, which further lead to the formation of neurotoxic neurofibrillary tangles (NFTs) (figure adapted and modified from [46])

1.1.2 Types of Alzheimer's Disease

There are two major types of AD, sporadic and hereditary (familial). Sporadic form is more common than familial, contributing to around 90% cases in age 60 and above [47].

1.1.2.1 Sporadic Alzheimer's Disease

Sporadic Alzheimer's disease is also called as Late-onset Alzheimer's disease (LOAD), which occurs after the age of 65 and is the most common form of the disease. Apolipoprotein (ApoE) is a major cholesterol-carrying protein and plays a role in lipid transport, metabolism, and redistribution [48]. There are three human isoforms of Apo E (E4, E3, and E2) encoded by alleles ε4, ε3, ε2 respectively, out of

which E3 is the most common isoform [49]. APOE ε4 is a major risk factor for sporadic AD development, APOE ε3 has lesser risk whereas APOE ε2 has a beneficial effect [50]. APOE ε4 has been found to be the first genetic risk factor for the development of sporadic AD [51]. APOE ε4 has been shown to alter the age of disease onset in allelic dependent manner. Mean onset age was found to be 84.3 in the absence of APOE ε4 whereas it reduced to 75.5 and 68.4 with 1 and 2 alleles of APOE4 ε4, respectively [52]. APOE ε4 allele is a crucial risk factor in developing disease, accounting for 50% of AD cases in the United States [4, 48, 52, 53]. ApoE isoforms differentially regulate Aβ clearance in the brain too [50] and APOE ε4 has been found to cause fibrillar Aβ deposition and neuritic plaques in transgenic mice [54].

1.1.2.2 **Familial Alzheimer's Disease**

Familial Alzheimer's disease (FAD) is a rare autosomal dominant disorder, called Early-onset Alzheimer's disease (EOAD). It is a hereditary form that usually occurs before the age of 65. Families with an autosomal dominant AD are associated with the mutations in 3 crucial genes AβPP, Presenilin 1 (PSEN1), Presenilin 2 (PSEN2) [4, 55-57]. Mutations in PSEN1 (chromosome 14) is the most common cause in familial AD as compared to PSEN2 (chromosome 1) mutations [58]. According to the Alzforum mutation database, PSEN1 has 221 pathogenic mutations followed by AβPP with 32 pathogenic mutations and PSEN2 has 19 pathogenic mutations [59]. PSEN1 gene encodes for presenilin 1 (PS1), which is the catalytic subunit of γ. Presenilin is a part of the γ secretase complex, which processes AβPP to form Aβ peptides of various lengths. Carriers of PSEN1 mutations suffer from early disease onset and shorter dementia as compared to AβPP mutation [60]. PSEN1 mutations have been found to increase $A\beta_{1-42}$ production and increase $A\beta_{1-42}/A\beta_{1-40}$ ratio [60]. PSEN1 mutations and increasing Aβ have been found to alter intracellular calcium signaling mediated by inositol 1,4,5-trisphosphate (IP3) leading to increased Aβ aggregation [61, 62]. PSEN2 mutation responsible for AD development was first identified in 1995 [63]. It was found to be a point mutation replacing isoleucine for asparagine (N141I). PSEN mutation has been found to increase γ secretase activity producing more amount of toxic $A\beta_{1-42}$ peptide [64]. PSEN2 mutation (N141I) and its

wild type been found to cause apoptosis through p53 pathway with subsequent PSEN1 downregulation [65]. Four mutations (T122P, N141I, M239I, and M239V) are known to increase Aβ production and three mutations (T122R, S130L, and M239I) have been found to alter calcium signaling [58]. There are various AβPP mutations such as Swedish (K670N/M671L), London (V717I), Dutch (E693Q), Arctic (E693G), Austrian (T714I), French (I715M), Florida (I716V), Indiana (V717F), Iowa (D694N), Flemish (A21G), Italian (E693K) and so on [66-68]. Swedish double mutation in AβPP increased BACE1 activity leading to increased Aβ synthesis [69]. London mutation occurring at γ secretase cleavage site on AβPP leads to increased BACE1 and γ secretase activity causing an increase in Aβ and sAPPβ. It has also been found to increase total tau and phosphorylated tau [70]. Dutch, Flemish, Italian and Arctic mutations have been found not only to increase Aβ fibril formation, but also increase the half life of Aβ peptide by increasing its resistance towards proteolytic degradation [71]. Austrian mutation has been found to alter γ secretase activity affecting $A\beta_{1-42}/A\beta_{1-40}$ ratio [72].

1.1.3 Diagnosis of Alzheimer's Disease

Currently, the diagnosis of AD is based upon a detailed patient's history, Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), Computed Tomography (CT), and Mini-Mental State Examination (MMSE) score. Guidelines given by the National Institute of Neurological and Communicative Diseases and Stroke (NINCDS) and the Alzheimer's disease and Related Disorders Association are used as the gold standard in the diagnosis of AD. According to these guidelines, AD cannot be diagnosed by laboratory tests. Instead, laboratory tests should be used to remove the probability of other neurological disorders such as cerebrovascular dementia, which might have similar symptoms like AD [73, 74].

1.1.3.1 **MRI/PET/CT scan**

MRI, PET or CT are used to rule out other medical conditions that show similar symptoms like AD but require different treatment. Along with traditional MRI/PET, structural and functional MRI and PET using fluoro- deoxy-glucose, amyloid tracers have been found to be useful in AD detection even in pre-symptomatic patients [75].

CT can be used to diagnose AD because it can exclude other disorders such as brain tumor, dementia associated with vascular disease, and so on. However, a quantitative CT scan would be useful for studying the course of AD development [73].

1.1.3.2 **MMSE**

MMSE, also known as the Folstein test, is a screening test that gives an idea about the overall status of cognitive impairment [76]. It consists of a list of questions based on cognitive function, for which scores are given that are used to categorize patients. It is used in mild cognitive impairment (MCI) cases to classify the patients into Alzheimer's group. MMSE has been found to be sensitive (27-89%) and specific (32-90%) for confirmation of AD in MCI. However, a lack of precision demands a better diagnostic test for AD [77].

Apart from above-mentioned methods, a decreased level of Aβ and increased phosphorylated tau in cerebrospinal fluid are being considered to diagnose AD [78].

1.1.4 Treatment of Alzheimer's Disease

AD is non-reversible, so current treatments focus on reducing the symptoms of the disease. Acetylcholinesterase inhibitors and NMDA antagonists are the commonly used group of drugs.

1.1.4.1 **Acetylcholinesterase inhibitors**

One of the oldest and essential theories for AD pathogenesis is the cholinergic hypothesis. It states that degeneration of cholinergic neurons in the forebrain leads to deficit in the cholinergic neurotransmission in the cerebral cortex of the brain, which contributes significantly to AD [4, 79]. Acetylcholinesterase enzyme degrades acetylcholine, which is an important neurotransmitter. Inhibiting acetylcholinesterase activity by using an inhibitor will increase the availability of acetylcholine and thus, improve neurotransmission. Drugs from this group include donepezil, rivastigmine, and galantamine [80-82].

1.1.4.2 **NMDA antagonists**

For proper neurotransmission, a balance between excitatory and inhibitory neurotransmission is crucial. Glutamate is an excitatory neurotransmitter acting through N-methyl-D-aspartate (NMDA) receptors. In AD, increased glutamatergic activity has been observed, which leads to neuronal dysfunction. NMDA antagonists bind to the glutamate receptor and prevent its activity, thus protecting neurons. A combination of these drugs with acetylcholine esterase inhibitors has been found to be more effective. Memantine is one of the widely used drugs from this group [83].

1.1.4.3 **Future directions for drug discovery**

Since, Aβ plaques and phosphorylated tau tangles cause neurodegeneration and synaptic loss, efforts are being directed towards the discovery of Aβ fibrillization inhibitors and anti-tau drugs. Inhibiting kinases activity by small molecules, there by reducing tau hyperphosphorylation is also under progress [39, 84]. Since secretases play a crucial role in determining AβPP processing's fate, small molecules that alter their activity are also gaining importance. Increasing α-secretase activity or decreasing β-secretase activity can help in reducing the formation of neurotoxic Aβ plaques. This has been supported by Luo *et al*, where they have shown that mice deficient in BACE1 resulted in a normal phenotype with no Aβ plaque production, indicating BACE1 as a therapeutic target for drug discovery [85]. Similar results were

obtained in many independent studies [86-88]. *In vitro* and animal study has found few candidate β secretase inhibitor drug molecules [89]. γ-secretase inhibitors have also been studied, but they have not been converted into therapeutic drugs due to their toxicity. Apart from AβPP processing, γ-secretases play other vital functions also such as cell signaling, Notch signaling. Hence, inhibiting its activity hampers other important cellular pathways, thus, limiting γ-secretase's use for drug discovery [90, 91]. A non-peptidic inhibitor with selective inhibition activity against AβPP processing has also been found [92].

1.2 Diabetes

Diabetes also called diabetes mellitus, is a chronic metabolic disease characterized by hyperglycemia either due to lack of insulin secretion or insulin resistance or both. The chronically elevated plasma glucose levels can cause various diabetic complications affecting important body organs such as kidneys (nephropathy), nervous system (neuropathy), heart (cardiomyopathy), eyes (retinopathy), brain (neurodegeneration) and so on [93]. Typical symptoms include polyphagia (increased hunger), polyurea (frequent urination), polydipsia (increased thirst), unexplained weight loss, and blurred vision [93].

1.2.1 Epidemiology

The rate of diabetes prevalence is increasing rapidly due to modernization, intake of unhealthy processed food, higher calorie intake, and sedentary lifestyle. Currently, 463 million adults have diabetes globally, and this number has tripled in the last 20 years as per the International Diabetes Federation (IDF) Diabetes Atlas. It has been projected that this number will rise to 578 million in 2030 and 700 million in 2045 [94]. Diabetes has been projected to be the 7^{th} leading cause of death by 2030, contributing to 3% of total deaths [95].

1.2.2 Insulin and its function

Insulin was discovered in 1921 by Fredrick Banting and Charles Best [96]. It is a peptide hormone secreted by β-cells of the islets of Langerhans in the pancreas and

plays an essential role in nutrient metabolism. It is a dipeptide having a molecular weight of 5808 Da. It has A (21 amino acids) and B (30 amino acids) chains bonded by one intra-chain and two inter-chain disulfide bonds. Its amino acid sequence was discovered in 1952 [97]. Insulin plays a crucial role in maintaining the blood glucose levels. When blood glucose concentration increases, insulin increases the translocation of its transporter GLUT4 onto the cell surface, leading to increased glucose uptake by tissue; thus, maintaining blood glucose level. Insulin upon binding to its cell surface insulin receptor (IR) leads to activation of tyrosine kinase, which phosphorylates insulin receptor substrates (IRS) (1 and 2). Phosphorylated IRS activates several intracellular signaling molecules such as phosphoinositide 3-kinase (PI3K), which promotes translocation of glucose transporter (GLUT4) on the plasma membrane, which increases glucose uptake; thus, maintaining blood glucose level. PI3K also activates forkhead box protein O1 (FOXO) transcription factor, which regulates transcription of various genes [98]. In type 2 diabetes, increased phosphorylation of serine residues on IRS inhibits tyrosine phosphorylation and increases IRS degradation.

1.2.3 Types of Diabetes

There are different types of diabetes Mellitus, majorly type 1 (insulin deficiency), type 2 (insulin resistance), gestational diabetes [99], and type 3 (Alzheimer's Disease) [100, 101].

1.2.3.1 Type 1 Diabetes mellitus (T1DM)

It is also called as insulin-dependent diabetes mellitus (IDDM) [102] or Juvenile-onset diabetes, and accounts only for 5-10% of total diabetes cases. It is usually immune-associated, characterized by auto-immune destruction of β-cells of the pancreas, resulting in reduced or no secretion of insulin [93] [103]. Various genetic and environmental factors are found to cause IDDM, with major histocompatibility complex (MHC) being the primary determinant [102]. It is detected in childhood as well as at later ages also. A lifetime insulin therapy is usually recommended for T1DM [104].

1.2.3.2 Type 2 Diabetes mellitus (T2DM)

It is also termed as non-insulin dependent diabetes mellitus (NIDDM). It is the most common form of diabetes observed [98]. It is characterized by hyperglycemia due to the combined effect of insulin resistance and insulin deficiency, resulting in dysregulation of carbohydrate, lipid, and protein metabolism [98, 105]. Various genetic, behavioral, and environmental risk factors contribute to the development of T2DM [106]. In 2010, 90% of all diabetic patients had T2DM [107, 108]. There are various modifiable (obesity, smoking, hypertension, physical inactivity and sedentary lifestyle) and non-modifiable (age, sex, family history, history of gestation diabetes and so on) risk factors for T2DM [105, 106]. It is characterized by chronic inflammation leading to micro and macrovascular complications [98].

1.2.3.3 Gestational Diabetes

It is a form of diabetes specifically observed during pregnancy [99, 109]. It usually occurs in 7% of pregnancies [110].

1.2.3.4 Type 3 Diabetes

Alzheimer's Disease is also called type 3 diabetes. Diabetes and AD share various common pathophysiological symptoms such as insulin resistance, increased advanced glycation end products, and brain atrophy. Diabetes is also one of the major risk

factors for the development of AD. Obesity and diabetes have been proven to be predisposing factors for future AD development [111].

1.2.4 Pathophysiology of Diabetes

There are various causative factors for the development of diabetes, such as heredity, sedentary lifestyle, physical activity, and so on. Inflammation and ROS have been found to be key players in diabetes [112]. Inflammation can cause β cell destruction (T1DM) and dysfunction (T2DM), both of which lead to hyperglycemia. If not treated, it can lead to micro and macro vascular complications. Microvascular complications include retinopathy, nephropathy, neuropathy whereas macrovascular complications includes coronary artery disease, stroke and so on [113]. Various pathways are activated in response to hyperglycemia, such as polyol pathway, hexosamine pathway, PKC pathway and glycation pathway. Persistent hyperglycemia leads to activation of the polyol pathway. In the first step of the polyol pathway, aldose reductase reduces glucose into sorbitol using NADPH as a cofactor. Accumulation of sorbitol causes osmotic stress. Sorbitol further gets converted into fructose by sorbitol dehydrogenase enzyme by using NAD^+ as a cofactor. Fructose accumulation causes increase in non-enzymatic reaction termed as glycation, leading to formation of advanced glycation end products (AGEs) which causes various diabetic complications. Fructose can further be converted into 3-deoxyglucose and fructose-3-phosphate, which are also glycating agents. Non-alcoholic fatty liver disease can occur due to overproduction of acetyl-CoA due to fructose metabolism by fructokinase. Redox imbalance occurring during polyol pathway causes a decrease in nitric oxide synthesis, vascular impairment and generation of ROS. It further leads to various diabetic complications such as retinopathy, nephropathy and neuropathy [114, 115]. ROS generated during the polyol pathway causes mitochondrial dysfunction. The majority of glucose is utilised by the glycolysis pathway. Glycolysis causes the conversion of glucose into pyruvate/lactate along with the production of adenosine triphosphate (ATP). Glucose is being taken up inside cells by glucose transporter (GLUT2/GLUT4). Upon entering inside cells, glucose gets converted into glucose-6-phosphate which then forms fructose-6-phosphate. It then undergoes phosphorylation to form fructose 1, 6 bisphosphate. It further forms dihydroxyacetone phosphate

(DHAP) and glyceraldehydes-3-phosphate, which in further multiple reactions forms pyruvate which then enters into tri carboxylic acid cycle (TCA). Major enzymes involved are, hexokinase, phosphofructokinase and pyruvate kinase [116]. Few of the fructose-6-phosphate molecules enter into the hexosamine pathway. It is acted upon by glutamine fructose 6 phosphate amidotransferase (GFAT) leading to glucosamine-phosphate formation, which further forms UDP (uridine diphosphate) *N*-acetyl glucosamine. *N*-acetylglucosamine group is then transferred to serine and threonine residues of transcription factors by enzyme O-GlcNAc transferase (OGT). It leads to the expression of genes that aggravates the disease such as TGFβ. The hexosamine pathway has been found to be involved in cardiomyocyte dysfunction, carotid artery disease, diabetic nephropathy, and so on [117-119]. Hyperglycemia also activates the formation of diacylglycerol, which is a cofactor, increasing expression of PKC isoforms (β, δ, α). Activation of PKC affects the expression of various genes involved in aggravating vascular complications of diabetes and diabetic nephropathy [120] [121]. Another vital pathway activated in hyperglycemia is the glycation pathway. In non insulin-sensitive cells, the rate of glycolysis increases in response to hyperglycemia**.** Fragmentation of glyceraldehydes-3-phosphate and DHAP leads to the formation of a toxic dicarbonyl compound termed as methylglyoxal (MGO) [122]. MGO is a potent AGE precursor that interacts with proteins and forms AGEs via glycation reaction. In hyperglycemia, glucose along with various glycating agents leads to formation of AGEs by glycation reaction and causes toxic effects.

1.2.4.1 **Role of Glycation in Diabetes**

The inevitable consequence of hyperglycemia is glycation, which is a non-enzymatic reaction of reducing sugars with the free amino group of lysine and arginine of proteins leading to the formation of a complex and heterogeneous group of compounds called advanced glycation end products (AGEs) (**Figure 1.3**) [123]. Glycation is also called a Maillard reaction. In the early stage, glucose after reaction with free lysine or arginine of proteins leads to the formation of a reversible unstable Schiff base, which further forms the Amadori product (ketamine). Schiff base and Amadori products can react with amino acids to form protein adducts. In late stage, Amadori further forms AGEs through various oxidation and dehydration reactions

[124, 125]. It further leads to the formation of irreversible AGEs [126]. Along with reducing sugars such as glucose, ribose, galactose, mannose, and fructose, glycolytic intermediates such as glyoxal, MGO and 3-deoxyglucosone also causes AGEs formation [127, 128]. Intracellularly, glucose upon auto-oxidation forms potent dicarbonyl glyoxal. Decomposition of amadori products leads to the formation of dicarbonyl 3-deoxyglucosone. Glyceraldehyde 3 phosphate and dihydroxyacetone phosphate, generated during glycolysis undergo fragmentation to form MGO. Glyoxal, 3-deoxyglucosone and MGO are potent AGE precursors and interact with proteins to form AGEs. There are different types of AGEs depending upon the glycating agent, such as N-ε-(carboxymethyl)lysine (CML), glyoxal lysine dimer (GOLD) by glyoxylic acid, N-ε-(carboxyethyl) lysine (CEL), methylglyoxal-derived lysine dimer (MOLD) by MGO and pyrraline, deoxyglucosone-lysine dimer (DOLD) by 3-deoxyglucosone and so on [123].

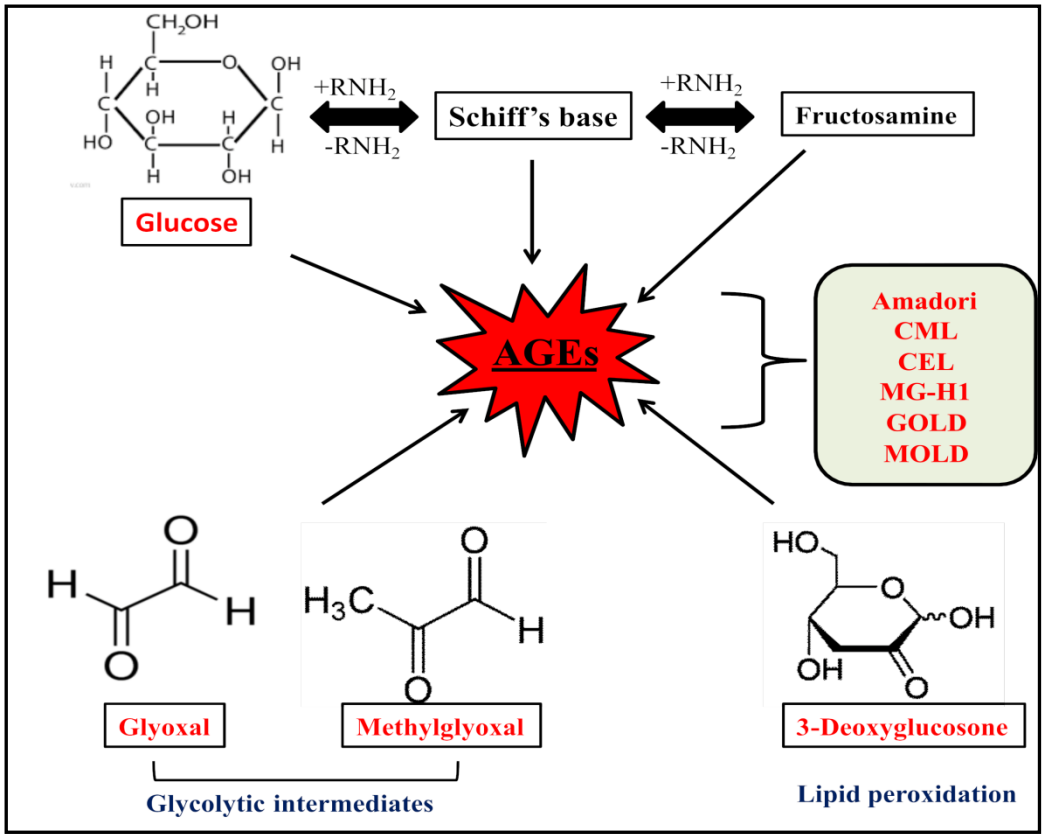

Figure 1. 3: AGEs formation

Glucose and other glycating agents react with the free lysine/arginine group of amino acids leading to the formation of toxic AGEs (Figure adapted and modified from [123])

AGEs form cross-links leading to protein aggregation along with increased oxidative stress [129]. Glycation of proteins causes a change in its conformation, function, and leads to aggregation [130]. Glycation has also been found to be responsible for the formation of protease-resistant proteins [131, 132]. Glycation of enzymes may alter the conformational structure affecting their enzymatic activity [133]. Glycation of extracellular matrix proteins can cause abnormal interaction with other membrane proteins and integrins which can activate several intracellular transducers. This activation leads to further activation of several transcription factors that can aggravate the disease. Similar activation can occur after the interaction of glycated plasma proteins with its cell surface receptor termed as RAGE. RAGE is a transmembrane protein belonging to the immunoglobulin superfamily, first isolated and characterized in 1992 [134]. It is a pattern recognition receptor and undergoes multiple splicing. Along with AGEs, there are different other ligands such as Aβ, S100/calgranulin, High mobility group box-1 (HMGB), and β2 integrin Mac-1 that bind to RAGE [135-137]. There are various forms of RAGE, such as full-length RAGE (fl RAGE), dominant negative RAGE (DN RAGE), soluble RAGE (sRAGE/esRAGE) (**Figure 1.4**) [138]. Full-length RAGE has a V domain-containing glycosylation site, C1 domain, C2 domain, a transmembrane domain, and cytoplasmic tail [139]. Soluble RAGE is present in free circulating form due to lack of transmembrane domain after proteolysis; thus, it does not initiate pathological cascade upon AGEs binding like fl RAGE and acts as scavenging receptor for circulating AGEs [140]. AGE-RAGE interaction in macrophages induces oxidative stress with an increase in Nf-κB activation. It further leads to the secretion of inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1α (IL-1α), tumor necrosis factor-α (TNF α), and various factors controlling inflammation, vasoconstriction, and coagulation [141]. It can lead to the synthesis of various cytokines and growth factors [142]. Interaction of AGEs with its receptor RAGE causes toxic effects such as the generation of ROS and inflammation [143, 144]. AGEs and fl RAGE interaction initiates a cascade of pathological events such as synthesis of pro-inflammatory cytokines via NF-κB in endothelial cells as well as in mice leading to inflammation [145, 146]. AGE-RAGE interaction causes NF- κB activation, which further leads to the synthesis of Vascular Cell Adhesion Molecule-1 (VCAM 1), Intercellular Adhesion Molecule-1 (ICAM1) along with the increased synthesis of proinflammatory cytokines IL-1α, IL-6, TNF-α.

It also causes increased Vascular Endothelial Growth Factor (VEGF), endothelin-1, and decreased nitric oxide (NO) synthesis [147]. In diabetes accelerated atherosclerosis, the AGE-RAGE axis also causes activation of NADPH oxidase causing ROS generation. Increased ROS along with activated MAPK/ERK and PKCα causes NF-κB mediated gene transcription. It further causes generation of pro-inflammatory and profibrotic cytokines and chemokines such as TGFβ, connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), TNF-α, IL-1, IL-6 [148]. In diabetic nephropathy, the AGE-RAGE axis causes ROS generation by increasing NADPH expression and reducing antioxidant enzymes. Increased expression of TGF-β, fibronectin and collagen also leads to fibrosis with increased RAGE expression. Increased oxidative stress, inflammation and fibrosis causes apoptosis and renal dysfunction [149].

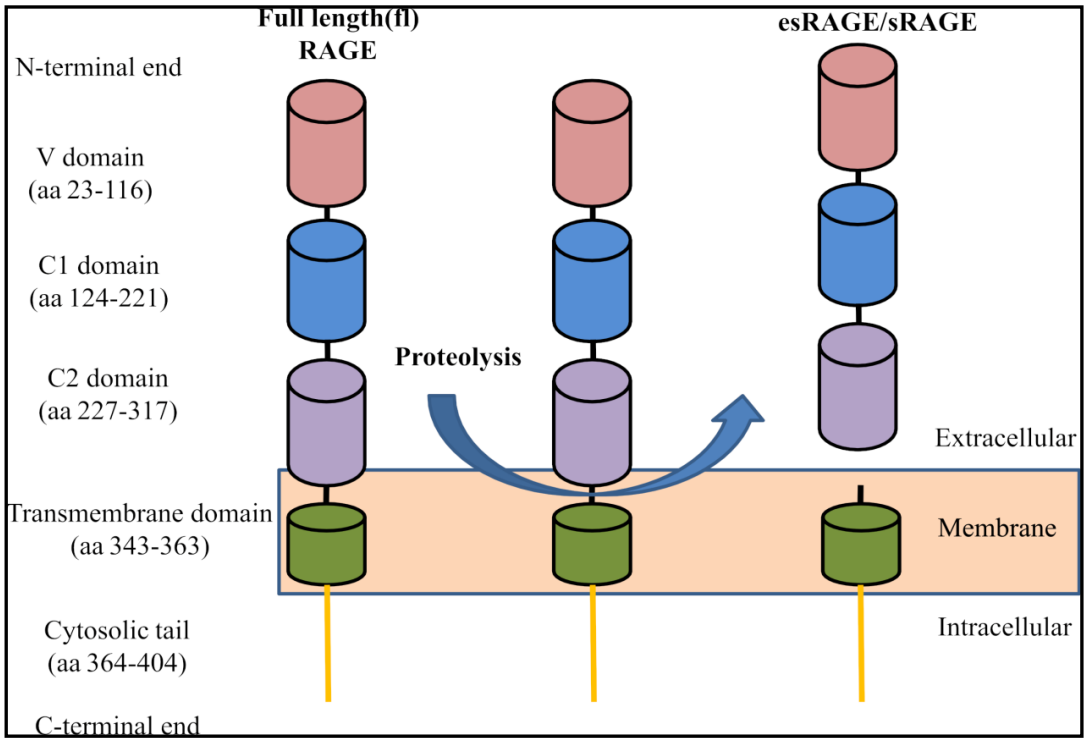

Figure 1. 4: RAGE and its isoforms

Full length RAGE is cleaved by various proteases leading to formation of soluble RAGE (Figure modified and adapted from [150])

Hyperglycemia can cause a variety of effects on its own as well as via toxic effects of AGEs. Glycation of long lived circulating proteins and matrix proteins causes oxidative stress. Activation of the glycation pathway has been found to be involved in

endothelial dysfunction, arterial stiffening, diabetic retinopathy, nephropathy and diabetic microvascular diseases [141, 142]. Diabetic retinopathy is characterized by increased proliferation of blood vessels, angiogenesis, hemorrhages, capillary basement membrane thickening, increased permeability, loss of pericytes, and so on in the retina. AGEs have been found to be deposited in blood vessel walls along with damaging pericytes due to AGE-RAGE interaction. As mentioned above, the glycation of proteins changes its function. Crystallin protein upon glycation reduces its function due to alteration in three-dimensional structure leading to aggravated diabetic cataract. Glycation of low density lipoprotein (LDL) increased its uptake by macrophages leading to hyperlipidemia and increased foam cell formation, thus increasing the complications of diabetic atherosclerosis. AGEs have been detected in kidney tissues of patients suffering from diabetic nephropathy. It has been found that AGEs stimulate TGF-β synthesis, thus causing increased collagen matrix synthesis leading to thickening of the neuronal basement membranes. Along with this, it further leads to altered filtration and loss of glomerular function. Myelin basic protein is important in myelination of nerves. Demyelination is one of the important factors in diabetic neuropathy. Glycated myelin protein is more prone for macrophage phagocytosis. Also, it stimulates macrophages to secrete various proteases which again increases demyelination [141].

1.2.5 Methylglyoxal

1.2.5.1 **MGO formation, toxicity, detoxification**

MGO, a dicarbonyl compound and a by-product of glycolysis having a molecular weight of 72 Da, is a potent AGE precursor [151]. Its levels are increased in diabetes, and it passes through the cell membrane by diffusion. It is found to be 20,000 fold more reactive than glucose in glycation reaction [152, 153]. Glycation reaction of MGO with proteins majorly leads to the formation of various adducts such as Nδ-(5-hydroxy-5-methyl-4-imidazol 2-yl)-ornithine (MG-H1), (Nδ-(5-hydroxy-4,6-dimethylpyrimidine-2-yl)- l-ornithine) (argpyrimidine), Nε-(1-carboxyethyl)lysine (CEL) and MGO derived lysine dimer (MOLD) [122, 154, 155]. Being highly potent, MGO has been reported to interact and modify proteins, lipids, and DNA [156, 157]. *In vivo*, MGO is formed majorly from the degradation of glyceraldehydes-3-

phosphate and to a lesser extent from acetone oxidation, threonine catabolism and non-enzymatic glucose degradation [158]. Exogenous sources include food and beverages, sterilization, and fermentation (**Figure 1.5**) [159]. MGO activates the NADPH oxidase enzyme, thereby inducing oxidative stress. It further activates c-Jun N-terminal kinase (JNK), Extracellular signal-regulated kinase (ERK), and p38 mitogen-activated protein kinase (p38 MAPK) leading to apoptosis [160]. MGO has been found to be elevated in various diabetic complications such as nephropathy and cardiomyopathy [161]. At the cellular level, MGO is responsible for proteasomal system dysfunction [162]. Exogenous treatment of MGO has been found to be toxic for SH-SY5Y, astrocytes, glia cells at different concentrations. Neurons have been found to be more susceptible to MGO mediated toxicity than astrocytes. Increased microglia resistance towards MGO can be explained by higher glutathione concentration, and increased glyoxalase-1 enzyme (GLO 1) and glyoxalase-2 enzyme (GLO 2) activities. MGO causes neuronal toxicity via oxidative stress and also through the formation of AGEs [122]. Along with neurons, MGO has been found to be toxic to blood cells such as RBCs, leukocytes, platelets. MGO caused hemolysis, decreased viability along with the increased level of glycated products [163]. In mammals, MGO is detoxified by the glyoxalase system, which converts toxic MGO into non-toxic D-lactate. Initially, MGO is converted into hemiacetal followed by conversion into S-D-Lactoylglutathione by GLO 1. It is further converted into D-lactate by glyoxalase-2 enzyme GLO 2. D-lactate is further converted into pyruvate, which then enters into the tricarboxylic acid (TCA) cycle [164].

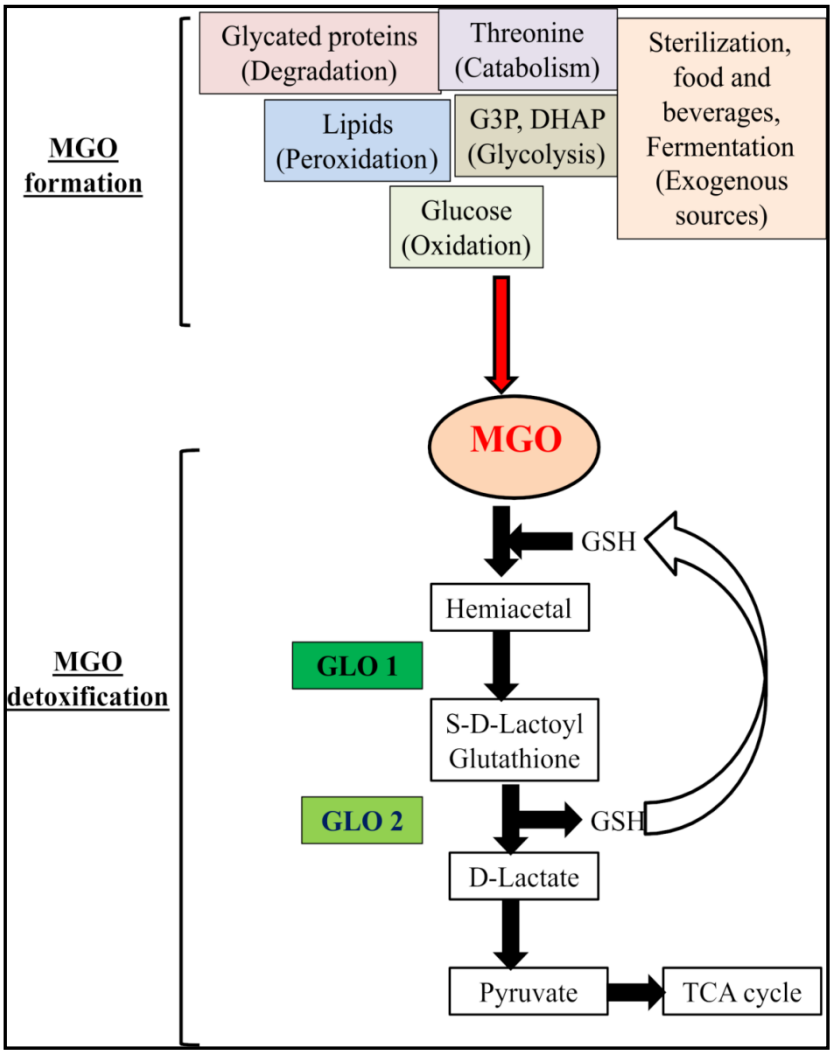

Figure 1. 5: Methylglyoxal formation and its detoxification

MGO formed in various ways is detoxified by glyoxalase 1 and glyoxalase 2 enzymes converting toxic MGO into non toxic D-Lactate. (Figure modified and adapted from [164])

1.2.6 Diagnosis of Diabetes

Diagnostic tests of diabetes follow the standard protocol by the American Diabetes Association. Diagnosis is based on blood glucose level, HbA_{1c} and oral glucose tolerance test (OGTT). Fasting plasma glucose (FPG) ≥ 126mg/dL (7.0 mmol/L) or postprandial glucose (PPG) ≥ 200mg/dL (11.1 mmol/L), HbA_{1c} ≥ 6.5% (48mmol/mol) or a random plasma glucose ≥ 200mg/dL (11.1 mmol/L) has been considered for diabetes detection [165].

1.2.7 Treatment of Diabetes

Adopting a healthy lifestyle, healthy protein-rich food, regular exercise, and meditation may help in the early stages of diabetes. In T1DM, insulin therapy is the treatment of choice. In T2DM, medications are given to maintain blood glucose level either by single or combination of drugs. There are different groups of drugs based on their mode of action. Choice and dosage of the drug are dependent on disease severity. Different classes include Biguanides (e.g. Metformin), Dipeptidyl peptidase inhibitor 4 inhibitors (e.g. Sitagliptin), Sodium-glucose cotransporter inhibitors (e.g. Canagliflozin), Sulfonylureas (e.g. Glimepiride), TZDs (e.g. Pioglitazone), GLP-1 agonists (e.g. Liraglutide) and so on [166-171]. Extracellular recombinant insulin treatment is also given in severe cases if oral drugs fail to maintain blood glucose levels.

1.3 Prevalence of Diabetes in AD patients

The etiology of AD is multifactorial and amongst various factors, diabetes is considered as the major risk factor for the development of AD. Subjects with diabetes in the early stages of life are more prone to develop AD [172, 173]. Besides, diabetic and AD subjects have common symptoms such as brain atrophy, reduced brain glucose metabolism, and insulin resistance; thus, AD is also termed as type-3 diabetes [174, 175]. In a longitudinal study, the risk of AD incidence has been found to be 65% higher in diabetic patients than in non-diabetic patients during observation of 5.5 years [172]. Similarly, a longitudinal study involving 2574 Japanese-American men also observed the synergistic effect of diabetes and the APOE ε4 allele on the development of AD [176]. In another study, diabetes was found to double the risk of dementia in a prospective population-based cohort study [177].

1.4 Diabetes and Alzheimer's Disease

1.4.1 Insulin

As mentioned above, insulin is a crucial player in maintaining normal blood glucose. Various studies suggest a connecting link between AD and insulin dysfunction. *In*

vitro studies have found a debatable role of insulin in tau phosphorylation. Short time insulin treatment leads to tau hyperphosphorylation, but a longer insulin treatment reduces it, both mediated by GSK3β kinase. Animal studies have shown that insulin deficiency caused due to STZ induction leads to increase in tau phosphorylation [178].

1.4.2 Methylglyoxal

This Highly reactive dicarbonyl has been found to be elevated in the cerebrospinal fluid of Alzheimer's patients than healthy matched controls [179]. MGO initiates apoptosis in neurons leading to neurodegeneration [180]. MGO has been found to increase reactive oxygen species (ROS), which is elevated in AD [155]. Serum level of MGO was higher in diabetic rats showing cognitive dysfunction [181]. MGO decreases mitochondrial membrane potential leading to reduced ATP synthesis [180, 182]. A higher level of serum MGO derivatives has been found to increase the cognitive decline [183]. The rate and size of synthetic Aβ peptide aggregation has been found to be increased in the presence of MGO [184]. MGO has also been shown to increase tau phosphorylation *in vitro* along with increased AGEs production [185]. Intracerebroventricular treatment of MGO in the rat model has been shown to cause cognitive decline [186]. Along with an increase in MGO production, its detoxification system (glyoxalase) also gets compromised in Alzheimer's [187]. GLO 1 expression was decreased along with concomitant increased AGE deposits in AD [122, 187]. Supporting this observation, restoring GLO 1 enzyme activity has been found to reduce cognitive dysfunction with a reduction in Aβ plaque load [188]. MGO has also been found to activate GSK-3β and p38 MAPK kinases leading to tau hyperphosphorylation along with an increase in AGEs and RAGE [185]. MGO has also been found to reduce PP-2A activity, thus increasing tau phosphorylation.

1.4.3 AGEs and RAGE

Since, Aβ plaques and NFT tangles are relatively stable, they are ideal substrates for *in vivo* glycation [155, 189]. The level of AGEs has been found to be more with Aβ plaques and NFTs. Immunohistochemical study of control and Alzheimer's patients

showed that AGEs level was increased in Alzheimer's and it co-localises with neurotoxic hyper phosphorylated tau tangles [190]. AGEs are known to induce oxidative stress and have been found to be co-localising with inducible nitric oxide synthase (iNOS) in advanced Alzheimer's patient's brains [191]. Hydroimidazolone, one of the AGEs formed by MGO has been found to be increased in the CSF of Alzheimer's patients [192]. CEL, MOLD and hydroimidazolone have also been found to be present in hyperphosphorylated tau deposits [193]. AGEs have been found to be colocalized with NFTs [190]. Several other studies have shown that Aβ plaques from AD brains have more AGE content than control samples [194, 195]. AGEs have also been found to alter AβPP processing and enhanced tau phosphorylation via the involvement of AGE-RAGE axis [138]. Increased AGEs have been found to cause tau phosphorylation by activation of CaMKII kinase [196]. Glycated Aβ has been found to be more neurotoxic than Aβ [197]. The AGE-RAGE axis also causes activation of various kinases such as GSK-3β which causes tau hyperphosphorylation [198].

Despite a century of AD discovery, the causes of the most prominent form of sporadic AD are not yet known clearly. Also, the treatments focus on preventing the further progression of AD and it is non reversible, making it one of the most severe diseases to study. We tried to understand the role of diabetes and MGO in AD development mainly by a proteomic approach. We expected that this study would help in identifying the role of diabetes and MGO in the manifestation of AD. Also, it will help in finding a more efficient drug target in the future.

With this background, following objectives were designed

1. To study the effect of MGO on the neuronal cell proteome *in vitro*

2. To study the effect of Diabetes and MGO in the development of Alzheimer's Disease in a rat model by proteomic approach

3. To study the role of glycation in the regulation of AβPP processing and amyloid β formation leading to Alzheimer's Disease

Chapter 2

To study the effect of MGO on the neuronal cell proteome *in vitro*

Chapter 2 To study the effect of MGO on the neuronal cell proteome *in vitro*

2.1 Introduction

Diabetes is characterized by elevated levels of blood glucose and dicarbonyls such as glyoxal and MGO, which causes further diabetic complications [199]. MGO is a highly active glycating agent causing protein glycation in various tissues and organs. Along with protein glycation, MGO induces ROS and causes cell damage. Diabetes is considered as a risk factor for AD development due to which AD is also called as type-3 diabetes. MGO plays an important role in AD pathogenesis. Higher serum MGO level is associated with cognitive decline in aged people [183]. MGO has been found to cause site specific tau phosphorylation in neuro-2a cells with subsequent upregulation of GSK-3β and p38 MAPK. MGO also increased AGEs formation with concomitant increase in RAGE expression [185]. MGO level has been found to be increased in AD patient's CSF as compared to control [179]. Neuronal cells are in close vicinity with CSF so higher MGO concentration can cause harmful effects. Proteomic analysis of MGO treated human aortal endothelial cells by high resolution mass spectrometry showed activation of unfolded protein response. MGO induced proteins are involved in protein folding, protein synthesis, and glycolysis [200]. Proteomic analysis of human neuroblastoma SH-SY5Y cells upon MGO treatment showed differential expression of actin, immunoglobulin lambda chain and protein phosphatase 2. These proteins have been found to play a major role in AD [201]. Despite these efforts, the exact role of MGO in AD development is not elucidated. AD is characterized by neurodegeneration, so studying neuronal proteome changes in the presence of MGO will help to understand the role of diabetes in the development of AD. This experiment aimed to study the effect of MGO on the differential expression of neuronal cell proteins by mass spectrometry and to study its involvement in the development of AD.

Mass spectrometry (MS) is an important advanced technique that helps understand the global expression of proteins in a cell or tissue. One of the most significant advantages of MS is its ability to identify proteins across a wide dynamic range so

that even a protein in less abundance can be detected. Along with identification, absolute or relative quantitation of proteins can be done [202]. In this study, we have analyzed the proteome of the neuro-2a cell line in the presence and absence of MGO treatment to study its role in neurodegeneration. The total protein from these samples was subjected for in-solution digestion followed by peptide identification on LC-MS^E using NanoAcquity ultra-performance liquid chromatography (UPLC). We believe that the results from this analysis may shed light on the involvement of diabetes, specifically MGO, in AD development.

2.2 Materials and Methods

Methodology used for studying the effect of MGO on neuronal cells is depicted in **Figure 2.1**.

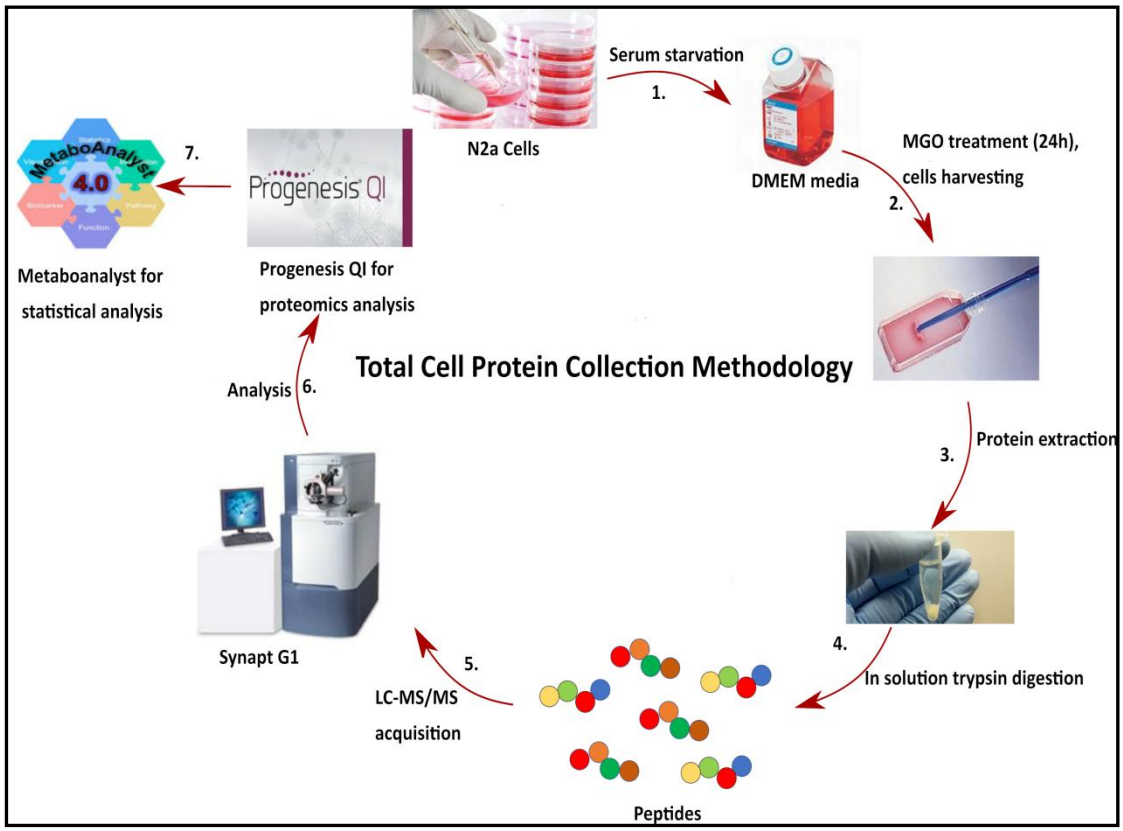

Figure 2.1: Workflow for studying *in vitro* MGO effect on neuronal cell proteome

2.2.1 Reagents

Dulbecco's Modified Eagle Medium (DMEM, Himedia, #AL183A), Fetal bovine serum (FBS, Himedia, #RM9955), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, #M5655), Proteomic grade porcine trypsin (Sigma, #T6567), Protease inhibitor cocktail (Sigma, #P8340), RapiGest (Waters, #186001860), ZipTip® (Millipore, #ZTC18S096) were used. All solvents for LC-MS^E were procured from J.T. Baker (J T. Baker, PA).

2.2.2 Cell culture

Mouse neuroblastoma cell line (Neuro-2a) was procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were grown in DMEM supplemented with 10% (v/v) heat-inactivated FBS. The culture was maintained at 37°C in a humidified atmosphere containing 5% (v/v) CO_2.

