Supplementary Material

Transcriptomic Analysis of Alzheimer's Disease Pathways in a Pakistani Population

Supplementary Table 1. Socio-demographic and lifestyle characteristics of the AD population:

	Total (n=132)	**Female (n=62)**	**Male (n=70)**	***p***
Age	79.29±8.31	79.93±8.37	78.72±8.27	0.97
BMI (kg/m^2)	20.48±2.20	19.82±1.87	22.00±2.00	0.004
Glucose Level (mg/dl)	119.15±25.01	118.80±23.49	119.47±26.43	0.63
HbA1c (mmol/mol)	5.33±0.72	5.19±0.71	5.45±0.70	0.019
Hypertension (%)	79	83	74	0.12
Cardiovascular Disease (%)	57	59	56	0.55
Family History of AD (%)	38	50	28	0.001
***Smoker (%)**	25	5	46	<0.0001
Illiterate (%)	56	58	54	0.56
Unemployed (%)	81	91	74	0.005
Living in an Industrial Area (%)	30	29	69	<0.0001
Residence Locality (%) (Rural/Urban)	54/46	51/49	56/44	0.47

*Among the participants in our smoker group, smoking habit was on average 7±10 years, with a daily consumption varying between 1 and 10 cigarettes per day.

Supplementary Table 2. Gene lists for TLDA card-based profiler array analysis using the TaqMan® Array (FAST Plate) from Applied Biosystems (Catalog # 4418715, Santa Clara, CA, USA) on the ABI 7900HT-Fast Real-time PCR system for quantification. The cards are designed with preselected ninety-six (96) genes, including a reference gene (GAPDH (Hs99999905_m1) and a manufacturer control (18s rRNA (Hs99999901_s1)

Gene ID	Gene Name	Gene Functions
Hs00194045_m1	*ABCA1*	With cholesterol as its substrate, this protein functions as a cholesterol efflux pump in the cellular lipid removal pathway. Mutations in both alleles of this gene cause Tangier disease and familial high-density lipoprotein (HDL) deficiency.
Hs00153853_m1	*ADAM10*	Members of the ADAM family are cell surface proteins with a unique structure possessing both potential adhesion and protease domains. This gene encodes and ADAM family member that cleaves many proteins including TNF-alpha and E-cadherin.
Hs00234224_m1	*ADAM17*	This gene is implicated in a variety of biologic processes involving cell-cell and cell-matrix interactions, including fertilization, muscle development, and neurogenesis. Elevated expression of this gene has been observed in specific cell types derived from psoriasis, rheumatoid arthritis, multiple sclerosis and Crohn's disease patients, suggesting that the encoded protein may play a role in autoimmune disease.
Hs00177638_m1	*ADAM9*	This gene encodes a member of the ADAM (a disintegrin and metalloprotease domain) family. Members of this family are membrane-anchored proteins structurally related to snake venom disintegrins and have been implicated in a variety of biological processes involving cell-cell and cell-matrix interactions, including fertilization, muscle development, and neurogenesis. The protein encoded by this gene interacts with SH3 domain-containing proteins, binds mitotic arrest deficient 2 beta protein, and is also involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor.
Hs00154104_m1	*APBA1*	The protein encoded by this gene is a member of the X11 protein family. It is a neuronal adapter protein that interacts with the Alzheimer's disease amyloid precursor protein (APP). It stabilizes APP and inhibits production of proteolytic APP fragments including the A beta peptide that is deposited in the brains of Alzheimer's disease patients. This gene product is believed to be involved in signal transduction processes. It is also regarded as a putative vesicular trafficking protein in the brain that can form a complex with the potential to couple synaptic vesicle exocytosis to neuronal cell adhesion.
Hs00194072_m1	*APBA2*	It is a neuronal adapter protein that interacts with the Alzheimer's disease amyloid precursor protein (APP). It stabilizes APP and inhibits production of proteolytic APP fragments including the A beta peptide that is deposited in the brains of Alzheimer's disease patients. This gene product is believed to be involved in signal transduction processes. It is also regarded as a putative vesicular trafficking protein in the brain that can form a complex with the potential to couple synaptic vesicle exocytosis to neuronal cell adhesion.

Hs00191660_m1	*APBA3*	The protein encoded by this gene is a member of the X11 protein family. It is an adapter protein that interacts with the Alzheimer's disease amyloid precursor protein. This gene product is believed to be involved in signal transduction processes. This gene is a candidate gene for Alzheimer's disease.
Hs00377427_m1	*APBB1*	It interacts with the Alzheimer's disease amyloid precursor protein (APP), transcription factor CP2/LSF/LBP1 and the low-density lipoprotein receptor-related protein. APP functions as a cytosolic anchoring site that can prevent the gene product's nuclear translocation. This encoded protein could play an important role in the pathogenesis of Alzheimer's disease.
Hs00300268_m1	*APBB2*	The protein encoded by this gene interacts with the cytoplasmic domains of amyloid beta (A4) precursor protein and amyloid beta (A4) precursor-like protein 2. This protein contains two phosphotyrosine binding (PTB) domains, which are thought to function in signal transduction. Polymorphisms in this gene have been associated with Alzheimer's disease.
Hs00195923_m1	*APBB3*	It is found in the cytoplasm and binds to the intracellular domain of the Alzheimer's disease beta-amyloid precursor protein (APP) as well as to other APP-like proteins. It is thought that the protein encoded by this gene may modulate the internalization of APP.
Hs00211268_m1	*APH1A*	This gene encodes a component of the gamma secretase complex that cleaves integral membrane proteins such as Notch receptors and beta-amyloid precursor protein.
Hs00229911_m1	*APH1B*	This gene encodes a multi-pass transmembrane protein that is a functional component of the gamma-secretase complex, which also contains presenilin and nicastrin. This protein represents a stabilizing cofactor for the presenilin holoprotein in the complex. The gamma-secretase complex catalyzes the cleavage of integral proteins such as notch receptors and beta-amyloid precursor protein.
Hs00193069_m1	*APLP1*	This gene encodes a member of the highly conserved amyloid precursor protein gene family. The encoded protein is a membrane-associated glycoprotein that is cleaved by secretases in a manner similar to amyloid beta A4 precursor protein cleavage.
Hs00155778_m1	*APLP2*	This gene encodes amyloid precursor- like protein 2 (APLP2), which is a member of the APP (amyloid precursor protein) family including APP, APLP1 and APLP2. This protein has been implicated in the pathogenesis of Alzheimer's disease.
Hs00171168_m1	*APOE*	The protein encoded by this gene is a major apoprotein of the chylomicron. It binds to a specific liver and peripheral cell receptor, and is essential for the normal catabolism of triglyceride-rich lipoprotein constituent.
Hs00169098_m1	*APP*	This gene encodes a cell surface receptor and transmembrane precursor protein that is cleaved by secretases to form a number of peptides. Some of these peptides are secreted and can bind to the acetyltransferase complex APBB1/TIP60 to promote transcriptional activation, while others form the protein basis of the amyloid plaques found in the brains of patients with Alzheimer’s disease.
Hs00201573_m1	*BACE1*	This transmembrane protease catalyzes the first step in the formation of amyloid beta peptide from amyloid precursor protein. Amyloid beta