2.2.2.1 **MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide)**

The effect of MGO on neuro-2a (N2a) cells was checked by the MTT assay. Cells were grown in sterile cell culture treated 96 well plate up to 80% confluency in medium containing 10% FBS, followed by starvation for four hours in DMEM with 0.25% BSA (starvation medium). MGO treatment was given in different concentrations (300 nM to 3 mM) for 24 h in a starvation medium. Cell health was monitored by looking at cell morphology under the microscope. After 24 h, the medium was aspirated, followed by two PBS washes. MTT stock was prepared by dissolving MTT powder in PBS (2.4 mM) and added to individual wells along with starvation medium and incubated for further four hours in the dark in the CO_2 incubator. After 4 h, the formation of formazan crystals was confirmed under a microscope. Crystals were dissolved by adding 50 µl of 100% DMSO into each well. After a 10 min incubation in the CO_2 incubator, absorbance was recorded at 550 nm (Thermo, Varioskan plate reader).

2.2.3 Total cell proteomic analysis

2.2.3.1 **Protein Extraction**

Cells were grown in P 90 dishes upto get 80% confluency in DMEM with 10% FBS. After confluency, cells were starved for four hours in DMEM with 0.25 % BSA. MGO treatment was given for 24 h in DMEM with 0.25% BSA. After treatment, the medium was aspirated and two PBS washes were given. Cell culture grade trypsin was added for 3-4 min to detach cells and cells were collected by centrifugation at 1600 rpm for 5 min. Pellet was washed with PBS two times and a protease inhibitor cocktail was added to avoid proteolysis. The cell pellet was used for protein extraction in mass spectrometer compatible detergent RapiGest at a final concentration of 0.1% in 50 mM ammonium bicarbonate. It was followed by incubation on ice for 30 min with intermittent vortexing after 5 min. Cell debris was removed by centrifugation at 17,000 rpm at 4ºC for 1 h and the supernatant containing proteins was collected. Protein estimation was performed using Bradford's Assay (Bio-Rad).

2.2.3.2 **Sample preparation for Liquid chromatography mass spectrometry (LC-MS^E)**

A total of 100 µg of protein was processed by in-solution digestion. Proteins were denatured at 80ºC for 20 min. It was followed by reduction with dithiothreitol (100 mM) at 60ºC for 15 min and alkylation with iodoacetamide (200 mM) at ambient temperature in the dark for 30 min. Proteomic grade porcine trypsin (4 µg) was added and kept at 37ºC for 18 h with shaking (200 rpm). Trypsin reaction was stopped by adding 2 µl of formic acid. The digest was briefly vortexed and kept on ice for 5 min to precipitate acid-labile RapiGest. The digest was centrifuged at 4°C, 20,000 rpm for 30 min and the supernatant was collected. Tryptic peptides were desalted by using ZipTip and concentrated by using a Speed-Vac vacuum concentrator. Peptides were reconstituted in 3% ACN with 0.1% formic acid before MS^E analysis.

2.2.3.3 **Liquid chromatography-mass spectrometry (LC-MS^E) acquisition and analysis**

Digested tryptic peptides were acquired on LC-MS^E using NanoAcquity ultra-performance liquid chromatography (UPLC), coupled to SYNAPT high definition mass spectrometer (Waters, Milford, MA, USA) with NanoLockSpray as the ion source. Mass spectra were acquired in biological duplicates and technical triplicates. The peptides were separated on Nano C18 reversed-phase column (i.d. 75 µm and length 250 mm) (Waters Corporation, USA). Binary solvent system (99.9% water and 0.1% formic acid (mobile phase A) and 99.9% acetonitrile and 0.1% formic acid (mobile phase B) was used for peptide separation. Peptide digest was injected into trapping; the column was washed with 0.1% solvent A (0.1% formic acid containing water) for 3 min at 5 µL/min flow rate. Sample elution was performed with a gradient of solvent B (0.1% formic acid containing acetonitrile) for 95 min (3 to 40%, 0-95 min) followed by column washing at 40 to 85% for 10 min and column equilibration at 85 to 3% for 15 min at a flow rate of 250 nl/min and column temperature at 40^{o}C. Lock mass calibrant peptide standard [Glu^1]-fibrinopeptide B (Sigma-Aldrich) was infused into ion source at the flow rate of 300 nl/min and LC-MS^E data was collected over an m/z range of 50 to 1999 at a scan time of 0.8 s and constant low energy of 10 V for MS mode.

The protein database of *Mus musculus* deposited in the UNIPROT as on 1st March 2020, (UniProt release 2020_01), consisting of both reviewed and unreviewed proteins, was used for peptide search and protein identification. Yeast enolase digest (SwissProt ID P00924) was used as an internal standard in the samples. The amino acid sequence of yeast enolase was included in the above mentioned protein sequence library for protein identification. Progenesis QI for proteomics (Nonlinear Dynamics, Newcastle, UK) was used to analyze the data acquired on MS Waters SYNAPT G1 HDMS platform. Proteins were identified containing at least 1 unique peptide. Protein abundance was determined for identified proteins based on intensities of uniquely identified peptides. Statistical analysis was performed using Metaboanalyst (https://www.metaboanalyst.ca/).

2.2.3.4 **Functional annotation**

Functional annotation of altered proteins was done using KAAS - KEGG Automatic Annotation Server online (https://www.genome.jp/kegg/kaas/). Involvement of differentially expressed proteins into various bioprocesses was studied by BINGO software.

2.3 Results and Discussions

2.3.1 MTT assay

MTT assay showed that MGO is neurotoxic in a concentration-dependent manner. MTT assay is based on the conversion of MTT to formazan crystals *in vivo*.

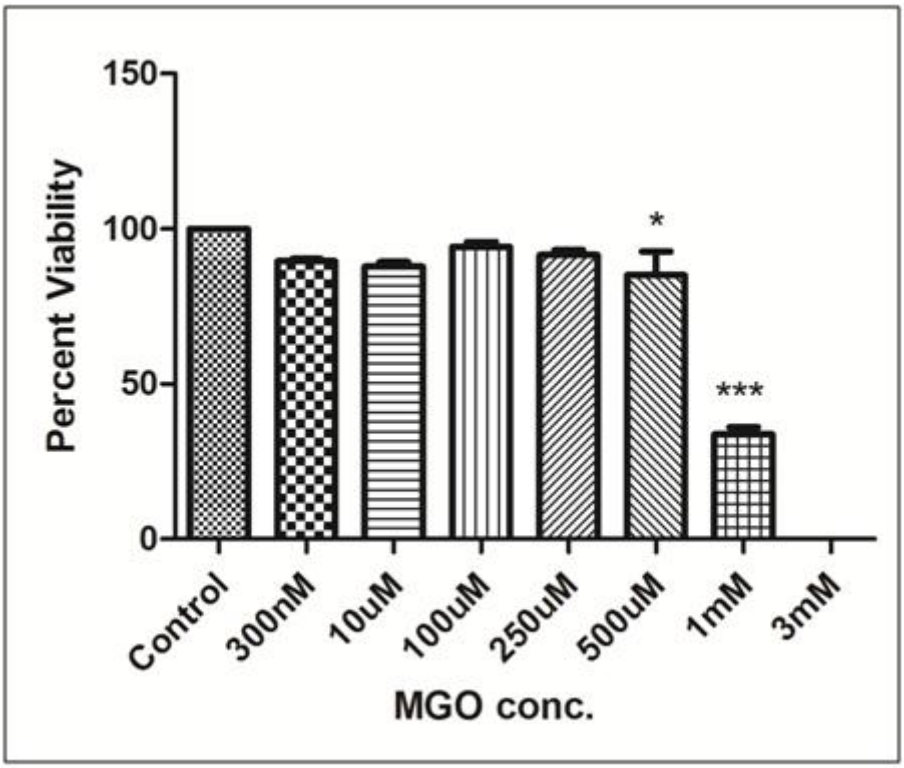

Figure 2.2: Neuro-2a MTT assay with different MGO concentrations for 24 h

(One-way ANOVA ,*$p < 0.05$, ***$p < 0.001$, data expressed as mean±SD)

It depicts the mitochondrial activity of cells that can be correlated with the viability of cells and thus evaluate the cytotoxic effect of MGO treatment. A higher rate of conversion of MTT to formazan crystals as seen by higher absorbance at 550 nm indicates a better metabolic state of cells [203]. MGO has been found to induce

apoptosis in neuronal cells via the mTOR/4E-BP1 pathway and activation of pro-apoptotic proteins such as Bax, caspase-1, cytochrome C [204]. Neurons have also been found to be susceptible to MGO induced cell death due to AGEs formation and generation of oxidative stress [122]. MGO has been found to cause apoptosis in SH-SY5Y neuronal cells at a concentration of 500 μM through the upregulation of apoptotic proteins [205]. The viability of insulin-producing RINm5F cells was found to be affected at 1 mM MGO concentration [206]. The primary culture of hippocampal neurons has been shown to have 100 μM MGO as IC_{50} [207]. MGO has shown an increase in neuronal viability, excitability and increased neurite extensions at lower concentrations (0-150 μM), whereas at higher concentrations (250-750 μM) it has been found to be cytotoxic [208]. In our study, it was found to be non-toxic till a concentration of 250 μM, since the cell's metabolic activity was unaffected while it was found to be decreasing at higher concentrations (**Figure 2.2**). A non-toxic MGO concentration of 10 μM was used for proteomic analysis as this concentration of MGO was found to be present in healthy CSF [179].

2.3.2 Differential protein expression upon MGO treatment

Neuro-2a cell proteins were digested in biological duplicates and were acquired in technical triplicates on the mass spectrometer and PCA was plotted for all replicates ($p < 0.05$). PCA plot showed good consistency and reproducibility between biological and technical replicates (**Figure 2.3 A**). The LC-MS^E analysis of neuro-2a cells in the presence or absence of MGO identified total 848 proteins with at least one unique peptide **(Appendix no. 1)**. For differential expression analysis, proteins with a fold change minimum of 1.3 fold and p value < 0.5 were considered. A total of 67 proteins were found to be upregulated and 57 proteins were downregulated upon MGO treatment (**Figure 2.3 B**). A list of differentially regulated proteins is given in **appendix no. 2**.

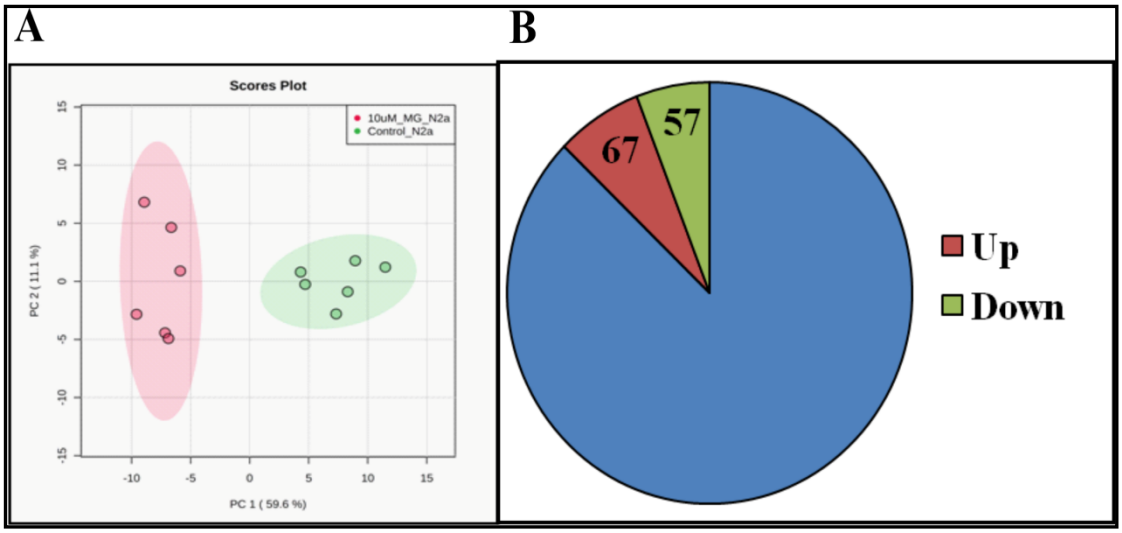

Figure 2.3: Differential protein expression upon MGO treatment.

(A) PCA plot showing consistency between biological and technical replicates (B) The pie chart indicates upregulation of 67 proteins and downregulation of 57 proteins upon MGO treatment out of 848 proteins quantified

2.3.3 Functional Annotation

KEGG KAAS functional analysis of differentially expressed proteins showed involvement of these proteins into various crucial pathways such as AD, diabetes, AGE-RAGE signaling, insulin secretion pathway, insulin signaling, synapse, calcium signaling, and apoptosis (**Figure 2.4**).

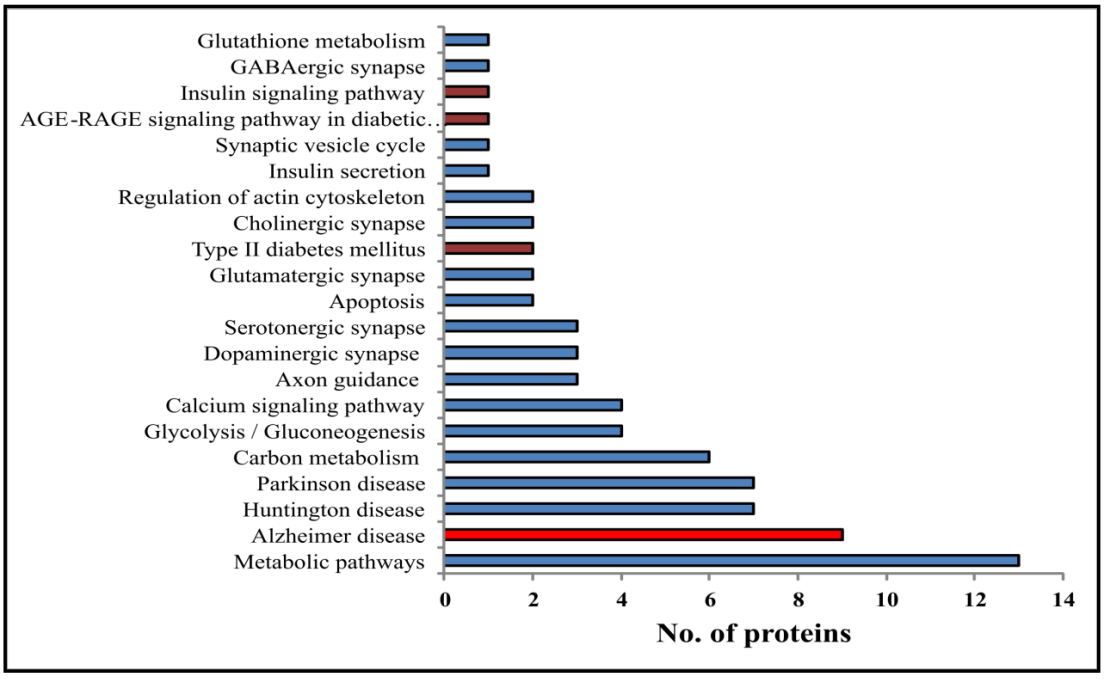

Figure 2.4: Functional analysis of MGO altered proteins by KEGG KAAS

Proteins mapped in AD are well known to be involved in AD. Proteins upregulated by MGO and involved in AD pathology include, voltage-gated calcium channel L type alpha-1C, inositol 1,4,5-trisphosphate receptor type 1 and 20S proteasome subunit.

Voltage-gated calcium channel L type alpha-1C has been found to be increased upon MGO treatment. These channels are responsible for calcium homeostasis inside cells and regulation of neuronal functions such as plasticity, transcriptional regulation, and so on. Its expression has been found to be increased in the presence of Aβ plaque deposition in the reactive astrocytes of transgenic mice carrying AβPP mutation [209]. Inositol 1,4,5-trisphosphate receptor ($InsP_3R$), a ligand-gated Ca^{+2} release channel is also upregulated upon MGO treatment. $InsP_3R$ is a receptor of Inositol 1,4,5-trisphosphate ($InsP_3$) which acts as a second messenger for endoplasmic reticulum (ER) Ca^{+2} release, thus plays a major role in Ca^{+2} homeostasis. Membrane G protein-coupled receptor gets stimulated upon extracellular ligand binding, which activates phospholipase C (PLC) which then hydrolyses phosphatidylinositol bi-phosphate to form $InsP_3$. $InsP_3$ then further interacts with $InsP_3R$ in the ER leading to increased Ca^{+2} release, causing an increase in cytoplasmic Ca^{+2} concentration, which promotes AβPP processing by β-secretases leading to enhanced Aβ production [210]. Cytoplasmic released Ca^{+2} also triggers apoptotic pathway via Bcl-2 protein family

[211, 212]. Cytoplasmic free Ca^{+2} concentration has been found to play an important role in learning and memory, synaptic transmission, membrane trafficking and so on [213]. Protein misfolding is one of the significant events leading to protein aggregation and has been found to worsen neurodegenerative diseases. The Ubiquitin Proteasomal System (UPS) removes misfolded proteins by tagging them with ubiquitin [214]. The proteasomal activity has been reduced in the AD brain compared to control [215]. The catalytic core of UPS is the 20S subunit, which is upregulated upon MGO treatment. This increase in expression can result from a compensatory mechanism for reduced activity, leading to enhanced protein aggregation.

Proteins such as reticulon 4, adenomatosis coli protein and kinesin were found to be downregulated upon MGO treatment. They have been known to play a role in AD. Reticulon is a group of proteins found to be interacting with BACE1 and altering its activity. Overexpression of reticulon 3, a member of the family, has been found to bind BACE1 and inhibit its activity leading to a reduction in Aβ production [216]. We have found downregulation of reticulon 4. Reticulon 4 also called NOGO-A, has various functions with limiting neurite outgrowth and plasticity of the nervous system [217]. Adenomatous polyposis coli (APC) is a part of the β catenin destruction complex which plays an essential role in the canonical Wnt pathway by maintaining cytoplasmic β catenin levels by degrading it [218]. This pathway regulates various cellular functions such as cell migration, neuronal polarity, cell polarity and so on. Levels of β catenin and Wnt signaling are downregulated in AD patients [219, 220]. Animals with APC protein knock-down studies were found to exhibit learning and memory impairment, increased synaptic spine density, and altered synaptic function [221]. Another downregulated protein upon MGO treatment is kinesin, which is involved in the anterograde transport of organelles in neurons. It is required for the normal functioning of neuronal cells [222]. Presenilin 1 has been found to cause GSK-3β mediated kinesin phosphorylation which leads to its release from membrane bound organelles which may compromise neuronal function [223]. It has also been found that the reduction in axonal transport can lead to tau hyperphosphorylation [224].

MGO altered proteins have also been shown to be involved in calcium signaling pathways. Calcium is an essential ion in neurotransmission. Synaptic vesicles in the

presynaptic terminal are loaded with neurotransmitters. Ca^{+2} ion is taken up from synaptic cleft through voltage-gated Ca^{+2} channel. In response to this, synaptic vesicles fuse with the membrane, and a neurotransmitter is released, which is then taken up by postsynaptic neurons [225, 226]. Another important observation was the involvement of proteins playing a role in various synapses such as GABAergic, glutamatergic, serotonergic, and dopaminergic. Synapse plays a crucial role in proper neurotransmission. Alternation in the synapse can lead to disturbances in neurotransmission. Synapse loss occurs in the early stages of AD development. [227, 228].

Proteins involved in diabetes are pyruvate kinase and voltage-dependent calcium channel L type alpha-1C. Pyruvate kinase is one of the major enzymes involved in glycolysis, which converts phosphoenolpyruvate to pyruvate. Increased activity of this enzyme has been found in AD brains [229]. This protein also serves as a pathway for insulin secretion in pancreatic β cells [230, 231]. Microglial cells exposed to AD plasma have shown an increased pyruvate kinase expression, suggesting an increase in anaerobic metabolism [232]. MGO treatment also caused upregulation of collagen type IV alpha 6 protein. This protein has been found to be involved in the AGE-RAGE signaling pathway. Expression of this protein is increased in type 1 diabetic rat's cortex as compared to diabetic RAGE null mice, indicating involvement in AGE-RAGE axis [233]. Further, an *in vitro* experiment has shown that AGEs induce collagen formation via JAK/STAT pathway [234]. Collagen content has also been found to be increased in cerebral microvessels isolated from AD [235].

BINGO analysis showed that MGO upregulated proteins were involved in the MGO metabolic process, response to reactive oxygen species, ATP metabolism, apoptosis, neurofilament cytoskeletal organization, and NMDA receptor activity regulation. MGO has been known to induce oxidative stress and cause apoptosis [236, 237]. MGO upregulated Protein/nucleic acid deglycase DJ-1 (Park 7). Park 7 is a major deglycase enzyme that acts on methylglyoxal and glyoxal glycated amino acids and repairs them. Park 7 has been found to be inactivated by MGO. Increased Park 7 expression can be a cell's response to combat an increased number of glycated proteins *in vivo* [238].

BINGO analysis showed that down regulated proteins were also found to be involved in various biological processes such as regulation of branching morphogenesis of nerves and positive regulation of synapse maturation (**Appendix no. 3**).

2.4 Conclusions

MGO, a potent glycating dicarbonyl compound is elevated in diabetes and AD patients. Herein, we have tried to study the effect of MGO on the global proteome of neuro-2a cells. MTT assay indicated that MGO is neurotoxic at higher concentrations. Despite earlier efforts to study neuronal proteome upn MGO treatment, the exact role of MGO in AD development is unknown. Herein we have tried to identify the maximum number of differentially expressed proteins by using sensitive LC-MS^E technique. Mass spectrometric analysis of neuro-2a proteins upon MGO treatment revealed that MGO alters the expression of proteins involved in AD, calcium signaling, neurotransmission and protein degradation which are involved in AD development. Along with AD, MGO treatment also leads to differential expression of proteins involved in insulin secretion, insulin signaling, and the AGE-RAGE pathway indicating its role in diabetes pathophysiology. Expression of proteins involved in MGO metabolic process, ATP generation, response to ROS, apoptosis, glutathione metabolic process was upregulated. On the contrary, proteins involved in regulation of branching morphogenesis of nerve, synapse maturation were downregulated. As MGO concentration is high in diabetes, it may cause these changes in the neuronal function leading to neurodegeneration which may ultimately become a risk factor for AD.

Chapter 3

To study the effect of Diabetes and MGO in the development of Alzheimer's Disease in a rat model by a proteomic approach.

Chapter 3 To study the effect of Diabetes and MGO in the development of Alzheimer's Disease in a rat model by a proteomic approach.

3.1 Introduction

Diabetes and AD are two of the major diseases affecting the aged population. Population based studies have shown a strong correlation between these two diseases, with diabetes being a risk factor for AD development. Diabetes and AD have been found to share various common pathological symptoms such as brain atrophy, insulin resistance, increased inflammation and so on [239]. Along with this, diabetes has been found to be a strong predisposing factor for AD development suggesting that studying pathological changes at the cellular level in diabetes can help to understand AD development. Insulin resistance has been found to increase neuritic plaque formation and cause cognitive impairment [240, 241]. Moderate insulin level has been shown to have a beneficial effect on the brain, whereas excess insulin reduces Aβ clearance from the brain [242]. Along with insulin resistance, insulin deficiency has also been found to induce cognitive impairment with a concomitant increase in neurotoxic Aβ peptide and tau hyperphosphorylation along with a decrease in p-Akt and phosphorylated GSK-3β in a transgenic mouse model [243]. Tau hyperphosphorylation has also been observed in STZ induced diabetes characterized by insulin deficiency along with PP-2A inhibition [244]. Type 1 diabetes-induced rats have been shown to cause cognitive impairment along with an increase in Aβ and tau hyperphosphorylation [245].

The result of insulin deficiency and insulin resistance is hyperglycemia, which increases protein glycation. Glycated albumin/Glycated haemoglobin ratio has been found to be associated with AD development in a population-based study [246]. It would be interesting to see the effect of hyperglycemia and insulin deficiency on the brain by proteomic analysis and other biochemical assays.

MGO is a toxic by-product of glycolysis whose levels are elevated in diabetes. MGO is one of the most potent glycating agents and forms AGEs. The effect of MGO on neuronal cells and in the pathophysiology of AD has been studied earlier. MGO has

also been found to increase the Aβ aggregation rate and size of aggregates [155]. MGO is detrimental to neurons via AGEs formation, as well as via generation of ROS [122]. MGO has been found to decrease mitochondrial membrane potential and increase intracellular ROS production in SHSY-5Y cells [182]. Huang *et al* have found that in neuro-2a cells, MGO activates proapoptotic mitogen-activated protein kinase (MAPK) signaling pathway involving JNK and p38 [247]. MGO has been found to induce cognitive deficit in the rat model [186]. Higher MGO level has also been found to be associated with poor memory and cerebral atrophy in older people [248]. Also, MGO concentration in cerebrospinal fluid (CSF) of AD patients is found to be higher than in control [179].

Despite efforts of studying the relationship between diabetes and AD, the exact mechanism is not understood. Neuronal proteomic changes upon MGO treatment indicated that MGO differentially regulates the expression of proteins involved in AD, calcium signaling, neurotransmission. Alterations in these important pathways can make diabetes a risk factor for AD development. Herein we have tried to study the effect of diabetes and MGO in the development of AD by using non-transgenic rats in the presence of two drugs, aminoguanidine (AMG) and telmisartan (TELMI).

AMG quenches MGO and reduces its toxicity. It is a selective inhibitor of inducible nitric oxide synthase (iNOS) [249]. AMG has been found to reduce anxiety in various conditions [250-252]. Telmisartan (commercially available as Micardis), belonging to the group of sartans, was selected due to its high bioavailability (42-58%) and more biological half-life (24 h) [253]. Telmisartan (TELMI) is an angiotensin II (Ang II) receptor blocker (ARB) which has been found to cross the blood brain barrier (BBB) [254]. It reduces excessive peripheral Ang II type 1 receptor (AT1R) activity and is used for hypertension treatment. TELMI has also been found to have various neuroprotective properties. Along with the renin angiotensin system, TELMI also has peroxisome proliferator-activated receptor-γ (PPAR-γ)–stimulating activity [255]. TELMI has a partial PPAR-γ agonistic activity in addition to its role in controlling hypertension through the renin-angiotensin system [256]. PPAR are a group of nuclear receptors that controls various transcription factors upon ligand binding. These transcription factors regulate various cellular processes such as inflammation,

adipogenesis, and maintenance of metabolic homeostasis [257]. PPAR-γ agonists have been found to be useful in restoring learning and memory deficits in AD rodent models [258, 259]. In this study, we have tried to explore the effect of AMG and TELMI drugs in MGO induced pathogenesis in rats by using behavioural study and proteomics.

We have used a label-free, untargeted DIA (data-independent acquisition) SWATH (sequential window acquisition of all theoretical fragments) method for understanding differential protein expression in the rat hippocampus region. Hippocampus is the region of the brain responsible for learning and memory. Hippocampus region was selected as it is responsible for learning and memory [260].

3.2 Materials and Methods

3.2.1 Reagents

MGO (Sigma, #M0252) and Aminoguanidine (Sigma, # 396494), Fructosamine kit (Abbexa, #abx098427), HbA1c measurement kit (NycoCard, #1042184), anti-Th 205/AT8 pTau antibody (AT8) (Thermo scientific, #MN1020), anti-Tau antibody (Dako, #A0024), anti-RAGE (Sigma, #R5278), anti-GAPDH (Sigma, #G8795), protease inhibitor cocktail (Sigma, #P8340), phosphatase inhibitor cocktail (Sigma, #P0044), Amersham ECL prime (GE Healthcare, #RPN2232), polyvinylidene difluoride (Millipore, #ISEQ10100) membranes were used. Telmisartan (Commercially available as Micardis tablet) was used.

3.2.2 Animal experiment

All the animal experiments were carried out at Experimental Animal Facility, Symbiosis School of Biological Sciences, Pune, India. Experiments were approved by the Institutional Animal Ethics committee (IAEC proposal no.-SSBS/AIEC/03-2017). Male Sprague Dawley rats were obtained from National Biosciences, Pune. Behaviour study was performed at Sinhgad Institute of Pharmacy (SIOP), Pune, India. They were supplied with a standard rodent chow diet and had access to water. These animals were housed in a room at an ambient temperature of 25 ± 2°C. A light and dark cycle of 12 ± 1 h was maintained. The protocol used and various treatments

given are depicted in **Figure 3.1**.

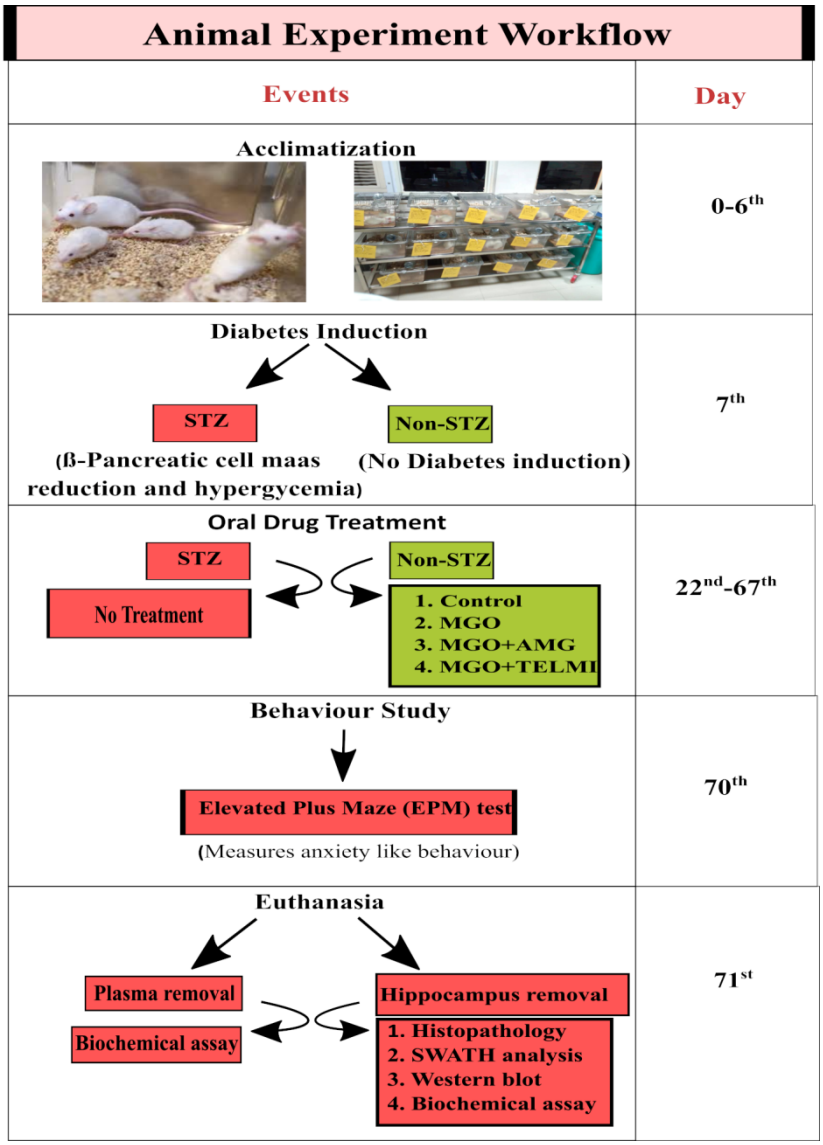

Figure 3.1: Experimental workflow of animal experiment

Diabetes was induced by injecting a single intraperitoneal injection of 55 mg/kg streptozotocin in 10 mM sodium citrate buffer pH (4.0) to 4 h starved rats whereas non diabetic rats received saline as a vehicle control. After 15 days, blood glucose and HbA_{1c} were measured to confirm the establishment of diabetes. Animals with blood glucose level of above 6.12 ± 1.7 mmol/L were considered as diabetic [261, 262]. Diabetic rats were used as a positive control and non-diabetic rats were given oral MGO treatment or in combination with TELMI and AMG.

MGO (50 mg/kg/day) [263] was given with or without TELMI (10 mg/kg/day) [264] and AMG (1 g/L/day) [265] for 45 days. MGO and TELMI were given orally and AMG was given through drinking water. Bodyweight, blood glucose, and HbA_{1c} were measured before and at the end of the treatment. After the behavior study, laboratory animals under experimentation were anesthetized with chloroform, and brain dissection was performed. The hippocampus region of the brain and blood samples were collected for further analysis (**Figure 3.1**).

3.2.3 Behavior study

Rats were acclimatized to the environment two days prior to behavior studies. The elevated plus maze (EPM) platform consisted of 2 opposing open arms (zone 1, 2) (10×50 cm) and 2 opposing closed arms (zone 3, 4) (50×20×50 cm) with side walls (15 cm). The platform is at the height of 40 cm from the ground. Distraction due to manual presence was avoided by placing a wall in between the experimental area and observer. Rats were placed on the central square platform (zone 5) (10×10 cm) and allowed to explore the maze freely for 5 min. Rat's behaviour was video-graphically recorded for 5 min by Maze Master (VJ instruments, India) software. Behavioural parameters such as number of entries, distance travelled, grooming and rearing were counted by the software.

3.2.4 Brain tissue processing

Rats were anesthetized, selectively hippocampus was isolated and washed with sterile chilled PBS to remove blood traces. Tissue was frozen immediately in liquid nitrogen followed by storage at -80°C until further use. Part of the hippocampal tissue was stored in formalin for histochemical analysis. For proteomic analysis, frozen tissue was washed once with 0.9% chilled saline to remove blood traces. It was followed by ice cold milli-Q washes. Tissue was homogenized in fine powder using mortar pestle in liquid nitrogen. Powdered tissue was weighed, and aliquots were kept in -80°C until further use.

3.2.4.1 **Sample preparation for mass spectrometry**

For mass spectrometric analysis, proteins were extracted by using 0.1% RapiGest SF (Waters Corporation, USA) in a 50 mM ammonium bicarbonate buffer. Tissue was homogenized for 30 min on ice with brief vortexing every 5 min. Homogenate was cleared by centrifugation at 17,000 rpm at 4ºC for 1 h and a clear supernatant was used for protein estimation by Bradford assay (Bio-Rad). Further, in solution trypsin digestion protocol was used as mentioned above in section no 2.2.3.2. Briefly 100 µg of protein was processed by in-solution digestion. Proteins were denatured at 80ºC for 20 min followed by reduction with dithiothreitol (100 mM) at 60ºC for 15 min and alkylation with iodoacetamide (200 mM) at ambient temperature in dark for 30 min. Trypsin (4 µg) was added and kept at 37ºC for 18 h. The reaction was stopped by acidifying the reaction mixture with 2 µl of formic acid. The digest was briefly vortexed and kept on ice for 5 min to precipitate acid-labile RapiGest. The digest was centrifuged at 4°C, 20,000 rpm for 30 min and supernatant was collected. Tryptic peptides were desalted by using ZipTip® and concentrated by using Speed-Vac vacuum concentrator. Peptides were reconstituted in 3% ACN with 0.1% formic acid before LC-MS^E analysis.

3.2.4.2 **Liquid Chromatography Mass spectrometry (SWATH-MS)**

All samples were analyzed on an AB Sciex Triple-TOF 5600 mass spectrometer coupled with micro LC 200 (Eksigent) in high-sensitivity mode. To generate the SWATH spectral library, peptide digests of each treatment were analyzed by LC-MS/MS in an Information Dependent Acquisition (IDA) mode and combining the results of all the treatments. Accumulation time for MS and MS/MS was set to 250 ms and 100 ms, respectively, and fragmentation was undertaken using rolling collision energy. MS scans were performed in the mass range of 350-1800 m/z, with a charge state 2 to 5, and MS/MS was triggered for ions exceeding 120 cps. SWATH-MS datasets were acquired in biological and technical triplicates. The desalted tryptic peptides were injected onto an Eksigent C18-RP HPLC column (100 × 0.3 mm,3 µm, 120 Å) at a flow rate of 8 µL/min over the following 120 min gradient. Solvent A (water with 0.1 % formic acid) and solvent B (ACN with 0.1 % formic acid): held at

97 % A for 5 min, 97-90 % A over 20 min, 90-70 % A over 70 min, 70-50 % A over 5 min, 50-10 % A over 1 min, at 10 % A for 7 min, 10-97% A over 1 min and held at 97 % A for 11 min. For SWATH-MS data acquisition, the instrument was tuned to optimize the quadrupole settings for the precursor ion selection window of 25 Da wide using 34 windows of 25 Da effective isolation width (with an additional 1 Da overlap) and with a dwell time of 70 ms to cover the mass range of 350-1200 m/z in 3.4 s. Before each cycle, an MS1 scan was acquired, and followed by the MS2 scan cycle (350–375 m/z precursor isolation window for the first scan, 474–500 m/z for the second, and 1174–1200 m/z for the last scan). The collision energy for each window was set using the collision energy of a 2+ ion centered in the middle of the window with a spread of 15 eV. To obtain spectral library from IDA runs, data was analyzed by ProteinPilot v5.0 software. *Rattus Norvegicus* database containing more than 16500 reviewed protein entries (2019) (UniProt release 2019_11) was used from UniProt. Trypsin was set as an enzyme used for digestion, cysteine alkylation was set to iodoacetamide and rapid ID was performed. A false discovery rate (FDR) was set to 1% for protein identification. The IDA analysis result was used as a spectral library (proteins with unique peptides) for PeakView v 2.2 software with SWATH extension and SWATH runs were processed using FDR 1%, mass error 50 ppm, the retention time window of 5 min, and 95% confidence interval. The number of peptides per protein was set to 10, and 6 was the number of transitions per peptide. SWATH data was exported to MarkerView™ v 1.2.1 for quantitative and statistical analysis. Proteins with fold change > 1.3 and p value < 0.05 were considered for further analysis.

3.2.4.3 **Functional Annotation**

DAVID (**D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery) software was used to understand the role of differentially expressed proteins in STZ and MGO. Further, functional annotation of proteins differentially regulated by MGO in rats was studied using Gene Set Enrichment Analysis (GSEA) software.

3.2.5 Plasma fructosamine assay

A total of 0.5 ml of blood was collected before sacrifice and mixed with ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. The blood sample

was centrifuged at 13,000 rpm and plasma was collected and stored in aliquots at -80ºC to avoid repeated freeze-thaw. Plasma fructosamine was performed as per the manufacturer's instructions. It has a detection range of 0.14-7 mmol/l. Fructosamine present in plasma reduces tetranitro blue tetrazolium chloride (NBT) into formazan under an alkaline condition which is measured by absorbance at 546 nm. Sample plasma fructosamine is calculated from the standard curve plotted by using different concentrations of the calibrator.

3.2.6 Western blotting

Hippocampal proteins were extracted in RIPA buffer (50 mM Tris HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton 100, 1 mM sodium orthovanadate) containing protease inhibitor and phosphatase inhibitor for 30 min on ice with brief vortexing after every 5 min. Tissue homogenate was cleared by centrifugation at 17,000 rpm, 4ºC for 1 h. Protein estimation from the supernatant was performed by Bradford's method. 40 µg of hippocampal proteins was separated on 10% SDS-PAGE gel. Proteins were then transferred onto polyvinylidene difluoride (PVDF) membrane. Ponceau S staining (0.1% w/v in 5% acetic acid) was used to monitor equal loading and uniform transfer of proteins and followed by incubation for 1 h at room temperature in a blocking buffer containing 3% BSA. It was then further incubated with primary antibody in the blocking buffer. Following antibody dilutions were used, Th 205/AT8 (1:1000), Tau (1:8000), RAGE (1:1500), GAPDH (1:1000). The membranes were either incubated with anti Mouse or anti Rabbit peroxidase-conjugated secondary antibody for 1 h at RT at a dilution of 1:5000 in PBST. Detection was performed using Amersham ECL prime as per the manufacturer's instructions. A harsh stripping buffer followed by blocking was used before reprobing with another primary antibody using the same protocol.

3.2.7 Hippocampal histochemistry

The brain tissue samples were collected from the respective experimental groups in 10% neutral buffered formalin as a tissue preservative for histology. The tissue sections of the brain, after 72 h of fixation, were grossed and processed on an

automated tissue processor for the histopathological protocol using ascending grades of alcohol and cleared in xylene. The tissue was embedded in paraffin blocks for micro-sectioning on an automated tissue microtome (Leica, Germany), and tissue sections of 5 µm were taken on glass slides. The tissue sections were stained by routine Hematoxylin and Eosin protocol and were observed under a binocular microscope with a microphotography unit (Nikon, Japan). The histopathological examination was also performed for observation of any pathological and cellular changes in the cerebrum and in the C-region of the brain (n=3).

3.3 Results and Discussions

3.3.1 Body parameters of rats

Bodyweight, blood glucose, and HbA_{1c} were measured before starting MGO treatment (**Table 3.1**). Diabetes was confirmed by measuring blood glucose and HbA_{1c}. Two-tailed t-tests was performed to calculate the significance of body weight, blood glucose and HbA_{1c} between control and diabetic group (*$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$). The average bodyweight of non-diabetic rats was 339.84 ± 39.55 g vs diabetic rats with 279.50 ± 64.86 g*, data expressed as mean (± SD). A significant decrease in body weight of diabetic rats was observed. Non-diabetic rats showed an increased blood glucose level of 87.71 ± 12.80 mg/dL as compared to diabetic rats with 537.20 ± 72.04 mg/dL***. Along with increased glucose level, diabetic rats showed an increase in HbA_{1c} value 7.03 ± 0.55 %*** as compared to non-diabetic rats 4.1 ± 0.12 %.

Table 3.1: Body parameters of rats after diabetes establishment

	Body weight (g)	**Blood glucose (mg/dL)**	**HbA_{1c} (%)**
Control (Non diabetic)(n=24)	339.84 ± 39.55	87.71 ± 12.80	4.1 ± 0.12
STZ (Diabetic)(n=6)	279.50 ± 64.86*	537.20 ± 72.04***	7.03 ± 0.55***
Data expressed as mean (± SD) (*$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$)			

3.3.2 Behavior study

Effect of STZ and MGO treatment (with or without AMG or TELMI) on rat behaviour was studied by the Elevated Plus Maze test (EPM). EPM detects the development of anxiety, which is observed in the early stages of AD development. Anxiety is detected by a reduced number of entries, and reduced distance travelled in open arms as compared to closed arms. A representative trajectory of rats in all groups is depicted in **Figure 3.2**. Control rats were found to explore open as well as closed arms indicating healthy condition, on the contrary STZ treated rats showed a significantly reduced number of entries w.r.t. control. STZ treatment has been found to lead to anxiety detectable by EPM [266, 267]. The number of entries into open arms in the STZ group were significantly decreased w.r.t. control indicating anxiety (**Figure 3.3 A**). Various studies have shown that deficiency of serotonin, adenylyl cyclase type VIII, and tuberoinfundibular peptide of 39 residues can lead to the development of anxiety in diabetic rats [267].

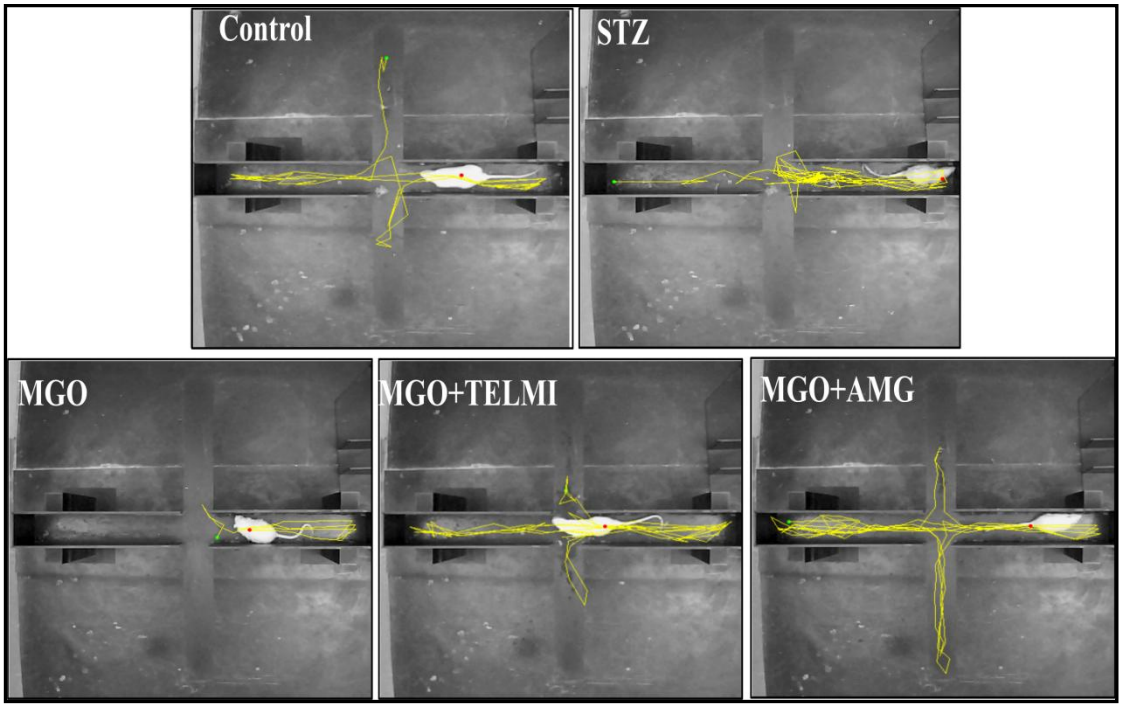

Figure 3.2: Representative trajectories of rats in EPM study

The role of MGO as an anxiety developer or anxiolytic is debatable. MGO is an agonist for $GABA_A$ receptors and a short-term MGO treatment has been found to reduce anxiety-like behavior in rats. Also increase in GLO1 expression increases

anxiety by detoxifying MGO [186, 267]. MGO treatment at low concentration has been found to reduce anxiety whereas higher dosage causes locomotor depression, ataxia, hypothermia [268]. MGO at low dose has been found to bind extracellular domain of tropomyosin receptor kinase B (TrkB) and mediate its dimerisation and autophosphorylation. This leads to expression of brain derived neurotrophic factors (BDNF), leading to reduced anxiety in the chronic mild stress rat model **[269].** Surprisingly, in our study, after chronic treatment of 45 days, we found increased anxiety in MGO treated rats as compared to vehicle control. MGO rats displayed a significantly reduced number of entries in open arms (**Figure 3.3 A**). This observation supports increased anxiety in diabetic patients despite having elevated MGO levels. Along with a reduced number of entries, STZ and MGO rats did not travel in open arms confirming anxiety development (**Figure 3.3 B**).

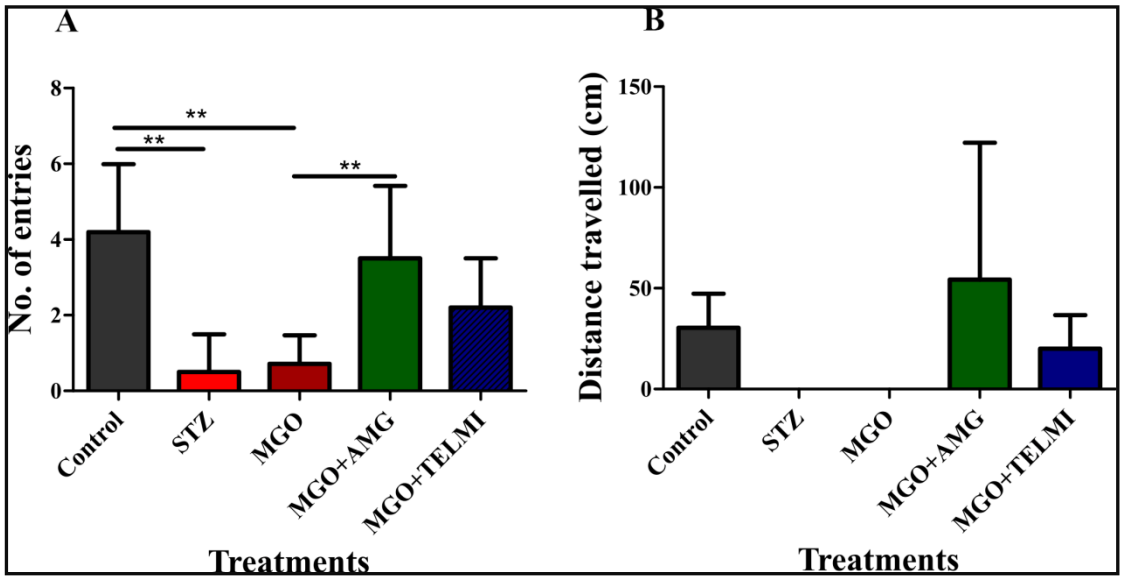

Figure 3.3: Elevated Plus Maze test of rats after STZ and MGO treatments

(A) Number of entries in open arms (B) Distance travelled in open arms
(n=5, One way ANOVA ,$^{**}p < 0.01$, data expressed as mean ± SD)

Co-treatment of AMG has been found to reduce anxiety as compared to MGO. AMG treatment significantly increased the number of entries in open arms w.r.t. MGO (**Figure 3.3**). AMG has shown its anti-anxiety effect in non diabetic stressed mice via inducible nitric oxide synthase cyclic guanosine monophosphate (iNOS-cGMP) pathway. iNOS leads to the production of nitric oxide (NO) which then increases cyclic guanosine monophosphate (cGMP), a secondary messenger that carries

neuronal communications and also increases anxiety. AMG acts by reducing NO synthesis, thereby reducing cGMP and anxiety. AMG has also been shown to inhibit the activity of guanylyl cyclase which is an important enzyme for cGMP formation [250]. Like AMG, co-treatment with TELMI also increased the total number of entries and distance travelled in open arms, indicating reduction in anxiety (**Figure 3.3**). TELMI has also been shown to reduce anxiety in diabetic rats by reducing levels of serum cortisol, NO, IL-6, IL-1β. Also, TELMI has found to increase expression of Peroxisome proliferator-activated receptors δ (PPARδ) and serotonin transporter (5 HTT) thereby reducing anxiety. PPARδ expression has been found to have anti-inflammatory, neuroprotective activity thereby reducing neuronal degeneration in AD along with reduced Aβ levels [270-272]. In our study, we have also found that co-treatment with AMG and TELMI treatment reduces anxiety developed by MGO.

3.3.3 Proteomic Data

3.3.3.1 **PCA plot and Venn diagram**

The principal component analysis was done to check the quality of different mass spectrometric acquisitions. **Figure 3.4 A** PCA plot shows consistency between biological and technical triplicates of mass spectrometric acquisition of hippocampal tissue. A total of 1692 proteins was identified with at least two unique peptides **(Appendix no. 4)**. **Figure 3.4 B** indicates the number of proteins common between MGO and STZ treatment. A total of 60 proteins were found to be common between STZ and MGO. Out of 60 proteins, 31 proteins were upregulated, and 29 proteins were downregulated w.r.t. control (**Figure 3.4 C, D)**. **Appendix no. 5** represents a list of commonly differentiated proteins between STZ and MGO w.r.t. Control. TELMI co-treatment restored the expression levels of 34 proteins (**Appendix no. 6**) and AMG co-treatment restored that of 33 proteins as compared to MGO treatment (**Appendix no. 7**).

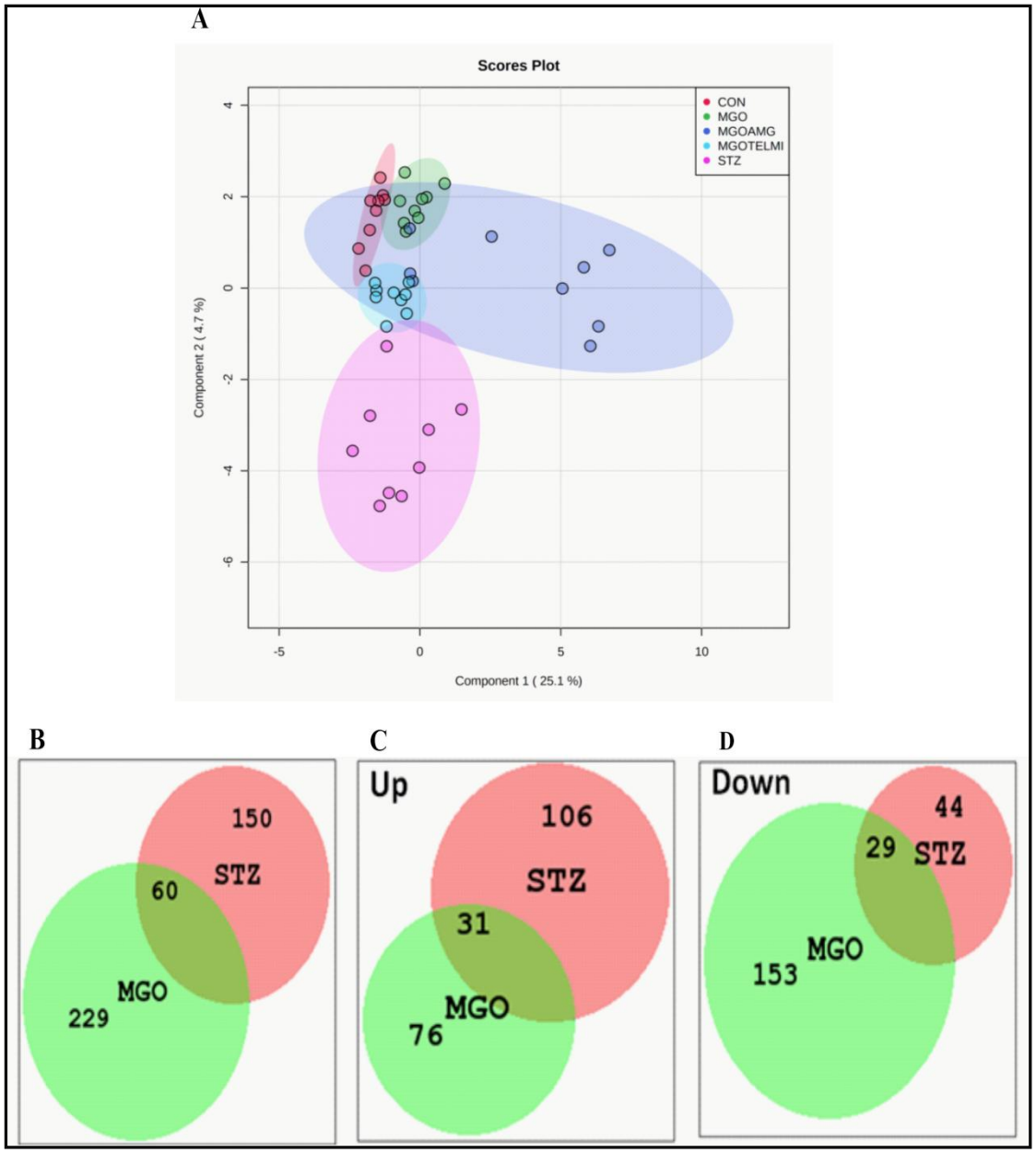

Figure 3.4: PCA and Venn diagram of rat hippocampal proteomic acquisition

(A) PCA plot showing consistency between biological and technical proteomic acquisitions (B) Venn diagram representing common proteins identified (C) Common upregulated proteins (D) Common downregulated proteins between STZ and MGO w.r.t. Control

3.3.3.2 **Functional analysis of proteins altered in STZ and MGO treatment**

Functional analysis was performed by the online DAVID software (**Figure 3.5**). Proteins upregulated in STZ treated rats were found to be involved in AD, glycolysis, glutathione metabolism, oxidative phosphorylation, and citrate cycle (**Figure 3.5 A**). Downregulated proteins were found to be involved in long term potentiation,

dopaminergic synapse, GABAergic synapse and synaptic vesicle cycle (**Figure 3.5 B**).

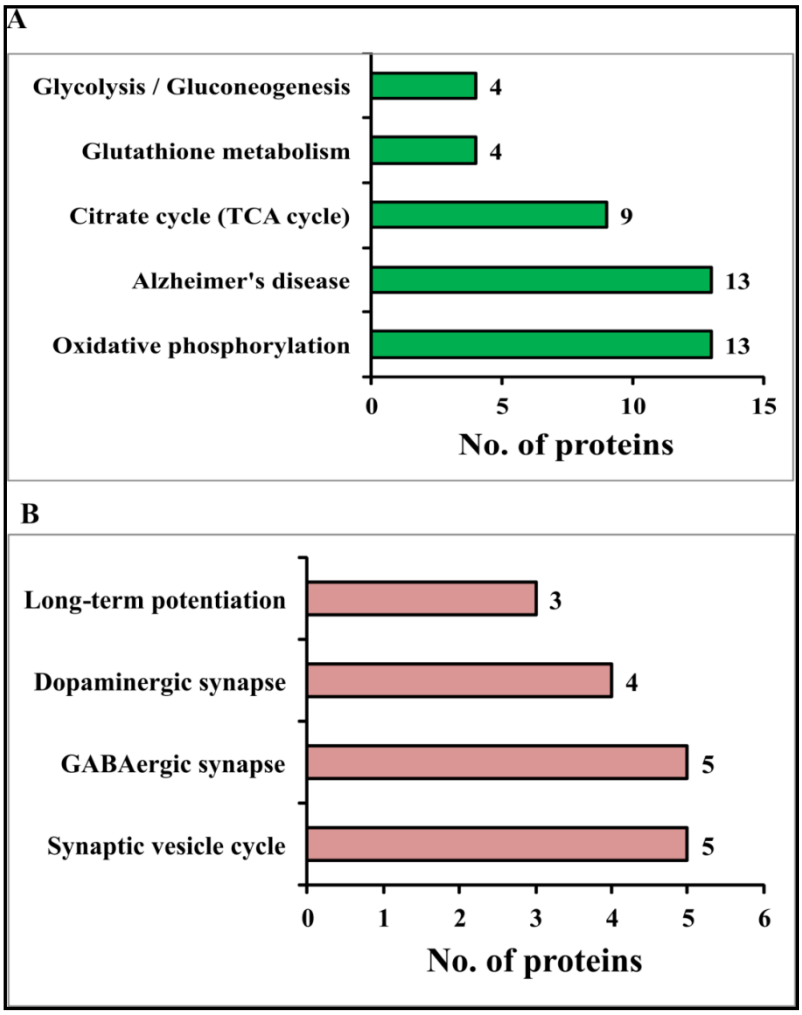

Figure 3.5: DAVID analysis of differentially expressed proteins in STZ treatment

(A) upregulated and (B) downregulated proteins w.r.t. Control

Proteins found to be involved in AD were mostly mitochondrial (Cytochrome b-c1 complex subunit 1, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5, NADH dehydrogenase [ubiquinone] flavoprotein 2, cytochrome b-c1 complex subunit 2, ATP synthase subunit gamma, cytochrome c oxidase subunit 5B, cytochrome c oxidase subunit 5A, cytochrome b-c1 complex subunit Rieske, succinate dehydrogenase [ubiquinone] iron-sulfur subunit,cytochrome b-c1 complex subunit 6, ATP synthase subunit beta, succinate dehydrogenase [ubiquinone] flavoprotein subunit) and calcineurin subunit B type 1. The Role of mitochondrial

dysfunction has been well studied in AD [273, 274]. MGO induces ROS production which might trigger mitochondrial dysfunction [275]. Also, dysfunctional mitochondria can produce more ROS through electron leakage during oxidative phosphorylation by producing H_2O_2, OH^-, and O_2^- [276]. Dysfunctional mitochondria have also been found to contribute to calcium homeostasis disturbances, which is crucial for proper neurotransmission [273]. Disturbances in mitochondrial functioning have also been reported to initiate apoptosis [276]. Diabetes is characterized by oxidative stress, which may trigger cellular response via the glutathione mechanism. Upregulation of proteins involved in the glutathione metabolism indicates its role as an antioxidant. The brain is one of the highest energy-requiring organs in the body. Upregulation of proteins involved in oxidative phosphorylation and citrate cycle indicated higher energy demand by the brain in diabetic conditions. Downregulated proteins in STZ were involved in the synaptic vesicle cycle, GABAergic synapse, dopaminergic synapse and long term potentiation (LTP). Synapses and synaptic vesicle cycle play an important role in neurotransmission. A study in the mouse model has shown that microglia and complement systems are involved in synapse loss in the early stages of AD [277]. Synapse is the intercellular region between two neurons where electrical/chemical transmission of impulses takes place. Proper neurotransmission largely depends upon the functioning of synapse [278]. Also, synaptic vesicle cycle is important as they carry neurotransmitters which upon fusion with the cell membrane, release it by exocytosis into the synaptic cleft. It is then taken up by specific receptors present on the neighbouring dendrite [279]. AD brains have been found to show dysregulation in LTP [280, 281]. Proteins involved in these pathways were found to be downregulated upon STZ treatment indicating improper functioning of neurotransmission system in diabetes.

Functional analysis of proteins altered after MGO (**Figure 3.6**) treatment found similar pathways like those after STZ treatment with few additional pathways.

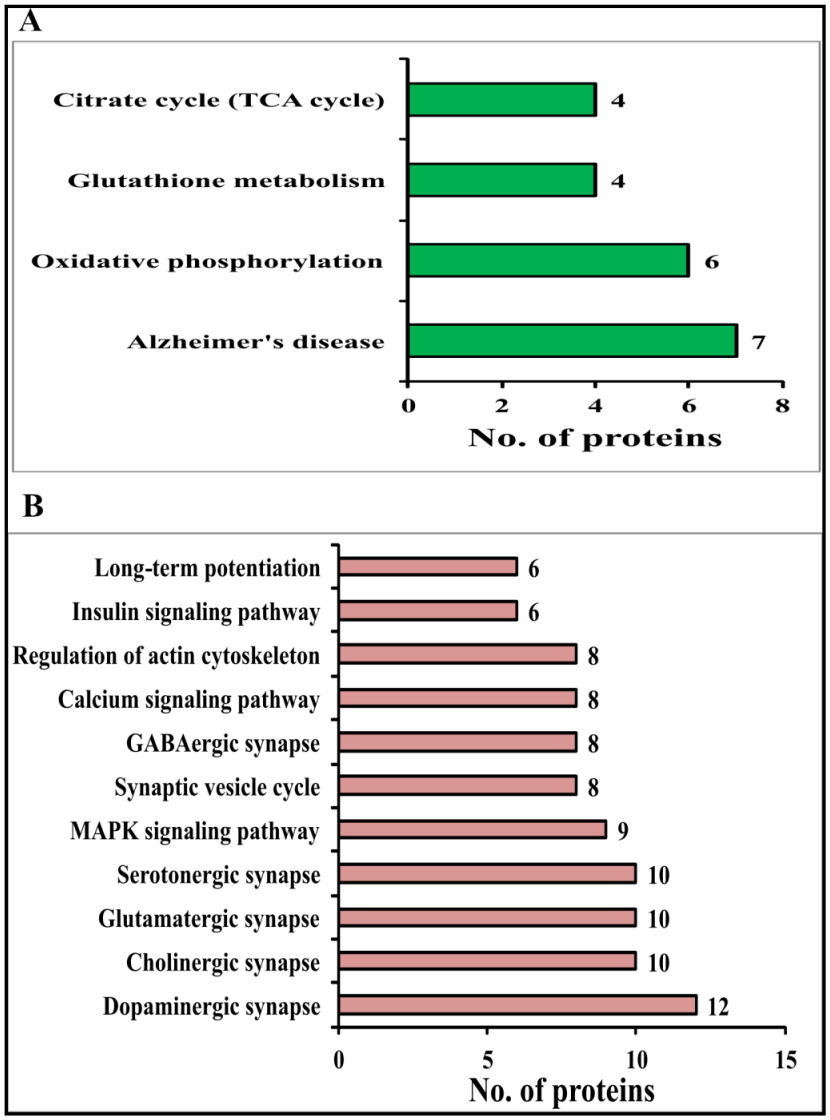

Figure 3.6: DAVID analysis of differentially expressed proteins in MGO treatment

(A) Upregulated (B) Downregulated proteins w.r.t. Control

Proteins altered after MGO treatment were found to be involved in the same pathways observed in STZ altered pathways except for glycolysis. Along with the same pathways observed after STZ treatment, downregulated proteins were found to be involved in insulin signaling, insulin secretion, regulation of actin cytoskeleton, MAPK signaling pathway, calcium signaling pathway, proteasome, and synapses (serotonergic, glutamatergic, cholinergic). Actin cytoskeleton helps neurons to hold its normal structure and carry out functions. Alteration in actin cytoskeleton has been reported in AD [282]. This observation was similar to *in vitro* neuro-2a proteomic

data upon MGO treatment (2.3.3). GSEA analysis also showed upregulation of proteins involved in mitochondrial matrix and downregulation of proteins involved in neuronal synapse (**Figure 3.7**).

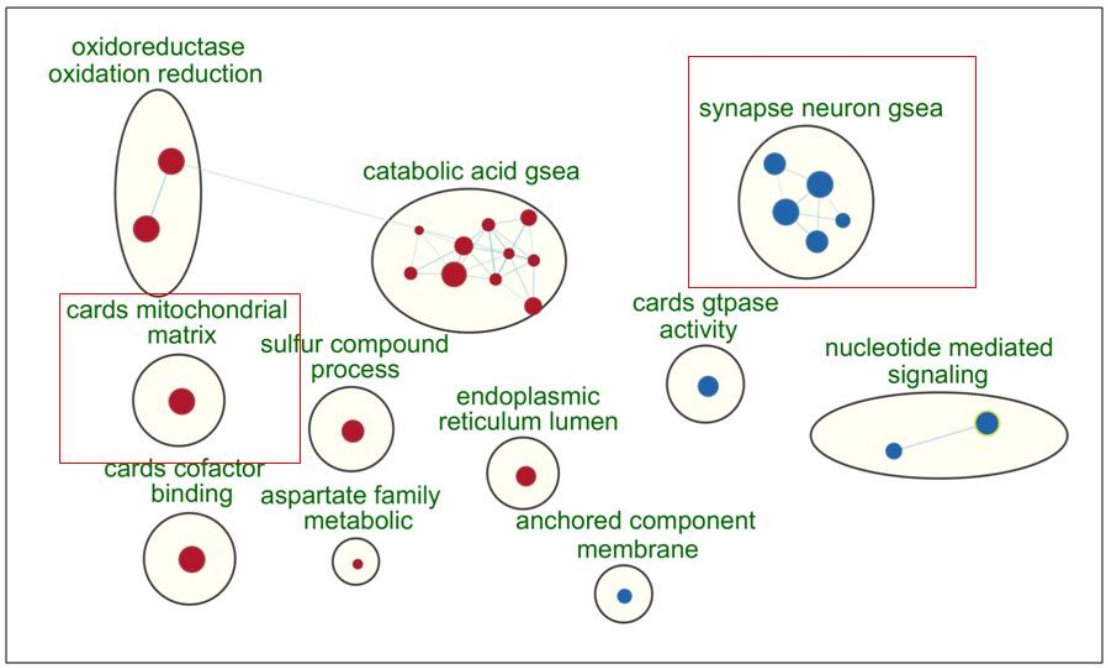

Figure 3.7: GSEA analysis of proteins altered in MGO treatment w.r.t. Control

3.3.3.3 Restoration of MGO altered proteins upon TELMI and AMG treatment

Co-treatment of TELMI, restored 34 proteins w.r.t. MGO treatment. TELMI was found to restore some of the dysregulated proteins such as mitochondrial proteins (Cytochrome c oxidase subunit 5B, [Pyruvate dehydrogenase [acetyl-transferring]]-phosphatase 1, Frataxin). Importantly TELMI restored expression of proteins playing a role in neurotransmission such as synaptosomal-associated protein 25, which has been reported to be decreased in AD [283]. This protein helps in the exocytosis of neurotransmitters [284]. Glutamate decarboxylase 2 plays an essential role in the production of principal inhibitory neurotransmitter γ aminobutyric acid (GABA). This enzyme carries out decarboxylation of glutamate, which leads to the formation of GABA. Expression of this enzyme has been found to be downregulated in AD [285]. Proper functioning of neurotransmission requires a balance between excitatory (mediated by glutamate) and inhibitory (majorly by GABA). This enzyme was downregulated upon MGO treatment, indicating lesser production of GABA. This may cause the development of anxiety, as seen in the EPM study [286]. The lower

level of GABA and glutamate have been observed in AD [287]. Another important enzyme playing a significant role in GABAergic synapse is Septin-11, which was downregulated upon MGO treatment. This protein has been found to play a role in neuronal architecture and GABAergic synaptic connectivity [288]. TELMI treatment restored septin-11 expression. TELMI was found to restore expression of tau as well, which plays an essential role in maintaining the neuronal architecture. One of the major lysosomal proteins involved in AD is acid ceramidase, which was found to be upregulated upon MGO treatment and downregulated by TELMI [289]. TELMI also restored proteins involved in maintaining cell homeostasis by regulating reactive oxygen species, apoptosis, chaperones, and degradation of ubiquitinated proteins such as Glutathione S-transferase, complement C3, 10 kDa heat shock protein, proteasome subunit beta type-5. The heat map of TELMI restored proteins is shown in **Figure 3.8**.

AMG co-treatment restored 33 proteins w.r.t. MGO treatment (**Figure 3.9**). AMG restored some of the proteins that were restored by TELMI such as acid ceramidase, proteasome subunit beta type-5, and glutamate decarboxylase 2. In addition, AMG exclusively restored a few proteins.

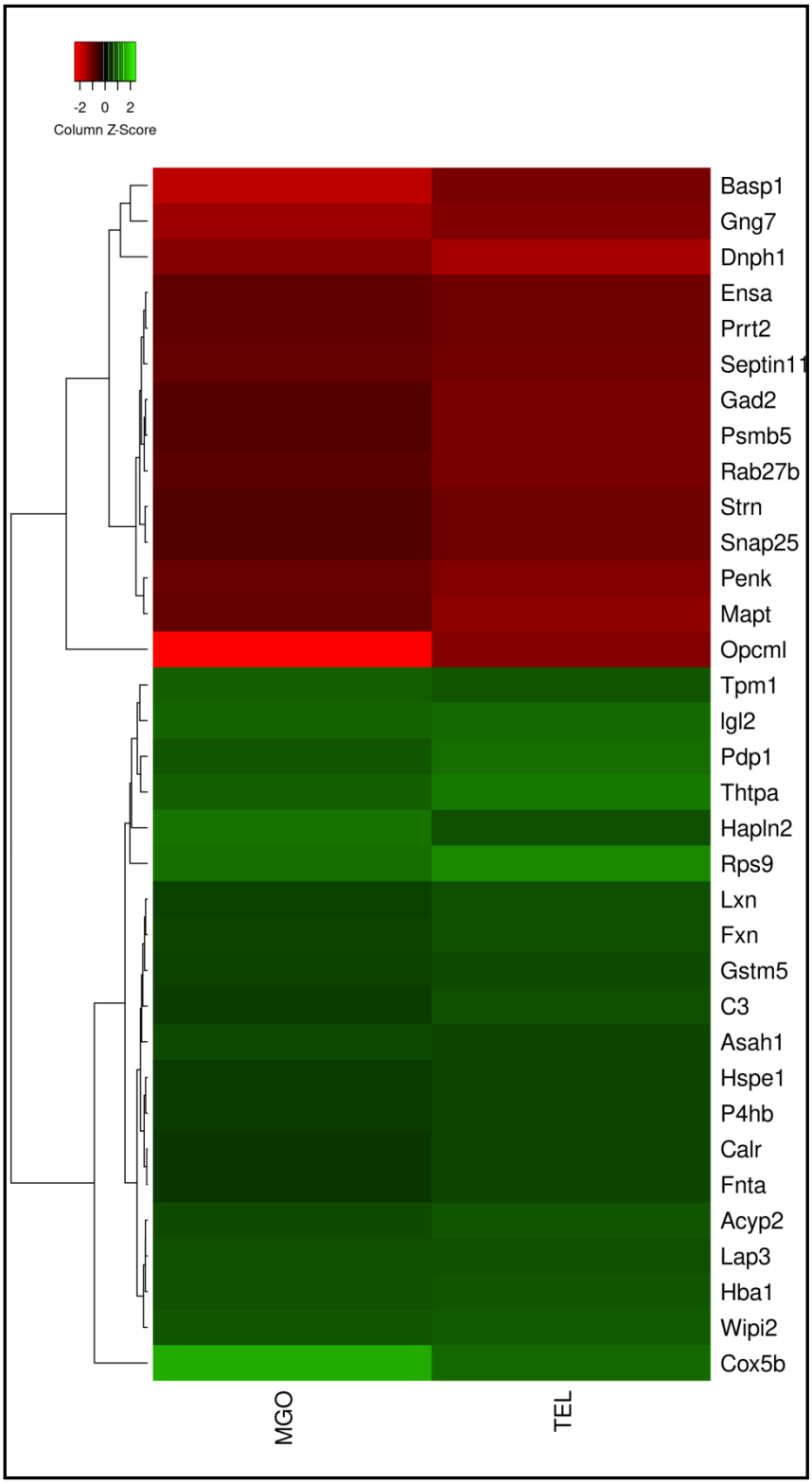

Figure 3.8: Heat map of proteins restored by TELMI co-treatment

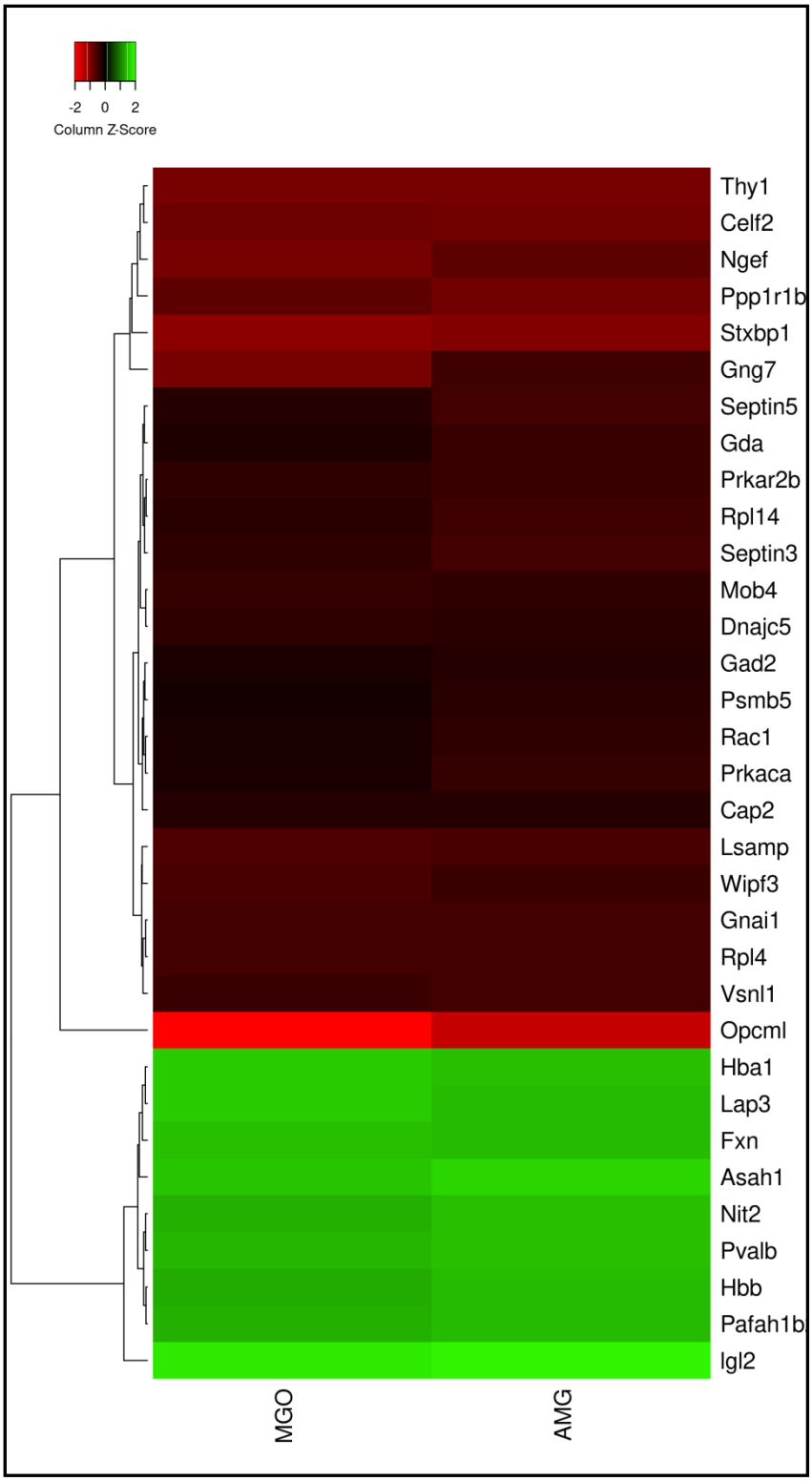

Figure 3.9: Heat map of proteins restored by AMG co-treatment

Important synaptic protein Septin-5 downregulated by MGO reported to be involved in AD was restored by AMG [290]. Neuronal-specific septin-3 plays a role in neurite

outgrowth, synaptic vesicle fusion, and recycling [291, 292]. Similarly, Protein phosphatase 1 regulatory subunit 1B was restored by AMG. Its role in tau dephosphorylation is in debate but it has been found to play an essential role in neuronal processes [293].

MGO is a potent precursor for AGEs formation. MGO can exert its toxic effect directly or by forming AGEs. To understand this, we have compared MGO altered proteins with earlier published data where the neuronal proteomic study has been performed after AGEs treatment [138]. In this study, treatment of neuronal cells with AGEs resulted in apoptosis along with upregulation of key proteins involved in AD such as cathepsin B and asparagine endopeptidase (AEP). It has been shown that cathepsin B has β-secretase activity leading to Aβ formation whereas AEP degrades tau protein and induces tau aggregation. This study also found that AGEs treatment upregulated RAGE formation and involvement of AGE-RAGE axis in the AD pathogenesis [294]. We found various proteins common between [138] and our study indicating involvement of the AGE-RAGE axis in MGO mediated observations (**Table 3.2**).

Mitochondrial proteins were found to be upregulated by both MGO and AGEs treatment. A total of ten upregulated proteins were found to be common after MGO and AGEs treatments. Mitochondrial proteins such as Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, ATP synthase subunit alpha, electron transfer flavoprotein subunit alpha, malate dehydrogenase, ES1 protein homolog were found to upregulated upon MGO and AGEs treatment. Acid ceramidase is a very important glycoprotein, which catalyzes conversion of ceramide into sphingosine and fatty acids. Alterations in lipid homeostasis have been reported in diabetes and AD [295]. Its expression and activity has been found to be elevated in AD brains. Acid ceramidase has been found to be colocalized with NFTs [296].

Table 3.2 List of common differentially expressed proteins after MGO and AGEs treatment

	Protein
Sr. No.	**Upregulated proteins**
1	Acid ceramidase
2	Peroxisomal multifunctional enzyme type 2
3	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial
4	Protein disulfide-isomerase
5	Secretogranin-2
6	ATP synthase subunit alpha
7	Glutathione S-transferase Mu 1
8	Electron transfer flavoprotein subunit alpha
9	Malate dehydrogenase,
10	ES1 protein homolog, mitochondrial
Sr. No.	**Downregulated proteins**
1	Protein SET
2	Nuclear migration protein nudC
3	26S proteasome non-ATPase regulatory subunit 1
4	Eukaryotic translation initiation factor 6
5	Actin-related protein 2
6	40S ribosomal protein S14
7	Cysteine-rich protein 2
8	Guanine nucleotide-binding protein G(o) subunit alpha
9	Brain acid soluble protein 1

3.3.4 Plasma fructosamine assay

To understand the effect of STZ and MGO treatments on the AGEs formation, we measured plasma fructosamine. Plasma fructosamine was normalised with total protein content (**Figure 3.10**).

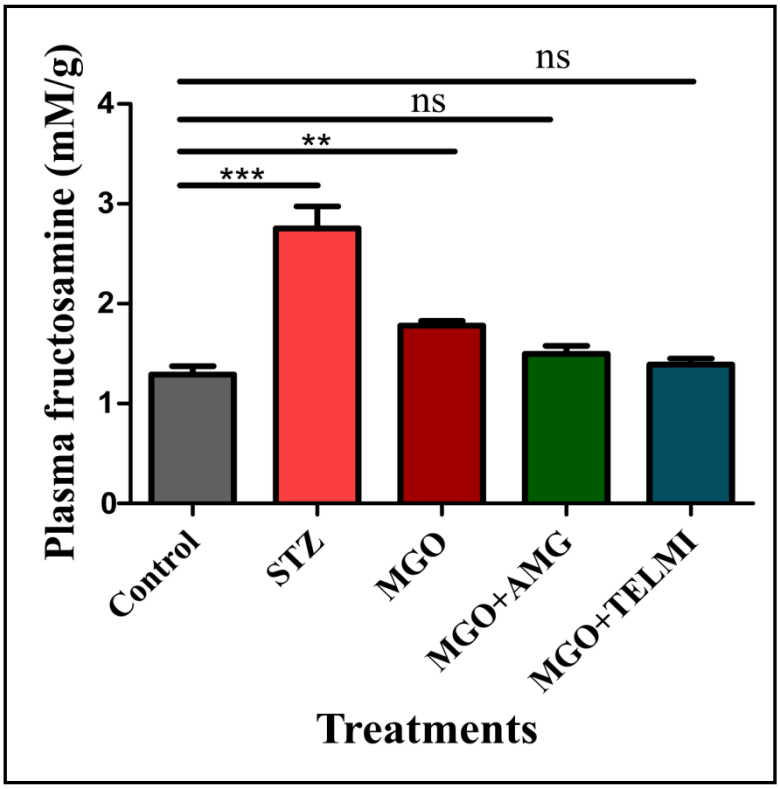

Figure 3.10: Plasma fructosamine assay

(n =3, One way ANOVA ,**$p < 0.01$, ***$p < 0.001$, data expressed as mean±SD)

Fructosamine is one of the intermediate products during AGEs formation. In the STZ treatment, due to a higher glucose level in the blood, an increased fructosamine content was observed w.r.t control. MGO treatment also showed a significant increase in fructosamine content w.r.t. control. Whereas MGO co-treatment with AMG and TELMI reduced fructosamine level similar to that of control. AMG is known to reduce fructosamine formation by quenching MGO [297]. TELMI has been known to downregulate RAGE expression via the PPAR γ pathway, but we found decreased fructosamine upon TELMI treatment. This observation needs to be further studied to get an mechanistic insight of TELMI mediated reduction in AGEs.

3.3.5 Effect of STZ and MGO treatments on RAGE expression

To understand the role of increased AGEs upon STZ and MGO treatments, we further studied the expression of RAGE in the hippocampus by western blotting (**Figure 3.11**). RAGE expression was normalized with GAPDH expression.

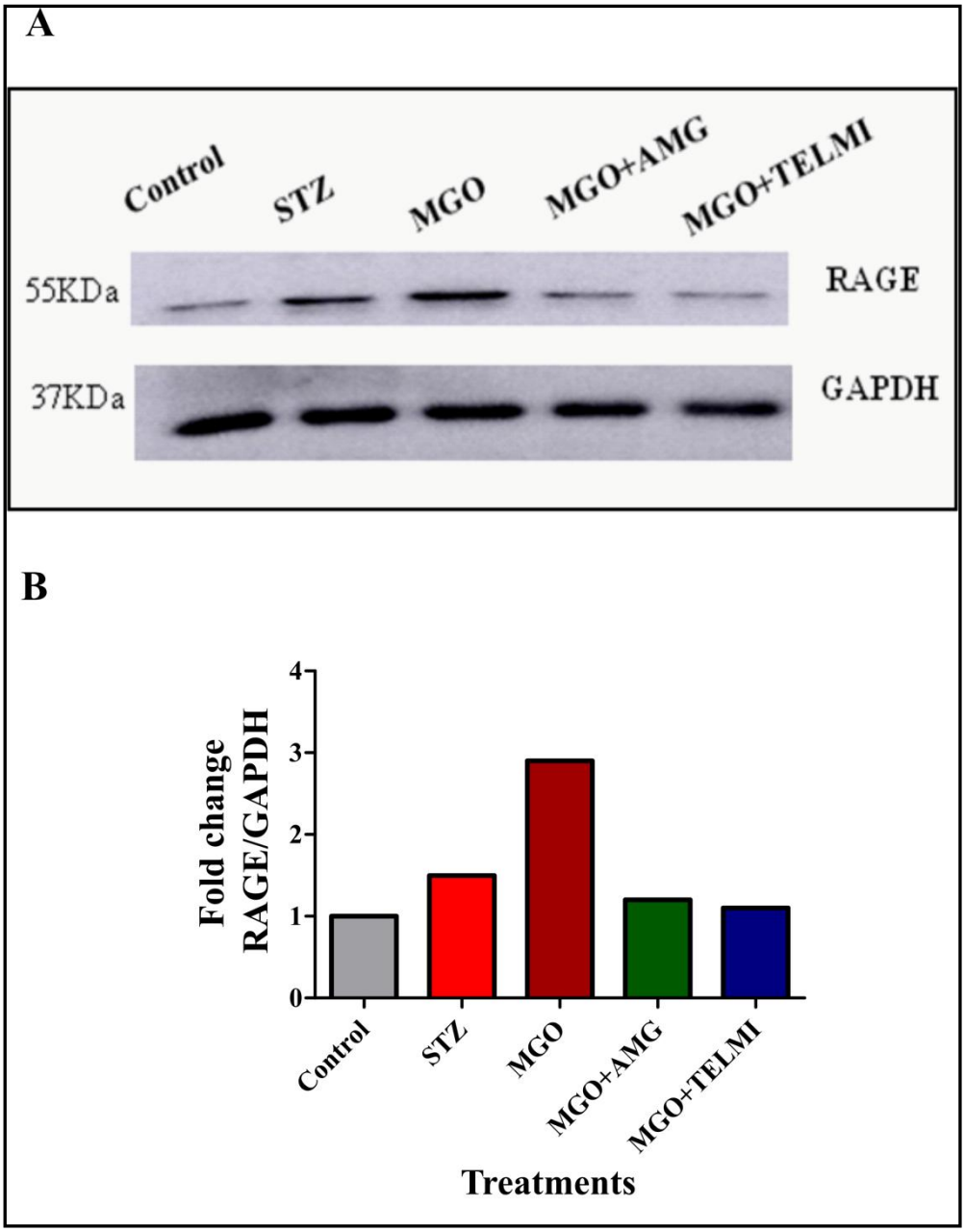

Figure 3.11: Western blot analysis for RAGE and GAPDH in hippocampus

(A) ECL image (B) Densitometric analysis of fold change RAGE/GAPDH

RAGE expression was found to be elevated in STZ and MGO treatment w.r.t. control. Co-treatments with both the drugs were found to reduce RAGE expression. TELMI is known to reduce RAGE expression by activation of the PPAR γ pathway [298]. AMG being a MGO quencher has reduced AGEs content, thereby reducing RAGE upregulation via AGE-RAGE axis [299].

3.3.6 Effect of STZ and MGO treatments on tau phosphorylation

Being one of the essential pathological hallmarks of AD, the effect of STZ and MGO treatment on tau phosphorylation was studied (**Figure 3.12**).

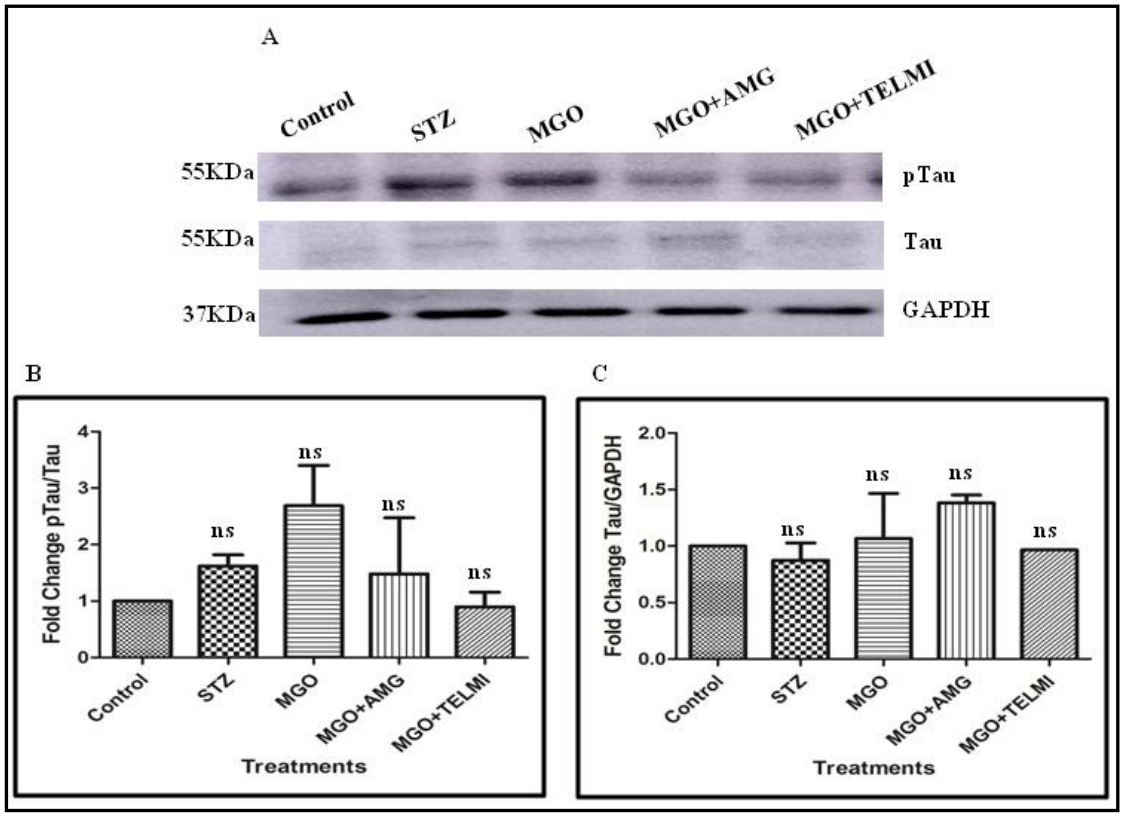

Figure 3.12: Western blot analysis for pTau in hippocampus

(A) ECL image (B) Densitometric analysis of fold change pTau/Tau (C) Densitometric analysis of fold change Tau/GAPDH

It was found to be increased in STZ and MGO treatment w.r.t. control. Total tau expression was normalised with GAPDH. This normalized factor was then used for phospho tau normalisation. STZ treatment has already been shown to increase tau phosphorylation. Two major kinases responsible for tau phosphorylation, Akt and GSK-3β have been found to alter their activities in response to blood glucose level.It has been postulated that hyperglycemia in diabetes might alter the balance between these kinases and phosphatase (PP-2A) leading to tau hyperphosphorylation [300]. Along with this, hyperglycemia can cause upregulation of the AGE-RAGE axis, causing increased expression of asparagine endopeptidase (AEP). AEP has been found to increase tau phosphorylation by inhibiting PP-2A activity [138, 301]. We also found an increase in tau phosphorylation upon MGO treatment. MGO activates

the AGE-RAGE axis, thereby activating kinases GSK-3β and p38 leading to tau phosphorylation [185]. This MGO increased tau phosphorylation was reduced upon AMG treatment, due to MGO quenching effect of AMG. TELMI has been reported to show neuroprotective effect by reducing Aβ accumulation and phosphorylated tau and reduced tau phosphorylation with its co-treatment was evident in our study as well [302, 303]. TELMI mediated reduction in tau phosphorylation can be because of reduction in AGEs and RAGE.

3.3.7 Hippocampal Histochemistry

Hippocampus is the first brain region to get affected in early stages of AD. It is responsible for memory as well as regulation of anxiety. Hippocampus region regulates anxiety [304]. This region has been found to show neuronal loss in AD, specifically in the regions called cornu ammonis (CA1) and CA3 regions [305, 306]. The effect of diabetes and MGO treatment on the structure CA1 region was studied by H and E staining (**Figure 3.13**). STZ and MGO have been found to cause a neuronal loss in the CA1 region as compared to control. Neurodegenerative changes have been reported in the CA1 region of the brain in STZ [307-309]. In STZ, mild vascular congestion in the brain tissue was observed along with mild neuronal degeneration with focal neuronal swelling. Focal loss of neuronal tissue with irregular arrangement along with a decreased thickness of small pyramidal cell layers at multiple foci was observed. H and E staining of tissue sections of the CA1 region of the brain showed that MGO causes minimal to mild neuronal degeneration with a reduced number of cells in the layer as well as the presence of dark stained neurons. Focal congestion with minimal to mild neuronal degeneration with decreased density of neuronal tissue was observed in the MGO treated group and it was observed that co treatment with AMG and TELMI treated groups showed comparatively lesser degenerative changes in neuronal tissue as compared to MGO.

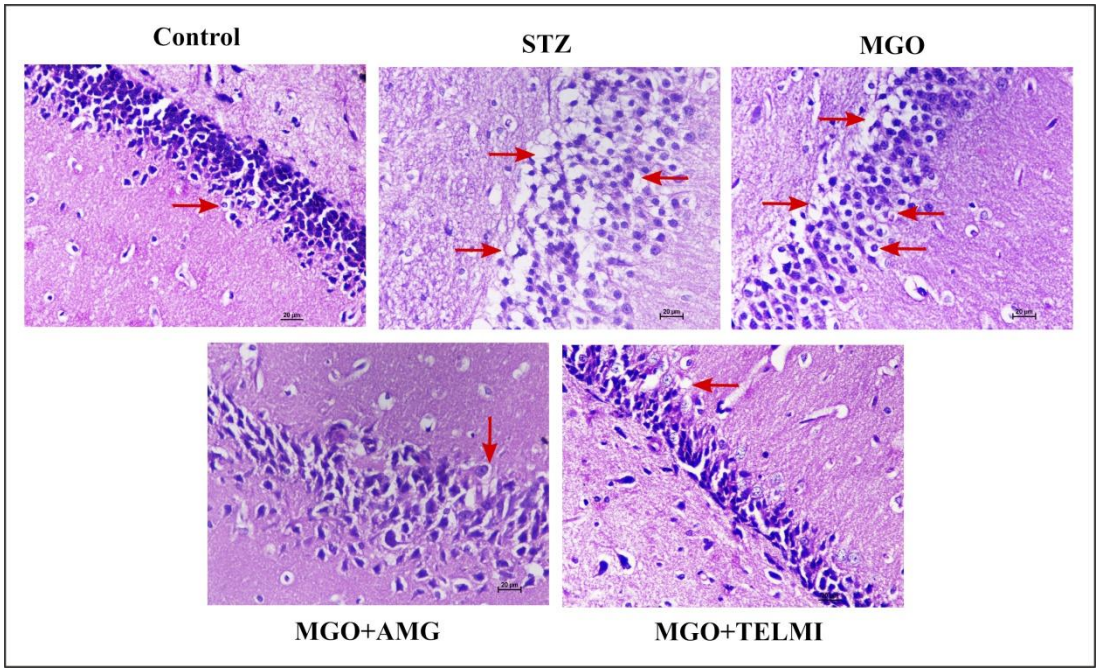

Figure 3.13: H & E staining of CA1 region of hippocampus

H & E staining of tissue sections of the CA1 region from the hippocampus of the brain showed that MGO causes minimal to mild neuronal degeneration with a reduced number of cells in the layer as well as the presence of darkly stained neurons. Focal congestion with minimal to mild neuronal degeneration with decreased neuronal tissue density was observed in the MGO treated group. It was observed that AMG and TELMI treated groups showed comparatively lesser degenerative changes in neuronal tissue as compared to MGO treated group.

3.4 Conclusions

STZ and MGO treatment lead to altered expression of proteins involved in key pathways of AD and diabetes in the rat model. It affected synapse, glutathione metabolism, and calcium signaling. STZ and MGO treatment also caused an increase in fructosamine with a concomitant increase in RAGE expression. We also found an increase in tau phosphorylation upon STZ and MGO treatment, which was reduced upon AMG and TELMI treatments. STZ developed anxiety in rats; also we found that MGO also caused anxiety which is contradictory to the reported role of MGO for showing anxiolytic effects. STZ and MGO caused neurodegenerative changes in the brain which were reduced in AMG, TELMI treated groups. This study has found that hyperglycemia and MGO cause alterations in protein expression involved in various

biological processes that increase the risk of AD development in diabetes. We have also found that AGEs formed due to hyperglycemia and MGO activate the AGE-RAGE pathway leading to increased tau phosphorylation. STZ and MGO also caused neurodegenerative changes in the hippocampus. This resulted in increased anxiety in diabetic and MGO treated rats, and these effects were reduced by AMG and TELMI.

Chapter 4

To study the role of glycation in regulation of AβPP processing and amyloid β formation leading to the Alzheimer's Disease

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Chapter 4 To study the role of glycation in regulation of AβPP processing and amyloid β formation leading to the Alzheimer's Disease

4.1 Introduction

Diabetes has been considered as one of the major risk factors for the development of AD. Subjects with diabetes in the early stages of life are more prone to develop AD than the subjects who develop diabetes in their later stages of life [176, 310]. Besides, diabetic and AD subjects have common features such as brain atrophy, reduced brain glucose metabolism and insulin resistance, thus AD is also termed as type-3 diabetes. In a longitudinal study for 5.5 years, the risk of the AD incident recorded 65% higher in diabetic than in the non-diabetic patients [176]. Similarly, another longitudinal study involving 2574 Japanese-American men also observed the synergistic effect of diabetes and the apoe4 allele on the risk of development of AD [310]. Diabetes was found to double the risk of dementia in a prospective population-based cohort study [177].

The inevitable consequence of hyperglycemic condition in type 2 diabetes is enhanced protein glycation. Protein glycation is a non-enzymatic reaction between reducing sugar such as glucose and free lysine (K) or arginine (R) group leading to the formation of a complex and heterogeneous group of compounds called advanced glycation end products (AGEs). Chronic hyperglycemic condition in diabetes promotes glycation of proteins.

Previous studies have shown that the glycation of proteins affects their function. Increased levels of glyoxal and methylglyoxal have been found to form AGE crosslinks of α crystalline protein which reduces its chaperone activity and exacerbates the condition [311]. Bisphosphoglycerate mutase isolated and purified from the diabetic patient's RBCs was found to be inactive due to glycation at K158 residue [312]. Similarly NaK–ATPase, aldehyde reductase, human muscle-specific enolase have been found to have reduced activity upon glycation [313-315]. Glycation of antioxidant enzymes such as catalase, copper-zinc superoxide dismutase,

glutathione reductase can suppress its antioxidant activity and increase oxidative stress [316, 317]. Various previous studies have shown that glycation inactivates enzymatic activity of heparin cofactor II [318], glucokinase [319], proteasome, glucose 6 phosphate dehydrogenase [320], glyceraldehyde 3 phosphate dehydrogenase (GAPDH) [317, 321], lactate dehydrogenase [317], cysteine proteases [322], paraoxonase [323, 324]. Total protease activity is reduced in diabetic conditions. Also, the chymotrypsin-like activity of proteasome decreases upon MGO treatment. This indicates that decreased proteasomal activity in diabetes maybe because of AGE modifications [325]. Cathepsin B activity has been found to be increased in AGE-treated neuro-2a cells [138]. Based on this information, we have asked two questions.

4.1.1 Question 1: Whether glycation of proteases influence AβPP processing

AβPP processing occurs either by α-secretases or by BACE1. α-secretases follow a non-amyloidogenic pathway leading to minimal accumulation of Aβ levels, whereas processing by BACE1 leads to amyloidogenic pathway resulting in enhanced accumulation of Aβ, a pathogenic hallmark of AD [17-19] (**Figure 1.1**). Matrix metalloproteinases (MMPs) ADAM9, 10, 17 have been found to have α-secretase activity [310-313]. α-secretases act at the extracellular domain of AβPP, precluding the formation of Aβ peptide, whereas cleavage by BACE1 generates N terminus of Aβ, which further aggregates to form neurotoxic Aβ plaques [19, 25]. Apart from BACE1, cathepsin B has also been found to have BACE1 like cleavage property [314].

As stated above glycation has been found to reduce the enzyme activities of crucial enzymes e.g. superoxide dismutase, glutathione s transferase, etc. involved in the regulation of oxidative stress [316, 317]. Curcumin amino acid conjugates that have been found to increase α-secretase activity might be through its antioxidant properties [326]. Oxidative stress generated by H_2O_2 and $FeCl_2$ has shown to down-regulate α-secretase activity and increase the expression and activity of BACE1 and γ secretase in human neuroblastoma cells [327]. Oxidative stress generated during aging has been found to increase BACE1 activity than α-secretase in a mouse model [328]. ADAM17

has shown to be glycosylated and phosphorylated but its effect on ADAM17 activity is still elusive [329]. The role of post-translational modifications of the key proteins involved in AD pathophysiology has been reviewed in great detail. It has been found that site-specific phosphorylation of BACE1 and AβPP can affect its fate and processing, altering AD pathophysiology [330]. Therefore, it is reasonable to speculate that glycation can affect enzyme activity by direct modification or indirectly through inducing oxidative stress and causing alterations in enzymatic activity.

4.1.2 Question 2: Whether AβPP glycation affects its processing

Along with the enzyme, post-translational modification of the substrate has been found to affect enzyme activity. Substrate glycation is known to alter MMP's activity [331]. As discussed above, glycated proteins are relatively resistant to proteolytic processing [320]. Protein glycation forms crosslinks leading to aggregation of proteins rendering them resistant to proteolytic action. This has been studied in collagen and Aβ senile plaques [131, 132]. Glycated bovine serum albumin has also been shown to be protease resistant which decreases in the presence of anti-glycation drug aminoguanidine [332]. Glycation has also been found to increase stability and influence the three-dimensional structure of proteins which may cause protease resistance [130, 333]. These pieces of evidence suggest that AβPP glycation can affect its processing by secretases. However, it would be worthwhile to study the interaction of glycated AβPP with α-secretases and BACE1, the lack of availability of glycated AβPP act as a hindrance for such computational study.

Therefore, we have performed computational study to understand the role of glycation in the increased Aβ production. We have investigated (1) The susceptibility of proteases to glycation and its effect on AβPP processing (2) The fate of AβPP processing if AβPP is glycated. Based on our investigation, we report two possibilities involving glycation mediated regulation of AβPP processing leading to enhanced production of Aβ, establishing a molecular link between diabetes and the progression of AD pathology.

4.2 Materials and Methods

4.2.1 NetGlycate analysis

To understand the possible effect of glycation on the proteases, we have analyzed the number of K and R of human α-secretases, BACE1 and cathepsin B. The sequences of ADAM9 (Accession No.: Q13443), ADAM10 (O14672), ADAM17 (P78536), BACE1 (P56817) and cathepsin B (P07858) were retrieved from UniProt and analysed by using NetGlycate 1.0 server.

4.2.2 Blind docking of proteases with glucose

To confirm our possibility, we performed the unbiased binding analysis by blind docking of proteases with glucose. Three dimensional structures of ADAM10 (PDB ID:6BE6), ADAM17 (PDB ID:2I47), BACE1 (PDB ID:2HIZ) and cathepsin B (PDB ID:6AY2) were accessed from PDB. Autodock 4.0 software was used to do the blind docking of these structures with glucose [334]. Receptor molecules were prepared by adding hydrogens, assigning Kollman and Gasteiger charges. This was followed by the conversion of receptor and ligand molecules from *.pdb to *.pdbqt. For blind docking, a grid box was set around the central atom of protein with the dimension that can cover all the molecules of the receptor protein. A detailed procedure is as explained earlier [335]. Analysis of the amino acid residues present in the close vicinity of glucose was carried out by PyMol Molecular Graphics Software (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC).

4.3 Results and Discussions

4.3.1 α-secretases are more susceptible for glycation

4.3.1.1 Net glycate analysis

Sequence analysis of these proteases suggests that α-secretases have more number of K, R the sites of glycation as compared to BACE1 and cathepsin B (**Figure 4.1 A**).

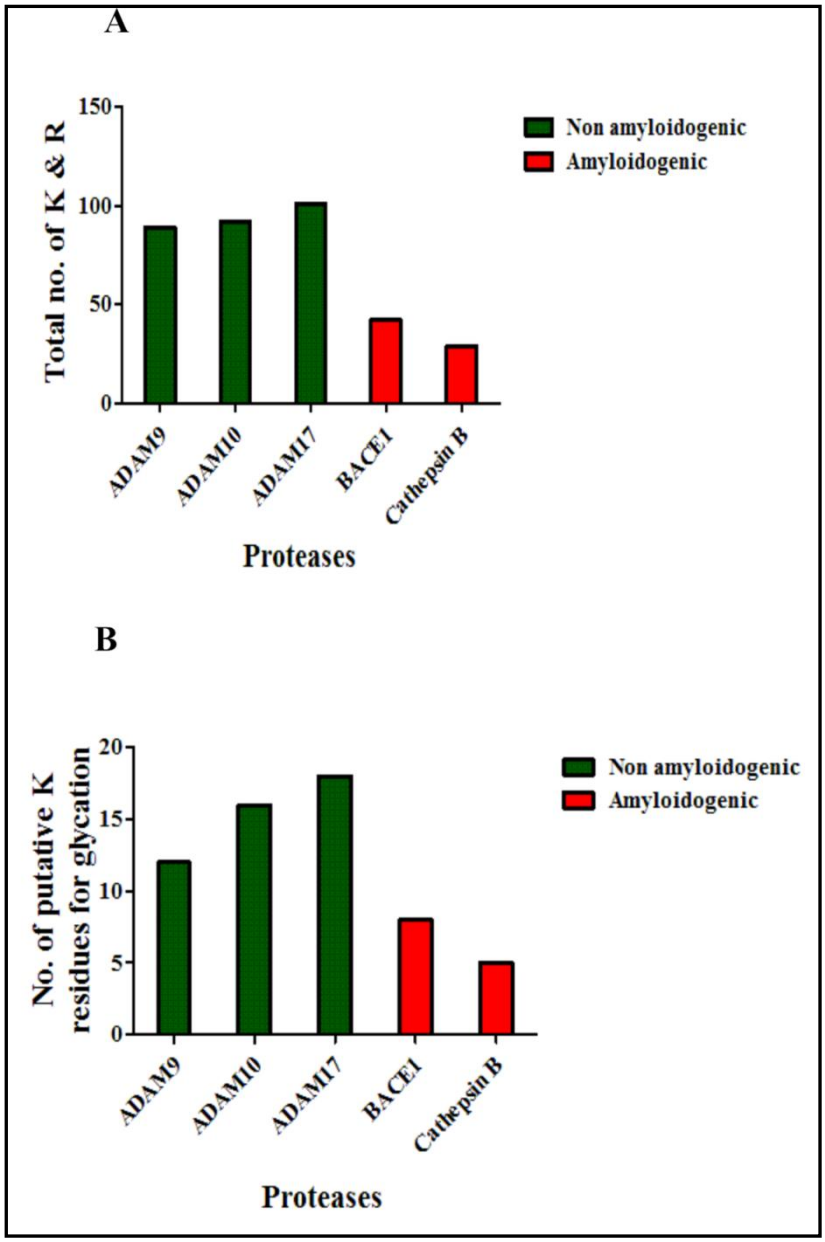

Figure 4.1: Analysis of K, R in proteases

UniProt (A) and NetGlycate analysis (B) of proteases

This indicates that α-secretases have more chance of getting glycated than BACE1 and cathepsinB. Further, we studied the number of probable K residues for glycation in these proteases by NetGlycate software. We found that α-secretases have more number of putative K residues for glycation than BACE1 and cathepsin B (**Figure 4.1 B**). Therefore, α-secretases may have more number of glycation modified residues than BACE1 and cathepsin B at a given physiological glucose concentration. Since glycation has been shown to reduce the activity of many enzymes including protease,

therefore, this study suggests that hyperglycemic condition in diabetes favors glycation of α-secretases due to more number of K and R residues possibly leading to its reduced activity. As a result, AβPP processing may shift towards an amyloidogenic pathway.

4.3.1.2 **Blind docking of proteases with glucose**

(a) Analysis of residues interacting with glucose

Binding energy (affinity) for the substrate ligand has been found to be ADAM10 (-5.8 Kcal/mol), ADAM17 (-5.9 Kcal/mol), BACE1 (-5.0 Kcal/mol), cathepsin B (-5.6 Kcal/mol). These results suggest that glucose has more or less similar binding affinity to α-secretases, BACE1, and cathepsin B. However a protein is known to be susceptible to glycation if it has more number of accessible K and R residues. Thus, the number of accessible K and R, their accessibility index along with the proximity of the glucose molecules with these residues provides a possible prediction of glycation propensities. Blind docking analysis was performed to understand the (A) Number of K, R residues interacting with glucose (B) Percentage of structures interacting with glucose (C) Percentage of structures with metal binding site interacting with glucose. It was found out that along with the number of K, R interacting with glucose, percentages of structures with active sites interacting with glucose was more for α secretases than BACE1 and cathepsin B **(Figure 4.2 A, B)**. Since α-secretases are metalloproteinases belonging to the metzincin family, hindrance in zinc metal binding sites can affect its activity. Fridman *et al* have shown that zinc-binding sites can be a good target for ADAMs activity [336]. Our study has found that all the metal-binding sites of ADAM10 and ADAM17 interact with glucose (**Figure 4.2 C**) which may affect Zn binding, reducing its activity. Also, hindrance in metal binding may reduce α-secretases activity. Interaction of glucose with the active site of secretases can also cause a reduction in its activity as a result, AβPP processing might drive towards amyloidogenic pathway by BACE1 and cathepsin B. α-secretases and BACE1 have a common intracellular AβPP pool to act on. It has already been predicted that the reduction in BACE1 activity would elevate α-secretases activity [337]. In a physiological glucose concentration, if α-secretases activity is getting down, the probability of AβPP processing by BACE1 and cathepsin

B increases leading to enhanced Aβ production. This might be one of the important mechanisms for diabetes being a risk factor for AD development. Nevertheless, it is also important to study the effect of glycation on BACE1 and cathepsin B.

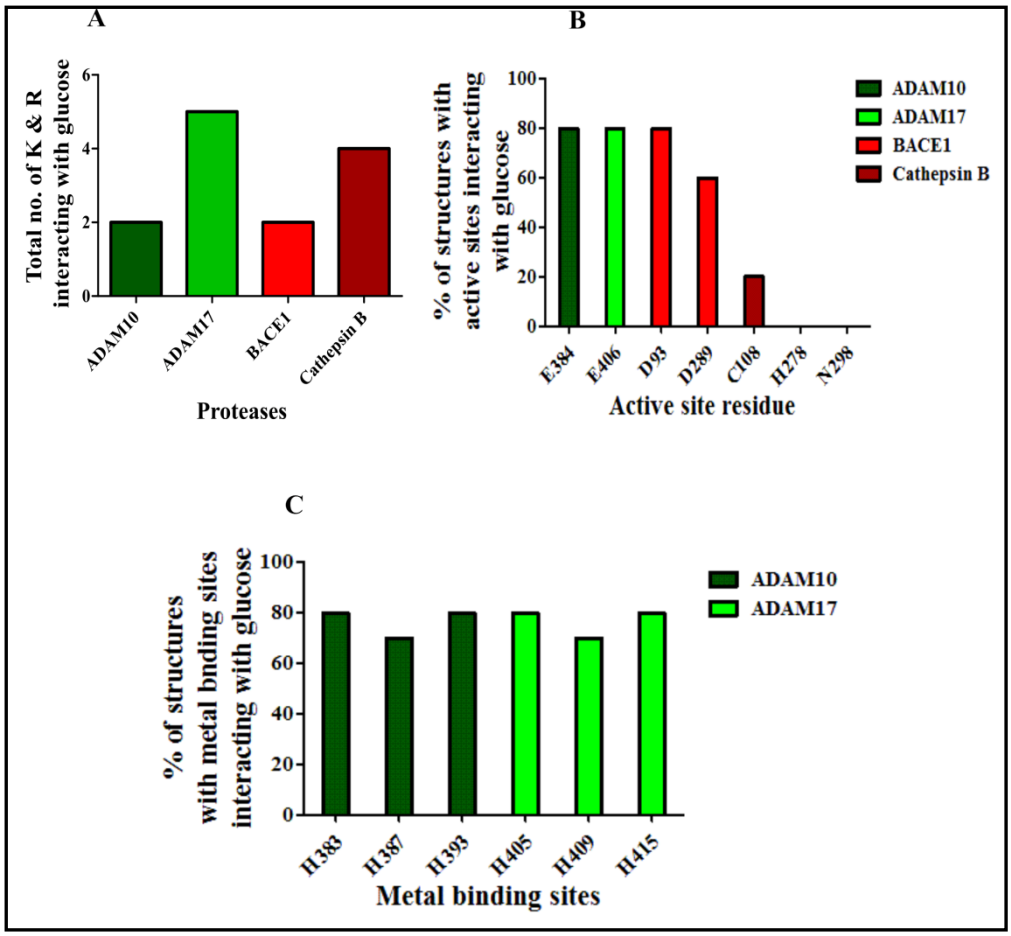

Figure 4.2: Blind docking of proteases with glucose

(A) Total number of K, R interacting with glucose (B) Percentage of protease structures with active sites interacting with glucose (C) Percentage of structures with metal binding sites interacting with glucose

(b) Active site volume of proteases

We have also studied the active site volume of these proteases upon glucose binding (**Figure 4.3**). The active site volumes could be one of the rate determining steps for the glycation. Calculation of the active site cavity volume of two important proteases ADAM17 and BACE1 by CASTp 3.0 software predicted 86.983 $Å^3$ and 651.932 $Å^3$ volume size respectively [338]. The larger cavity of BACE1 indicates the probability

of substrate binding despite bound glucose. Whereas, the active site of ADAM17 is concise, leaving no space for substrate binding upon interaction with glucose.

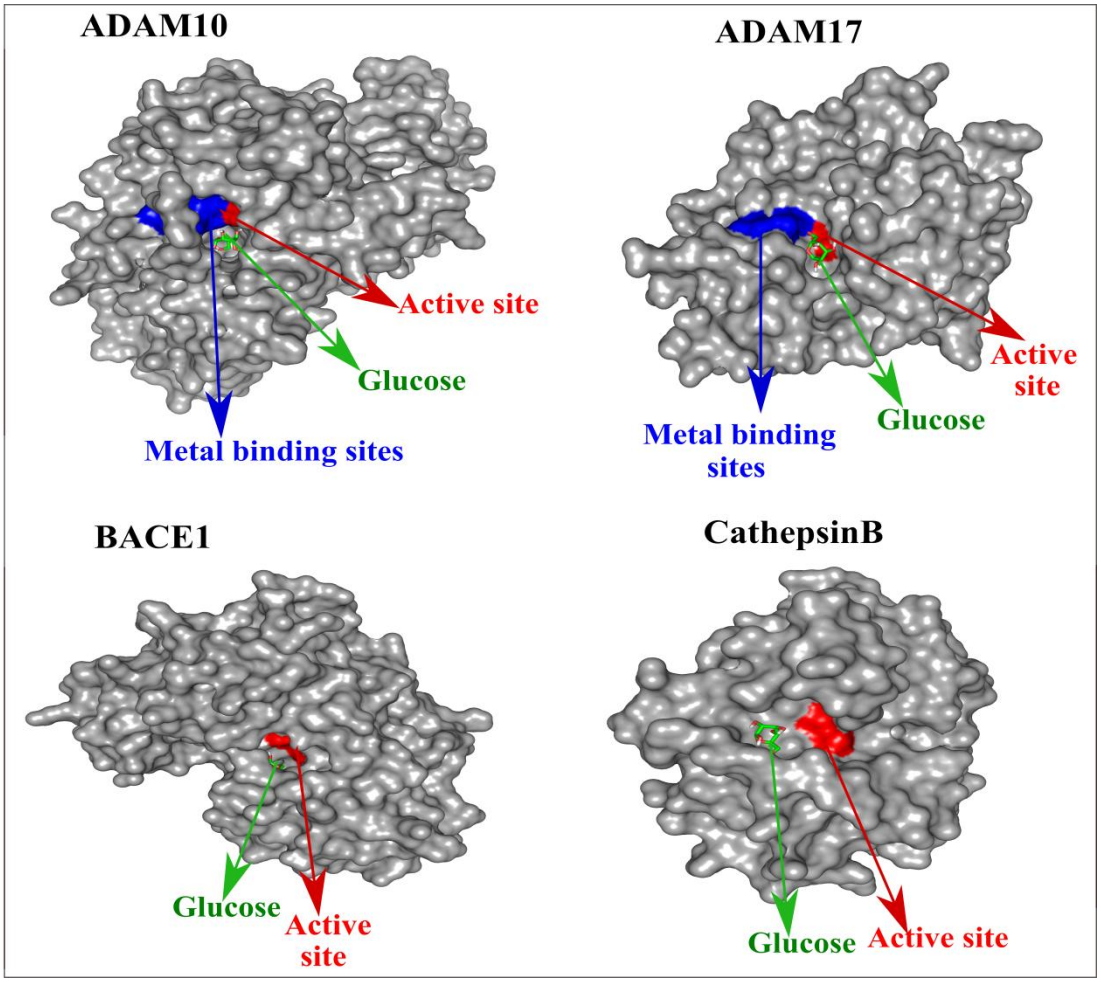

Figure 4.3: Comparison of active site volumes of proteases upon glucose binding

4.3.2 AβPP glycation may affect its processing

As discussed above, α-secretases act on AβPP at a site precluding Aβ formation**.** In diabetic condition, glycation of AβPP at α-secretases cleavage site i.e **K**/LVFF may resist α-secretases mediated AβPP processing (**Figure 4.4**), while glycation at BACE1/cathepsin B cleavage site **K**M/DAEF may not affect its processing as that is not the exact cleavage site of BACE1/cathepsin B. Therefore, it is possible that AβPP processing may shift towards amyloidogenic processing by BACE1/cathepsin B forming Aβ plaques.

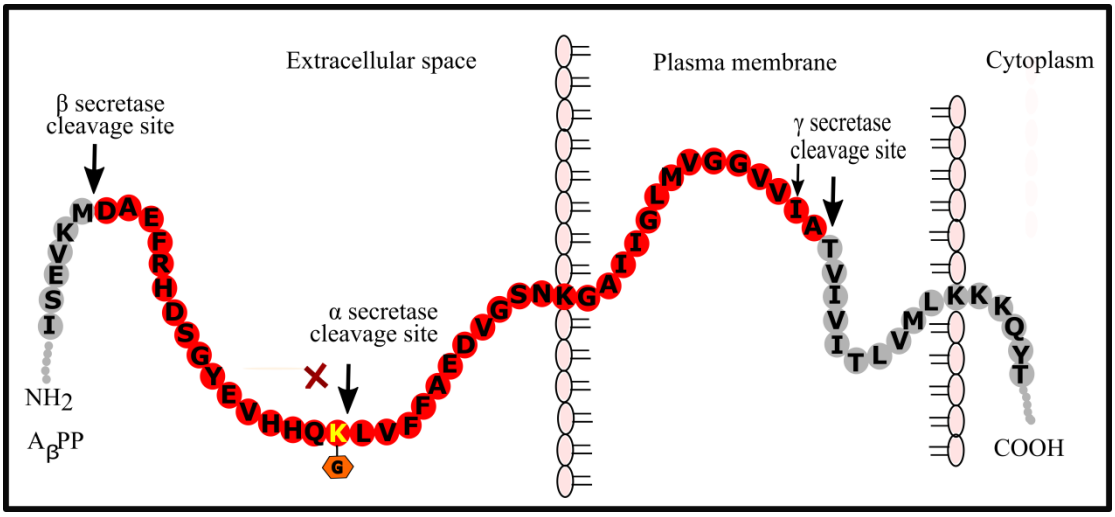

Figure 4.4: Cleavage sites of secretases on AβPP

4.4 Conclusions

Diabetes is a predisposing factor for AD. Despite many efforts, the exact mechanism is not known. This computational analysis suggests two glycation mediated mechanisms by which Aβ production might be enhanced. One pathway might be glycation of α-secretases leading to a reduction in their activity. NetGlycate analysis and blind docking study have found that α-secretases have more number of K, R residues for glycation than BACE1 and cathepsin B. Also, active site residues and all the metal-binding sites of α-secretases interacts with glucose which may reduce α-secretases activity by glycation or by affecting its metal binding capacity resulting in a shift in AβPP processing towards amyloidogenic pathway (**Figure 4.5**).

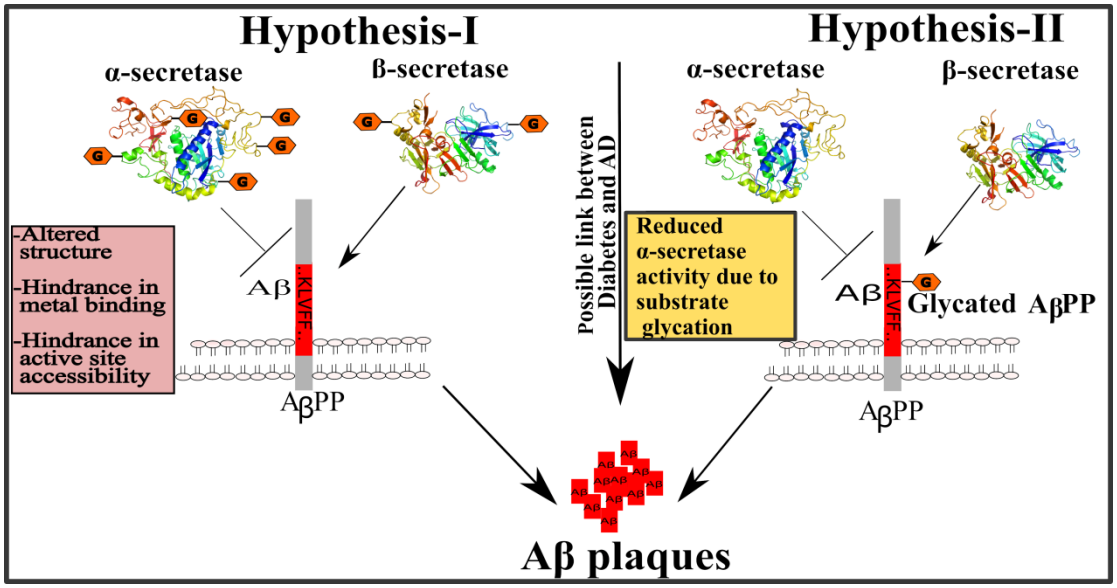

Figure 4.5: Summary of the both the possibilities for the regulation of Aβ accumulation by glycation

Alternatively, AβPP may get glycated at the α-secretases cleavage site, preventing its processing. Glycation at the cleavage site may reduce AβPP processing by α-secretases and increase the Aβ burden. These possibilities need to be tested in model animals to understand the role of glycation in AβPP processing by secretases. The summary of both the possibilities is presented in **Figure 4.5**.

Thesis Summary

AD, one of the most prevalent neurodegenerative diseases, is characterized by Aβ aggregation and phosphorylated tau protein tangles. Diabetes is considered as a major risk factor for AD. Diabetes is characterized by hyperglycemia, leading to the formation of AGEs by non-enzymatic glycation reaction, which interacts with its cell surface receptor RAGE causing further inflammation and cell damage. The current study was performed to understand the role of diabetes in the development of AD. We studied the effect of STZ induced diabetes, and MGO, a highly reactive glycating agent, whose concentration increases in diabetes.

In the first chapter, *in vitro* treatment of neuronal cells with MGO resulted in a concentration-dependent increase in cell death. Proteomic analysis was performed at a non-toxic concentration of MGO, and it revealed that MGO alters the expression of various proteins involved in AD, diabetes, calcium signaling, protein degradation, insulin secretion, apoptosis, and so on. BINGO analysis further suggested that MGO upregulated proteins were involved in various biological processes such as the MGO metabolic process, apoptosis, ATP metabolism, cytoskeleton, and NMDA receptor activity regulation. MGO down regulated proteins were involved in branching morphogenesis of nerves and positive regulation of synapse maturation

In the chapter 2, we have checked the effect of hyperglycemia and MGO in the development of AD-like symptoms in the Sprague Dawley rat model. STZ and MGO treated rats developed anxiety in the EPM test, as evidenced by the decreased number and decreased distance traveled in open arms. Anxiety was reduced upon co-treatment of MGO with AMG (MGO quencher) and TELMI (angiotensin receptor blocker). Further proteomic analysis of the hippocampus region followed by functional analysis by DAVID software revealed that STZ treatment upregulated proteins involved in AD, glycolysis, glutathione metabolism, citrate cycle, and oxidative phosphorylation. STZ down regulated proteins were involved in the synaptic vesicle cycle, various synapses, and long-term potentiation.

Similarly, MGO treatment caused upregulation of proteins involved in AD, oxidative phosphorylation, glutathione mechanism, and citrate cycle. MGO down regulated proteins were found to play a role in different synapses, calcium signaling, synaptic vesicle cycle, actin cytoskeleton, insulin secretion, insulin signaling pathway, proteasome, and long term potentiation. Few of the MGO altered proteins were found to be restored by co-treatment with TELMI and AMG. Co-treatment of MGO with TELMI restored 34 proteins. Restored proteins were found to play a role in mitochondrial functioning, neurotransmission, maintaining intracellular ROS levels, proteasomal system, and so on. Co-treatment of MGO with AMG restored 33 proteins with some of the proteins the same as that of the restoration by TELMI. AMG exclusively restored few other proteins playing a role in maintaining neuronal structure. Disturbances in the above cell processes have been reported to play a role in AD. Further, we have also checked plasma fructosamine levels to understand the effect of STZ and MGO treatments on AGEs formation. We found that STZ and MGO treatments caused an increase in fructosamine w.r.t. control, whereas AMG and TELMI treatment reduces it. STZ and MGO also caused an increase in RAGE expression, which was reduced in the presence of AMG and TELMI. We further checked the effect of STZ and MGO treatment on tau phosphorylation, one of the essential pathological hallmarks of AD. It was found to be increased upon STZ and MGO treatments, whereas AMG and TELMI treatment reduced it. The effect of STZ and MGO on the CA 1 region hippocampus structure was studied by H & E staining. STZ and MGO treatments were found to cause neurodegenerative changes.

In the 3rd chapter, we have performed a computational study to understand the molecular mechanisms for increased Aβ in diabetic conditions. Here, we have suggested two possibilities. In the 1st possibility, we have found that α-secretases are more prone to glycation induced inactivation than BACE1 and cathepsin B due to more number of K and R, as well as putative glycation sites by NetGlycate analysis. Further blind docking with glucose also shows that active site and metal-binding sites of α secretases interact more with glucose than those of BACE1 and cathepsin B. Hindrance in the active site and metal-binding sites due to bound glucose can lead to decreased activities of α-secretases. Also, the binding pocket volume of ADAM17

was found to be small as compared to BACE1. The 2nd possibility suggests that glycation of lysine residue at α-secretase cleavage site on AβPP, prevents its processing by α-secretases. It may be because of alteration in three dimensional structure. Both these possibilities may lead to increased Aβ production in diabetic subjects. In summary, the investigation carried out in this thesis describes the role of diabetes , methylglyoxal and glycation in the development of AD.

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Appendix

Appendix 1: List of proteins identified in neuro-2a cells upon MGO treatment

Sr. No.	Acc.No.	Protein	Unique Peptides
1	Q3TH56	Uncharacterized protein	38
2	Q3V2S4	IF rod domain-containing protein	29
3	W6PPR4	480-kDa ankyrinG	24
4	B2RQQ5	Microtubule-associated protein 1B	22
5	P05202	Aspartate aminotransferase_ mitochondrial	21
6	Q91V38	Heat shock protein 90_ beta (Grp94)_ member 1	21
7	P00924	Enolase 1	21
8	E9Q1F2	Actin_ cytoplasmic 1	20
9	P56480	ATP synthase subunit beta_ mitochondrial	20
10	E9PVZ8	Golgi autoantigen_ golgin subfamily b_ macrogolgin 1	19
11	Q3UAJ1	Peptidyl-prolyl cis-trans isomerase	17
12	B7FAV1	Filamin_ alpha (Fragment)	16
13	P16045	Galectin-1	16
14	Q3UZQ3	Elongation factor 1-alpha	15
15	G3X981	Peripherin	15
16	Q3UMM1	Tubulin beta chain	15
17	P38647	Stress-70 protein_ mitochondrial	14
18	Q61667	Histone H4 (Fragment)	13
19	Q3UDU4	L-lactate dehydrogenase	12
20	Q5FW97	Enolase 1_ alpha non-neuron	12
21	Q921L4	Histone H2B	12
22	B2MWM9	Calreticulin	11
23	P27773	Protein disulfide-isomerase A3	11
24	Q99KI0	Aconitate hydratase_ mitochondrial	11
25	Q3U2G2	Heat shock 70 kDa protein 4	10
26	Q3TSG8	Uncharacterized protein	10
27	F8VPV0	Pericentrin	10
28	A0A0A0MQA5	Tubulin alpha chain (Fragment)	10
29	O08614	Cytoskeletal protein	9
30	Q8VDD5	Myosin	9
31	Q3T984	Uncharacterized protein	9
32	Q3U4U6	T-complex protein 1 subunit gamma	9
33	P08249	Malate dehydrogenase_ mitochondrial	9
34	Q9JKR6	Hypoxia up-regulated protein 1	9
35	Q91V92	ATP-citrate synthase	9
36	Q3TXW2	Uncharacterized protein	9
37	Q80U36	MKIAA0325 protein (Fragment)	9
38	E9Q0U7	Heat shock protein 105 kDa	9

39	S4R257	Glyceraldehyde-3-phosphate dehydrogenase (Fragment)	9
40	B9EIU1	Glutamyl-prolyl-tRNA synthetase	9
41	A0A2I3BPQ5	Zinc finger CCCH domain-containing protein 13	8
42	B2RY15	Tln2 protein	8
43	B2RXX1	Cep110 protein	8
44	B7ZC50	Heat shock protein HSP 90-alpha (Fragment)	8
45	P26039	Talin-1	8
46	B2RQQ1	40S ribosomal protein S21	8
47	B2RX08	Spectrin beta chain	8
48	E9PX29	Spectrin beta chain	8
49	P80316	T-complex protein 1 subunit epsilon	8
50	Q3THQ5	Uncharacterized protein	8
51	Q3V0Z8	Uncharacterized protein (Fragment)	8
52	Q922B0	Ubc protein	8
53	Q8C5Q5	T-complex protein 1 subunit eta	8
54	Q4U4S6	Xin actin-binding repeat-containing protein 2	8
55	Q3UJZ8	T-complex protein 1 subunit delta	8
56	A0AUV1	Histone H2A (Fragment)	8
57	Q3UKW2	Calmodulin-1	7
58	A0A140LIN9	Dynein heavy chain 3_ axonemal	7
59	P52480	Pyruvate kinase PKM	7
60	K3W4R2	Myosin-14	7
61	A0A0A0MQE8	Rho GTPase-activating protein 21	7
62	Q3UH59	Myosin-10	7
63	Q9CWJ9	Bifunctional purine biosynthesis protein PURH	7
64	Q3TX47	Tr-type G domain-containing protein	7
65	E9PWT1	Transformation/transcription domain-associated protein	7
66	E9Q7G0	Nuclear mitotic apparatus protein 1	7
67	Q9WVA4	Transgelin-2	7
68	E9Q2E4	HECT domain E3 ubiquitin protein ligase 4	6
69	B2CY77	Laminin receptor (Fragment)	6
70	Q45VK7	Cytoplasmic dynein 2 heavy chain 1	6
71	Q3V2C6	IF rod domain-containing protein	6
72	B1ART2	Vacuolar protein sorting 13D	6
73	Q8BNF8	Uncharacterized protein	6
74	A2A4P0	ATP-dependent RNA helicase DHX8	6
75	B2RRE0	A kinase (PRKA) anchor protein (Gravin) 12	6
76	P63038	60 kDa heat shock protein_ mitochondrial	6
77	A0A5F8MPY2	Uncharacterized protein	6
78	P50396	Rab GDP dissociation inhibitor alpha	6

79	Q5SUC3	Uncharacterized protein	6
80	E9PWE8	Dihydropyrimidinase-related protein 3	6
81	Q3TW28	Uncharacterized protein	6
82	D3Z6F5	ATP synthase subunit alpha	6
83	Q545V3	Uncharacterized protein	6
84	P17751	Triosephosphate isomerase	6
85	Q5SX49	Profilin	6
86	A0A1D5RLV3	Protein unc-80 homolog	6
87	B9EHN0	Ubiquitin-like modifier activating enzyme 1	6
88	P68510	14-3-3 protein eta	5
89	A0A1B0GRY3	Predicted gene 45861	5
90	G3X9L9	Myomegalin	5
91	O08807	Peroxiredoxin-4	5
92	P68040	Receptor of activated protein C kinase 1	5
93	Q3TT41	Uncharacterized protein	5
94	Q80TQ3	MKIAA0845 protein (Fragment)	5
95	Q8CJ40-2	Isoform 2 of Rootletin	5
96	G5E924	Heterogeneous nuclear ribonucleoprotein L (Fragment)	5
97	Q9DCN2	NADH-cytochrome b5 reductase 3	5
98	A0A0G2JF23	Malate dehydrogenase (Fragment)	5
99	Q3TKG0	HATPase_c domain-containing protein (Fragment)	5
100	P08032	Spectrin alpha chain_ erythrocytic 1	5
101	Q05CG9	Psmd1 protein (Fragment)	5
102	P70296	Phosphatidylethanolamine-binding protein 1	5
103	A1E281	Beta-actin (Fragment)	5
104	Q3UXC2	Uncharacterized protein	5
105	Q4FZE6	40S ribosomal protein S7	5
106	O08553	Dihydropyrimidinase-related protein 2	5
107	Q9CZD3	Glycine--tRNA ligase	5
108	Q8BVI9	Uncharacterized protein	5
109	B2RTM0	Histone H4	5
110	Q8BPH1	14_3_3 domain-containing protein	5
111	Q3U8U8	Polyadenylate-binding protein	4
112	A0A2X0U2I4	DOCK11	4
113	D3YV98	Fructose-bisphosphate aldolase A (Fragment)	4
114	Q68FD5	Clathrin heavy chain 1	4
115	Q9CXW3	Calcyclin-binding protein	4
116	Q5CZY9	Rps16 protein	4
117	E9QQ10	A-kinase anchor protein 9	4
118	Q3ULW0	GTP-binding nuclear protein Ran	4

119	Q1XGY5	Fras1 related extracellular matrix protein 2	4
120	Q3TJG6	CS domain-containing protein	4
121	E9PX70	Collagen alpha-1(XII) chain	4
122	B2RSN3	Tubulin beta chain	4
123	E9Q8T7	Dynein heavy chain 1_ axonemal	4
124	H7BX26	Centrosomal protein of 170 kDa	4
125	P10639	Thioredoxin	4
126	A0PJF4	Ahnak protein (Fragment)	4
127	Q3UBR0	Uncharacterized protein (Fragment)	4
128	Q61753	D-3-phosphoglycerate dehydrogenase	4
129	B2RQG2	PHD finger protein 3	4
130	E9Q512	Thyroid hormone receptor interactor 11	4
131	B1AXW5	Peroxiredoxin-1 (Fragment)	4
132	A0A1L1SQR4	Dedicator of cytokinesis protein 6	4
133	F8VQL9	Nuclear receptor corepressor 2	4
134	Q99PT1	Rho GDP-dissociation inhibitor 1	4
135	O89052	Alpha-tubulin (Fragment)	4
136	E9Q0A4	Kinesin-like protein KIF21B	4
137	P09411	Phosphoglycerate kinase 1	4
138	J3QMG3	Voltage-dependent anion-selective channel protein 3	4
139	Q3UD67	AA_TRNA_LIGASE_II_ALA domain-containing protein	4
140	Q6B822	Histone H4 (Fragment)	4
141	Q8CF78	Uncharacterized protein	4
142	P46660	Alpha-internexin	4
143	Q3U9G2	Uncharacterized protein	3
144	E9QKK1	Centromere-associated protein E	3
145	P12970	60S ribosomal protein L7a	3
146	Q3UAG2	6-phosphogluconate dehydrogenase_ decarboxylating	3
147	Q6KCD5	Nipped-B-like protein	3
148	Q3UFS6	Pyruvate carboxylase	3
149	B2RSU7	GRIP and coiled-coil domain containing 2	3
150	Q5SX39	Myosin-4	3
151	A0A2I3BQ03	14-3-3 protein zeta/delta (Fragment)	3
152	B7U582	Heat shock protein 70-2	3
153	F8WHL2	Coatomer subunit alpha	3
154	Q3KNY0	Immunoglobulin-like and fibronectin type III domain-containing protein 1	3
155	Q3U2W2	Uncharacterized protein	3
156	Q3UEI4	Pyruvate kinase (Fragment)	3
157	A0A0U1RNK7	Dedicator of cytokinesis protein 7	3
158	P11881	Inositol 1_4_5-trisphosphate receptor type 1	3

159	P49717	DNA replication licensing factor MCM4	3
160	D3Z1U0	Citron Rho-interacting kinase	3
161	S4R2R5	Ankyrin-2	3
162	B7ZWF1	Ddx3x protein	3
163	Q91VW5	Golgin subfamily A member 4	3
164	Q5RIM6	Nuclear receptor corepressor 1	3
165	E9Q0F0	Keratin 78	3
166	A8IP69	14-3-3 protein gamma subtype	3
167	A2AQB2	Nebulin	3
168	A3KML3	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein_ theta polypeptide	3
169	G5E898	Periplakin	3
170	Q80YC2	Hsp90ab1 protein (Fragment)	3
171	E9Q3D6	Heat shock protein HSP 90-beta (Fragment)	3
172	H3BJZ7	Protein unc-13 homolog A	3
173	A2AX52	Collagen alpha-4(VI) chain	3
174	Q8R4B4	Down syndrome cell adhesion molecule-like protein (Fragment)	3
175	Q6P1J1	Crmp1 protein	3
176	Q01149	Collagen alpha-2(I) chain	3
177	E9QNN1	ATP-dependent RNA helicase A	3
178	Q9ERC8	Down syndrome cell adhesion molecule homolog	3
179	Q3UEM8	Uncharacterized protein (Fragment)	3
180	E9Q1M1	PDZ domain-containing 2	3
181	Q3TST6	Fibrillar collagen NC1 domain-containing protein (Fragment)	3
182	Q3UZG3	Uncharacterized protein	3
183	A0A2I3BQS6	E3 ubiquitin-protein ligase UBR5	3
184	A0A0R4J2A0	Protein phosphatase Slingshot homolog 2	3
185	Q80YR5	Scaffold attachment factor B2	3
186	Q3TAS8	Amidohydro-rel domain-containing protein	3
187	P00342	L-lactate dehydrogenase C chain	3
188	A1L347	Heat shock protein 1-like	3
189	Q3TWE3	Protein disulfide-isomerase	3
190	Q3TWL8	Uncharacterized protein	3
191	A0A5F8MPN8	Malate dehydrogenase_ cytoplasmic	3
192	Q6UL10	AHNAK (Fragment)	3
193	Q3TCS0	Uncharacterized protein	3
194	A0A0G2JDE3	Adhesion G protein-coupled receptor L2	3
195	Q1W5W7	Putative gag-pol protein	3
196	D4AFX7	DnaJ heat shock protein family (Hsp40) member C13	3
197	Q9DCV7	Keratin_ type II cytoskeletal 7	3

198	A0A1S6GWH4	SAM_MT_RSMB_NOP domain-containing protein	3
199	C1KG51	Truncated profilaggrin/filaggrin flaky tail mutant form	3
200	B2RVP5	Histone H2A	3
201	Q9R0P9	Ubiquitin carboxyl-terminal hydrolase isozyme L1	3
202	E9PYH2	Cytosolic acyl coenzyme A thioester hydrolase	3
203	Q3TV20	Glutamine amidotransferase type-2 domain-containing protein	3
204	Q3UBP6	Uncharacterized protein	3
205	A0A1D5RLV7	WD repeat and FYVE domain-containing protein 3	3
206	P70168	Importin subunit beta-1	3
207	Q544Y7	Cofilin 1_ non-muscle	3
208	Q9CZN7	Serine hydroxymethyltransferase_ mitochondrial	3
209	Q3TTX0	Uncharacterized protein	3
210	O89061	Delta-aminolevulinic acid dehydratase (Fragment)	3
211	A0A140T8T2	Slit homolog 2 protein	3
212	Q5U438	Nucleophosmin 1	3
213	A0A494BBG8	Xaa-Pro aminopeptidase 1	3
214	Q1WWK3	Hist1h1b protein (Fragment)	3
215	Q9DCR1	Uncharacterized protein (Fragment)	2
216	Q5SWU9	Acetyl-CoA carboxylase 1	2
217	D3Z0X5	Pleckstrin homology-like domain family B member 1	2
218	A0A5F8MPW9	Paternally-expressed gene 3 protein	2
219	B2RXX9	Myosin_ heavy polypeptide 7_ cardiac muscle_ beta	2
220	Q3UGZ4	Spectrin beta chain	2
221	P97351	40S ribosomal protein S3a	2
222	P80317	T-complex protein 1 subunit zeta	2
223	F6RND9	Myosin phosphatase Rho-interacting protein (Fragment)	2
224	A5HLW0	PCF11 (Fragment)	2
225	Q9CVR0	Tubulin beta chain (Fragment)	2
226	A0A1Y7VIS3	Iroquois-class homeodomain protein IRX-1 (Fragment)	2
227	A0A286YCV9	Kinesin family member 13B	2
228	Q3U630	AA_TRNA_LIGASE_II domain-containing protein	2
229	B7ZP22	Heterogeneous nuclear ribonucleoprotein A2/B1	2
230	A0A1L1SSN6	Pyruvate kinase PKM (Fragment)	2
231	H3BIW6	Phosphoinositide phospholipase C	2
232	Q99LC5	Electron transfer flavoprotein subunit alpha_ mitochondrial	2
233	B1ARR7	Alpha-enolase (Fragment)	2
234	Q8BKX6	Serine/threonine-protein kinase SMG1	2
235	E9Q652	Regulator of G-protein-signaling 12	2

236	Q3TCH2	Ubiquitin carboxyl-terminal hydrolase (Fragment)	2
237	Z4YL23	Fer-1-like protein 4	2
238	Q9D9Y2	Uncharacterized protein	2
239	A0A2R8VHG2	Poly(rC)-binding protein 2 (Fragment)	2
240	O70349	Uncharacterized protein SKI	2
241	A0A140LIV1	Sperm flagellar protein 2	2
242	Q3U417	Uncharacterized protein	2
243	B7ZWN1	Kif20b protein	2
244	P15864	Histone H1.2	2
245	A0A171KXD3	Protein arginine N-methyltransferase 1	2
246	Q9CPY7	Cytosol aminopeptidase	2
247	Q3U741	Probable ATP-dependent RNA helicase DDX17	2
248	A0A5F8MPJ5	Xin actin-binding repeat-containing protein 2	2
249	P26638	Serine--tRNA ligase_ cytoplasmic	2
250	Q91XQ0-2	Isoform 2 of Dynein heavy chain 8_ axonemal	2
251	P05532	Mast/stem cell growth factor receptor Kit	2
252	Q5U445	cDNA sequence BC085271	2
253	P11983	T-complex protein 1 subunit alpha	2
254	A0A0R4J1H6	Golgin subfamily A member 3	2
255	Q3UK56	KH type-2 domain-containing protein	2
256	A0A3B2WBH9	Tight junction protein ZO-2	2
257	I7ENE7	Ubiquitin carboxyl-terminal hydrolase (Fragment)	2
258	Q3U548	UTP--glucose-1-phosphate uridylyltransferase	2
259	Q8R055	DNA ligase	2
260	Q8C6S9	Cilia- and flagella-associated protein 54	2
261	B8QI34	Liprin-alpha-2	2
262	Q6P5E5	Structural maintenance of chromosomes protein	2
263	E9QAS4	Chromodomain-helicase-DNA-binding protein 4	2
264	A0A494B982	Guanine deaminase	2
265	Q71LX8	Heat shock protein 84b	2
266	Q3TIQ2	Uncharacterized protein	2
267	J3QPN0	Zinc finger protein 518B	2
268	G3X940	Histone acetyltransferase	2
269	A0A3G0YW97	Frmpd3 (Fragment)	2
270	B7ZWN9	Senp6 protein	2
271	E9PX68	Solute carrier family 4 (anion exchanger)_ member 1_ adaptor protein	2
272	P20782	Acetylcholine receptor subunit epsilon	2

273	A0A1B0GS91	Supervillin	2
274	A2AKX3	Probable helicase senataxin	2
275	A0A2I3BRM1	Regulating synaptic membrane exocytosis protein 2 (Fragment)	2
276	A2ARW8	Predicted gene 14399	2
277	E9Q286	Little elongation complex subunit 1	2
278	P62918	60S ribosomal protein L8	2
279	V9GWT9	Transcription factor 24	2
280	Q5SZM8	Zinc finger protein 184 (Kruppel-like)	2
281	P08551	Neurofilament light polypeptide	2
282	F6Q404	Protein disulfide-isomerase A3 (Fragment)	2
283	E9QK89	Mediator of DNA damage checkpoint protein 1	2
284	P62821	Ras-related protein Rab-1A	2
285	B2RSV4	Splicing factor 3b_ subunit 3	2
286	A0A087WR97	TAR DNA-binding protein 43 (Fragment)	2
287	Q4VAG4	Ribosomal protein L22	2
288	B7ZNJ5	Odz3 protein	2
289	A2ARP8	Microtubule-associated protein 1A	2
290	F8WIR1	Cathepsin D	2
291	Q4VBF8	Signal-induced proliferation-associated 1-like protein 1	2
292	Q5NCP6	Peripheral-type benzodiazepine receptor-associated protein 1	2
293	A0A1D5RLE0	Dedicator of cytokinesis protein 9	2
294	E9Q309	Centrosome-associated protein 350	2
295	Q07417	Short-chain specific acyl-CoA dehydrogenase_ mitochondrial	2
296	E9QPX1	Collagen alpha-1(XVIII) chain	2
297	G3X9W0	Heterogeneous nuclear ribonucleoprotein D0	2
298	Q6DI58	Rpl12 protein (Fragment)	2
299	B1AR69	Myosin_ heavy polypeptide 13_ skeletal muscle	2
300	P99024	Tubulin beta-5 chain	2
301	Q3TIV6	Lysine--tRNA ligase	2
302	Q6NWV5	Pgam1 protein (Fragment)	2
303	B2RXQ4	Nek1 protein	2
304	A0A0R4J0Z1	Protein disulfide-isomerase A4	2
305	Q3TGQ4	Ribosomal_L7Ae domain-containing protein	2
306	Q6PDN3	Myosin light chain kinase_ smooth muscle	2
307	Q3TIP8	Chloride intracellular channel protein	2
308	Q3THH1	Uncharacterized protein	2
309	A0A1D5RLY0	Dynamin-binding protein	2
310	Q80U35	Rho guanine nucleotide exchange factor 17	2
311	Q3TGW8	TPR_REGION domain-containing protein (Fragment)	2

312	P51881	ADP/ATP translocase 2	2
313	Q3TJU7	Uncharacterized protein (Fragment)	2
314	A0A1W2P7W3	Receptor-type tyrosine-protein phosphatase beta	2
315	Q8BVT7	RIKEN cDNA 4921511C20 gene	2
316	E9Q6I3	Protein Shroom3	2
317	P11087	Collagen alpha-1(I) chain	2
318	B2RUG9	Adenomatosis polyposis coli	2
319	E9QPK4	Serine/threonine-protein kinase ATR	2
320	Q564E8	Ribosomal protein L4	2
321	E9Q7P2	Voltage-dependent T-type calcium channel subunit alpha	2
322	Q542D9	Uncharacterized protein	2
323	P35441	Thrombospondin-1	2
324	Q8CDP0	Cytosolic carboxypeptidase 3	2
325	P70670	Nascent polypeptide-associated complex subunit alpha_ muscle-specific form	2
326	A0A0R5RP28	Sodium channel protein	2
327	Q0VGU4	Neurosecretory protein VGF	2
328	B0QZL1	Alpha-enolase (Fragment)	2
329	A0A1D5RMI8	Alstrom syndrome protein 1 homolog	2
330	O88207-2	Isoform 2 of Collagen alpha-1(V) chain	2
331	E9QPR7	Down syndrome cell adhesion molecule-like protein 1 homolog	2
332	E9QPE7	Myosin-11	2
333	Q05816	Fatty acid-binding protein 5	2
334	A2AIK5	Homeobox protein aristaless-like 4	2
335	A2AFS0	Serine--tRNA ligase_ cytoplasmic (Fragment)	2
336	Q8BH59	Calcium-binding mitochondrial carrier protein Aralar1	2
337	P11531	Dystrophin	2
338	Q3UHQ5	TOG domain-containing protein	2
339	A2BDQ4	Ephrin type-A receptor 7	2
340	A0A087WP83	Vigilin	2
341	F8WGF2	Nitric oxide synthase_ brain	2
342	A0A1W2P711	Melanoma inhibitory activity protein 2	2
343	F8VQ95	Transforming acidic coiled-coil-containing protein 1	2
344	Q99LT6	Eef2 protein (Fragment)	2
345	P14869	60S acidic ribosomal protein P0	2
346	Q571C7	Transcription factor TFIIIB component B" homolog	2
347	Q9CY58-4	Isoform 4 of Plasminogen activator inhibitor 1 RNA-binding protein	2
348	F8WHM5	Golgi apparatus protein 1 (Fragment)	2
349	Q09XV5	Chromodomain-helicase-DNA-binding protein 8	2

350	P40142	Transketolase	2
351	A2AVJ7	Ribosome-binding protein 1	2
352	Q8K4K8	Antioxidant protein	2
353	A2AFG7	Neural cell adhesion molecule L1	2
354	G4V4Z1	Gag-pro-pol polyprotein	2
355	A2AIS0	Voltage-dependent N-type calcium channel subunit alpha	2
356	A0A1B0GRM4	Liprin-alpha-3 (Fragment)	2
357	P15532	Nucleoside diphosphate kinase A	2
358	A0A0J9YUD5	Nucleoporin 205	2
359	E9Q4D4	Polycystic kidney disease protein 1-like 2	2
360	A0A0G2JG60	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 3	2
361	E9PYH0	Versican core protein	2
362	Q8BV79	TPR and ankyrin repeat-containing protein 1	2
363	A0A1Y7VLA4	Protein TASOR 2 (Fragment)	2
364	Q01853	Transitional endoplasmic reticulum ATPase	2
365	B1ATR2	Testis-expressed protein 2	2
366	A0A1W2P768	H3 clustered histone 14	2
367	H7BX15	Adhesion G protein-coupled receptor L1	2
368	D3Z0R8	Tetratricopeptide repeat domain 7	2
369	Q4KL76	Heat shock protein 1 (Chaperonin 10)	2
370	P23116	Eukaryotic translation initiation factor 3 subunit A	2
371	A0A140LJ69	Protein unc-13 homolog B	2
372	Q3TFF0	Uncharacterized protein	2
373	A2AFQ0	E3 ubiquitin-protein ligase HUWE1	2
374	E9QKU9	Tetratricopeptide repeat protein 23	2
375	A0A0G2JE32	Ubiquitin-conjugating enzyme E2 D3 (Fragment)	2
376	B9EKS2	Jumonji domain containing 1B	2
377	D3YW29	RNA exonuclease 5	2
378	Q9DBW2	Rho-GAP domain-containing protein (Fragment)	2
379	A0A338P6N4	Beta/gamma crystallin domain-containing protein 3	2
380	B1AVK5	Collagen_ type IV_ alpha 6	2
381	A0A1D5RLD1	Unconventional myosin-IXb	2
382	Q58E35	Ribosomal protein_ large_ P1	2
383	A6PWD6	Predicted gene 5431	2
384	A0A5F8MPR4	Xin actin-binding repeat-containing protein 2	2
385	Q9Z1D2	Breast cancer type 1 susceptibility protein homolog	2
386	A0A1D5RMC4	RIKEN cDNA 4932438A13 gene (Fragment)	2

387	P26350	Prothymosin alpha	2
388	F6QNN0	Coiled-coil domain-containing 180 (Fragment)	2
389	P62631	Elongation factor 1-alpha 2	2
390	Q4VAB7	Nuclear receptor subfamily 1_ group D_ member 2	2
391	Q8K083	Zinc finger protein 536	2
392	A0A1B0GSS6	Predicted gene 3854	2
393	A3KGI3	Kinetochore scaffold 1	2
394	E0CZ27	Histone H3 (Fragment)	2
395	Q6ZPT2	MKIAA1352 protein (Fragment)	2
396	E9PW69	Proteasome subunit alpha type (Fragment)	2
397	A2AL50	Alkylglycerone-phosphate synthase	2
398	A0A0U1RPN8	Fructose-bisphosphate aldolase A (Fragment)	2
399	Q148T5	Tnc protein	2
400	Q3TDD9	Protein phosphatase 1 regulatory subunit 21	2
401	Q8VHY0	Chondroitin sulfate proteoglycan 4	1
402	A0A087WR13	Dynein_ axonemal_ heavy chain 7C	1
403	Q3U775	Uncharacterized protein	1
404	P31245	Homeobox protein Hox-A2	1
405	Q3TSA3	Protein kinase domain-containing protein	1
406	D3Z3D0	Nuclear-interacting partner of ALK	1
407	A0A0R4J1N7	Ankyrin-1	1
408	A0A1L1SUD9	Coiled-coil domain-containing protein 187	1
409	A2ADB0	Msx2-interacting protein	1
410	A0A5F8MPH5	Synaptotagmin-like protein 2	1
411	F6TDX7	R3H domain-containing protein 2 (Fragment)	1
412	F8WGU3	Epithelial-splicing regulatory protein 1	1
413	P70699	Lysosomal alpha-glucosidase	1
414	Q9CRT0	Tubulin_C domain-containing protein (Fragment)	1
415	B0V2N7	Annexin (Fragment)	1
416	B2RWY4	Whirlin	1
417	A0A087WQ86	Dedicator of cytokinesis protein 10	1
418	Q68FE7	Transmembrane protein 151B	1
419	D3Z7F0	L-lactate dehydrogenase (Fragment)	1
420	F6XS92	Podocan (Fragment)	1
421	Q62261	Spectrin beta chain_ non-erythrocytic 1	1
422	P08121	Collagen alpha-1(III) chain	1
423	E9PYP2	Neurobeachin-like 1	1
424	F6RPJ9	Insulin-degrading enzyme (Fragment)	1

425	Q91WN1	DnaJ homolog subfamily C member 9	1
426	A0A571BEB6	Sodium channel protein type 3 subunit alpha (Fragment)	1
427	Q4FK36	Destrin	1
428	A0A0J9YUE9	Dynamin-1	1
429	H7BX49	WD repeat-containing protein 90	1
430	P46061	Ran GTPase-activating protein 1	1
431	A0A1L1SU37	Pyruvate kinase PKM (Fragment)	1
432	D3Z2F2	60 kDa heat shock protein_ mitochondrial (Fragment)	1
433	A0A0U1RNJ2	Cardiotrophin-1	1
434	A0A1D5RMD4	Kinesin-like protein KIF16B	1
435	A0A087WQS0	Tensin 1	1
436	Q14BY7	Regulating synaptic membrane exocytosis	1
437	A0A571BG59	Tenascin XB	1
438	A0A0R4J084	SUN domain-containing protein 5	1
439	E9PWX9	Transmembrane protease serine 5	1
440	Q790I0	Valyl-tRNA-synthetase G7a/Bat6	1
441	A0A0R4J036	Neurofilament medium polypeptide	1
442	Q8R010	Aminoacyl tRNA synthase complex-interacting multifunctional protein 2	1
443	Q3U7E6	Uncharacterized protein	1
444	B2RY77	Phosphodiesterase	1
445	A2A891-4	Isoform 4 of Calmodulin-binding transcription activator 1	1
446	Q91WU5	Arsenite methyltransferase	1
447	Q3UXU7	Vang-like protein	1
448	Q3TQ38	WD_REPEATS_REGION domain-containing protein	1
449	A0A0J9YTV0	Eukaryotic translation initiation factor 4 gamma 1 (Fragment)	1
450	Q8BZW9	Uncharacterized protein	1
451	A0A2L2P590	Discs large MAGUK scaffold protein 2 gamma	1
452	Q8C1L7	40S rib	1
453	H3BLK8	Zinc finger homeobox protein 4	1
454	A2RRY2	2310046A06Rik protein	1
455	E9Q263	Protein unc-13 homolog B	1
456	Q3UK83	Uncharacterized protein	1
457	A1YYM3	Fibroblast growth factor receptor	1
458	A0A1L1SSA4	Centrosomal protein of 164 kDa	1
459	Q6A099	MKIAA0248 protein (Fragment)	1
460	A3KGJ7	Centrosome-associated protein CEP250	1

461	A0A2I3BQF4	60S ribosomal protein L30	1
462	Q61213	Gag	1
463	A0A2I3BQU5	SR-related CTD-associated factor 11	1
464	Q9D2G2	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex_ mitochondrial	1
465	E9Q2X6	Structural maintenance of chromosomes protein	1
466	A0A1W2P7Z3	Ubiquitin-conjugating enzyme E2 N	1
467	Q9WVS5	Chaperonin containing TCP-1 theta subunit	1
468	G3UWZ0	Bromodomain adjacent to zinc finger domain protein 1A	1
469	Q3UGP2	F-box domain-containing protein	1
470	Q3U9U3	Tubulin beta chain	1
471	P63213	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2	1
472	E9Q7L1	URB2 ribosome biogenesis 2 homolog (S. cerevisiae)	1
473	P60335	Poly(rC)-binding protein 1	1
474	P35700	Peroxiredoxin-1	1
475	Q64152	Transcription factor BTF3	1
476	A2AP88	Zinc finger CCCH domain-containing protein 6	1
477	Q924A2	Protein capicua homolog	1
478	Q3U6K9	Phosphoserine aminotransferase	1
479	G5E829	Plasma membrane calcium-transporting ATPase 1	1
480	Q3V065	DUF3496 domain-containing protein	1
481	G5E8B1	Tyrosine-protein phosphatase non-receptor type 13	1
482	I6L9I7	Cbx3 protein	1
483	Q5DTT2	PH and SEC7 domain-containing protein 1	1
484	A0A2R8VHK2	Probable ATP-dependent RNA helicase DDX46 (Fragment)	1
485	Q3YAB4	Potassium channel interacting protein 1	1
486	Q3TQP7	Uncharacterized protein	1
487	Q61768	Kinesin-1 heavy chain	1
488	A0A5H1ZRL7	Transformation related protein 53 binding protein 1	1
489	A0A087WPS5	Centrosomal protein of 70 kDa	1
490	A0A0A6YW46	Filaggrin	1
491	P62315	Small nuclear ribonucleoprotein Sm D1	1
492	A2A8V4	Zinc finger protein 993 (Fragment)	1
493	A0A0R4J1E9	Hypoxia-inducible factor 1-alpha	1
494	A0A0G2JDV6	Ubiquitin-associated protein 2-like	1
495	A0A1D5RMH0	Storkhead-box protein 2	1
496	A2AU62	RNA-binding protein Raly (Fragment)	1

497	A1BN54	Alpha actinin 1a	1
498	G3UX48	Protein PRRC2A	1
499	A0A1B0GSX7	Nuclear pore complex protein Nup98-Nup96	1
500	Q5DQR4	Syntaxin-binding protein 5-like	1
501	Q8C196	Carbamoyl-phosphate synthase [ammonia]_ mitochondrial	1
502	B1AQN2	Receptor-type tyrosine-protein phosphatase T	1
503	A2AQA1	Zinc finger protein 345	1
504	E0CXJ3	Eukaryotic translation initiation factor 2 subunit 2 (Fragment)	1
505	A0A0G2JDF8	Serrate RNA effector molecule homolog (Fragment)	1
506	B7ZWM7	Ankrd11 protein	1
507	B9EKT6	IQ motif containing GTPase activating protein 3	1
508	A0A2Z4SX77	MHC class II antigen D (Fragment)	1
509	F8VQ75	Kinesin-like protein KIF13A	1
510	Q3TDV6	Uncharacterized protein	1
511	D3YV82	Zinc finger protein 715 (Fragment)	1
512	A2A7B5	PR domain-containing 2_ with ZNF domain	1
513	G5E8G3	Opioid-binding protein/cell adhesion molecule-like	1
514	Q571M5	MKIAA4011 protein (Fragment)	1
515	E0CXG4	Protein GREB1	1
516	A0A087WS46	Eukaryotic translation elongation factor 1 beta 2	1
517	P62737	Actin_ aortic smooth muscle	1
518	E9Q842	Neuron navigator 2	1
519	Q99P72	Reticulon-4	1
520	Q8BYH5	SEC7 domain-containing protein (Fragment)	1
521	A0A140LJ91	Zinc finger protein 568 (Fragment)	1
522	P58774-2	Isoform 2 of Tropomy	1
523	E4NKG5	RNA granule protein 140	1
524	G3X9M2	SKI/DACH domain-containing protein 1	1
525	Q8BI29	Specifically androgen-regulated gene protein	1
526	Q3TXQ1	Uncharacterized protein	1
527	S4R1I4	Centrosomal protein of 112 kDa	1
528	A0A087WQT9	Voltage-dependent L-type calcium channel subunit alpha	1
529	B2RUM8	RNA helicase	1
530	A0A087WRL5	TBC1 domain family member 2B	1
531	A0A0N4SW47	Zinc finger protein 775 (Fragment)	1
532	A2AU83	Predicted gene 14124	1
533	L7N248	Predicted gene 6871	1
534	Q99JM0	Chd4 protein (Fragment)	1

535	Q9R1M5	NACHT_ LRR and PYD domains-containing protein 5	1
536	A0A0R4J031	Tripartite motif-containing protein 35	1
537	A0A0G2JDJ5	Farnesyl pyrophosphate synthase (Fragment)	1
538	Q8BX05	Putative glycerol kinase 5	1
539	G3X9Y9	Unconventional myosin-Vb	1
540	A2A4L0	Pleckstrin homology domain-containing family H member 3 (Fragment)	1
541	Q2VIS4	Filaggrin-2	1
542	A0A0U1RNJ1	Fatty acid synthase	1
543	Q3TWN8	Delta-1-pyrroline-5-carboxylate synthase	1
544	A0A140T8I6	Epithelial-stromal interaction protein 1	1
545	Q3UKQ2	Uncharacterized protein	1
546	E3VVQ8	Supervillin muscle-specific isoform	1
547	P32443	Homeobox protein MOX-2	1
548	H3BL56	Rho-related GTP-binding protein RhoC	1
549	Q2VPQ9	Chromatin modification-related protein MEAF6	1
550	B2RQR2	Mpdz protein	1
551	Q059T5	Mannoside acetylglucosaminyltransferase 5	1
552	A0A0J9YVH8	Ras/Rap GTPase-activating protein SynGAP (Fragment)	1
553	A0A0R4J0G0	Phosphoenolpyruvate carboxykinase [GTP]_ mitochondrial	1
554	Q8VEH6	COBW domain-containing protein 1	1
555	P47708	Rabphilin-3A	1
556	A0A5F8MPY3	Focal adhesion kinase 1	1
557	S4R1U4	Ankyrin-3 (Fragment)	1
558	E9Q470	Serine/threonine-protein kinase WNK4	1
559	A0A0R4J1E2	Elongation factor 1-delta	1
560	Q3TWV0	IF rod domain-containing protein	1
561	A2AH85	116 kDa U5 small nuclear ribonucleoprotein component	1
562	Q3TCI7	L-lactate dehydrogenase	1
563	E9Q1U1	Coiled-coil domain-containing protein 171	1
564	D3Z5N5	1-phosphatidylinositol 3-phosphate 5-kinase	1
565	P52800	Ephrin-B2	1
566	B2RXQ2	Ppfia1 protein	1
567	B9EKJ7	Tankyrase 1 binding protein 1	1
568	Q6DFV1	Condensin-2 complex subunit G2	1
569	Q99M73	Keratin_ type II cuticular Hb4	1
570	Q8BJQ7	Glycine cleavage system P protein	1
571	A0A140LJJ5	A-kinase anchor protein 13	1

572	Q76MZ3	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	1
573	A0A2I3BQA5	Leucine-rich repeat and guanylate kinase domain-containing protein	1
574	A1ILG8	Chorein	1
575	Q3UHT7	ERC protein 2	1
576	G3X963	ATPase family AAA domain-containing protein 2	1
577	Q3U046	WW domain-containing protein (Fragment)	1
578	Q9Z329	Inositol 1_4_5-trisphosphate receptor type 2	1
579	Q99JX6	Annexin	1
580	P36895	Bone morphogenetic protein receptor type-1A	1
581	A0A0A0MQG2	Spectrin beta chain_ non-erythrocytic 1 (Fragment)	1
582	A0A2X0SFE3	ECT2 (Fragment)	1
583	A0A0A0MQM2	Phosphoinositide phospholipase C	1
584	A0A589P973	Neuron navigator 1	1
585	A0A0J9YUB5	Zinc finger protein 433 (Fragment)	1
586	A0A1B0GRB7	VPS10 domain-containing receptor SorCS1	1
587	B2RUR8	OTU domain-containing protein 7B	1
588	Q8BN57	Protein C3orf33 homolog	1
589	Q3UX10	Tubulin alpha chain-like 3	1
590	B2RUJ7	Xanthine dehydrogenase	1
591	D3YZV5	SHC-transforming protein 1 (Fragment)	1
592	B2RQD1	Cat eye syndrome chromosome region_ candidate 6 homolog (Human)	1
593	A0A1B0GRV3	MHC class II regulatory factor RFX1	1
594	Q8BTJ9	Aamy domain-containing protein	1
595	A0A076N9U7	Celsr3	1
596	Q3ULG5	DNA helicase	1
597	Q61484	Desmoyokin (Fragment)	1
598	E9Q9B0	Kinesin-like protein KIF1C	1
599	Q99PA7	Mage-k1	1
600	Q8K2B3	Succinate dehydrogenase [ubiquinone] flavoprotein subunit_ mitochondrial	1
601	A0A2X0SSQ6	TIAM2 (Fragment)	1
602	Q5DTH5	Teashirt homolog 1	1
603	P60867	40S ribosomal protein S20	1
604	B9EKJ1	Spna2 protein	1
605	A0A087WQG8	DNA mismatch repair protein	1
606	A0A2I3BRL8	Predicted gene 7324	1

607	Q3U0T9	Rab35	1
608	G3X8T2	Zinc finger CCCH domain-containing protein 18	1
609	F6WJB7	Ubiquitin carboxyl-terminal hydrolase 34 (Fragment)	1
610	B1AR17	Chromodomain helicase DNA-binding protein 3	1
611	Q925L0	Protocadherin-betaV	1
612	A0A494B953	E3 ubiquitin-protein ligase NEDD4-like	1
613	A2A5Y4	KAT8 regulatory NSL complex subunit 1	1
614	Q9CTX4	Uncharacterized protein (Fragment)	1
615	Q91Z58	Uncharacterized protein C6orf132 homolog	1
616	Q91XV3	Brain acid soluble protein 1	1
617	A0A2I3BQB9	Roundabout homolog 2 (Fragment)	1
618	A0A411ACW5	Voltage-dependent calcium channel alpha2/delta-2 transcript variant 3	1
619	F8VQD7	Receptor-type tyrosine-protein phosphatase gamma	1
620	Q6RI64	Proteasome subunit beta	1
621	A2AQ07	Tubulin beta-1 chain	1
622	C6EQK4	L1 unspliced fusion gene protein	1
623	A2AHX7	Bcl-2-like protein 1 (Fragment)	1
624	E9PWT2	Zinc finger protein 229	1
625	E9PZF5	Acidic leucine-rich nuclear phosphoprotein 32 family member E (Fragment)	1
626	A0A0G2JGS4	Calcium/calmodulin-dependent protein kinase type II subunit delta	1
627	H3BJ30	Cleavage and polyadenylation-specificity factor subunit 6	1
628	E9PWQ3	Collagen_ type VI_ alpha 3	1
629	A0A0R4J0B6	Broad substrate-specificity ATP-binding cassette transporter ABCG2	1
630	B7ZMV3	3110001K24Rik protein (Fragment)	1
631	Q5DTK7	MKIAA4158 protein (Fragment)	1
632	A9C471	Baculoviral IAP repeat-containing protein 1b	1
633	A2A9R4	ELAV-like protein 4 (Fragment)	1
634	P97807	Fumarate hydratase_ mitochondrial	1
635	K3W4P8	Sodium channel protein	1
636	Q05793	Basement membrane-specific heparan sulfate proteoglycan core protein	1
637	E9Q0B6	Dynein_ axonemal_ heavy chain 6	1
638	Q52KI6	Zinc finger protein 780B	1
639	E9PUN0	Neurexin-2	1
640	E9PWL1	Voltage-dependent T-type calcium channel subunit alpha	1
641	A0A0R4J0X7	Glyceraldehyde-3-phosphate dehydrogenase	1
642	A0A087WR50	Fibronectin	1
643	Q640L5	Coiled-coil domain-containing protein 18	1

644	P47963	60S ribosomal protein L13	1
645	A0A1L1SV25	Alpha-actinin-4	1
646	E9Q9C3	Afadin	1
647	A0A0A0MQD9	PR domain-containing protein 11	1
648	A1Z198-2	Isoform 2 of NACHT_ LRR and PYD domains-containing protein 1b allele 2	1
649	B2RWX5	Centrosomal protein 250	1
650	Q80TY5	Vacuolar protein sorting-associated protein 13B	1
651	A0A1B4Z9N9	Breast cancer 1_ early onset (Fragment)	1
652	Q9JL35	High mobility group nucleosome-binding domain-containing protein 5	1
653	E9Q3Y4	Lipopolysaccharide-responsive and beige-like anchor protein	1
654	A0A087WNV6	5'-3' exoribonuclease 1	1
655	Q3TXD3	PKS_ER domain-containing protein	1
656	E9QK83	Small subunit processome component 20 homolog	1
657	Q8C2T9	Uncharacterized protein	1
658	A0A1Y7VM45	Pre-mRNA-processing factor 39 (Fragment)	1
659	D3YVM5	60S acidic ribosomal protein P0 (Fragment)	1
660	F8WGW3	S1 RNA-binding domain-containing protein 1	1
661	Q9JM52	Misshapen-like kinase 1	1
662	Q3UDN8	Uncharacterized protein (Fragment)	1
663	Q03265	ATP synthase subunit alpha_ mitochondrial	1
664	D3YXK2	Scaffold attachment factor B1	1
665	B1AZI6	THO complex subunit 2	1
666	E9Q9I2	Disks large homolog 5	1
667	Q3U6K8	Uncharacterized protein	1
668	E9Q8F8	ATP-binding cassette_ sub-family A (ABC1)_ member 14	1
669	D3Z4A4	Peroxiredoxin-2 (Fragment)	1
670	Q0VAV5	RAS protein activator like 2	1
671	E3WH32	Swi5 protein (Fragment)	1
672	A0A2X0SZ45	SRGAP2 (Fragment)	1
673	A0A087WQE8	Kinesin-like protein KIF1A	1
674	D6RHT6	E3 ubiquitin-protein ligase TTC3 (Fragment)	1
675	P24457	Cytochrome P450 2D11	1
676	Q3TIG8	Uncharacterized protein	1
677	A0A0A6YWJ1	Transforming protein RhoA (Fragment)	1
678	Q9ERD7	Tubulin beta-3 chain	1
679	Q9ERU9	E3 SUMO-protein ligase RanBP2	1
680	A2RSJ4	UHRF1-binding protein 1-like	1

681	A0A0R4J1W4	Tectonic-3	1
682	Q155P7	LEK1	1
683	A2AT70	Titin (Fragment)	1
684	Q3V2V4	Uncharacterized protein	1
685	D3Z6R1	Predicted gene 7361	1
686	Q8CDK2-5	Isoform 5 of Cytosolic carboxypeptidase 2	1
687	Q8K3G7	Reticulon	1
688	D3Z1A5	A disintegrin-like and metallopeptidase (reprolysin type) with thromb	1
689	P62932	F-box only protein 40	1
690	H3BLL4	Heterogeneous nuclear ribonucleoprotein K	1
691	Q543H0	Uncharacterized protein	1
692	A0A068EW80	Promyelocytic leukemia	1
693	Q6ZPE7	MKIAA3015 protein (Fragment)	1
694	P11679	Keratin_ type II cytoskeletal 8	1
695	A0A068BD65	Putative retinoid X receptor beta isoform 2	1
696	Q3TRH8	F-actin-capping protein subunit beta	1
697	P13542	Myosin-8	1
698	O08648	Mitogen-activated protein kinase kinase kinase 4	1
699	A0A1D5RLQ9	Non-specific serine/threonine protein kinase	1
700	Q5DTX3	MKIAA1490 protein (Fragment)	1
701	A2AW41	Heterogeneous nuclear ribonucleoprotein R (Fragment)	1
702	A2ANL9	Teneurin-1	1
703	P05201	Aspartate aminotransferase_ cytoplasmic	1
704	B0LAC5	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (Fragment)	1
705	Q7TQ39	Gag protein	1
706	P59240	Nephrocystin-4	1
707	Q3TZL2	Uncharacterized protein (Fragment)	1
708	Q80TB2	MKIAA1621 protein (Fragment)	1
709	B2RRC9	Coagulation factor VIII	1
710	A0A3Q4EHZ1	Cap-specific mRNA (nucle	1
711	F8WHT3	Protein PRRC2B	1
712	Q3TRW3	Staphylococcal nuclease domain-containing protein	1
713	B7ZP47	Wapal protein	1
714	D3YXM8	Mitogen-activated protein kinase kinase kinase	1
715	Q1WWL0	Cgrrf1 protein (Fragment)	1
716	Q9CZS1	Aldehyde dehydrogenase X_ mitochondrial	1
717	E9QMD3	Zinc finger homeobox protein 3	1
718	P40201	Chromodomain-helicase-DNA-binding protein 1	1

719	Q8BZQ2	Cysteine-rich secretory protein LCCL domain-containing 2	1
720	B9EIX4	C-type lectin domain-containing protein	1
721	A0A0N4SVM5	Eukaryotic translation initiation factor 4A3-like 1 (Fragment)	1
722	A2A813	Protein/nucleic acid deglycase DJ-1	1
723	Q9CWZ5	Uncharacterized protein	1
724	E9PWJ7	ATP-binding cassette_ sub-family A (ABC1)_ member 16	1
725	A0A571BG24	LIM and calponin homology domains-containing protein 1	1
726	Q99N18	Cytochrome P450 CYP4F15	1
727	P28481	Collagen alpha-1(II) chain	1
728	A4FUV6	Met protein (Fragment)	1
729	Q3UNT7	ANK_REP_REGION domain-containing protein	1
730	B2M1R6	Heterogeneous nuclear ribonucleoprotein K	1
731	A0A0R4J259	Heterogeneous nuclear ribonucleoprotein Q	1
732	Q3UXI9	Uncharacterized protein	1
733	E9PWB6	Mucin 5_ subtypes A and C_ tracheobronchial/gastric	1
734	A0A0R4J034	Pyridoxal-dependent decarboxylase domain-containing protein 1	1
735	F6QKE4	Immunoglobulin-like and fibronectin type III domain-containing protein 1 (Fragment)	1
736	Q80TV8-2	Isoform 2 of CLIP-associating protein 1	1
737	A0A0R4J1M7	Sodium channel protein	1
738	A0A0G2JGB7	Leucine-rich glioma-inactivated protein 1	1
739	Q80YG4	SEC63	1
740	B1ATP7	Zinc phosphodiesterase ELAC protein 2	1
741	P05063	Fructose-bisphosphate aldolase C	1
742	Q5FWB7	Fructose-bisphosphate aldolase	1
743	P80314	T-complex protein 1 subunit beta	1
744	F2Z3U3	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1	1
745	E9PV66	Myosin XVIIIb	1
746	G3X8Q1	Calcineurin-binding protein 1	1
747	B9EJ77	Tanc1 protein	1
748	E9QAE4	Histone-lysine N-methyltransferase	1
749	E0CY23	Heat shock 70 kDa protein 4L (Fragment)	1
750	A2ICR0	PHEX	1
751	A0A0R4J0K2	Cytoskeleton-associated protein 5	1
752	E9Q0T8	Dynein_ axonemal_ heavy chain 7A	1
753	Q58E64	Elongation factor 1-alpha	1
754	A0A1B0GRH2	Receptor-type tyrosine-protein phosphatase	1
755	Q9QZ84	LEK1 (Fragment)	1

756	A0A1L1SQZ0	tRNA (guanine(10)-N2)-methyltransferase homolog (Fragment)	1
757	A0A0G2JGW3	Zinc finger CCHC domain-containing protein 8 (Fragment)	1
758	A0A0R4J0B1	Non-specific serine/threonine protein kinase	1
759	A0A1B0GRJ0	Rab11 family-interacting protein 1	1
760	A2AHW8	GTPase-activating Rap/Ran-GAP domain-like protein 3	1
761	O88493	Type VI collagen alpha 3 subunit	1
762	A0A140LIF9	Aldehyde dehydrogenase family 1 member A3	1
763	A0A0A1HAM8	80K protein	1
764	A0A0A6YWS6	UTP25 small subunit processome component	1
765	Q3UA58	14_3_3 domain-containing protein (Fragment)	1
766	A0A0R4J211	Rho guanine nucleotide exchange factor 25	1
767	Q9ES52	Phosphatidylinositol 3_4_5-trisphosphate 5-phosphatase 1	1
768	Q80TB8	Synaptic vesicle membrane protein VAT-1 homolog-like	1
769	A2A5N2	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein_ beta polypeptide	1
770	P54728	UV excision repair protein RAD23 homolog B	1
771	P97929	Breast cancer type 2 susceptibility protein homolog	1
772	E0CYH7	Filamin A-interacting protein 1-like	1
773	Q99LF1	Akirin-1	1
774	Q6P208	Regulator of G-protein signaling 17	1
775	Q5H8C4	Vacuolar protein sorting-associated protein 13A	1
776	A6PWD2	Forkhead-associated domain-containing protein 1	1
777	A0A494B952	Isochorismatase domain-containing protein 1	1
778	Q3UC72	Rab GDP dissociation inhibitor (Fragment)	1
779	P63276	40S ribosomal protein S17	1
780	A0A0J9YUW5	Sodium channel protein	1
781	G3XA08	Zinc finger protein 26	1
782	Q6P5E4	UDP-glucose:glycoprotein glucosyltransferase 1	1
783	D3YZB0	Family with sequence similarity 129_ member C	1
784	A0A0N4SUJ8	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 (Fragment)	1
785	P32067	Lupus La protein homolog	1
786	P52480-2	Isoform M1 of Pyruvate kinase PKM	1
787	Q70FJ1	A-kinase anchor protein 9	1
788	A2CG77	Histone-lysine N-methyltransferase EHMT2 (Fragment)	1
789	E9PW83	Family with sequence similarity 184_ member A	1
790	Q5SSW2	Proteasome activator complex subunit 4	1
791	B7ZNH9	Phosphoinositide phospholipase C	1

792	A2AC24	WD repeat-containing protein 13 (Fragment)	1
793	Q6IMP4	Pannexin-2	1
794	P03975	IgE-binding protein	1
795	Q3TAI8	Uncharacterized protein	1
796	K7N6S0	Spermatogenesis-associated glutamate (E)-rich protein 4B (Fragment)	1
797	M0QWQ9	Predicted gene 17174	1
798	P28738	Kinesin heavy chain isoform 5C	1
799	A0A1Y7VMN0	Rho-associated protein kinase 2 (Fragment)	1
800	P43274	Histone H1.4	1
801	P70206	Plexin-A1	1
802	A0A5F8MQ58	Periaxin (Fragment)	1
803	G1K381	Ephrin type-A receptor 6	1
804	A0A2R8W6V7	Myosin-9 (Fragment)	1
805	Q63870	Collagen alpha-1(VII) chain	1
806	O70479	BTB/POZ domain-containing adapter for CUL3-mediated RhoA degradation protein 2	1
807	P62141	Serine/threonine-protein ph	1
808	P58771	Tropomyosin alpha-1 chain	1
809	Q9JJD8	Brain cDNA_ clone MNCb-1272_ similar to Mus musculus chaperonin subunit 2 (beta) (Cct2)_ mRNA	1
810	P08228	Superoxide dismutase [Cu-Zn]	1
811	P35922-12	Isoform 12 of Synaptic functional regulator FMR1	1
812	E9Q0X7	Lysyl oxidase homolog 3	1
813	F6U3N7	Predicted gene 21977 (Fragment)	1
814	F7AXU7	PHD finger protein 12 (Fragment)	1
815	P27659	60S ribosomal protein L3	1
816	F6SXE5	Gametocyte-specific factor 1 (Fragment)	1
817	H3BKH3	DNA-binding protein SATB	1
818	A0A494BAN8	Dynein heavy chain 8_ axonemal (Fragment)	1
819	A0PJE6	Pccb protein (Fragment)	1
820	A2A9K3	Centrosomal protein of 85 kDa	1
821	Q4FK74	Atp5d protein	1
822	V9GXW1	KICSTOR complex protein SZT2 (Fragment)	1
823	E9PUA2	Terminal uridylyltransferase 7	1
824	A0A0R4J032	Complement component C9	1
825	P06745	Glucose-6-phosphate isomerase	1
826	E9QK36	WD repeat-containing protein 62	1
827	Q3UC67	AMP-binding domain-containing protein	1
828	A0A571BEL9	T-complex protein 1 subunit zeta	1

829	A2ATQ5	Anaphase-promoting complex subunit 1	1
830	Q60805	Tyrosine-protein kinase Mer	1
831	A0A1Y7VL99	Rab GDP dissociation inhibitor (Fragment)	1
832	A2AM56	ATP-binding cassette_ sub-family A (ABC1)_ member 8b	1
833	P97855	Ras GTPase-activating protein-binding protein 1	1
834	Q810U4	Neuronal cell adhesion molecule	1
835	O88282	B-cell CLL/lymphoma 6 member B protein	1
836	A0A1L1SV73	Ubiquitin carboxyl-terminal hydrolase 47	1
837	A0A0R4J140	Clustered mitochondria protein homolog	1
838	F4YJP8	Transient receptor potential M3 protein beta 2 variant	1
839	E9Q9G8	Intraflagellar transport protein 122 homolog	1
840	B1AV35	Dystrophin-related protein 2	1
841	A0A0R4J1C3	Predicted gene_ 43951	1
842	Q8VCI1	Zinc finger protein 566	1
843	Q8BFZ3	Beta-actin-like protein 2	1
844	P63038-2	Isoform 2 of 60 kDa heat shock protein_ mitochondrial	1
845	B1AT03	DNA ligase	1
846	F8WI35	Histone H3	1
847	Q8VI59	Pecanex-like protein 3	1
848	B2RQC6	CAD protein	1

Appendix 2: Proteins differentially expressed upon MGO treatment in neuro-2a cells

Acc. No.	Protein	Gene name	Fold change MGO to Control
Upregulated proteins			
A2AHW8	GTPase-activating Rap/Ran-GAP domain-like protein 3	Garnl3	1.31
A0A494B952	Isochorismatase domain-containing protein 1	Isoc1	1.31
A0A0G2JGB7	Leucine-rich glioma-inactivated protein 1	Lgi1	1.32
Q05816	Fatty acid-binding protein 5	Fabp5	1.32
Q80YR5	Scaffold attachment factor B2	Safb2	1.32
Q544Y7	Cofilin 1_ non-muscle	Cfl1	1.33
A0A494BBG8	Xaa-Pro aminopeptidase 1	Xpnpep1	1.33
Q14BY7	Regulating synaptic membrane exocytis 3	Rims3	1.34
P35922-12	Isoform 12 of Synaptic functional regulator FMR1	Fmr1	1.35
A0A0R4J1W4	Tectonic-3	Tctn3	1.37
A0A0N4SUJ8	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 (Fragment)	Rpn1	1.37
A0A3Q4EHZ1	Cap-specific mRNA (nucleide-2'-O-)-methyltransferase 1 (Fragment)	Cmtr1	1.37
A0A1Y7VLA4	Protein TASOR 2 (Fragment)	Tasor2	1.37
P08228	Superoxide dismutase [Cu-Zn]	Sod1	1.38
A9C471	Baculoviral IAP repeat-containing protein 1b	Naip2	1.38
B7ZP47	Wapal protein	Wapl	1.39
B9EKJ1	Spna2 protein	Sptan1	1.39
E9PW69	Proteasome subunit alpha type (Fragment)	Psma4	1.40
B1ATP7	Zinc phphodiesterase ELAC protein 2	Elac2	1.44
G3X8Q1	Calcineurin-binding protein 1	Cabin1	1.44
A0A0N4SVM5	Eukaryotic translation initiation factor 4A3-like 1 (Fragment)	Eif4a3l1	1.45
A0A5F8MQ58	Periaxin (Fragment)	Prx	1.45
A0A494BAN8	Dynein heavy chain 8_ axonemal (Fragment)	Dnah8	1.45
A2A813	Protein/nucleic acid deglycase DJ-1	Park7	1.45
Q6ZPT2	MKIAA1352 protein (Fragment)	Lars	1.46
O08553	Dihydropyrimidinase-related protein 2	Dpysl2	1.47
P24457	Cytochrome P450 2D11	Cyp2d11	1.47
F6SXE5	Gametocyte-specific factor 1 (Fragment)	Gtsf1	1.48

A0A140LJ69	Protein unc-13 homolog B	Unc13b	1.48
E9Q0A4	Kinesin-like protein KIF21B	Kif21b	1.48
P09411	Phosphoglycerate kinase 1	Pgk1	1.51
Q8CDK2-5	Isoform 5 of Cytosolic carboxypeptidase 2	Agbl2	1.51
B9EJ77	Tanc1 protein	Tanc1	1.52
A2A9K3	Centrosomal protein of 85 kDa	Cep85	1.54
A0A0J9YUD5	Nucleoporin 205	Nup205	1.54
A0A0A6YWS6	UTP25 small subunit processome component	Utp25	1.56
A2AL50	Alkylglycerone-phosphate synthase	Agps	1.57
A2CG77	Histone-lysine N-methyltransferase EHMT2 (Fragment)	Ehmt2	1.57
Q543H0	Uncharacterized protein	Srm	1.58
Q99PA7	Mage-k1	4930550L24Rik	1.58
A0A087WQT9	Voltage-dependent L-type calcium channel subunit alpha	Cacna1c	1.60
P05063	Fructose-bisphosphate aldolase C	Aldoc	1.60
P52480-2	Isoform M1 of Pyruvate kinase PKM	Pkm	1.64
P06745	Glucose-6-phosphate isomerase	Gpi	1.67
A3KGI3	Kinetochore scaffold 1	Knl1	1.68
Q8BPH1	14_3_3 domain-containing protein	Ywhae	1.68
Q70FJ1	A-kinase anchor protein 9	Akap9	1.68
A0A1Y7VL99	Rab GDP dissociation inhibitor (Fragment)	Gdi2	1.74
A0A0R4J211	Rho guanine nucleotide exchange factor 25	Arhgef25	1.75
A2AC24	WD repeat-containing protein 13 (Fragment)	Wdr13	1.79
E9QK36	WD repeat-containing protein 62	Wdr62	1.80
A2ATQ5	Anaphase-promoting complex subunit 1	Anapc1	1.80
P13542	Myosin-8	Myh8	1.84
A0A0J9YUB5	Zinc finger protein 433 (Fragment)	Zfp433	1.92
P70206	Plexin-A1	Plxna1	2.03
A6PWD2	Forkhead-associated domain-containing protein 1	Fhad1	2.07
A0A0U1RPN8	Fructose-bisphosphate aldolase A (Fragment)	Aldoa	2.18
E0CY23	Heat shock 70 kDa protein 4L (Fragment)	Hspa4l	2.25
Q3UC67	AMP-binding domain-containing protein	Acsl5	2.51
A0A571BEL9	T-complex protein 1 subunit zeta	Cct6a	2.55
Q8BFZ3	Beta-actin-like protein 2	Actbl2	2.60
A2AM56	ATP-binding cassette_ sub-family A (ABC1)_ member 8b	Abca8b	3.10
Q148T5	Tnc protein	Tnc	3.21
A0A1L1	Ubiquitin carboxyl-terminal hydrolase 47	Usp47	4.27

SV73			
B1AVK5	Collagen_ type IV_ alpha 6	Col4a6	4.31
P46660	Alpha-internexin	Ina	13.07
Q9Z329	Inositol 1_4_5-trisphosphate receptor type 2	Itpr2	39.17
Downregulated proteins			
B2RQC6	CAD protein	Cad	-31.53
Q8VI59	Pecanex-like protein 3	Pcnx3	-17.32
M0QWQ9	Predicted gene 17174	Gm17174	-13.16
B1AT03	DNA ligase	Lig3	-9.59
A0A0R4J140	Clustered mitochondria protein homolog	Cluh	-3.91
A0A0R4J0K2	Cytoskeleton-associated protein 5	Ckap5	-3.56
Q8VCI1	Zinc finger protein 566	Zfp566	-3.36
Q3TZL2	Uncharacterized protein (Fragment)	Sfpq	-3.31
V9GXW1	KICSTOR complex protein SZT2 (Fragment)	Szt2	-2.60
O88282	B-cell CLL/lymphoma 6 member B protein	Bcl6b	-2.53
Q7TQ39	Gag protein	gag	-2.47
H3BLL4	Heterogeneous nuclear ribonucleoprotein K	Hnrnpk	-2.28
P03975	IgE-binding protein	Iap	-2.27
P62631	Elongation factor 1-alpha 2	Eef1a2	-2.14
B2M1R6	Heterogeneous nuclear ribonucleoprotein K	Hnrnpk	-2.13
A0A1B0GRH2	Receptor-type tyrosine-protein phosphatase	Ptpre	-2.06
E0CZ27	Histone H3 (Fragment)	H3f3a	-2.00
Q1WWK3	Hist1h1b protein (Fragment)	H1f5	-1.94
Q5SSW2	Proteasome activator complex subunit 4	Psme4	-1.89
A0A0U1RNJ1	Fatty acid synthase	Fasn	-1.86
E9QMD3	Zinc finger homeobox protein 3	Zfhx3	-1.83
D3Z0R8	Tetratricopeptide repeat domain 7	Ttc7	-1.81
P28738	Kinesin heavy chain isoform 5C	Kif5c	-1.79
Q921L4	Histone H2B	LOC665622	-1.76
A0A1B0GSS6	Predicted gene 3854	Gm3854	-1.72
Q9Z1D2	Breast cancer type 1 susceptibility protein homolog	Brca1	-1.67
B2RTM0	Histone H4	Hist2h4	-1.66
P62932	F-box only protein 40	Fbxo40	-1.65
P43274	Histone H1.4	H1-4	-1.64
Q9CZN7	Serine hydroxymethyltransferase_ mitochondrial	Shmt2	-1.61
Q80TY5	Vacuolar protein sorting-associated protein 13B	Vps13b	-1.58
Q61667	Histone H4 (Fragment)	N/A	-1.56
A0A087	Vigilin	Hdlbp	-1.55

WP83			
Q8BV79	TPR and ankyrin repeat-containing protein 1	Trank1	-1.51
H7BX15	Adhesion G protein-coupled receptor L1	Adgrl1	-1.50
P47963	60S ribosomal protein L13	Rpl13	-1.50
D3YVM5	60S acidic ribosomal protein P0 (Fragment)	Rplp0	-1.49
E9Q0B6	Dynein_ axonemal_ heavy chain 6	Dnah6	-1.49
Q8K3G7	Reticulon	Rtn4	-1.48
Q6P5E4	UDP-glucose:glycoprotein glucosyltransferase 1	Uggt1	-1.48
P11679	Keratin_ type II cytkeletal 8	Krt8	-1.45
Q6B822	Histone H4 (Fragment)	N/A	-1.40
Q8BVI9	Uncharacterized protein	Slc25a4	-1.39
J3QMG3	Voltage-dependent anion-selective channel protein 3	Vdac3	-1.37
P60867	40S ribosomal protein S20	Rps20	-1.37
B2RUG9	Adenomatosis polyposis coli	Apc	-1.37
Q8BH59	Calcium-binding mitochondrial carrier protein Aralar1	Slc25a12	-1.36
P62315	Small nuclear ribonucleoprotein Sm D1	Snrpd1	-1.34
Q4KL76	Heat shock protein 1 (Chaperonin 10)	Hspe1	-1.34
Q3UDN8	Uncharacterized protein (Fragment)	Trim28	-1.33
A0A0G2JDE3	Adhesion G protein-coupled receptor L2	Adgrl2	-1.33
B2RVP5	Histone H2A	H2az2	-1.33
Q8K2B3	Succinate dehydrogenase [ubiquinone] flavoprotein subunit_ mitochondrial	Sdha	-1.32
Q542D9	Transferrin receptor protein 1	Tfrc	-1.30
B2RQD1	Cat eye syndrome chromosome region_ candidate 6 homolog (Human)	Tmem121b	-1.30
D4AFX7	DnaJ heat shock protein family (Hsp40) member C13	Dnajc13	-1.30
E9PYP2	Neurobeachin-like 1	Nbeal1	-1.30

Appendix 3:Biological processes of MGO altered neuro2a proteins

Biological Process	Acc.No.	Protein	Gene name
Upregulated Proteins			
ATP generation from ADP	P06745	Glucose-6-phosphate isomerase	Gpi1
	P05063	Fructose-bisphosphate aldolase C	Aldoc
	P09411	Phosphoglycerate kinase 1	Pgk1
Methylglyoxal metabolic process	A2A813	Protein/nucleic acid deglycase DJ-1	Park7
	P06745	Glucose-6-phosphate isomerase	Gpi
Cellular response to reactive oxygen species	P08228	Superoxide dismutase [Cu-Zn]	Sod1
	A2A813	Protein/nucleic acid deglycase DJ-1	Park7
	Q544Y7	Cofilin 1_ non-muscle	Cfl1
Response to hydrogen peroxide	P08228	Superoxide dismutase [Cu-Zn]	Sod1
	A2A813	Protein/nucleic acid deglycase DJ-1	Park7
	Q544Y7	Cofilin 1_ non-muscle	Cfl1
ATP metabolic process	P13542	Myosin-8	Myh8
	P06745	Glucose-6-phosphate isomerase	Gpi1
	P05063	Fructe-bisphphate aldolase C	Aldoc
	P09411	Phosphoglycerate kinase 1	Pgk1-rs7
Neurofilament cytoskeleton organization	P08228	Superoxide dismutase [Cu-Zn]	Sod1
	P46660	Alpha-internexin	Ina
Positive regulation of oxidative stress-induced intrinsic apoptotic signaling pathway	P08228	Superoxide dismutase [Cu-Zn]	Sod1
	A2A813	Protein/nucleic acid deglycase DJ-1	Park7
Regulation of NMDA receptor activity	A2A813	Protein/nucleic acid deglycase DJ-1	Park7
	Q544Y7	Cofilin 1_ non-muscle	Cfl1

Regulation of superoxide metabolic process	P08228	Superoxide dismutase [Cu-Zn]	Sod1
	A2A813	Protein/nucleic acid deglycase DJ-1	Park7
Negative regulation of neuron apoptotic process	P08228	Superoxide dismutase [Cu-Zn]	Sod1
	A2A813	Protein/nucleic acid deglycase DJ-1	Park7
	P06745	Glucose-6-phosphate isomerase	Gpi1
Glutathione metabolic process	Sod1	Superoxide dismutase [Cu-Zn]	P08228
	Park7	Protein/nucleic acid deglycase DJ-1	A2A813
Downregulated Proteins			
Regulation of branching morphogenesis of a nerve	B2M1R6	Heterogeneous nuclear ribonucleoprotein K	Hnrnpk
	Q8K3G7	Reticulon	Rtn4
Positive regulation of synapse maturation	B2M1R6	Heterogeneous nuclear ribonucleoprotein K	Hnrnpk
	H7BX15	Adhesion G protein-coupled receptor L1	Adgrl1

Appendix 4: list of identified proteins in rat hippocampal IDA library

Sr. No.	Acc. No.	Protein	Peptides (95%)
1	P16086	Spectrin alpha chain, non-erythrocytic 1	175
2	P11442	Clathrin heavy chain 1	124
3	P38650	Cytoplasmic dynein 1 heavy chain 1	123
4	P15146-2	Isoform 2 of Microtubule-associated protein 2	110
5	P15146	Microtubule-associated protein 2	109
6	P34926	Microtubule-associated protein 1A	105
7	P85108	Tubulin beta-2A chain	78
8	P12785	Fatty acid synthase	76
9	Q3KRE8	Tubulin beta-2B chain	74
10	Q6P9T8	Tubulin beta-4B chain	73

11	P47942	Dihydropyrimidinase-related protein 2	66
12	P69897	Tubulin beta-5 chain	66
13	P11980	Pyruvate kinase PKM	65
14	Q9QWN8	Spectrin beta chain, non-erythrocytic 2	64
15	Q4QRB4	Tubulin beta-3 chain	63
16	P11980-2	Isoform M2 of Pyruvate kinase PKM	63
17	P15205	Microtubule-associated protein 1B	62
18	P21575	Dynamin-1	62
19	P21575-4	Isoform 4 of Dynamin-1	62
20	P63018	Heat shock cognate 71 kDa protein	62
21	P21575-2	Isoform 2 of Dynamin-1	61
22	P21575-8	Isoform 8 of Dynamin-1	61
23	P21575-5	Isoform 5 of Dynamin-1	61
24	P21575-6	Isoform 6 of Dynamin-1	60
25	P07335	Creatine kinase B-type	58
26	P04797	Glyceraldehyde-3-ph	58
27	P21575-7	Isoform 7 of Dynamin-1	58
28	Q9ER34	Aconitate hydratase, mitochondrial	57
29	P82995	Heat shock protein HSP 90-alpha	55
30	P53534	Glycogen phosphorylase, brain form (Fragment)	55
31	Q62910	Synaptojanin-1	54
32	Q62910-2	Isoform 2 of Synaptojanin-1	54
33	Q62910-4	Isoform 4 of Synaptojanin-1	53
34	Q62910-5	Isoform 5 of Synaptojanin-1	53
35	P46462	Transitional endoplasmic reticulum ATPase	52
36	Q5U300	Ubiquitin-like modifier-activating enzyme 1	52
37	P63039	60 kDa heat shock protein, mitochondrial	52
38	P61765-2	Isoform 2 of Syntaxin-binding protein 1	51
39	P61765	Syntaxin-binding protein 1	51
40	P05065	Fructose-bisphosphate aldolase A	50
41	P68370	Tubulin alpha-1A chain	50
42	O88600	Heat shock 70 kDa protein 4	49
43	P50398	Rab GDP dissociation inhibitor alpha	48
44	P34058	Heat shock protein HSP 90-beta	48
45	P09117	Fructose-bisphosphate aldolase C	48
46	Q9QXQ0	Alpha-actinin-4	47
47	P06687	Sodium/potassium-transporting ATPase subunit alpha-3	47
48	P09951	Synapsin-1	47
49	Q6P6V0	Glucose-6-phosphate isomerase	47
50	P09812	Glycogen phosphorylase, muscle form	47
51	P10719	ATP synthase subunit beta, mitochondrial	47

52	P63259	Actin, cytoplasmic 2	47
53	P60711	Actin, cytoplasmic 1	47
54	Q5XIF6	Tubulin alpha-4A chain	47
55	Q9QUL6	Vesicle-fusing ATPase	46
56	P09951-2	Isoform IB of Synapsin-1	46
57	P62944	AP-2 complex subunit beta	46
58	P62944-2	Isoform 2 of AP-2 complex subunit beta	46
59	Q62950	Dihydropyrimidinase-related protein 1	46
60	P42123	L-lactate dehydrogenase B chain	46
61	Q63560	Microtubule-associated protein 6	45
62	P10860	Glutamate dehydrogenase 1, mitochondrial	45
63	P52873	Pyruvate carboxylase, mitochondrial	44
64	P12839	Neurofilament medium polypeptide	44
65	P04764	Alpha-enolase	44
66	P47819	Glial fibrillary acidic protein	44
67	P62260	14-3-3 protein epsilon	44
68	P62815	V-type proton ATPase subunit B, brain isoform	44
69	P05197	Elongation factor 2	43
70	P13233	2',3'-cyclic-nucleotide 3'-phosphodiesterase	43
71	P05708	Hexokinase-1	42
72	P47860	ATP-dependent 6-phosphofructokinase, platelet type	42
73	P23565	Alpha-internexin	41
74	Q5XI78	2-oxoglutarate dehydrogenase, mitochondrial	40
75	P19527	Neurofilament light polypeptide	40
76	P48721	Stress-70 protein, mitochondrial	40
77	P07323	Gamma-enolase	40
78	P18484	AP-2 complex subunit alpha-2	39
79	P04642	L-lactate dehydrogenase A chain	39
80	P50137	Transketolase	38
81	Q66HA8	Heat shock protein 105 kDa	38
82	P16617	Phosphoglycerate kinase 1	38
83	P50399	Rab GDP dissociation inhibitor beta	38
84	Q9WTT6	Guanine deaminase	36
85	P47858	ATP-dependent 6-phosphofructokinase, muscle type	36
86	P00507	Aspartate aminotransferase, mitochondrial	36
87	Q00981	Ubiquitin carboxyl-terminal hydrolase isozyme L1	36
88	Q9JK11	Reticulon-4	35
89	O35814	Stress-induced-phosphoprotein 1	35
90	P13221	Aspartate aminotransferase, cytoplasmic	35
91	Q63537-2	Isoform IIb of Synapsin-2	35
92	P97536	Cullin-associated NEDD8-dissociated protein 1	34

93	P02770	Serum albumin	34
94	P06761	Endoplasmic reticulum chaperone BiP	34
95	P63102	14-3-3 protein zeta/delta	34
96	Q62952-2	Isoform 2 of Dihydropyrimidinase-related protein 3	34
97	Q62952	Dihydropyrimidinase-related protein 3	34
98	Q9Z1P2	Alpha-actinin-1	34
99	P06685	Sodium/potassium-transporting ATPase subunit alpha-1	34
100	P16638	ATP-citrate synthase	33
101	P16638-2	Isoform 2 of ATP-citrate synthase	33
102	P63329-2	Isoform 2 of Serine/threonine-protein phosphatase 2B catalytic subunit alpha isoform	33
103	Q05140-2	Isoform 2 of Clathrin coat assembly protein AP180	33
104	P50554	4-aminobutyrate aminotransferase, mitochondrial	33
105	Q63537	Synapsin-2	33
106	P68035	Actin, alpha cardiac muscle 1	33
107	P63329	Serine/threonine-protein phosphatase 2B catalytic subunit alpha isoform	33
108	P50475	Alanine--tRNA ligase, cytoplasmic	32
109	Q9JHU0	Dihydropyrimidinase-related protein 5	32
110	Q05140	Clathrin coat assembly protein AP180	32
111	P68511	14-3-3 protein eta	32
112	P52303	AP-1 complex subunit beta-1	32
113	P52303-2	Isoform B of AP-1 complex subunit beta-1	32
114	P68255	14-3-3 protein theta	32
115	P62738	Actin, aortic smooth muscle	32
116	P63269	Actin, gamma-enteric smooth muscle	32
117	P06686	Sodium/potassium-transporting ATPase subunit alpha-2	32
118	Q6AY56	Tubulin alpha-8 chain	32
119	P31000	Vimentin	31
120	P11598	Protein disulfide-isomerase A3	31
121	P85972	Vinculin	31
122	P13264	Glutaminase kidney isoform, mitochondrial	31
123	P51650	Succinate-semialdehyde dehydrogenase, mitochondrial	31
124	Q63198	Contactin-1	30
125	P35213	14-3-3 protein beta/alpha	30
126	P35213-2	Isoform Short of 14-3-3 protein beta/alpha	30
127	Q5XIM9	T-complex protein 1 subunit beta	29
128	O08838	Amphiphysin	29
129	Q62951	Dihydropyrimidinase-related protein 4 (Fragment)	29
130	P14408	Fumarate hydratase, mitochondrial	29
131	P14408-2	Isoform Cytoplasmic of Fumarate hydratase, mitochondrial	29
132	Q5RKI0	WD repeat-containing protein 1	28
133	O35303	Dynamin-1-like protein	28

134	O35303-4	Isoform 4 of Dynamin-1-like protein	28
135	Q62717	Calcium-dependent secretion activator 1	28
136	Q05546	Tenascin-R	28
137	P28023	Dynactin subunit 1	28
138	P15999	ATP synthase subunit alpha, mitochondrial	28
139	P25113	Phosphoglycerate mutase 1	28
140	O88989	Malate dehydrogenase, cytoplasmic	28
141	P20651	Serine/threonine-protein phosphatase 2B catalytic subunit beta isoform	28
142	O35303-5	Isoform 5 of Dynamin-1-like protein	27
143	O35303-2	Isoform 2 of Dynamin-1-like protein	27
144	Q63028	Alpha-adducin	27
145	P01026	Complement C3	26
146	Q66HD0	Endoplasmin	26
147	P11884	Aldehyde dehydrogenase, mitochondrial	26
148	Q68FS4	Cytosol aminopeptidase	26
149	Q68FS4-2	Isoform 2 of Cyt	26
150	P10760	Adenosylhomocysteinase	26
151	Q8VHF5	Citrate synthase, mitochondrial	26
152	O35567	Bifunctional purine biosynthesis protein PURH	25
153	Q63028-2	Isoform 2 of Alpha-adducin	25
154	P12346	Serotransferrin	25
155	P19332-4	Isoform Tau-D of Microtubule-associated protein tau	25
156	P19332-5	Isoform Tau-E of Microtubule-associated protein tau	25
157	O08839	Myc box-dependent-interacting protein 1	25
158	O08839-2	Isoform AMPH2-2 of Myc box-dependent-interacting protein 1	25
159	Q08163	Adenylyl cyclase-associated protein 1	25
160	P16884	Neurofilament heavy polypeptide	25
161	P27139	Carbonic anhydrase 2	25
162	Q9ERH3	WD repeat-containing protein 7	24
163	Q9ERH3-2	Isoform 2 of WD repeat-containing protein 7	24
164	O08839-5	Isoform AMPH2-5 of Myc box-dependent-interacting protein 1	24
165	P62632	Elongation factor 1-alpha 2	24
166	P47728	Calretinin	24
167	P30835	ATP-dependent 6-phosphofructokinase, liver type	24
168	Q99NA5	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	24
169	P08009	Glutathione S-transferase Yb-3	24
170	P61983	14-3-3 protein gamma	24
171	P62630	Elongation factor 1-alpha 1	24
172	P19332-3	Isoform Tau-C of Microtubule-associated protein tau	24
173	Q64560	Tripeptidyl-peptidase 2	23

174	Q9JLT0	Myosin-10	23
175	P10687	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-1	23
176	P97685	Neurofascin	23
177	P13596	Neural cell adhesion molecule 1	23
178	Q63041	Alpha-1-macroglobulin	23
179	P28037	Cytosolic 10-formyltetrahydrofolate dehydrogenase	23
180	P85970	Actin-related protein 2/3 complex subunit 2	23
181	Q9Z0W5	Protein kinase C and casein kinase substrate in neurons protein 1	23
182	P48500	Triosephosphate isomerase	23
183	P02091	Hemoglobin subunit beta-1	23
184	P14046	Alpha-1-inhibitor 3	22
185	O35095	Neurochondrin	22
186	P28480	T-complex protein 1 subunit alpha	22
187	P85834	Elongation factor Tu, mitochondrial	22
188	P85845	Fascin	22
189	P18418	Calreticulin	22
190	Q920L2	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	22
191	P09606	Glutamine synthetase	22
192	P04636	Malate dehydrogenase, mitochondrial	22
193	P25809	Creatine kinase U-type, mitochondrial	22
194	P04692-5	Isoform 5 of Tropomyosin alpha-1 chain	22
195	P10111	Peptidyl-prolyl cis-trans isomerase A	22
196	P14659	Heat shock-related 70 kDa protein 2	22
197	P19332-7	Isoform Tau-G of Microtubule-associated protein tau	22
198	P30427-2	Isoform 2 of Plectin	21
199	P30427	Plectin	21
200	P30427-4	Isoform 4 of Plectin	21
201	P30427-3	Isoform 3 of Plectin	21
202	Q63610	Tropomyosin alpha-3	21
203	Q68FQ0	T-complex protein 1 subunit epsilon	21
204	Q4V8B0	Oxidation resistance protein 1	21
205	P04785	Protein disulfide-isomerase	21
206	Q63617	Hypoxia up-regulated protein 1	21
207	Q64548	Reticulon-1	21
208	P0C1X8	AP2-associated protein kinase 1	21
209	P38652	Phosphoglucomutase-1	21
210	P08461	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	21
211	Q6Q0N1	Cytosolic non-specific dipeptidase	21
212	P04905	Glutathione S-transferase Mu 1	21
213	Q64428	Trifunctional enzyme subunit alpha, mitochondrial	21

214	P61265	Syntaxin-1B	21
215	P11517	Hemoglobin subunit beta-2	21
216	Q01986	Dual specificity mitogen-activated protein kinase kinase 1	21
217	P11348	Dihydropteridine reductase	21
218	O35077	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	20
219	B3GNI6	Septin-11	20
220	B3GNI6-3	Isoform 3 of Septin-11	20
221	B3GNI6-2	Isoform 2 of Septin-11	20
222	Q6P502	T-complex protein 1 subunit gamma	20
223	P56574	Isocitrate dehydrogenase [NADP], mitochondrial	20
224	P63004	Platelet-activating factor acetylhydrolase IB subunit alpha	20
225	P26284	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	20
226	O35179	Endophilin-A1	20
227	Q5HZV9	Protein phosphatase 1 regulatory subunit 7	20
228	Q63416	Inter-alpha-trypsin inhibitor heavy chain H3	20
229	Q9WVC0	Septin-7	20
230	P0DP31	Calmodulin-3	20
231	P0DP30	Calmodulin-2	20
232	P0DP29	Calmodulin-1	20
233	Q08877-9	Isoform 8 of Dynamin-3	20
234	Q08877-7	Isoform 7 of Dynamin-3	20
235	Q08877-13	Isoform 12 of Dynamin-3	20
236	Q08877-12	Isoform 11 of Dynamin-3	20
237	Q08877-11	Isoform 10 of Dynamin-3	20
238	Q08877-10	Isoform 9 of Dynamin-3	20
239	Q63610-2	Isoform 2 of Tropomyosin alpha-3 chain	20
240	Q63610-3	Isoform 3 of Tropomyosin alpha-3 chain	20
241	P10362	Secretogranin-2	19
242	P52481	Adenylyl cyclase-associated protein 2	19
243	P63086	Mitogen-activated protein kinase 1	19
244	O35331	Pyridoxal kinase	19
245	P20059	Hemopexin	19
246	Q6AY84	Secernin-1	19
247	D3ZQG6	Tripartite motif-containing protein 2	19
248	O35244	Peroxiredoxin-6	19
249	O35987	NSFL1 cofactor p47	19
250	Q68FY0	Cytochrome b-c1 complex subunit 1, mitochondrial	19
251	P45592	Cofilin-1	19
252	Q5I0G4	Glycine--tRNA ligase	19
253	P60881	Synaptosomal-associated protein 25	19
254	Q6AYH5	Dynactin subunit 2	19

255	B5DFN2	S-adenosylhomocysteine hydrolase-like protein 1	19
256	Q64559	Cytosolic acyl coenzyme A thioester hydrolase	19
257	Q64559-1	Isoform 1 of Cytosolic acyl coenzyme A thioester hydrolase	19
258	Q63716	Peroxiredoxin-1	19
259	P31044	Phosphatidylethanolamine-binding protein 1	19
260	P04906	Glutathione S-transferase P	19
261	Q08877	Dynamin-3	19
262	Q08877-6	Isoform 6 of Dynamin-3	19
263	Q08877-5	Isoform 5 of Dynamin-3	19
264	Q08877-4	Isoform 4 of Dynamin-3	19
265	Q08877-3	Isoform 3 of Dynamin-3	19
266	Q08877-2	Isoform 2 of Dynamin-3	19
267	P04692-2	Isoform 2 of Tropomyosin alpha-1 chain	19
268	P04692-3	Isoform 3 of Tropomyosin alpha-1 chain	19
269	Q02253	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	18
270	Q63598	Plastin-3	18
271	P12369	cAMP-dependent protein kinase type II-beta regulatory subunit	18
272	P52296	Importin subunit beta-1	18
273	Q05764	Beta-adducin	18
274	Q05764-2	Isoform 2 of Beta-adducin	18
275	P22062	Protein-L-isoaspartate(D-aspartate) O-methyltransferase	18
276	P11275	Calcium/calmodulin-dependent protein kinase type II subunit alpha	18
277	B2GV06	Succinyl-CoA:3-ketoacid coenzyme A transferase 1, mitochondrial	18
278	Q6AYS7	Aminoacylase-1A	18
279	Q9QYU4	Ketimine reductase mu-crystallin	18
280	Q6DGG0	Peptidyl-prolyl cis-trans isomerase D	18
281	Q6RJR6	Reticulon-3	18
282	P85973	Purine nucle	18
283	P59215	Guanine nucleotide-binding protein G(o) subunit alpha	18
284	P39069	Adenylate kinase isoenzyme 1	18
285	P07171	Calbindin	18
286	P37805	Transgelin-3	18
287	Q63270	Cytoplasmic aconitate hydratase	18
288	Q8VBU2	Protein NDRG2	18
289	P49432	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	18
290	P35704	Peroxiredoxin-2	18
291	P70566	Tropomodulin-2	18
292	Q5SGE0	Leucine-rich PPR motif-containing protein, mitochondrial	17
293	P97686-3	Isoform 3 of Neuronal cell adhesion molecule	17

294	P97686-2	Isoform 2 of Neuronal cell adhesion molecule	17
295	Q9WTP0-2	Isoform L of Band 4.1-like protein 1	17
296	Q9WTP0	Band 4.1-like protein 1	17
297	Q7TPB1	T-complex protein 1 subunit delta	17
298	Q6P6R2	Dihydrolipoyl dehydrogenase, mitochondrial	17
299	Q07266	Drebrin	17
300	P85969	Beta-soluble NSF attachment protein	17
301	Q5PPJ9	Endophilin-B2	17
302	P85515	Alpha-centractin	17
303	Q68FX0	Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial	17
304	Q4FZT9	26S proteasome non-ATPase regulatory subunit 2	17
305	O88761	26S proteasome non-ATPase regulatory subunit 1	17
306	Q99PD4	Actin-related protein 2/3 complex subunit 1A	17
307	P61980	Heterogeneous nuclear ribonucleoprotein K	17
308	Q62656	Receptor-type tyrosine-protein phosphatase zeta	17
309	Q62656-3	Isoform 3 of Receptor-type tyrosine-protein phosphatase zeta	17
310	A0JPJ7	Obg-like ATPase 1	17
311	P17764	Acetyl-CoA acetyltransferase, mitochondrial	17
312	P13697	NADP-dependent malic enzyme	17
313	P25286	V-type proton ATPase 116 kDa subunit a isoform 1	17
314	P25286-2	Isoform II of V-type proton ATPase 116 kDa subunit a isoform 1	17
315	Q4FZY0	EF-hand domain-containing protein D2	17
316	P09527	Ras-related protein Rab-7a	17
317	Q3T1K5	F-actin-capping protein subunit alpha-2	17
318	Q07009	Calpain-2 catalytic subunit	17
319	P01946	Hemoglobin subunit alpha-1/2	17
320	P59215-2	Isoform Alpha-2 of Guanine nucleotide-binding protein G(o) subunit alpha	17
321	P04692-6	Isoform 6 of Tropomyosin alpha-1 chain	17
322	P97686	Neuronal cell adhesion molecule	17
323	P97686-2	Isoform 2 of Neuronal cell adhesion molecule	17
324	P60881-2	Isoform 2 of Synaptosomal-associated protein 25	17
325	O09175	Aminopeptidase B	16
326	P41499	Tyrosine-protein phosphatase non-receptor type 11	16
327	Q07266-2	Isoform E1 of Drebrin	16
328	Q9ESW0	DNA damage-binding protein 1	16
329	Q4V7C7	Actin-related protein 3	16
330	P07943	Aldo-keto reductase family 1 member B1	16
331	Q5RKI1	Eukaryotic initiation factor 4A-II	16
332	Q01066	Calcium/calmodulin-dependent 3',5'-cyclic nucleotide ph	16
333	Q63622	Disks large homolog 2	16
334	Q63622-4	Isoform 4 of Disks large homolog 2	16

335	Q63622-3	Isoform 3 of Disks large homolog 2	16
336	Q63622-2	Isoform 2 of Disks large homolog 2	16
337	P21707	Synaptotagmin-1	16
338	Q63347	26S proteasome regulatory subunit 7	16
339	P55068	Brevican core protein	16
340	P62198	26S proteasome regulatory subunit 8	16
341	Q9JLJ3	4-trimethylaminobutyraldehyde dehydrogenase	16
342	Q64057	Alpha-aminoadipic semialdehyde dehydrogenase	16
343	Q6PCU2	V-type proton ATPase subunit E 1	16
344	P25093	Fumarylacetoacetase	16
345	Q05175	Brain acid soluble protein 1	16
346	P02688	Myelin basic protein	16
347	P02688-2	Isoform 2 of Myelin basic protein	16
348	P32551	Cytochrome b-c1 complex subunit 2, mitochondrial	16
349	Q63569	26S proteasome regulatory subunit 6A	16
350	P07895	Superoxide dismutase [Mn], mitochondrial	16
351	Q03626	Murinoglobulin-1	16
352	P04692	Tropomyosin alpha-1 chain	16
353	P41499-2	Isoform 2 of Tyrosine-protein phosphatase non-receptor type 11	15
354	Q08602	Geranylgeranyl transferase type-2 subunit alpha	15
355	Q6AYN4	Phytanoyl-CoA hydroxylase-interacting protein-like	15
356	P05370	Glucose-6-phosphate 1-dehydrogenase	15
357	P24155	Thimet oligopeptidase	15
358	Q63622-7	Isoform 7 of Disks large homolog 2	15
359	Q63622-6	Isoform 6 of Disks large homolog 2	15
360	Q5FVI6	V-type proton ATPase subunit C 1	15
361	Q5M7W5	Microtubule-associated protein 4	15
362	P0DJJ3	SH3-containing GRB2-like protein 3-interacting protein 1	15
363	P84079	ADP-ribosylation factor 1	15
364	P61206	ADP-ribosylation factor 3	15
365	Q5M7U6	Actin-related protein 2	15
366	Q63525	Nuclear migration protein nudC	15
367	P04182	Ornithine aminotransferase, mitochondrial	15
368	P02688-5	Isoform 5 of Myelin basic protein	15
369	Q6AYT3	tRNA-splicing ligase RtcB homolog	15
370	Q05683	Glutamate decarboxylase 2	15
371	P13383	Nucleolin	15
372	P54690	Branched-chain-amino-acid aminotransferase, cyt	15
373	Q63413	Spliceosome RNA helicase Ddx39b	15
374	Q5RJL0	Ermin	15
375	Q497B0	Omega-amidase NIT2	15

376	Q9WVR7	Protein phosphatase 1F	15
377	O35964	Endophilin-A2	15
378	P63012	Ras-related protein Rab-3A	15
379	P00564	Creatine kinase M-type	15
380	Q03626-2	Isoform 2 of Murinoglobulin-1	15
381	Q6P799	Serine--tRNA ligase, cytoplasmic	14
382	Q68FR6	Elongation factor 1-gamma	14
383	P51635	Aldo-keto reductase family 1 member A1	14
384	Q3MIE4	Synaptic vesicle membrane protein VAT-1 homolog	14
385	Q9QYF3	Unconventional myosin-Va	14
386	P84092	AP-2 complex subunit mu	14
387	P04904	Glutathione S-transferase alpha-3	14
388	Q5I0D1	Glyoxalase domain-containing protein 4	14
389	Q5M7W5-2	Isoform 2 of Microtubule-associated protein 4	14
390	P29266	3-hydroxyisobutyrate dehydrogenase, mitochondrial	14
391	O08651	D-3-phosphoglycerate dehydrogenase	14
392	P35465	Serine/threonine-protein kinase PAK 1	14
393	P20717	Protein-arginine deiminase type-2	14
394	Q68FS2	COP9 signalosome complex subunit 4	14
395	Q5FVJ0	Protein RUFY3	14
396	Q5FVJ0-2	Isoform 2 of Protein RUFY3	14
397	P13803	Electron transfer flavoprotein subunit alpha, mitochondrial	14
398	P03994	Hyaluronan and proteoglycan link protein 1	14
399	Q5XI32	F-actin-capping protein subunit beta	14
400	P47727	Carbonyl reductase [NADPH] 1	14
401	O08557	N(G),N(G)-dimethylarginine dimethylaminohydrolase 1	14
402	P55067	Neurocan core protein	14
403	Q920J4	Thioredoxin-like protein 1	14
404	P20760	Ig gamma-2A chain C region	14
405	Q91Y81	Septin-2	14
406	Q5RJQ4	NAD-dependent protein deacetylase sirtuin-2	14
407	Q2PQA9	Kinesin-1 heavy chain	14
408	P28075	Proteasome subunit beta type-5	14
409	P36876	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	14
410	P13668	Stathmin	14
411	P62138	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	14
412	P13676	Acylamino-acid-releasing enzyme	14
413	Q641Y8	ATP-dependent RNA helicase DDX1	14
414	P12368	cAMP-dependent protein kinase type II-alpha regulatory subunit	14
415	P63100	Calcineurin subunit B type 1	14

416	P63100-2	Isoform 2 of Calcineurin subunit B type 1	14
417	P43244	Matrin-3	14
418	Q63570	26S proteasome regulatory subunit 6B	14
419	P21708	Mitogen-activated protein kinase 3	14
420	P21708-2	Isoform 2 of Mitogen-activated protein kinase 3	14
421	Q6IE52	Murinoglobulin-2	14
422	Q03555-3	Isoform 2 of Gephyrin	13
423	Q03555	Gephyrin	13
424	Q03555-6	Isoform 5 of Gephyrin	13
425	Q03555-5	Isoform 4 of Gephyrin	13
426	Q03555-4	Isoform 3 of Gephyrin	13
427	P41562	Isocitrate dehydrogenase [NADP] cytoplasmic	13
428	Q5XIT1	Microtubule-associated protein RP/EB family member 3	13
429	Q68FP1	Gelsolin	13
430	Q68FP1-2	Isoform 2 of Gelsolin	13
431	Q4KMA2	UV excision repair protein RAD23 homolog B	13
432	P56571	ES1 protein homolog, mitochondrial	13
433	Q64542-3	Isoform ZA of Plasma membrane calcium-transporting ATPase 4	13
434	Q64542-2	Isoform XA of Plasma membrane calcium-transporting ATPase 4	13
435	P24329	Thiosulfate sulfurtransferase	13
436	Q5GFD9	Protein IMPACT	13
437	P07936	Neuromodulin	13
438	P46844	Biliverdin reductase A	13
439	Q32Q06	AP-1 complex subunit mu-1	13
440	Q8R491	EH domain-containing protein 3	13
441	Q3T1J1	Eukaryotic translation initiation factor 5A-1	13
442	P63331	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	13
443	P62716	Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform	13
444	P11507	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	13
445	P11507-2	Isoform 2 of Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	13
446	O55096	Dipeptidyl peptidase 3	13
447	P15650	Long-chain specific acyl-CoA dehydrogenase, mitochondrial	13
448	O70351	3-hydroxyacyl-CoA dehydrogenase type-2	13
449	P50503	Hsc70-interacting protein	13
450	P16446	Phosphatidylinositol transfer protein alpha isoform	13
451	P02650	Apolipoprotein E	13
452	P32851	Syntaxin-1A	13
453	Q6AYK6	Calcyclin-binding protein	13
454	Q9Z0V6	Thioredoxin-dependent peroxide reductase, mitochondrial	13

455	P08413	Calcium/calmodulin-dependent protein kinase type II subunit beta	13
456	P62986	Ubiquitin-60S ribosomal protein L40	13
457	P62982	Ubiquitin-40S ribosomal protein S27a	13
458	P0CG51	Polyubiquitin-B	13
459	Q63429	Polyubiquitin-C	13
460	P0DMW1	Heat shock 70 kDa protein 1B	13
461	P0DMW0	Heat shock 70 kDa protein 1A	13
462	Q03555-2	Isoform 1 of Gephyrin	12
463	Q9EPH8	Polyadenylate-binding protein 1	12
464	P30349	Leukotriene A-4 hydrolase	12
465	P15178	Aspartate--tRNA ligase, cytoplasmic	12
466	Q9R063	Peroxiredoxin-5, mitochondrial	12
467	Q9R063-2	Isoform Cytoplasmic+peroxisomal of Peroxiredoxin-5, mitochondrial	12
468	F1LMZ8	26S proteasome non-ATPase regulatory subunit 11	12
469	Q9JJM9-2	Isoform 2 of Septin-5	12
470	Q5XI73	Rho GDP-dissociation inhibitor 1	12
471	Q8VIF7	Methanethiol oxidase	12
472	P27653	C-1-tetrahydrofolate synthase, cytoplasmic	12
473	Q99MZ8	LIM and SH3 domain protein 1	12
474	P62909	40S ribosomal protein S3	12
475	Q5HZA6	Prolyl endopeptidase-like	12
476	Q5HZA6-2	Isoform 2 of Prolyl endopeptidase-like	12
477	P62994	Growth factor receptor-bound protein 2	12
478	P31232	Transgelin	12
479	P18420	Proteasome subunit alpha type-1	12
480	Q499N5	Medium-chain acyl-CoA ligase ACSF2, mitochondrial	12
481	Q4FZT2	Protein phosphatase methylesterase 1	12
482	B2RYW9	Fumarylacetoacetate hydrolase domain-containing protein 2	12
483	Q91ZN1	Coronin-1A	12
484	Q63081	Protein disulfide-isomerase A6	12
485	P20650	Protein phosphatase 1A	12
486	Q01205	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	12
487	Q6P7A9	Lysosomal alpha-glucosidase	12
488	P05712	Ras-related protein Rab-2A	12
489	Q5XHZ0	Heat shock protein 75 kDa, mitochondrial	12
490	P14604	Enoyl-CoA hydratase, mitochondrial	12
491	Q63768	Adapter molecule crk	12
492	P41565	Isocitrate dehydrogenase [NAD] subunit gamma 1, mitochondrial	12
493	P26772	10 kDa heat shock protein, mitochondrial	12
494	P62824	Ras-related protein Rab-3C	12

495	Q4KM49	Tyrosine--tRNA ligase, cytoplasmic	12
496	Q8VIJ5	Protein O-GlcNAcase	12
497	Q6P7Q4	Lactoylglutathione lyase	12
498	P09034	Argininosuccinate synthase	12
499	B1WC61	Complex I assembly factor ACAD9, mitochondrial	12
500	Q4KM73	UMP-CMP kinase	12
501	Q9EQS0	Transaldolase	12
502	Q6IFW6	Keratin, type I cytoskeletal 10	12
503	Q62936	Disks large homolog 3	12
504	Q62936-2	Isoform Short of Disks large homolog 3	12
505	Q5XFX0	Transgelin-2	12
506	P09495	Tropomyosin alpha-4 chain	12
507	Q63754	Beta-synuclein	12
508	Q9JJM9	Septin-5	12
509	P02688-4	Isoform 4 of Myelin basic protein	12
510	P02688-3	Isoform 3 of Myelin basic protein	12
511	Q63083	Nucleobindin-1	11
512	P62994-2	Isoform 2 of Growth factor receptor-bound protein 2	11
513	P61107	Ras-related protein Rab-14	11
514	Q02356	AMP deaminase 2	11
515	Q66HF1	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	11
516	Q66HL2	Src substrate cortactin	11
517	P62193	26S proteasome regulatory subunit 4	11
518	P07632	Superoxide dismutase [Cu-Zn]	11
519	Q9Z339	Glutathione S-transferase omega-1	11
520	B0BNN3	Carbonic anhydrase 1	11
521	Q62696	Disks large homolog 1	11
522	Q66HF8	Aldehyde dehydrogenase X, mitochondrial	11
523	O70196	Prolyl endopeptidase	11
524	P37285	Kinesin light chain 1	11
525	P37285-3	Isoform B of Kinesin light chain 1	11
526	P37285-2	Isoform A of Kinesin light chain 1	11
527	Q62847	Gamma-adducin	11
528	Q62847-2	Isoform 1 of Gamma-adducin	11
529	O08618	Phosphoribosyl pyrophosphate synthase-associated protein 2	11
530	Q04462	Valine--tRNA ligase	11
531	P08932	T-kininogen 2	11
532	P19804	Nucleoside diphosphate kinase B	11
533	P68182	cAMP-dependent protein kinase catalytic subunit beta	11
534	Q8VD52	Pyridoxal phosphate phosphatase	11
535	Q6PST4	Atlastin-1	11

536	Q68A21	Transcriptional activator protein Pur-beta	11
537	P63245	Receptor of activated protein C kinase 1	11
538	O35264	Platelet-activating factor acetylhydrolase IB subunit beta	11
539	Q9Z1B2	Glutathione S-transferase Mu 5	11
540	B2GUZ5	F-actin-capping protein subunit alpha-1	11
541	Q9WVB1	Ras-related protein Rab-6A	11
542	P07483	Fatty acid-binding protein, heart	11
543	B0BNF1	Septin-8	11
544	P84083	ADP-ribosylation factor 5	11
545	Q63941	Ras-related protein Rab-3B	11
546	P58775-2	Isoform 2 of Tropomyosin beta chain	11
547	P58775	Tropomyosin beta chain	11
548	Q9JJM9-3	Isoform 3 of Septin-5	11
549	O54975	Xaa-Pro aminopeptidase 1	10
550	P31977	Ezrin	10
551	B2RYG6	Ubiquitin thioesterase OTUB1	10
552	P97697	Inositol monophosphatase 1	10
553	Q9Z1N4	3'(2'),5'-bisphosphate nucleotidase 1	10
554	Q63092	CaM kinase-like vesicle-associated protein	10
555	Q924N5	Long-chain-fatty-acid--CoA ligase ACSBG1	10
556	A7VJC2	Heterogeneous nuclear ribonucleoproteins A2/B1	10
557	A7VJC2-4	Isoform B1b of Heterogeneous nuclear ribonucleoproteins A2/B1	10
558	A7VJC2-3	Isoform A2b of Heterogeneous nuclear ribonucleoproteins A2/B1	10
559	A7VJC2-2	Isoform A2 of Heterogeneous nuclear ribonucleoproteins A2/B1	10
560	Q9WU34	Neuronal-specific septin-3	10
561	Q8VHV7	Heterogeneous nuclear ribonucleoprotein H	10
562	P12711	Alcohol dehydrogenase class-3	10
563	Q68FU3	Electron transfer flavoprotein subunit beta	10
564	P13437	3-ketoacyl-CoA thiolase, mitochondrial	10
565	P04177	Tyrosine 3-monooxygenase	10
566	Q8K4M9	Oxysterol-binding protein-related protein 1	10
567	O88377	Phosphatidylinositol 5-phosphate 4-kinase type-2 beta	10
568	Q6P7B0	Tryptophan--tRNA ligase, cytoplasmic	10
569	P54311	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	10
570	Q01062	cGMP-dependent 3',5'-cyclic phosphodiesterase	10
571	Q5XIG8	Serine-threonine kinase receptor-associated protein	10
572	P19468	Glutamate--cysteine ligase catalytic subunit	10
573	O88767	Protein/nucleic acid deglycase DJ-1	10
574	P85968	6-phosphogluconate dehydrogenase, decarboxylating	10
575	Q5QD51-2	Isoform 2 of A-kinase anchor protein 12	10

576	Q5QD51	A-kinase anchor protein 12	10
577	Q5QD51-3	Isoform 3 of A-kinase anchor protein 12	10
578	Q9QXU9	ProSAAS	10
579	D3ZAA9	MAGUK p55 subfamily member 2	10
580	Q9JHL4	Drebrin-like protein	10
581	Q9JHL4-3	Isoform 3 of Drebrin-like protein	10
582	Q9JHL4-2	Isoform 2 of Drebrin-like protein	10
583	Q9QZR6	Septin-9	10
584	Q9QZR6-2	Isoform 2 of Septin-9	10
585	Q5U318	Astrocytic phosphoprotein PEA-15	10
586	P55161	Nck-associated protein 1	10
587	P06866	Haptoglobin	10
588	P06866-2	Isoform 2 of Haptoglobin	10
589	Q62868	Rho-associated protein kinase 2	10
590	Q9ERB4	Versican core protein (Fragments)	10
591	Q9ERB4-3	Isoform Vint of Versican core protein	10
592	Q9ERB4-2	Isoform V3 of Versican core protein	10
593	P18265	Glycogen synthase kinase-3 alpha	10
594	P61203	COP9 signalosome complex subunit 2	10
595	Q9WVK7	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	10
596	Q99MI7	NEDD8-activating enzyme E1 catalytic subunit	10
597	P53042	Serine/threonine-protein phosphatase 5	10
598	Q7M0E3	Destrin	10
599	Q5I0D7	Xaa-Pro dipeptidase	10
600	Q9QX69	Glutathione S-transferase LANCL1	10
601	P24268	Cathepsin D	10
602	P51647	Retinal dehydrogenase 1	10
603	Q5U2Z3	Nucleosome assembly protein 1-like 4	10
604	Q9EPC6	Profilin-2	10
605	P31016	Disks large homolog 4	10
606	B0BNE5	S-formylglutathione hydrolase	10
607	P62963	Profilin-1	10
608	Q5XI22	Acetyl-CoA acetyltransferase, cytosolic	10
609	Q568Z9	Phytanoyl-CoA hydroxylase-interacting protein	10
610	P86252	Transcriptional activator protein Pur-alpha (Fragments)	10
611	P18266	Glycogen synthase kinase-3 beta	10
612	O35763	Moesin	10
613	P04631	Protein S100-B	10
614	O55012	Phosphatidylinositol-binding clathrin assembly protein	10
615	O55012-2	Isoform 2 of Phosphatidylinositol-binding clathrin assembly protein	10
616	Q05982	Nucleoside diphosphate kinase A	10

617	Q6AY09	Heterogeneous nuclear ribonucleoprotein H2	10
618	P11505	Plasma membrane calcium-transporting ATPase 1	10
619	P11505-6	Isoform K of Plasma membrane calcium-transporting ATPase 1	10
620	P11505-4	Isoform C of Plasma membrane calcium-transporting ATPase 1	10
621	P11505-1	Isoform D of Plasma membrane calcium-transporting ATPase 1	10
622	P11505-5	Isoform E of Plasma membrane calcium-transporting ATPase 1	10
623	P11505-2	Isoform A of Plasma membrane calcium-transporting ATPase 1	10
624	P63088	Serine/threonine-protein phosphatase PP1-gamma catalytic subunit	10
625	P63088-2	Isoform 2 of Serine/threonine-protein phosphatase PP1-gamma catalytic subunit	10
626	O08873	MAP kinase-activating death domain protein	9
627	Q9WU34-3	Isoform 3 of Neuronal-specific septin-3	9
628	Q9WU34-2	Isoform 2 of Neuronal-specific septin-3	9
629	Q8VHV7-2	Isoform 2 of Heterogeneous nuclear ribonucleoprotein H	9
630	Q8CG45	Aflatoxin B1 aldehyde reductase member 2	9
631	P12007	Isovaleryl-CoA dehydrogenase, mitochondrial	9
632	P41542	General vesicular transport factor p115	9
633	P48004	Proteasome subunit alpha type-7	9
634	P48004-2	Isoform RC6-IS of Proteasome subunit alpha type-7	9
635	Q9Z2F5	C-terminal-binding protein 1	9
636	Q924S5	Lon protease homolog, mitochondrial	9
637	P62762	Visinin-like protein 1	9
638	P15087	Carboxypeptidase E	9
639	P02625	Parvalbumin alpha	9
640	Q99PF5	Far upstream element-binding protein 2	9
641	Q9JHL4-4	Isoform 4 of Drebrin-like protein	9
642	Q02563	Synaptic vesicle glycoprotein 2A	9
643	P17425	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	9
644	P60901	Proteasome subunit alpha type-6	9
645	Q8VI04	Isoaspartyl peptidase/L-asparaginase	9
646	P62898	Cytochrome c, somatic	9
647	P52555	Endoplasmic reticulum resident protein 29	9
648	P62828	GTP-binding nuclear protein Ran	9
649	Q62703	Reticulocalbin-2	9
650	P13086	Succinate--CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	9
651	P37377-2	Isoform Syn2 of Alpha-synuclein	9
652	P37377	Alpha-synuclein	9
653	Q6NYB7	Ras-related protein Rab-1A	9
654	P10960	Prosaposin	9

655	D3ZQL7	Tubulin polymerization-promoting protein	9
656	P47709	Rabphilin-3A	9
657	Q9JLZ1	Glutaredoxin-3	9
658	P38983	40S ribosomal protein SA	9
659	P85971	6-phosphogluconolactonase	9
660	P24587	A-kinase anchor protein 5	9
661	P70645	Bleomycin hydrolase	9
662	P61203-2	Isoform 2 of COP9 signal	9
663	B0BNA5	Coactosin-like protein	9
664	P18088	Glutamate decarboxylase 1	9
665	Q6AYG3	Exopolyphosphatase PRUNE1	9
666	Q5XIE6	3-hydroxyisobutyryl-CoA hydrolase, mitochondrial	9
667	P54921	Alpha-soluble NSF attachment protein	9
668	Q62784	Inositol polyphosphate-4-phosphatase type I A	9
669	Q62784-2	Isoform 2 of Inositol polyphosphate-4-phosphatase type I A	9
670	P18421	Proteasome subunit beta type-1	9
671	A0A0G2JTR4	Active breakpoint cluster region-related protein	9
672	O89049	Thioredoxin reductase 1, cytoplasmic	9
673	P21531	60S ribosomal protein L3	9
674	O35180	Endophilin-A3	9
675	Q9Z0W7	Chloride intracellular channel protein 4	9
676	P19139	Casein kinase II subunit alpha	9
677	P31596-2	Isoform Glt-1A of Excitatory amino acid transporter 2	9
678	P31596	Excitatory amino acid transporter 2	9
679	Q8CFN2	Cell division control protein 42 homolog	9
680	Q6P6T4	Echinoderm microtubule-associated protein-like 2	9
681	P60892	Ribose-phosphate pyrophosphokinase 1	9
682	O35509	Ras-related protein Rab-11B	9
683	P11030	Acyl-CoA-binding protein	9
684	P17220	Proteasome subunit alpha type-2	9
685	P49242	40S ribosomal protein S3a	9
686	P62959	Histidine triad nucleotide-binding protein 1	9
687	Q68FR9-2	Isoform 2 of Elongation factor 1-delta	9
688	Q68FR9	Elongation factor 1-delta	9
689	P10536	Ras-related protein Rab-1B	9
690	Q62829	Serine/threonine-protein kinase PAK 3	9
691	P62142	Serine/threonine-protein phosphatase PP1-beta catalytic subunit	9
692	P27791	cAMP-dependent protein kinase catalytic subunit alpha	9
693	P54313	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	9
694	P11730	Calcium/calmodulin-dependent protein kinase type II subunit gamma	9

695	P11730-3	Isoform C of Calcium/calmodulin-dependent protein kinase type II subunit gamma	9
696	P11730-2	Isoform B of Calcium/calmodulin-dependent protein kinase type II subunit gamma	9
697	P15791	Calcium/calmodulin-dependent protein kinase type II subunit delta	9
698	P15791-7	Isoform Delta 7 of Calcium/calmodulin-dependent protein kinase type II subunit delta	9
699	P15791-6	Isoform Delta 6 of Calcium/calmodulin-dependent protein kinase type II subunit delta	9
700	P15791-4	Isoform Delta 4 of Calcium/calmodulin-dependent protein kinase type II subunit delta	9
701	P15791-5	Isoform Delta 5 of Calcium/calmodulin-dependent protein kinase type II subunit delta	9
702	P15791-3	Isoform Delta 3 of Calcium/calmodulin-dependent protein kinase type II subunit delta	9
703	P15791-2	Isoform Delta 2 of Calcium/calmodulin-dependent protein kinase type II subunit delta	9
704	P01835	Ig kappa chain C region, B allele	9
705	Q9QVC8	Peptidyl-prolyl cis-trans isomerase FKBP4	8
706	B5DF89	Cullin-3	8
707	Q63692	Hsp90 co-chaperone Cdc37	8
708	P11915	Non-specific lipid-transfer protein	8
709	P27605	Hypoxanthine-guanine phosphoribosyltransferase	8
710	P07633	Propionyl-CoA carboxylase beta chain, mitochondrial	8
711	P36972	Adenine phosphoribosyltransferase	8
712	Q5MJ12	F-box/LRR-repeat protein 16	8
713	P29066	Beta-arrestin-1	8
714	Q5PPH0	Enolase-phosphatase E1	8
715	Q9QUR2	Dynactin subunit 4	8
716	Q9QUR2-2	Isoform 2 of Dynactin subunit 4	8
717	Q6IMY8	Heterogeneous nuclear ribonucleoprotein U	8
718	Q64640	Adenosine kinase	8
719	P08082	Clathrin light chain B	8
720	O35824	DnaJ homolog subfamily A member 2	8
721	Q63009	Protein arginine N-methyltransferase 1	8
722	B0BN85	Protein SGT1 homolog	8
723	O88794	Pyridoxine-5'-phosphate oxidase	8
724	Q4V7C6	GMP synthase [glutamine-hydrolyzing]	8
725	Q5RJR2	Twinfilin-1	8
726	Q5XIM7	Lysine--tRNA ligase	8
727	P14173	Aromatic-L-amino-acid decarboxylase	8
728	Q6AYR6	Haloacid dehalogenase-like hydrolase domain-containing protein 2	8
729	P55053	Fatty acid-binding protein 5	8
730	Q6J4I0	Protein phosphatase 1 regulatory subunit 1B	8
731	Q711G3	Isoamyl acetate-hydrolyzing esterase 1 homolog	8

732	O88588	Phosphofurin acidic cluster sorting protein 1	8
733	O88339	Epsin-1	8
734	P07340	Sodium/potassium-transporting ATPase subunit beta-1	8
735	Q4VSI4	Ubiquitin carboxyl-terminal hydrolase 7	8
736	Q6P6T4-2	Isoform 2 of Echinoderm microtubule-associated protein-like 2	8
737	P45479	Palmitoyl-protein thioesterase 1	8
738	P27952	40S ribosomal protein S2	8
739	P07722	Myelin-associated glycoprotein	8
740	P07722-2	Isoform S-MAG of Myelin-associated glycoprotein	8
741	O08875	Serine/threonine-protein kinase DCLK1	8
742	P00787	Cathepsin B	8
743	P42676	Neurolysin, mitochondrial	8
744	P50408	V-type proton ATPase subunit F	8
745	Q5I6B8	Phosphatidylinositol 4-phosphate 5-kinase type-1 gamma	8
746	Q64119	Myosin light polypeptide 6	8
747	P97834	COP9 signalosome complex subunit 1	8
748	D3ZDK7	Glycerol-3-phosphate phosphatase	8
749	A2RUW1	Toll-interacting protein	8
750	Q62658	Peptidyl-prolyl cis-trans isomerase FKBP1A	8
751	O35828	Coronin-7	8
752	F1LQ48	Heterogeneous nuclear ribonucleoprotein L	8
753	P21670	Proteasome subunit alpha type-4	8
754	Q63544	Gamma-synuclein	8
755	Q63945	Protein SET	8
756	Q63945-2	Isoform 2 of Protein SET	8
757	P04256	Heterogeneous nuclear ribonucleoprotein A1	8
758	Q91Y78	Ubiquitin carboxyl-terminal hydrolase isozyme L3	8
759	Q5XHY5	Threonine--tRNA ligase 1, cytoplasmic	8
760	Q3B8Q0	Microtubule-associated protein RP/EB family member 2	8
761	Q99N27	Sorting nexin-1	8
762	Q9EQX9	Ubiquitin-conjugating enzyme E2 N	8
763	P63041	Complexin-1	8
764	P11506-8	Isoform ZB of Plasma membrane calcium-transporting ATPase 2	8
765	P11506-7	Isoform YB of Plasma membrane calcium-transporting ATPase 2	8
766	P11506-6	Isoform XB of Plasma membrane calcium-transporting ATPase 2	8
767	P11506-1	Isoform WB of Plasma membrane calcium-transporting ATPase 2	8
768	P11506-9	Isoform WC of Plasma membrane calcium-transporting ATPase 2	8
769	P11506-5	Isoform ZA of Plasma membrane calcium-transporting ATPase 2	8

770	P11506-4	Isoform YA of Plasma membrane calcium-transporting ATPase 2	8
771	P11506-3	Isoform XA of Plasma membrane calcium-transporting ATPase 2	8
772	P11506-2	Isoform WA of Plasma membrane calcium-transporting ATPase 2	8
773	P11506-12	Isoform ZC of Plasma membrane calcium-transporting ATPase 2	8
774	P11506-11	Isoform YC of Plasma membrane calcium-transporting ATPase 2	8
775	P11506-10	Isoform XC of Plasma membrane calcium-transporting ATPase 2	8
776	P29101	Synaptotagmin-2	8
777	P04897	Guanine nucleotide-binding protein G(i) subunit alpha-2	8
778	Q9R0I8	Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha	8
779	P01048	T-kininogen 1	8
780	P30904	Macrophage migration inhibitory factor	8
781	P27791-2	Isoform 2 of cAMP-dependent protein kinase catalytic subunit alpha	8
782	Q8CFN2-2	Isoform 2 of Cell division control protein 42 homolog	8
783	Q5U206	Calmodulin-like protein 3	8
784	P08082-2	Isoform Non-brain of Clathrin light chain B	7
785	P40329	Arginine--tRNA ligase, cytoplasmic	7
786	P20156	Neurosecretory protein VGF	7
787	P20788	Cytochrome b-c1 complex subunit Rieske, mitochondrial	7
788	P14480	Fibrinogen beta chain	7
789	Q80Z30	Protein phosphatase 1E	7
790	Q9QXU8	Cytoplasmic dynein 1 light intermediate chain 1	7
791	Q64536	[Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 2, mitochondrial	7
792	Q5BK81	Prostaglandin reductase 2	7
793	P17475	Alpha-1-antiproteinase	7
794	Q5I0D5	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	7
795	Q7TP47	Heterogeneous nuclear ribonucleoprotein Q	7
796	P01015	Angiotensinogen	7
797	Q811X6	Lambda-crystallin homolog	7
798	O70593	Small glutamine-rich tetratricopeptide repeat-containing protein alpha	7
799	Q63100	Cytoplasmic dynein 1 intermediate chain 1	7
800	Q63100-3	Isoform 2 of Cytoplasmic dynein 1 intermediate chain 1	7
801	Q5M7A7	CB1 cannabinoid receptor-interacting protein 1	7
802	P01836	Ig kappa chain C region, A allele	7
803	P47875	Cysteine and glycine-rich protein 1	7
804	P35565	Calnexin	7
805	P63159	High mobility group protein B1	7
806	Q5XIT9	Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial	7

807	P53678	AP-3 complex subunit mu-2	7
808	Q6PEC0	Bis(5'-nucleosyl)-tetraphosphatase [asymmetrical]	7
809	Q6IRK9	Carboxypeptidase Q	7
810	Q68FX1	Mannose-6-phosphate isomerase	7
811	F1LU71	Methylglutaconyl-CoA hydratase, mitochondrial	7
812	P02401	60S acidic ribosomal protein P2	7
813	P08592	Amyloid-beta A4 protein	7
814	P08592-2	Isoform APP695 of Amyloid-beta A4 protein	7
815	Q9R1T5	Aspartoacylase	7
816	P19234	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	7
817	P08644	GTPase KRas	7
818	P08644-2	Isoform 2B of GTPase KRas	7
819	P05369	Farnesyl pyrophosphate synthase	7
820	Q6IMF3	Keratin, type II cytoskeletal 1	7
821	Q566R0	Acyl-coenzyme A thioesterase THEM4	7
822	Q9QZA2	Programmed cell death 6-interacting protein	7
823	D4AAT7	ATP-dependent (S)-NAD(P)H-hydrate dehydratase	7
824	Q63450	Calcium/calmodulin-dependent protein kinase type 1	7
825	Q9JJ50	Hepatocyte growth factor-regulated tyrosine kinase substrate	7
826	Q9JJ50-2	Isoform 2 of Hepatocyte growth factor-regulated tyrosine kinase substrate	7
827	Q9JJ19	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	7
828	P20761	Ig gamma-2B chain C region	7
829	Q99MS0	SEC14-like protein 2	7
830	P62775	Myotrophin	7
831	Q08415	Kynurenine--oxoglutarate transaminase 1, mitochondrial	7
832	Q08415-2	Isoform 2 of Kynurenine--oxoglutarate transaminase 1, mitochondrial	7
833	Q6MG60	N(G),N(G)-dimethylarginine dimethylaminohydrolase 2	7
834	P08081	Clathrin light chain A	7
835	Q5BJU7	Wiskott-Aldrich syndrome protein family member 1	7
836	P37996	ADP-ribosylation factor-like protein 3	7
837	P62243	40S ribosomal protein S8	7
838	P04762	Catalase	7
839	Q5U2U2	Crk-like protein	7
840	P09456	cAMP-dependent protein kinase type I-alpha regulatory subunit	7
841	P04094	Proenkephalin-A	7
842	Q62636	Ras-related protein Rap-1b	7
843	P28073	Proteasome subunit beta type-6	7
844	P21533	60S ribosomal protein L6	7
845	Q63797	Proteasome activator complex subunit 1	7
846	Q5FVH2	Phospholipase D3	7

847	P10824	Guanine nucleotide-binding protein G(i) subunit alpha-1	7
848	Q6PEC1	Tubulin-specific chaperone A	7
849	P62329	Thymosin beta-4	7
850	P23358	60S ribosomal protein L12	7
851	Q4V8C3	Echinoderm microtubule-associated protein-like 1	7
852	Q4V8C3-2	Isoform 2 of Echinoderm microtubule-associated protein-like 1	7
853	P52759	2-iminobutanoate/2-iminopropanoate deaminase	7
854	P11506-14	Isoform 2 of Plasma membrane calcium-transporting ATPase 2	7
855	Q62813	Limbic system-associated membrane protein	7
856	Q62813-2	Isoform 2 of Limbic system-associated membrane protein	7
857	Q9Z2Q1	Protein transport protein Sec31A	7
858	Q9Z2Q1-2	Isoform 2 of Protein transport protein Sec31A	7
859	Q6AXY0	Glutathione S-transferase A6	7
860	P14942	Glutathione S-transferase alpha-4	7
861	P62628	Dynein light chain roadblock-type 1	7
862	P27682	Neuroendocrine protein 7B2	7
863	P20759	Ig gamma-1 chain C region	7
864	P35281	Ras-related protein Rab-10	7
865	Q4V8B0-2	Isoform 2 of Oxidation resistance protein 1	7
866	P20171	GTPase HRas	7
867	Q04970	GTPase NRas	7
868	P62836	Ras-related protein Rap-1A	7
869	P61751	ADP-ribosylation factor 4	7
870	P11506	Plasma membrane calcium-transporting ATPase 2	7
871	P11506-14	Isoform 2 of Plasma membrane calcium-transporting ATPase 2	7
872	P14480-2	Isoform 2 of Fibrinogen beta chain	6
873	Q63100-4	Isoform 3 of Cytoplasmic dynein 1 intermediate chain 1	6
874	P82471	Guanine nucleotide-binding protein G(q) subunit alpha	6
875	O35952	Hydroxyacylglutathione hydrolase, mitochondrial	6
876	O35952-2	Isoform 2 of Hydroxyacylglutathione hydrolase, mitochondrial	6
877	P61589	Transforming protein RhoA	6
878	P18298	S-adenosylmethionine synthase isoform type-2	6
879	P19945	60S acidic ribosomal protein P0	6
880	Q6PCT3	Tumor protein D54	6
881	Q4QQT4	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	6
882	P35435	ATP synthase subunit gamma, mitochondrial	6
883	P34064	Proteasome subunit alpha type-5	6
884	P04041	Glutathione peroxidase 1	6
885	M0RC99	Ras-related protein Rab-5A	6

886	P32232	Cystathionine beta-synthase	6
887	P32232-3	Isoform IV of Cystathionine beta-synthase	6
888	P32232-2	Isoform III of Cystathionine beta-synthase	6
889	P02680	Fibrinogen gamma chain	6
890	P02680-2	Isoform Gamma-A of Fibrinogen gamma chain	6
891	Q6URK4	Heterogeneous nuclear ribonucleoprotein A3	6
892	Q6URK4-2	Isoform 2 of Heterogeneous nuclear ribonucleoprotein A3	6
893	Q5XII0	Mammalian ependymin-related protein 1	6
894	P11960	2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial (Fragment)	6
895	Q60587	Trifunctional enzyme subunit beta, mitochondrial	6
896	Q920Q0	Paralemmin-1	6
897	P17105	Inositol-trisphosphate 3-kinase A	6
898	B0BN93	26S proteasome non-ATPase regulatory subunit 13	6
899	P69682	Adaptin ear-binding coat-associated protein 1	6
900	Q6PEC4	S-phase kinase-associated protein 1	6
901	P62870	Elongin-B	6
902	Q7M767	Ubiquitin-conjugating enzyme E2 variant 2	6
903	P14882	Propionyl-CoA carboxylase alpha chain, mitochondrial	6
904	P0C2X9	Delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial	6
905	P21913	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	6
906	Q3ZB98	Breast carcinoma-amplified sequence 1 homolog	6
907	Q3ZB98-6	Isoform 6 of Breast carcinoma-amplified sequence 1 homolog	6
908	Q3ZB98-5	Isoform 5 of Breast carcinoma-amplified sequence 1 homolog	6
909	Q3ZB98-4	Isoform 4 of Breast carcinoma-amplified sequence 1 homolog	6
910	Q3ZB98-3	Isoform 3 of Breast carcinoma-amplified sequence 1 homolog	6
911	Q3ZB98-2	Isoform 2 of Breast carcinoma-amplified sequence 1 homolog	6
912	G3V9R8	Heterogeneous nuclear ribonucleoprotein C	6
913	P14841	Cystatin-C	6
914	P45953	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	6
915	A2VCX1	TIP41-like protein	6
916	P51583	Multifunctional protein ADE2	6
917	Q562C6	Leucine zipper transcription factor-like protein 1	6
918	Q62651	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	6
919	P34067	Proteasome subunit beta type-4	6
920	Q62915	Peripheral plasma membrane protein CASK	6
921	P54319	Phospholipase A-2-activating protein	6
922	Q5XI72	Eukaryotic translation initiation factor 4H	6
923	Q9Z269	Vesicle-associated membrane protein-associated protein B	6
924	D3ZW55	Inosine triphosphate pyrophosphatase	6
925	O55156	CAP-Gly domain-containing linker protein 2	6

926	P13635	Ceruloplasmin	6
927	P49187	Mitogen-activated protein kinase 10	6
928	P49187-2	Isoform 2 of Mitogen-activated protein kinase 10	6
929	P51146	Ras-related protein Rab-4B	6
930	Q9JIX3	Bis(5'-adenosyl)-triphosphatase	6
931	Q9Z244	GMP reductase 1	6
932	P46413	Glutathione synthetase	6
933	P05545	Serine protease inhibitor A3K	6
934	P62083	40S ribosomal protein S7	6
935	Q1HCL7	NAD kinase 2, mitochondrial	6
936	Q6AYK3	Inositol-3-phosphate synthase 1	6
937	Q9QYL8	Acyl-protein thioesterase 2	6
938	P18422	Proteasome subunit alpha type-3	6
939	P23928	Alpha-crystallin B chain	6
940	Q62698	Cytoplasmic dynein 1 light intermediate chain 2	6
941	Q9Z2L9	Protein NDRG4	6
942	Q9Z2G8	Nucleosome assembly protein 1-like 1	6
943	P62703	40S ribosomal protein S4, X isoform	6
944	Q68FW9	COP9 signalosome complex subunit 3	6
945	P67874	Casein kinase II subunit beta	6
946	P13084	Nucleophosmin	6
947	Q9WTV5	26S proteasome non-ATPase regulatory subunit 9	6
948	Q9QY17	Protein kinase C and casein kinase substrate in neurons 2 protein	6
949	Q9QY17-4	Isoform 4 of Protein kinase C and casein kinase substrate in neurons 2 protein	6
950	Q9QY17-3	Isoform 3 of Protein kinase C and casein kinase substrate in neurons 2 protein	6
951	Q9QY17-2	Isoform 2 of Protein kinase C and casein kinase substrate in neurons 2 protein	6
952	Q66HR2	Microtubule-associated protein RP/EB family member 1	6
953	P49911	Acidic leucine-rich nuclear phosphoprotein 32 family member A	6
954	P40112	Proteasome subunit beta type-3	6
955	P18666	Myosin regulatory light chain 12B	6
956	P13832	Myosin regulatory light chain RLC-A	6
957	B0BNM1	NAD(P)H-hydrate epimerase	6
958	Q9QZM5	Abl interactor 1	6
959	P38659	Protein disulfide-isomerase A4	6
960	P01041	Cystatin-B	6
961	O89046	Coronin-1B	6
962	P05371	Clusterin	6
963	P60203	Myelin proteolipid protein	6
964	Q9ESB5	N-terminal EF-hand calcium-binding protein 1	6

965	Q6RUV5	Ras-related C3 botulinum toxin substrate 1	6
966	Q6AYR2	Protein NDRG3	6
967	P49088	Asparagine synthetase [glutamine-hydrolyzing]	6
968	P25409	Alanine aminotransferase 1	6
969	P85125	Caveolae-associated protein 1	6
970	P61972	Nuclear transport factor 2	6
971	P23965	Enoyl-CoA delta isomerase 1, mitochondrial	6
972	Q9JJ54	Heterogeneous nuclear ribonucleoprotein D0	6
973	Q9JJ54-4	Isoform 4 of Heterogeneous nuclear ribonucleoprotein D0	6
974	Q9JJ54-3	Isoform 3 of Heterogeneous nuclear ribonucleoprotein D0	6
975	Q9JJ54-2	Isoform 2 of Heterogeneous nuclear ribonucleoprotein D0	6
976	Q4G017	Nischarin	6
977	P20767	Ig lambda-2 chain C region	6
978	Q8R431	Monoglyceride lipase	6
979	Q04631	Protein farnesyltransferase/geranylgeranyltransferase type-1 subunit alpha	6
980	P60841	Alpha-endosulfine	6
981	P60841-2	Isoform 2 of Alpha-endosulfine	6
982	P62839	Ubiquitin-conjugating enzyme E2 D2	6
983	O35550	Rab GTPase-binding effector protein 1	6
984	P84087	Complexin-2	6
985	Q64303	Serine/threonine-protein kinase PAK 2	6
986	Q812E9	Neuronal membrane glycoprotein M6-a	6
987	Q812E9-2	Isoform 2 of Neuronal membrane glycoprotein M6-a	6
988	P35815	Protein phosphatase 1B	6
989	P35815-2	Isoform 2 of Protein phosphatase 1B	6
990	Q641Z6	EH domain-containing protein 1	6
991	P81377	cAMP-dependent protein kinase type I-beta regulatory subunit	6
992	P09626	Potassium-transporting ATPase alpha chain 1	6
993	Q64548-2	Isoform RTN1-S of Reticulon-1	6
994	Q9Z2L9-4	Isoform 4 of Protein NDRG4	6
995	P61078	Ubiquitin-conjugating enzyme E2 D3	6
996	Q07205	Eukaryotic translation initiation factor 5	5
997	O55171	Acyl-coenzyme A thioesterase 2, mitochondrial	5
998	Q6AY86	Vacuolar protein sorting-associated protein 26A	5
999	Q792I0	Protein lin-7 homolog C	5
1000	Q9JLA3	UDP-glucose:glycoprotein glucosyltransferase 1	5
1001	P29315	Ribonuclease inhibitor	5
1002	O35796	Complement component 1 Q subcomponent-binding protein, mitochondrial	5
1003	P63045	Vesicle-associated membrane protein 2	5
1004	P62744	AP-2 complex subunit sigma	5

1005	D4A4T9	Cysteine and histidine-rich domain-containing protein 1	5
1006	P70619	Glutathione reductase (Fragment)	5
1007	P97756	Calcium/calmodulin-dependent protein kinase kinase 1	5
1008	Q7TQ94	Deaminated glutathione amidase	5
1009	Q7TQ94-2	Isoform 2 of Deaminated glutathione amidase	5
1010	Q6I7R3	Isochorismatase domain-containing protein 1	5
1011	Q5HZE4	Methylthioribose-1-phosphate isomerase	5
1012	P70483	Striatin	5
1013	Q9JJ31	Cullin-5	5
1014	P63029	Translationally-controlled tumor protein	5
1015	P09216	Protein kinase C epsilon type	5
1016	Q6P4Z6	Leucine carboxyl methyltransferase 1	5
1017	P29314	40S ribosomal protein S9	5
1018	O88637	Ethanolamine-phosphate cytidylyltransferase	5
1019	O88637-2	Isoform 2 of Ethanolamine-ph	5
1020	Q9Z2L9-3	Isoform 3 of Protein NDRG4	5
1021	Q9Z2L9-2	Isoform 2 of Protein NDRG4	5
1022	Q02589	[Protein ADP-ribosylarginine] hydrolase	5
1023	P56558	UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase 110 kDa subunit	5
1024	P13084-2	Isoform B23.2 of Nucleophosmin	5
1025	Q9JJP9	Ubiquilin-1	5
1026	Q66HA6	ADP-ribosylation factor-like protein 8B	5
1027	Q6AXQ0	SUMO-activating enzyme subunit 1	5
1028	Q80W98	Small glutamine-rich tetratricopeptide repeat-containing protein beta	5
1029	P62246	40S ribosomal protein S15a	5
1030	P41123	60S ribosomal protein L13	5
1031	P62882	Guanine nucleotide-binding protein subunit beta-5	5
1032	Q4G061	Eukaryotic translation initiation factor 3 subunit B	5
1033	P29476	Nitric oxide synthase, brain	5
1034	P29476-3	Isoform PNNOS of Nitric oxide synthase, brain	5
1035	P29476-2	Isoform N-NOS-2 of Nitric oxide synthase, brain	5
1036	D4A6L0	Probable G-protein coupled receptor 158	5
1037	F1LQY6	N-terminal EF-hand calcium-binding protein 2	5
1038	P60123	RuvB-like 1	5
1039	P63036	DnaJ homolog subfamily A member 1	5
1040	Q6P4Z9	COP9 signalosome complex subunit 8	5
1041	Q9JHW0	Proteasome subunit beta type-7	5
1042	Q9Z270	Vesicle-associated membrane protein-associated protein A	5
1043	P35467	Protein S100-A1	5
1044	Q6JE36	Protein NDRG1	5
1045	Q80U96	Exportin-1	5

1046	P20762	Ig gamma-2C chain C region	5
1047	Q4KM45	UPF0687 protein C20orf27 homolog	5
1048	B2RZ78	Vacuolar protein sorting-associated protein 29	5
1049	B2RZ78-2	Isoform 2 of Vacuolar protein sorting-associated protein 29	5
1050	Q5BJP3	Ubiquitin-fold modifier 1	5
1051	P55051	Fatty acid-binding protein, brain	5
1052	P06214	Delta-aminolevulinic acid dehydratase	5
1053	Q9Z214	Homer protein homolog 1	5
1054	Q9Z214-2	Isoform 2 of Homer protein homolog 1	5
1055	P11240	Cytochrome c oxidase subunit 5A, mitochondrial	5
1056	P41498	Low molecular weight ph	5
1057	Q8R478	WW domain-binding protein 2	5
1058	Q6AYG5	Ethylmalonyl-CoA decarboxylase	5
1059	Q58FK9	Kynurenine--oxoglutarate transaminase 3	5
1060	G3V7P1	Syntaxin-12	5
1061	P38656	Lupus La protein homolog	5
1062	Q6AYD3	Proliferation-associated protein 2G4	5
1063	Q7TP54	Rho family-interacting cell polarization regulator 2	5
1064	P02793	Ferritin light chain 1	5
1065	P24368	Peptidyl-prolyl cis-trans isomerase B	5
1066	Q8K4F7	m7GpppX diphosphatase	5
1067	Q62871-2	Isoform 2B of Cytoplasmic dynein 1 intermediate chain 2	5
1068	Q62871-3	Isoform 2C of Cytoplasmic dynein 1 intermediate chain 2	5
1069	P18395	Cold shock domain-containing protein E1	5
1070	P84076	Neuron-specific calcium-binding protein hippocalcin	5
1071	Q5RJZ6	Short coiled-coil protein	5
1072	Q8K3X8	Heat shock factor-binding protein 1	5
1073	P19103	Protein phosphatase 1 regulatory subunit 1A	5
1074	P11762	Galectin-1	5
1075	P20595	Guanylate cyclase soluble subunit beta-1	5
1076	Q5RJP0	Aldo-keto reductase family 1 member B7	5
1077	Q5M7A4	Ubiquitin-like modifier-activating enzyme 5	5
1078	O88831-2	Isoform 2 of Calcium/calmodulin-dependent protein kinase kinase 2	5
1079	O88831	Calcium/calmodulin-dependent protein kinase kinase 2	5
1080	Q6QLM7	Kinesin heavy chain isoform 5A	5
1081	Q62785	28 kDa heat- and acid-stable phosphoprotein	5
1082	P86172	NmrA-like family domain-containing protein 1 (Fragments)	5
1083	O35263	Platelet-activating factor acetylhydrolase IB subunit gamma	5
1084	P15651	Short-chain specific acyl-CoA dehydrogenase, mitochondrial	5
1085	P62747	Rho-related GTP-binding protein RhoB	5
1086	P05544	Serine protease inhibitor A3L	5

1087	Q9Z0V5	Peroxiredoxin-4	5
1088	Q9Z2L9-6	Isoform 6 of Protein NDRG4	5
1089	Q9Z2L9-5	Isoform 5 of Protein NDRG4	5
1090	Q63862	Myosin-11 (Fragments)	5
1091	Q63862-3	Isoform 3 of Myosin-11	5
1092	Q63862-2	Isoform 2 of Myosin-11	5
1093	P35280	Ras-related protein Rab-8A	5
1094	D3ZDK2	Ubiquitin-conjugating enzyme E2 D1	5
1095	P49186	Mitogen-activated protein kinase 9	5
1096	P49186-2	Isoform Alpha-1 of Mitogen-activated protein kinase 9	5
1097	Q5M9G3	Caprin-1	4
1098	Q63228	Glia maturation factor beta	4
1099	Q63564	Synaptic vesicle glycoprotein 2B	4
1100	Q64611	Cysteine sulfinic acid decarboxylase	4
1101	P27867	Sorbitol dehydrogenase	4
1102	P08503	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial	4
1103	P11232	Thioredoxin	4
1104	Q9QYU2	Elongation factor Ts, mitochondrial	4
1105	P57113	Maleylacetoacetate isomerase	4
1106	P97710	Tyrosine-protein phosphatase non-receptor type substrate 1	4
1107	P63322	Ras-related protein Ral-A	4
1108	Q9EPB1	Dipeptidyl peptidase 2	4
1109	Q566C7	Diphosphoinositol polyphosphate phosphohydrolase 1	4
1110	P62804	Histone H4	4
1111	Q641W2	UPF0160 protein MYG1, mitochondrial	4
1112	P18297	Sepiapterin reductase	4
1113	P70600	Protein-tyrosine kinase 2-beta	4
1114	P70600-3	Isoform 3 of Protein-tyrosine kinase 2-beta	4
1115	Q1WIM1	Cell adhesion molecule 4	4
1116	P60522	Gamma-aminobutyric acid receptor-associated protein-like 2	4
1117	P30009	Myristoylated alanine-rich C-kinase substrate	4
1118	Q5U1Z2	Trafficking protein particle complex subunit 3	4
1119	Q1WIM3	Cell adhesion molecule 3	4
1120	P33124-2	Isoform 2 of Long-chain-fatty-acid--CoA ligase 6	4
1121	P01830	Thy-1 membrane glycoprotein	4
1122	O70511	Ankyrin-3	4
1123	O70511-3	Isoform 3 of Ankyrin-3	4
1124	O70511-2	Isoform 2 of Ankyrin-3	4
1125	Q9WUC4	Copper transport protein ATOX1	4
1126	P50878	60S ribosomal protein L4	4
1127	Q5M827	Pirin	4

1128	Q9WV63	Kinesin-like protein KIF2A	4
1129	Q9WV63-2	Isoform 2 of Kinesin-like protein KIF2A	4
1130	Q5U2R0	Methionine adenosyltransferase 2 subunit beta	4
1131	Q66X93	Staphylococcal nuclease domain-containing protein 1	4
1132	Q6DGG1	Protein ABHD14B	4
1133	B0BNM9	Glycolipid transfer protein	4
1134	P22734	Catechol O-methyltransferase	4
1135	P22734-2	Isoform 2 of Catechol O-methyltransferase	4
1136	P61227	Ras-related protein Rap-2b	4
1137	O09178	AMP deaminase 3	4
1138	Q6AYD3-2	Isoform 2 of Proliferation-associated protein 2G4	4
1139	P62755	40S ribosomal protein S6	4
1140	O88778	Protein bassoon	4
1141	Q6AYQ8	Acylpyruvase FAHD1, mitochondrial	4
1142	P40307	Proteasome subunit beta type-2	4
1143	Q5M821	Protein phosphatase 1H	4
1144	Q62848	ADP-ribosylation factor GTPase-activating protein 1	4
1145	Q62848-2	Isoform 2 of ADP-ribosylation factor GTPase-activating protein 1	4
1146	B0BN18	Prefoldin subunit 2	4
1147	P22063	Contactin-2	4
1148	Q62871	Cytoplasmic dynein 1 intermediate chain 2	4
1149	P97532	3-mercaptopyruvate sulfurtransferase	4
1150	Q62688	Inactive phospholipase C-like protein 1	4
1151	P63047	Sulfotransferase 4A1	4
1152	P29410	Adenylate kinase 2, mitochondrial	4
1153	P29410-2	Isoform 2 of Adenylate kinase 2, mitochondrial	4
1154	Q6AXT5	Ras-related protein Rab-21	4
1155	Q9Z1A5	NEDD8-activating enzyme E1 regulatory subunit	4
1156	Q5FWY5	AH receptor-interacting protein	4
1157	Q6GMN2	Brain-specific angiogenesis inhibitor 1-associated protein 2	4
1158	Q6GMN2-2	Isoform 2 of Brain-specific angiogenesis inhibitor 1-associated protein 2	4
1159	Q4V7D2	Protein rogdi homolog	4
1160	Q5I0P2	Glycine cleavage system H protein, mitochondrial	4
1161	Q8VIL3	ZW10 interactor	4
1162	P17074	40S ribosomal protein S19	4
1163	Q63468	Phosphoribosyl pyrophosphate synthase-associated protein 1	4
1164	P10868	Guanidinoacetate N-methyltransferase	4
1165	Q63396	Activated RNA polymerase II transcriptional coactivator p15	4
1166	P63055	Calmodulin regulator protein PCP4	4
1167	P35434	ATP synthase subunit delta, mitochondrial	4
1168	Q641Y5	Ubiquitin-like modifier-activating enzyme ATG7	4

1169	P14925	Peptidylglycine alpha-amidating monooxygenase	4
1170	P14925-5	Isoform PAM-3B of Peptidylglycine alpha-amidating monooxygenase	4
1171	P14925-4	Isoform PAM-3A of Peptidylglycine alpha-amidating monooxygenase	4
1172	P14925-3	Isoform PAM-3 of Peptidylglycine alpha-amidating monooxygenase	4
1173	P14925-2	Isoform PAM-2 of Peptidylglycine alpha-amidating monooxygenase	4
1174	P14925-6	Isoform PAM-4 of Peptidylglycine alpha-amidating monooxygenase	4
1175	P14925-7	Isoform PAM-5 of Peptidylglycine alpha-amidating monooxygenase	4
1176	O35314	Secretogranin-1	4
1177	P50430	Arylsulfatase B	4
1178	Q6AYE2	Endophilin-B1	4
1179	Q05962	ADP/ATP translocase 1	4
1180	Q66H61	Glutamine--tRNA ligase	4
1181	Q91Z79	Liprin-alpha-3	4
1182	Q8R511	Formin-binding protein 1	4
1183	Q8R511-7	Isoform 7 of Formin-binding protein 1	4
1184	Q8R511-6	Isoform 6 of Formin-binding protein 1	4
1185	Q8R511-5	Isoform 5 of Formin-binding protein 1	4
1186	Q8R511-4	Isoform 4 of Formin-binding protein 1	4
1187	Q8R511-3	Isoform 3 of Formin-binding protein 1	4
1188	Q8R511-2	Isoform 2 of Formin-binding protein 1	4
1189	P29411	GTP:AMP phosphotransferase AK3, mitochondrial	4
1190	Q5EGY4	Synaptobrevin homolog YKT6	4
1191	P47868	Secretogranin-3	4
1192	O08629	Transcription intermediary factor 1-beta	4
1193	Q64598	Histone H2A type 1-F	4
1194	Q4FZT6	Histone H2A type 3	4
1195	Q00728	Histone H2A type 4	4
1196	P0CC09	Histone H2A type 2-A	4
1197	P0C170	Histone H2A type 1-E	4
1198	P0C169	Histone H2A type 1-C	4
1199	P02262	Histone H2A type 1	4
1200	A9UMV8	Histone H2A.J	4
1201	B0BN94	Protein FAM136A	4
1202	Q923S8	4'-phosphopantetheine phosphatase	4
1203	P24049	60S ribosomal protein L17	4
1204	Q80Z29	Nicotinamide phosphoribosyltransferase	4
1205	P39032	60S ribosomal protein L36	4
1206	P70580	Membrane-associated progesterone receptor component 1	4
1207	P97852	Peroxisomal multifunctional enzyme type 2	4

1208	P62853	40S ribosomal protein S25	4
1209	O35458	Vesicular inhibitory amino acid transporter	4
1210	P00762	Anionic trypsin-1	4
1211	P20673	Argininosuccinate lyase	4
1212	P35332	Hippocalcin-like protein 4	4
1213	Q9EST6	Acidic leucine-rich nuclear ph	4
1214	Q6AY63	ADP-sugar pyroph	4
1215	Q9WUH4	Four and a half LIM domains protein 1	4
1216	Q794E4	Heterogeneous nuclear ribonucleoprotein F	4
1217	P56932	Serine/threonine-protein ph	4
1218	P05714	Ras-related protein Rab-4A	4
1219	Q62812	Myosin-9	4
1220	Q5PQN0	Neurocalcin-delta	4
1221	P56536	Kinesin heavy chain isoform 5C (Fragment)	4
1222	P62749	Hippocalcin-like protein 1	4
1223	Q9Z250	Protein lin-7 homolog A	4
1224	P21807	Peripherin	4
1225	P63025	Vesicle-associated membrane protein 3	4
1226	P70600-2	Isoform 2 of Protein-tyr	3
1227	P33124	Long-chain-fatty-acid--CoA ligase 6	3
1228	P05426	60S ribosomal protein L7	3
1229	O88658	Kinesin-like protein KIF1B	3
1230	O88658-2	Isoform 2 of Kinesin-like protein KIF1B	3
1231	Q62848-3	Isoform 3 of ADP-ribosylation factor GTPase-activating protein 1	3
1232	P62168	Neuronal calcium sensor 1	3
1233	D3ZYW7	Frataxin, mitochondrial	3
1234	Q80WA4	RNA-binding protein Nova-1 (Fragment)	3
1235	P23514	Coatomer subunit beta	3
1236	Q68FX9	NAD-dependent protein deacylase sirtuin-5, mitochondrial	3
1237	Q78P75	Dynein light chain 2, cytoplasmic	3
1238	A1L108	Actin-related protein 2/3 complex subunit 5-like protein	3
1239	A1L108-2	Isoform 2 of Actin-related protein 2/3 complex subunit 5-like protein	3
1240	Q8K1Q0	Glycylpeptide N-tetradecanoyltransferase 1	3
1241	P83868	Prostaglandin E synthase 3	3
1242	P97576	GrpE protein homolog 1, mitochondrial	3
1243	Q00715	Histone H2B type 1	3
1244	P12001	60S ribosomal protein L18	3
1245	Q6AYT0	Quinone oxidoreductase	3
1246	P37397	Calponin-3	3
1247	Q4KLF8	Actin-related protein 2/3 complex subunit 5	3
1248	Q6AYT5	Damage-control phosphatase ARMT1	3

1249	O35764	Neuronal pentraxin receptor	3
1250	Q99JD4	CLIP-associating protein 2	3
1251	Q498R7	CXXC motif containing zinc binding protein	3
1252	P62919	60S ribosomal protein L8	3
1253	Q71UE8	NEDD8	3
1254	Q6AZ50	Ubiquitin-like-conjugating enzyme ATG3	3
1255	P62076	Mitochondrial import inner membrane translocase subunit Tim13	3
1256	Q03114	Cyclin-dependent-like kinase 5	3
1257	P35745	Acylphosphatase-2	3
1258	B5DF91	ELAV-like protein 1	3
1259	P24942	Excitatory amino acid transporter 1	3
1260	P24942-2	Isoform GLAST-1A of Excitatory amino acid transporter 1	3
1261	O08697	ADP-ribosylation factor-like protein 2	3
1262	P62271	40S ribosomal protein S18	3
1263	Q5PPG6	Nucleosome assembly protein 1-like 5	3
1264	P62501	TSC22 domain family protein 1	3
1265	Q7TP52	Carboxymethylenebutenolidase homolog	3
1266	Q6AYK8	Eukaryotic translation initiation factor 3 subunit D	3
1267	P10354	Chromogranin-A	3
1268	Q9WUS0	Adenylate kinase 4, mitochondrial	3
1269	P35738	2-oxoisovalerate dehydrogenase subunit beta, mitochondrial	3
1270	P18886	Carnitine O-palmitoyltransferase 2, mitochondrial	3
1271	P62250	40S ribosomal protein S16	3
1272	A0JPM9	Eukaryotic translation initiation factor 3 subunit J	3
1273	P70470	Acyl-protein thioesterase 1	3
1274	Q9WU82	Catenin beta-1	3
1275	Q6IUR5	Neudesin	3
1276	Q5XIE0	Acidic leucine-rich nuclear ph	3
1277	Q498E0	Thioredoxin domain-containing protein 12	3
1278	P35234	Tyrosine-protein phosphatase non-receptor type 5	3
1279	P46101	Dipeptidyl aminopeptidase-like protein 6	3
1280	P46101-2	Isoform DPPX-S of Dipeptidyl aminopeptidase-like protein 6	3
1281	P62425	60S ribosomal protein L7a	3
1282	Q1M168	Caytaxin	3
1283	P60905	DnaJ homolog subfamily C member 5	3
1284	Q66HG4	Aldose 1-epimerase	3
1285	P36201	Cysteine-rich protein 2	3
1286	P17077	60S ribosomal protein L9	3
1287	Q78PB6	Nuclear distribution protein nudE-like 1	3
1288	Q78PB6-2	Isoform 2 of Nuclear distribution protein nudE-like 1	3
1289	A8WCF8	Tumor protein p63-regulated gene 1-like protein	3

1290	Q9WU49	Calcium-regulated heat stable protein 1	3
1291	Q07803	Elongation factor G, mitochondrial	3
1292	Q6PDU1	Serine/arginine-rich splicing factor 2	3
1293	Q9JHB5	Translin-associated protein X	3
1294	P06399	Fibrinogen alpha chain	3
1295	P06399-2	Isoform 2 of Fibrinogen alpha chain	3
1296	P62278	40S ribosomal protein S13	3
1297	Q499N6	UBX domain-containing protein 1	3
1298	P68101	Eukaryotic translation initiation factor 2 subunit 1	3
1299	Q8K4K5	Lethal(2) giant larvae protein homolog 1	3
1300	P47245	Nardilysin	3
1301	P47245-2	Isoform 2 of Nardilysin	3
1302	Q2TL32	E3 ubiquitin-protein ligase UBR4	3
1303	F1M386	Rap guanine nucleotide exchange factor 2	3
1304	B1H267	Sorting nexin-5	3
1305	Q66H80	Coatomer subunit delta	3
1306	Q64537	Calpain small subunit 1	3
1307	Q9Z0J5	Thioredoxin reductase 2, mitochondrial	3
1308	Q9Z0J5-2	Isoform 2 of Thioredoxin reductase 2, mitochondrial	3
1309	P32736	Opioid-binding protein/cell adhesion molecule	3
1310	P32736-2	Isoform 2 of Opioid-binding protein/cell adhesion molecule	3
1311	P09895	60S ribosomal protein L5	3
1312	Q5XIG0	ADP-ribose pyrophosphatase, mitochondrial	3
1313	P61150	Fibroblast growth factor 12	3
1314	P61150-2	Isoform 2 of Fibroblast growth factor 12	3
1315	E9PSK7	C-Jun-amino-terminal kinase-interacting protein 3	3
1316	Q05695	Neural cell adhesion molecule L1	3
1317	Q05695-2	Isoform 2 of Neural cell adhesion molecule L1	3
1318	Q8CGV7	Thiamine-triphosphatase	3
1319	P49803	Regulator of G-protein signaling 7	3
1320	Q794F9	4F2 cell-surface antigen heavy chain	3
1321	Q5XIN6	Mitochondrial proton/calcium exchanger protein	3
1322	P68403-2	Isoform Beta-II of Protein kinase C beta type	3
1323	F1MA98	Nucleoprotein TPR	3
1324	Q9R085	Ubiquitin carboxyl-terminal hydrolase 15	3
1325	Q5EB77	Ras-related protein Rab-18	3
1326	Q2TA68	Dynamin-like 120 kDa protein, mitochondrial	3
1327	Q2TA68-3	Isoform 3 of Dynamin-like 120 kDa protein, mitochondrial	3
1328	Q2TA68-2	Isoform 2 of Dynamin-like 120 kDa protein, mitochondrial	3
1329	Q9QYW3	MOB-like protein phocein	3
1330	P61314	60S ribosomal protein L15	3

1331	P62914	60S ribosomal protein L11	3
1332	Q8CH84	ELAV-like protein 2	3
1333	Q5U1X1	Oligoribonuclease, mitochondrial	3
1334	Q6P7S1	Acid ceramidase	3
1335	Q9ESI7	Neuronal migration protein doublecortin	3
1336	P80299	Bifunctional epoxide hydrolase 2	3
1337	P60868	40S ribosomal protein S20	3
1338	Q9ES40	PRA1 family protein 3	3
1339	P58405	Striatin-3	3
1340	P58405-2	Isoform 2 of Striatin-3	3
1341	P31647	Sodium- and chloride-dependent GABA transporter 3	3
1342	O88350	Serine hydrolase RBBP9	3
1343	P31399	ATP synthase subunit d, mitochondrial	3
1344	Q63798	Proteasome activator complex subunit 2	3
1345	Q03346	Mitochondrial-processing peptidase subunit beta	3
1346	Q704S8	Carnitine O-acetyltransferase	3
1347	D3ZFB6	Proline-rich transmembrane protein 2	3
1348	Q792H5	CUGBP Elav-like family member 2	3
1349	Q792H5-4	Isoform 4 of CUGBP Elav-like family member 2	3
1350	Q792H5-3	Isoform 3 of CUGBP Elav-like family member 2	3
1351	Q792H5-2	Isoform 2 of CUGBP Elav-like family member 2	3
1352	P21571	ATP synthase-coupling factor 6, mitochondrial	3
1353	Q09073	ADP/ATP translocase 2	3
1354	Q9JI66	Electrogenic sodium bicarbonate cotransporter 1	3
1355	Q9JI66-3	Isoform 3 of Electrogenic sodium bicarbonate cotransporter 1	3
1356	Q9JI66-2	Isoform 2 of Electrogenic sodium bicarbonate cotransporter 1	3
1357	Q63666	Vesicle-associated membrane protein 1	3
1358	Q63666-4	Isoform 4 of Vesicle-associated membrane protein 1	3
1359	Q63666-3	Isoform 3 of Vesicle-associated membrane protein 1	3
1360	Q63666-2	Isoform 2 of Vesicle-associated membrane protein 1	3
1361	Q4FZT8	SPRY domain-containing protein 4	3
1362	Q2IBD4	Cortactin-binding protein 2	3
1363	Q2IBD4-2	Isoform 2 of Cortactin-binding protein 2	3
1364	Q8VHK7	Hepatoma-derived growth factor	3
1365	Q63633	Solute carrier family 12 member 5	3
1366	Q63633-2	Isoform 2 of Solute carrier family 12 member 5	3
1367	P22057	Prostaglandin-H2 D-isomerase	3
1368	P19132	Ferritin heavy chain	3
1369	Q5PPK9	EARP and GARP complex-interacting protein 1	3
1370	P11497	Acetyl-CoA carboxylase 1	3
1371	P11497-2	Isoform 2 of Acetyl-CoA carboxylase 1	3

1372	P12075	Cytochrome c oxidase subunit 5B, mitochondrial	3
1373	Q63279	Keratin, type I cytoskeletal 19	3
1374	Q6IFV3	Keratin, type I cytoskeletal 15	3
1375	Q03344	ATPase inhibitor, mitochondrial	3
1376	P12749	60S ribosomal protein L26	3
1377	B0BND0	Glycerophosphocholine cholinephosphodiesterase ENPP6	3
1378	Q9Z1Z3	Epsin-2	3
1379	P14270-8	Isoform 5 of cAMP-specific 3',5'-cyclic phosphodiesterase 4D	3
1380	P14270	cAMP-specific 3',5'-cyclic phosphodiesterase 4D	3
1381	P14270-9	Isoform 8 of cAMP-specific 3',5'-cyclic phosphodiesterase 4D	3
1382	P14270-6	Isoform 9 of cAMP-specific 3',5'-cyclic phosphodiesterase 4D	3
1383	P14270-4	Isoform 7 of cAMP-specific 3',5'-cyclic phosphodiesterase 4D	3
1384	P14270-1	Isoform 33 of cAMP-specific 3',5'-cyclic phosphodiesterase 4D	3
1385	Q99ND9	RWD domain-containing protein 1	3
1386	P62907	60S ribosomal protein L10a	3
1387	P15865	Histone H1.4	3
1388	Q3KRD5	Mitochondrial import receptor subunit TOM34	3
1389	P07825	Synaptophysin	3
1390	P62074	Mitochondrial import inner membrane translocase subunit Tim10	3
1391	P22791	Hydroxymethylglutaryl-CoA synthase, mitochondrial	3
1392	Q9R037	WD repeat-containing protein 44	3
1393	Q5U316	Ras-related protein Rab-35	3
1394	P81795	Eukaryotic translation initiation factor 2 subunit 3, X-linked	3
1395	P12928	Pyruvate kinase PKLR	3
1396	P12928-2	Isoform L-type of Pyruvate kinase PKLR	3
1397	F1LXF1	Breakpoint cluster region protein	3
1398	Q07310	Neurexin-3	3
1399	Q07310-9	Isoform 9 of Neurexin-3	3
1400	Q07310-8	Isoform 8 of Neurexin-3	3
1401	Q07310-7	Isoform 7 of Neurexin-3	3
1402	Q07310-6	Isoform 6 of Neurexin-3	3
1403	Q07310-5	Isoform 5 of Neurexin-3	3
1404	Q07310-4	Isoform 4 of Neurexin-3	3
1405	Q07310-3	Isoform 3 of Neurexin-3	3
1406	Q07310-2	Isoform 2 of Neurexin-3	3
1407	Q07310-19	Isoform 19 of Neurexin-3	3
1408	Q07310-18	Isoform 18 of Neurexin-3	3
1409	Q07310-17	Isoform 17 of Neurexin-3	3
1410	Q07310-16	Isoform 16 of Neurexin-3	3
1411	Q07310-15	Isoform 15 of Neurexin-3	3
1412	Q07310-14	Isoform 14 of Neurexin-3	3

1413	Q07310-13	Isoform 13 of Neurexin-3	3
1414	Q07310-12	Isoform 12 of Neurexin-3	3
1415	Q07310-11	Isoform 11 of Neurexin-3	3
1416	Q07310-10	Isoform 10 of Neurexin-3	3
1417	Q63372-9	Isoform 9a of Neurexin-1	3
1418	Q63372-8	Isoform 8a of Neurexin-1	3
1419	Q63372-7	Isoform 7a of Neurexin-1	3
1420	Q63372-6	Isoform 6a of Neurexin-1	3
1421	Q63372-5	Isoform 5a of Neurexin-1	3
1422	Q63372-4	Isoform 4a of Neurexin-1	3
1423	Q63372-3	Isoform 3a of Neurexin-1	3
1424	Q63372-1	Isoform 2a of Neurexin-1	3
1425	Q63372-13	Isoform 13a of Neurexin-1	3
1426	Q63372-12	Isoform 12a of Neurexin-1	3
1427	Q63372-11	Isoform 11a of Neurexin-1	3
1428	Q63372-10	Isoform 10a of Neurexin-1	3
1429	P48679	Prelamin-A/C	3
1430	P38406	Guanine nucleotide-binding protein G(olf) subunit alpha	3
1431	G3V9R3	Sulfotransferase 1 family member D1	3
1432	Q63421	Calcium/calmodulin-dependent 3',5'-cyclic nucleotide ph	3
1433	Q9Z252	Protein lin-7 homolog B	3
1434	O09032	ELAV-like protein 4	3
1435	Q5BJ92	Serine/threonine-protein phosphatase 4 catalytic subunit	3
1436	Q712U5	cAMP-regulated phosphoprotein 19	3
1437	Q712U5-2	Isoform ARPP-16 of cAMP-regulated phosphoprotein 19	3
1438	Q63372	Neurexin-1	3
1439	Q63372-9	Isoform 9a of Neurexin-1	3
1440	Q63372-8	Isoform 8a of Neurexin-1	3
1441	Q63372-7	Isoform 7a of Neurexin-1	3
1442	Q63372-6	Isoform 6a of Neurexin-1	3
1443	Q63372-5	Isoform 5a of Neurexin-1	3
1444	Q63372-4	Isoform 4a of Neurexin-1	3
1445	Q63372-3	Isoform 3a of Neurexin-1	3
1446	Q63372-1	Isoform 2a of Neurexin-1	3
1447	Q63372-13	Isoform 13a of Neurexin-1	3
1448	Q63372-12	Isoform 12a of Neurexin-1	3
1449	Q63372-11	Isoform 11a of Neurexin-1	3
1450	Q63372-10	Isoform 10a of Neurexin-1	3
1451	Q9ES39	Nuclear distribution protein nudE homolog 1	2
1452	P0C0S7	Histone H2A.Z	2
1453	Q5PPN5	Tubulin polymerization-promoting protein family member 3	2

1454	Q7TP36	Protein Shroom2	2
1455	Q5QJC9	BAG family molecular chaperone regulator 5	2
1456	Q9JHZ4	GRIP1-associated protein 1	2
1457	Q9JHZ4-2	Isoform 2 of GRIP1-associated protein 1	2
1458	P68403	Protein kinase C beta type	2
1459	Q920A6	Retinoid-inducible serine carboxypeptidase	2
1460	Q63507	60S ribosomal protein L14	2
1461	Q6XVN8	Microtubule-associated proteins 1A/1B light chain 3A	2
1462	Q9ESM2	Hyaluronan and proteoglycan link protein 2	2
1463	Q62940	E3 ubiquitin-protein ligase NEDD4	2
1464	P63249	cAMP-dependent protein kinase inhibitor alpha	2
1465	O35142	Coatomer subunit beta'	2
1466	Q9JKS6	Protein piccolo	2
1467	Q9JKS6-3	Isoform 3 of Protein piccolo	2
1468	Q9JKS6-2	Isoform 2 of Protein piccolo	2
1469	P30713	Glutathione S-transferase theta-2	2
1470	Q561S0	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial	2
1471	P84850	D-2-hydroxyglutarate dehydrogenase, mitochondrial	2
1472	P97615	Thioredoxin, mitochondrial	2
1473	P19627	Guanine nucleotide-binding protein G(z) subunit alpha	2
1474	Q8K4G6	ADP-ribose glycohydrolase MACROD1 (Fragment)	2
1475	P20069	Mitochondrial-processing peptidase subunit alpha	2
1476	Q9WVE9	Intersectin-1	2
1477	P18445	60S ribosomal protein L27a	2
1478	Q4KLH4	Paraspeckle component 1	2
1479	Q62634	Vesicular glutamate transporter 1	2
1480	Q62634-2	Isoform 2 of Vesicular glutamate transporter 1	2
1481	P43138	DNA-(apurinic or apyrimidinic site) lyase	2
1482	Q6MG49	Large proline-rich protein BAG6	2
1483	Q6MG49-2	Isoform 2 of Large proline-rich protein BAG6	2
1484	P18757	Cystathionine gamma-lyase	2
1485	Q9EQV6	Tripeptidyl-peptidase 1	2
1486	Q8CGU9	Tryptophan 5-hydroxylase 2	2
1487	B2RZ37	Receptor expression-enhancing protein 5	2
1488	Q08603	Geranylgeranyl transferase type-2 subunit beta	2
1489	P21775	3-ketoacyl-CoA thiolase A, peroxisomal	2
1490	P07871	3-ketoacyl-CoA thiolase B, peroxisomal	2
1491	P48508	Glutamate--cysteine ligase regulatory subunit	2
1492	Q9WVA1	Mitochondrial import inner membrane translocase subunit Tim8 A	2
1493	Q4FZU2	Keratin, type II cytoskeletal 6A	2
1494	Q09167	Serine/arginine-rich splicing factor 5	2

1495	P04644	40S ribosomal protein S17	2
1496	Q64361	Latexin	2
1497	Q6AY71	Protein C8orf37 homolog	2
1498	Q5PQN7	Protein LZIC	2
1499	P97519	Hydroxymethylglutaryl-CoA lyase, mitochondrial	2
1500	Q566Q8	UPF0696 protein C11orf68 homolog	2
1501	P84586	RNA-binding motif protein, X chrom	2
1502	D4AE41	RNA binding motif protein, X-linked-like-1	2
1503	Q4V898	RNA-binding motif protein, X chrom	2
1504	Q4KLZ6	Triokinase/FMN cyclase	2
1505	P63081	V-type proton ATPase 16 kDa proteolipid subunit	2
1506	D3Z8L7	Ras-related protein R-Ras	2
1507	Q6MGD0	Protein CutA	2
1508	Q6AXU6	Jupiter microtubule associated homolog 1	2
1509	Q63690	Apoptosis regulator BAX	2
1510	Q62876	Synaptogyrin-1	2
1511	Q4V8E4	Cilia- and flagella-associated protein 36	2
1512	Q02765	Cathepsin S	2
1513	P63326	40S ribosomal protein S10	2
1514	P48199	C-reactive protein	2
1515	P19944	60S acidic ribosomal protein P1	2
1516	P13471	40S ribosomal protein S14	2
1517	P04550	Parathymosin	2
1518	P00763	Anionic trypsin-2	2
1519	B2GV54	Neutral cholesterol ester hydrolase 1	2
1520	Q4G009	Malignant T-cell-amplified sequence 1	2
1521	P07151	Beta-2-microglobulin	2
1522	P80254	D-dopachrome decarboxylase	2
1523	P83732	60S ribosomal protein L24	2
1524	P62850	40S ribosomal protein S24	2
1525	P62850-3	Isoform 3 of 40S ribosomal protein S24	2
1526	P62850-2	Isoform 2 of 40S ribosomal protein S24	2
1527	P62859	40S ribosomal protein S28	2
1528	F1M5N7	Kinesin-like protein KIF21B	2
1529	Q62639	GTP-binding protein Rheb	2
1530	Q5I0C3	Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial	2
1531	P05765	40S ribosomal protein S21	2
1532	Q4V8K5	BRO1 domain-containing protein BROX	2
1533	Q66H12	Alpha-N-acetylgalactosaminidase	2
1534	O88506	STE20/SPS1-related proline-alanine-rich protein kinase	2
1535	Q9R1T3	Cathepsin Z	2

1536	O88483	[Pyruvate dehydrogenase [acetyl-transferring]]-phosphatase 1, mitochondrial	2
1537	P62856	40S ribosomal protein S26	2
1538	P62832	60S ribosomal protein L23	2
1539	Q4KM74	Vesicle-trafficking protein SEC22b	2
1540	Q8VHU4	Elongator complex protein 1	2
1541	P29418	ATP synthase subunit epsilon, mitochondrial	2
1542	Q6IFV1	Keratin, type I cytoskeletal 14	2
1543	Q6IFU8	Keratin, type I cytoskeletal 17	2
1544	Q4V8F9	Hydroxysteroid dehydrogenase-like protein 2	2
1545	O54783	Choline/ethanolamine kinase	2
1546	P32362	Uroporphyrinogen decarboxylase (Fragment)	2
1547	B5DFC8	Eukaryotic translation initiation factor 3 subunit C	2
1548	P42930	Heat shock protein beta-1	2
1549	Q9QYJ6-4	Isoform 4 of cAMP and cAMP-inhibited cGMP 3',5'-cyclic ph	2
1550	Q9QYJ6-3	Isoform 3 of cAMP and cAMP-inhibited cGMP 3',5'-cyclic ph	2
1551	Q9QYJ6	cAMP and cAMP-inhibited cGMP 3',5'-cyclic ph	2
1552	Q9QYJ6-2	Isoform 2 of cAMP and cAMP-inhibited cGMP 3',5'-cyclic ph	2
1553	Q6IG02	Keratin, type II cytoskeletal 2 epidermal	2
1554	P24054	SPARC-like protein 1	2
1555	P63324	40S ribosomal protein S12	2
1556	A0A0G2JV04	ADP-ribosylation factor-binding protein GGA3	2
1557	Q5BKC9	Ephexin-1	2
1558	Q7TP98	Interleukin enhancer-binding factor 2	2
1559	P05982	NAD(P)H dehydrogenase [quinone] 1	2
1560	Q64620	Serine/threonine-protein phosphatase 6 catalytic subunit	2
1561	Q5XID1	Anamorsin	2
1562	Q66H20	Polypyrimidine tract-binding protein 2	2
1563	Q66H20-2	Isoform 2 of Polypyrimidine tract-binding protein 2	2
1564	P61354	60S ribosomal protein L27	2
1565	P52504	NADH dehydrogenase [ubiquinone] iron-sulfur protein 6, mitochondrial	2
1566	O88370	Phosphatidylinositol 5-phosphate 4-kinase type-2 gamma	2
1567	P29147	D-beta-hydroxybutyrate dehydrogenase, mitochondrial	2
1568	P84060	Dystrobrevin beta	2
1569	Q63065	[Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 1, mitochondrial	2
1570	Q5M7T9	Threonine synthase-like 2	2
1571	P63035	Cytohesin-2	2
1572	Q8K4Y5	Leucine-rich glioma-inactivated protein 1	2
1573	P84817-2	Isoform 2 of Mitochondrial fission 1 protein	2
1574	P84817-3	Isoform 3 of Mitochondrial fission 1 protein	2
1575	P84817	Mitochondrial fission 1 protein	2

1576	Q924K2	FAS-associated factor 1	2
1577	P21139	Alpha-mannosidase 2C1	2
1578	Q6P9U8	Eukaryotic translation initiation factor 3 subunit H	2
1579	Q4QQV8	Charged multivesicular body protein 5	2
1580	Q157S1	CREB-regulated transcription coactivator 1	2
1581	Q5XIU5	Proteasome inhibitor PI31 subunit	2
1582	Q6AY57	WD repeat domain phosphoinositide-interacting protein 2	2
1583	D4ABY2	Coatomer subunit gamma-2	2
1584	Q4KM65	Cleavage and polyadenylation specificity factor subunit 5	2
1585	Q1WIM2	Cell adhesion molecule 2	2
1586	Q1WIM2-2	Isoform 2 of Cell adhesion molecule 2	2
1587	D3ZUQ0	RILP-like protein 1	2
1588	P08649	Complement C4	2
1589	Q6AXS5	Plasminogen activator inhibitor 1 RNA-binding protein	2
1590	Q6AXS5-2	Isoform 2 of Plasminogen activator inhibitor 1 RNA-binding protein	2
1591	P26453	Basigin	2
1592	P26453-2	Isoform 2 of Basigin	2
1593	P61149	Fibroblast growth factor 1	2
1594	Q6LED0	Histone H3.1	2
1595	P84245	Histone H3.3	2
1596	P26817	Beta-adrenergic receptor kinase 1	2
1597	P07897	Aggrecan core protein	2
1598	P07897-2	Isoform 2 of Aggrecan core protein	2
1599	P62078	Mitochondrial import inner membrane translocase subunit Tim8 B	2
1600	Q9Z327	Synaptopodin	2
1601	Q9Z327-2	Isoform 2 of Synaptopodin	2
1602	Q9Z327-3	Isoform 3 of Synaptopodin	2
1603	P01244	Somatotropin	2
1604	Q3KR86	MICOS complex subunit Mic60 (Fragment)	2
1605	Q62881	Nucleolar protein 3	2
1606	P97829-2	Isoform 2 of Leukocyte surface antigen CD47	2
1607	Q99PS8	Histidine-rich glycoprotein	2
1608	Q5U211	Sorting nexin-3	2
1609	Q6P7R8	Very-long-chain 3-oxoacyl-CoA reductase	2
1610	A2RRU1	Glycogen [starch] synthase, muscle	2
1611	Q496Z0	Elongator complex protein 2	2
1612	P14668	Annexin A5	2
1613	Q9ES53	Ubiquitin recognition factor in ER-associated degradation protein 1	2
1614	Q4QQS3	Protein OSCP1	2
1615	P63319	Protein kinase C gamma type	2

1616	Q71UF4	Histone-binding protein RBBP7	2
1617	Q9JI85	Nucleobindin-2	2
1618	P0C5H9	Mesencephalic astrocyte-derived neurotrophic factor	2
1619	P97546	Neuroplastin	2
1620	P97546-3	Isoform 3 of Neuroplastin	2
1621	P97546-1	Isoform 1 of Neuroplastin	2
1622	C9WPN6	Eukaryotic translation initiation factor 2 subunit 3, Y-linked	2
1623	Q5U2R7	LRP chaperone MESD	2
1624	P23593	Apolipoprotein D	2
1625	Q71TY3	40S ribosomal protein S27	2
1626	P62845	40S ribosomal protein S15	2
1627	Q5U2Q3	Ester hydrolase C11orf54 homolog	2
1628	Q6MFY6	E3 ubiquitin-protein ligase PPP1R11	2
1629	Q6MFY6-2	Isoform 2 of E3 ubiquitin-protein ligase PPP1R11	2
1630	P61959	Small ubiquitin-related modifier 2	2
1631	O35274	Neurabin-2	2
1632	Q99ML5	Prenylcysteine oxidase	2
1633	Q9JID2	Guanine nucleotide-binding protein subunit alpha-11	2
1634	P19356	Porphobilinogen deaminase	2
1635	P19356-2	Isoform 2 of Porphobilinogen deaminase	2
1636	P14200	Pro-MCH	2
1637	Q5MPA9-3	Isoform 3 of Serine/threonine-protein kinase DCLK2	2
1638	Q5MPA9	Serine/threonine-protein kinase DCLK2	2
1639	Q5MPA9-2	Isoform 2 of Serine/threonine-protein kinase DCLK2	2
1640	Q04940	Neurogranin	2
1641	D3ZHV2	Microtubule-actin cross-linking factor 1	2
1642	P83941	Elongin-C	2
1643	P84100	60S ribosomal protein L19	2
1644	Q63648	Merlin (Fragment)	2
1645	O70277	Tripartite motif-containing protein 3	2
1646	P97888	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B gamma isoform	2
1647	Q62625	Microtubule-associated proteins 1A/1B light chain 3B	2
1648	Q62625-2	Isoform 2 of Microtubule-associated proteins 1A/1B light chain 3B	2
1649	Q99P74	Ras-related protein Rab-27B	2
1650	Q6MG61	Chloride intracellular channel protein 1	2
1651	P62268	40S ribosomal protein S23	2
1652	Q9JJK1	Neuronal membrane glycoprotein M6-b	2
1653	P56603	Secretory carrier-associated membrane protein 1	2
1654	O70441	Synapsin-3	2
1655	Q925G1	Hepatoma-derived growth factor-related protein 2	2
1656	Q925G1-2	Isoform 2 of Hepatoma-derived growth factor-related protein	2

		2	
1657	Q923W4	Hepatoma-derived growth factor-related protein 3	2
1658	Q62844	Tyrosine-protein kinase Fyn	2
1659	F1LM93	Tyrosine-protein kinase Yes	2
1660	P50545	Tyrosine-protein kinase HCK	2
1661	P50545-2	Isoform 2 of Tyrosine-protein kinase HCK	2
1662	Q01621	Proto-oncogene tyrosine-protein kinase LCK	2
1663	P31662	Sodium-dependent neutral amino acid transporter SLC6A17	2
1664	P35571	Glycerol-3-phosphate dehydrogenase, mitochondrial	2
1665	Q5FVM4	Non-POU domain-containing octamer-binding protein	2
1666	P30337	N-chimaerin	2
1667	Q64591	2,4-dienoyl-CoA reductase, mitochondrial	2
1668	Q06647	ATP synthase subunit O, mitochondrial	2
1669	Q6MG06	Guanine nucleotide-binding protein-like 1	2
1670	P24050	40S ribosomal protein S5	2
1671	Q6AYC4	Macrophage-capping protein	2
1672	Q4V7A0	WD repeat-containing protein 61	2
1673	Q920P0	L-xylulose reductase	2
1674	G3V928	Prolow-density lipoprotein receptor-related protein 1	2
1675	O35831	Cyclin-dependent kinase 17	2
1676	Q5M819	Phosphoserine phosphatase	2
1677	P62282	40S ribosomal protein S11	2
1678	Q63345	Myelin-oligodendrocyte glycoprotein	2
1679	P04639	Apolipoprotein A-I	2
1680	F1LQX4	Rho GTPase-activating protein 44	2
1681	F1LQX4-3	Isoform 3 of Rho GTPase-activating protein 44	2
1682	F1LQX4-2	Isoform 2 of Rho GTPase-activating protein 44	2
1683	Q5BJY9	Keratin, type I cytoskeletal 18	2
1684	D4A0X3	AP2-interacting clathrin-endocytosis protein	2
1685	P62332	ADP-ribosylation factor 6	2
1686	Q7TSP2	REVERSED Kinesin-like protein KIF15	2
1687	Q8CFG6	Voltage-dependent calcium channel subunit alpha-2/delta-2	2
1688	Q6IG12	Keratin, type II cytoskeletal 7	2
1689	P08934-2	Isoform LMW of Kininogen-1	2
1690	O35832	Cyclin-dependent kinase 18	2
1691	Q9Z1J8	SEC14-like protein 3	2
1692	D3ZW91	POC1 centriolar protein homolog B	2

Appendix 5: list of commonly differentiated proteins between STZ and MGO w.r.t. Control

Acc. No.	Protein Description	Gene	STZ/Control	MGO/Control
P21913	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	Sdhb	2.62	1.58
P12075	Cytochrome c oxidase subunit 5B, mitochondrial	Cox5b	2.28	3.85
D3ZYW7	Frataxin, mitochondrial	Fxn	2.12	1.71
P18757	Cystathionine gamma-lyase	Cth	2.09	1.40
P32551	Cytochrome b-c1 complex subunit 2, mitochondrial	Uqcrc2	2.02	1.42
Q9Z1B2	Glutathione S-transferase Mu 5	Gstm5	1.89	1.64
Q04631	Protein farnesyltransferase/geranylgeranyltransferase type-1 subunit alpha	Fnta	1.83	1.39
P15651	Short-chain specific acyl-CoA dehydrogenase, mitochondrial	Acads	1.75	1.64
P63100	Calcineurin subunit B type 1	Ppp3r1	1.74	1.32
P13635	Ceruloplasmin	Cp	1.69	1.38
Q5XI78	2-oxoglutarate dehydrogenase, mitochondrial	Ogdh	1.69	1.37
P08503	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial	Acadm	1.64	1.59
P10719	ATP synthase subunit beta, mitochondrial	Atp5f1b	1.63	1.32
O35263	Platelet-activating factor acetylhydrolase IB subunit gamma	Pafah1b3	1.62	1.82
P35435	ATP synthase subunit gamma, mitochondrial	Atp5f1c	1.60	1.48
Q64361	Latexin	Lxn	1.57	1.65
P11960	2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial (Fragment)	Bckdha	1.56	1.39
O88483	[Pyruvate dehydrogenase [acetyl-transferring]]-phosphatase 1, mitochondrial	Pdp1	1.55	2.07
P11915	Non-specific lipid-transfer protein	Scp2	1.55	1.60
P13803	Electron transfer flavoprotein subunit alpha, mitochondrial	Etfa	1.53	1.38
Q5XIE6	3-hydroxyisobutyryl-CoA hydrolase, mitochondrial	Hibch	1.48	1.31
Q1WIM1	Cell adhesion molecule 4	Cadm4	1.46	1.33
P01015	Angiotensinogen	Agt	1.44	1.41
Q9EPB1	Dipeptidyl peptidase 2	Dpp7	1.43	1.30
P97532	3-mercaptopyruvate sulfurtransferase	Mpst	1.42	1.39
P04785	Protein disulfide-isomerase	P4hb	1.40	1.52
P50554	4-aminobutyrate aminotransferase, mitochondrial	Abat	1.39	1.32
P04905	Glutathione S-transferase Mu 1	Gstm1	1.32	1.39
Q497B0	Omega-amidase NIT2	Nit2	1.31	1.53
P04636	Malate dehydrogenase, mitochondrial	Mdh2	1.30	1.37

P10362	Secretogranin-2	Scg2	1.30	1.46
Q9QYF3	Unconventional myosin-Va	Myo5a	-1.29	-1.43
Q9JJ54	Heterogeneous nuclear ribonucleoprotein D0	Hnrnpd	-1.31	-1.30
Q62717	Calcium-dependent secretion activator 1	Cadps	-1.33	-1.32
Q6PST4	Atlastin-1	Atl1	-1.35	-1.51
Q6IMY8	Heterogeneous nuclear ribonucleoprotein U	Hnrnpu	-1.37	-1.39
B3GNI6	Septin-11	Septin11	-1.39	-1.79
Q5M7U6	Actin-related protein 2	Actr2	-1.40	-1.59
Q05683	Glutamate decarboxylase 2	Gad2	-1.45	-1.51
P54921	Alpha-soluble NSF attachment protein	Napa	-1.46	-1.55
Q5XI32	F-actin-capping protein subunit beta	Capzb	-1.47	-1.40
Q9ERH3	WD repeat-containing protein 7	Wdr7	-1.48	-1.80
Q5MJ12	F-box/LRR-repeat protein 16	Fbxl16	-1.49	-1.60
P25286	V-type proton ATPase 116 kDa subunit a isoform 1	Atp6v0a1	-1.50	-2.07
Q4QQS3	ProteinCP1	Oscp1	-1.53	-1.62
A0A0G2JTR4	Active breakpoint cluster region-related protein	Abr	-1.55	-1.73
Q9Z0J8	Neuronal growth regulator 1	Negr1	-1.58	-2.29
Q9JJ19	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	Slc9a3r1	-1.60	-1.31
Q6P9T8	Tubulin beta-4B chain	Tubb4b	-1.63	-1.32
Q6URK4	Heterogeneous nuclear ribonucleoprotein A3	Hnrnpa3	-1.67	-1.36
P19332-3	Isoform Tau-C of Microtubule-associated protein tau	Mapt	-1.70	-1.80
Q01986	Dual specificity mitogen-activated protein kinase kinase 1	Map2k1	-1.89	-1.90
P43425	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-7	Gng7	-1.90	-2.90
Q01066	Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1B	Pde1b	-1.90	-1.74
O35820	2'-deoxynucleoside 5'-phosphate N-hydrolase 1	Dnph1	-1.92	-2.44
Q5BKC9	Ephexin-1	Ngef	-1.93	-2.92
Q9QUL6	Vesicle-fusing ATPase	Nsf	-2.01	-1.83
O08875	Serine/threonine-protein kinase DCLK1	Dclk1	-2.15	-2.39
Q6J4I0	Protein phosphatase 1 regulatory subunit 1B	Ppp1r1b	-2.19	-2.52
Q64548-2	Isoform RTN1-S of Reticulon-1	Rtn1	-2.78	-2.08

Appendix 6: MGO+TELMI restored proteins w.r.t. control

Acc. no.	Protein	Gene Name	MGO/Control	MGO TELMI/Control

P12075	Cytochrome c oxidase subunit 5B, mitochondrial	Cox5b	3.85	1.88
Q9ESM2	Hyaluronan and proteoglycan link protein 2	Hapln2	2.65	1.48
P29314	40S ribosomal protein S9	Rps9	2.57	2.31
P20767	Ig lambda-2 chain C region	lgl2	2.32	1.81
Q8CGV7	Thiamine-triphosphatase	Thtpa	2.21	2.11
O88483	[Pyruvate dehydrogenase [acetyl-transferring]]-phosphatase 1, mitochondrial	Pdp1	2.07	1.90
Q6AY57	WD repeat domain phosphoinositide-interacting protein 2	Wipi2	1.99	1.60
Q68FS4	Cytosol aminopeptidase	Lap3	1.90	1.50
P01946	Hemoglobin subunit alpha-1/2	Hba1	1.88	1.57
Q6P7S1	Acid ceramidase	Asah1	1.79	1.31
D3ZYW7	Frataxin, mitochondrial	Fxn	1.71	1.45
Q64361	Latexin	Lxn	1.65	1.47
Q9Z1B2	Glutathione S-transferase Mu 5	Gstm5	1.64	1.37
P04785	Protein disulfide-isomerase	P4hb	1.52	1.34
P01026	Complement C3	C3	1.51	1.48
Q04631	Protein farnesyltransferase/geranylgeranyltransferase type-1 subunit alpha	Fnta	1.39	1.33
P04692-2	Isoform 2 of Tropomyosin alpha-1 chain	Tpm1	2.21	1.55
P35745	Acylphosphatase-2	Acyp2	1.83	1.51
P26772	10 kDa heat shock protein, mitochondrial	Hspe1	1.47	1.30
P18418	Calreticulin	Calr	1.37	1.34
P60881	Synaptosomal-associated protein 25	Snap25	-1.32	-1.30
P28075	Proteasome subunit beta type-5	Psmb5	-1.47	-1.43
Q05683	Glutamate decarboxylase 2	Gad2	-1.51	-1.49
Q99P74	Ras-related protein Rab-27B	Rab27b	-1.60	-1.47
D3ZFB6	Proline-rich transmembrane protein 2	Prrt2	-1.69	-1.34
B3GNI6	Septin-11	Septin11	-1.79	-1.36
P19332-3	Isoform Tau-C of Microtubule-associated protein tau	Mapt	-1.80	-1.75
P43425	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-7	Gng7	-2.90	-1.56
Q05175	Brain acid soluble protein 1	Basp1	-3.54	-1.49

P32736	Opioid-binding protein/cell adhesion molecule	Opcml	-4.99	-1.62
P70483	Striatin	Strn	-1.40	-1.32
P60841	Alpha-endosulfine	Ensa	-1.63	-1.32
P04094	Proenkephalin-A	Penk	-1.84	-1.62
O35820	2'-deoxynucleoside 5'-phosphate N-hydrolase 1	Dnph1	-2.44	-2.16

Appendix 7: MGO+AMG restored proteins w.r.t. control

Acc. No.	Protein	Gene Name	MGO/Control	MGO AMG/Control
P20767	Ig lambda-2 chain C region	lgl2	2.32	1.95
Q68FS4	Cytosol aminopeptidase	Lap3	1.90	1.33
P01946	Hemoglobin subunit alpha-1/2	Hba1	1.88	1.40
Q6P7S1	Acid ceramidase	Asah1	1.79	1.64
D3ZYW7	Frataxin, mitochondrial	Fxn	1.71	1.34
P02625	Parvalbumin alpha	Pvalb	1.58	1.36
Q497B0	Omega-amidase NIT2	Nit2	1.53	1.40
O35264	Platelet-activating factor acetylhydrolase IB subunit beta	Pafah1b2	1.46	1.33
P02091	Hemoglobin subunit beta-1	Hbb	1.42	1.30
P28075	Proteasome subunit beta type-5	Psmb5	-1.47	-1.38
Q05683	Glutamate decarboxylase 2	Gad2	-1.51	-1.30
P43425	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-7	Gng7	-2.90	-1.61
P32736	Opioid-binding protein/cell adhesion molecule	Opcml	-4.99	-3.20
P27791	cAMP-dependent protein kinase catalytic subunit alpha	Prkaca	-1.54	-1.48
Q6RUV5	Ras-related C3 botulinum toxin substrate 1	Rac1	-1.57	-1.42
Q9WTT6	Guanine deaminase	Gda	-1.64	-1.56
P52481	Adenylyl cyclase-associated protein 2	Cap2	-1.66	-1.29
Q9JJM9-2	Isoform 2 of Septin-5	Septin5	-1.70	-1.65
Q63507	60S ribosomal protein L14	Rpl14	-1.80	-1.61
P12369	cAMP-dependent protein kinase type II-beta regulatory subunit	Prkar2b	-1.82	-1.57
Q9WU34	Neuronal-specific septin-3	Septin3	-1.83	-1.69
P60905	DnaJ homolog subfamily C member 5	Dnajc5	-1.84	-1.38

Q9QYW3	MOB-like protein phocein	Mob4	-1.89	-1.41
P62762	Visinin-like protein 1	Vsnl1	-1.99	-1.67
P50878	60S ribosomal protein L4	Rpl4	-2.12	-1.69
P10824	Guanine nucleotide-binding protein G(i) subunit alpha-1	Gnai1	-2.18	-1.67
Q9Z0G8	WAS/WASL-interacting protein family member 3	Wipf3	-2.24	-1.54
Q62813	Limbic system-associated membrane protein	Lsamp	-2.31	-1.72
Q6J4I0	Protein phosphatase 1 regulatory subunit 1B	Ppp1r1b	-2.52	-2.21
Q792H5	CUGBP Elav-like family member 2	Celf2	-2.78	-2.21
P01830	Thy-1 membrane glycoprotein	Thy1	-2.90	-2.27
Q5BKC9	Ephexin-1	Ngef	-2.92	-1.99
P61765	Syntaxin-binding protein 1	Stxbp1	-3.28	-2.42

ABSTRACT

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AcSIR academic centre/CSIR Lab:NCL, Pune **Name of the Supervisor(s):Dr. Mahesh Kulkarni**

Title of the thesis: Investigation of Diabetes as a Risk Factor for Development of Alzheimer's Disease

AD is one of the most prevalent neurodegenerative diseases, characterized by Aβ aggregation and phosphorylated tau protein tangles. Diabetes acts as a major risk factor for AD. Hyperglycemic condition in diabetes leads to the formation of advanced glycation end products (AGEs) that interact with cell surface receptor RAGE causing oxidative stress, inflammation, and cell damage. The current study was performed to understand the role of diabetes in AD development. *In vitro* treatment of neuronal cells with methylglyoxal (MGO), a reactive dicarbonyl formed during diabetes showed concentration dependent increase in neurotoxicity. Neuronal proteomic analysis at a non-toxic concentration of MGO revealed that it alters the expression of various proteins involved in AD, diabetes, and calcium signaling. Further, we have checked the effect of hyperglycemia and MGO in the development of AD like symptoms in the rat model. We found that streptozotocin(STZ) and MGO develops anxiety in EPM test, and it decreases in presence of drugs, aminoguanidine (AMG) and telmisartan (TELMI). Further proteomic analysis of the hippocampus region revealed that STZ treatment upregulated proteins involved in AD, glycolysis and glutathione metabolism, and downregulated proteins are involved in synapses and synaptic vesicle cycle. Similarly, MGO treatment caused upregulation of proteins involved in AD, oxidative phosphorylation, and glutathione mechanism. MGO down regulated proteins have been found to play a role in different synapses, calcium signaling, and synaptic vesicle cycle. Few of these proteins were found to be restored by co-treatment with AMG and TELMI. We found that STZ and MGO treatments cause an increase in plasma fructosamine, RAGE expression, and tau phosphorylation, which was reduced in the presence of AMG and TELMI. STZ and MGO treatments were found to cause neurodegenerative changes in the CA1 region of the hippocampus. Further, we have performed a computational study to understand the molecular mechanisms for increased Aβ in diabetic conditions. Here, we suggest two possibilities. In the 1st possibility, we have found that α secretases are more prone to glycation induced inactivation due to more number of K and R, as well as putative glycation sites by NetGlycate analysis. Further blind docking with glucose also shows that active site and metal-binding sites of α secretases interact more with glucose than those of BACE1 and cathepsin B. Also, the binding pocket volume of ADAM17 was found to be small as compared to BACE1. We also suggest that glycation of lysine residue at α-secretase cleavage site on AβPP, prevents its processing by α secretases. It may be because of alteration in three dimensional structure. Both these possibilities may lead to increased Aβ production in diabetic subjects.

Curriculum Vitae of Gouri Patil

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Current Position: **Ph. D. student** at **CSIR-National Chemical Laboratory**, Pune

Education:

- **Master of Science in Microbiology** (2008-2010) University of Pune (*first class*)
- **Bachelor of Science in Industrial Microbiology** (2006-2008) Shivaji University, Kolhapur (*Distinction*)
- **Higher Secondary Certificate** (2005) Maharashtra State Board of Secondary and Higher Education (*Distinction*)
- **Secondary School Certificate Exam** (2003) Maharashtra State Board of Secondary and Higher Education (*Distinction*)

Research Experience:

- **Research Fellow** at **CSIR-National Chemical Laboratory:** January 2014 – January 2021

Project title "**Investigation of Diabetes as a Risk Factor for Development of Alzheimer's Disease"** under the supervision of Dr. Mahesh J. Kulkarni

- **Project Assistant II** at **CSIR-National Chemical Laboratory:** October 2010 – April 2013

Project title "**Comparison of placental proteomic patterns in pregnancy complications**" under the supervision of Dr. Ashok Giri

- **Project Trainee** at **DBT-National Centre for Cell Science, Pune:** July 2009-April 2010
  Project title "**Role of SMAR1 in DNA double strand break repair**" under the supervision of Dr. Samit Chattopadhyay.

Publications:

- **Patil GV**, Joshi RS, Kazi RS, Kulsange SE, Kulkarni MJ: **A possible role of glycation in the regulation of amyloid β precursor protein processing leading to amyloid β accumulation**. *Medical Hypotheses* 2020, **142**:109799.
- Kazi RS, Banarjee RM, Deshmukh AB, **Patil GV**, Jagadeeshaprasad MG, Kulkarni MJ: **Glycation inhibitors extend yeast chronological lifespan by reducing advanced glycation end products and by back regulation of proteins involved in mitochondrial respiration**. *Journal of Proteomics* 2017, **156**:104-112.
- Batkulwar KB, Bansode SB, **Patil GV**, Godbole RK, Kazi RS, Chinnathambi S, Shanmugam D, Kulkarni MJ: **Investigation of phosphoproteome in RAGE signaling**. *PROTEOMICS* 2015, **15**(2-3):245-259.
- Mary S, **Patil GV**, Kulkarni AV, Kulkarni MJ, Joshi SR, Mehendale SS, Giri AP: **Dynamic proteome in enigmatic preeclampsia: An account of molecular mechanisms and biomarker discovery**. *PROTEOMICS – Clinical Applications* 2012, **6**(1-2):79-90.

Awards & Achievements:

- Recipient of **Lady Tata Memorial Trust Fellowship for Ph. D.** for the duration of 2013-2017
- Received Special recognition award for oral presentation at 4th International Diabetes Summit, arranged by Chellaram diabetes Institute, Pune in 2020
- Qualified **Graduate Aptitude Test in Engineering** (**GATE)** with **AIR 104** in 2013
- Qualified University Grant Commission (UGC) **NET for lectureship** in 2013
- Shivaji University, Kolhapur, **Merit Scholarship** 2006-07 (Amount Rs. 10,000/-) holder
- Shivaji University, Kolhapur, **Dakshina Scholarship** 2008-2009 holder
- Yashwantrao Chavan Institute of Science, Satara, 2nd prize in intercollegiate elocution competition in 2006

Technical Skills:

- **Microbial & Animal Cell Culture:** Growth & maintenance of bacterial and yeast cultures, Maintenance of mammalian cell lines, Cell based assays, Immunofluorescence, Flow cytometry

- **Proteomics:** Immunoprecipitation, Western & dot blotting, SDS-PAGE, MALDI, LC-MS acquisition methods like MS^E, SWATH, PRM & data analysis using softwares like Protein Lynx Global Server (PLGS), Proteome Discoverer, ProteinPilot, PeakView, MarkerView
- **Molecular biology:** DNA & RNA isolation, PCR, qPCR
- **Biophysical:** CD spectroscopy, UV-VIS & Fluorescence spectroscopy
- **Bioinformatics and biostatistical tools:** Cytoscape, GraphPad Prism
- **Other softwares and tools:** Inkscape, ImageJ
- **Animal experiments:** Diabetes induction, Behavior study

Workshops attended:

- **Basic R for Life Sciences** conducted by BioSakshat at Venture Centre, NCL Innovation Park, Pune during June 29th - July 1st 2017
- **Proteomics Informatics Course** conducted by Institute of Systems Biology as a part of **Targeted Proteomics Workshop and International Symposium-Trans Proteomic Pipeline Workshop** at Indian Institute of Technology, Mumbai during 10th - 14th December 2015
- Workshop on **Insights in Biology** at CSIR-NCL on 29th October 2015

Conferences/Seminars Attended:

- **6th Annual meeting of Proteomics Society, India (PSI)** at IIT, Mumbai during 7th-9th December 2014
- **7th Annual meeting of Proteomics Society, India (PSI)** at VIT, Vellore during 3rd -5th December 2015
- **International Diabetes Summit** by Chellaram Diabetes Institute, Pune during 10th-12th March 2017
- **2nd International Diabetes Summit** by Chellaram Diabetes Institute, Pune during 9th-11th March 2018
- **4th International Diabetes Summit** by Chellaram Diabetes Institute, Pune during 6th-8th March 2020

Poster Presentations:

- Presented at CSIR, NCL during National Science Day Celebrations during 25th-26th February 2014
- Presented at 7th Annual Meeting of Proteomics Society, India at VIT Vellore during 3rd-5th December 2015
- Presented at CSIR, NCL during National Science Day Celebrations during 25th-26th February 2016
- Presented at ICAR-National Dairy Research Institute, Haryana at 11th Annual Meeting of Proteomics Society during 2nd -4th December 2019

Details of the publications emanating from the thesis work

1) List of publication(s) in SCI Journal(s) (published & accepted) emanating from the thesis work, with complete bibliographic details.

I. **Patil GV**, Joshi RS, Kazi RS, Kulsange SE, Kulkarni MJ: **A possible role of glycation in the regulation of amyloid β precursor protein processing leading to amyloid β accumulation**. *Medical Hypotheses* 2020, **142**:109799.

2) List of Papers with abstract presented (oral/poster) at national/international conferences/seminars with complete details.

I. Presented poster entitled "Role of methylglyoxal in the development of Alzheimer's Disease" at 11th Annual Meeting of Proteomics Society, India held during 2nd -4th December 2019 at ICAR-National Dairy Research Institute, Karnal, Haryana, India

Abstract

Alzheimer's disease (AD) is one of the common neurodegenerative diseases in aged population. Extracellular amyloid beta (Aβ) plaques and intracellular Tau protein tangles are the pathological hallmarks of AD. Diabetes is an important risk factor for AD. Hyperglycemia in diabetes causes non enzymytic protein glycation. Methylglyoxal(MGO) is a potent glycating agent. We have studied the effect of methylglyoxal treatment on the development of AD by behaviour studies such as actophotometer, novel object recognition test and elevated plus maze test. To understand the effect on protein expression, we have analysed hippocampal proteomics by IDA-SWATH mass spectrometry. A total of 3873 proteins were identified. SWATH analysis revealed that proteins involved in mitochondrial functioning and neurotransmission have found to be altered upon methylglyoxal treatment. Further we have studied hippocampal sections by histochemistry. Neurodegeneration has been observed in MGO treated rats. Tau Phosphorylation has been found to be increased in MGO treated rats as compared to Control which is reduced in presence of anti glycation drug aminoguanidine. Further experiments are going on to quantify Aβ 1-42 in the hippocampus. This study will help in understanding the mechanism of methylglyoxal in diabetes induced Alzheimer's disease.

II. **Oral presentation entitled** "Role of methylglyoxal in the development of Alzheimer's Disease" at 4th International Diabetes Summit-2020 from 6th-8th March 2020 at Pune hosted by Chellaram Diabetes Institute (CDI), Pune **(Received special recognition award)**

Abstract

Alzheimer's disease (AD) is one of the common neurodegenerative diseases in aged population. Extracellular amyloid beta (Aβ) plaques and intracellular tau protein tangles are the pathological hallmarks of AD. Diabetes is an important risk factor for AD. Hyperglycemia in diabetes causes non enzymytic protein glycation. Methylglyoxal (MGO) is a potent glycating agent. We have studied the effect of MGO on the development of AD in rat model by hippocampus proteomics, behaviour study, histochemistry and pTau western blotting. To understand the MGO effect on protein expression, we have analysed hippocampal proteome by IDA-SWATH mass spectrometry. A total of 3873 proteins have been identified. SWATH analysis revealed that proteins involved in mitochondrial functioning, apoptosis, oxidative stress are altered upon methylglyoxal treatment. Proteins involved in excitatory and inhibitory neurotransmission have been found to be down regulated upon MGO treatment. Elevated plus maze test have shown increased anxiety in MGO treated rats. H and E staining of hippocampal sections shows neurodegeneration and vascular changes in MGO treated rats. Tau Phosphorylation has been found to be increased in MGO treated rats which is reduced in presence of anti glycation drug aminoguanidine and ARB blocker telmisartan. Plasma fructosamine levels have been found to be increased in MGO treated rats along with increased RAGE expression in hippocampus. Further experiments are going on to quantify Aβ 1-42 in the hippocampus. This study will help in understanding the mechanism of methylglyoxal in diabetes induced Alzheimer's disease.