		peptides are the main constituent of amyloid beta plaques, which accumulate in the brains of human Alzheimer's disease patients.
Hs00273238_m1	*BACE2*	This gene encodes an integral membrane glycoprotein that functions as an aspartic protease. The encoded protein cleaves amyloid precursor protein into amyloid beta peptide, which is a critical step in the etiology of Alzheimer's disease and Down syndrome.
Hs00559804_m1	*CAPN1*	This gene encodes the large subunit of the ubiquitous enzyme, calpain 1. Several transcript variants encoding two different isoforms have been found for this gene.
Hs00263337_m1	*CASP3*	The protein encoded by this gene is a cysteine-aspartic acid protease that plays a central role in the execution-phase of cell apoptosis. This protein itself is processed by caspases 8, 9, and 10. It is the predominant caspase involved in the cleavage of amyloid-beta 4A precursor protein, which is associated with neuronal death in Alzheimer's disease.
Hs00154250_m1	*CASP6*	Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. This protein is processed by caspases 7, 8 and 10, and is thought to function as a downstream enzyme in the caspase activation cascade.
Hs00364293_m1	*CDC2*	The protein encoded by this gene is a member of the Ser/Thr protein kinase family. This protein is a catalytic subunit of the highly conserved protein kinase complex known as M-phase promoting factor (MPF), which is essential for G1/S and G2/M phase transitions of eukaryotic cell cycle. Mitotic cyclins stably associate with this protein and function as regulatory subunits.
Hs00358991_g1	*CDK5*	This gene predominantly expressed at high levels in mammalian postmitotic central nervous system neurons, functions in diverse processes such as synaptic plasticity and neuronal migration through phosphorylation of proteins required for cytoskeletal organization, endocytosis and exocytosis, and apoptosis.
Hs00243655_s1	*CDK5R1*	The protein encoded by this gene (p35) is a neuron-specific activator of cyclin-dependent kinase 5 (CDK5); the activation of CDK5 is required for proper development of the central nervous system. The p25 form accumulates in the brain neurons of patients with Alzheimer's disease. This accumulation correlates with an increase in CDK5 kinase activity, and may lead to aberrantly phosphorylated forms of the microtubule-associated protein tau, which contributes to Alzheimer's disease.
Hs00268179_s1	*SLC18A3*	This gene is a member of the vesicular amine transporter family. The encoded transmembrane protein transports acetylcholine into secretory vesicles for release into the extracellular space. Acetylcholine transport utilizes a proton gradient established by a vacuolar ATPase.
Hs00265195_s1	*CHRM1*	Muscarinic receptors influence many effects of acetylcholine in the central and peripheral nervous system. The muscarinic cholinergic receptor 1 is involved in mediation of vagally-induced bronchoconstriction and in the acid secretion of the gastrointestinal tract.
Hs00265216_s1	*CHRM3*	The muscarinic cholinergic receptors belong to a larger family of G protein-coupled receptors. The functional diversity of these receptors is defined by the binding of acetylcholine and includes cellular

		responses such as adenylate cyclase inhibition, phosphoinositide degeneration, and potassium channel mediation. Muscarinic receptors influence many effects of acetylcholine in the central and peripheral nervous system.
Hs00181247_m1	*CHRNA4*	This gene encodes a nicotinic acetylcholine receptor, which belongs to a superfamily of ligand-gated ion channels that play a role in fast signal transmission at synapses. Mutations in this gene cause nocturnal frontal lobe epilepsy type 1. Polymorphisms in this gene that provide protection against nicotine addiction have been described.
Hs00793391_m1	*CSNK1A1*	CSNK1A1 (Casein Kinase 1 Alpha 1) is a Protein Coding gene. Diseases associated with CSNK1A1 include Enterobiasis and Colorectal Cancer. Among its related pathways are Misspliced GSK3beta mutants stabilize beta-catenin and Noncanonical Wnt signaling pathway.
Hs00157194_m1	*CTSB*	This enzyme is a lysosomal cysteine protease with both endopeptidase and exopeptidase activity that may play a role in protein turnover. It is also known as amyloid precursor protein secretase and is involved in the proteolytic processing of amyloid precursor protein (APP). Incomplete proteolytic processing of APP has been suggested to be a causative factor in Alzheimer's disease, the most common cause of dementia.
Hs00175188_m1	*CTSC*	This gene encodes a member of the peptidase C1 family and lysosomal cysteine proteinase that appears to be a central coordinator for activation of many serine proteinases in cells of the immune system. Defects in the encoded protein have been shown to be a cause of Papillon-Lefevre syndrome, an autosomal recessive disorder.
Hs00157205_m1	*CTSD*	The encoded preproprotein is proteolytically processed to generate multiple protein products. Mutations in this gene play a causal role in neuronal ceroid lipofuscinosis-10 and may be involved in the pathogenesis of several other diseases, including breast cancer and possibly Alzheimer's disease.
Hs00175195_m1	*CTSG*	he encoded protease has a specificity similar to that of chymotrypsin C, and may participate in the killing and digestion of engulfed pathogens, and in connective tissue remodeling at sites of inflammation.
Hs00189461_m1	*BPTF*	This gene was identified by the reactivity of its encoded protein to a monoclonal antibody prepared against brain homogenates from patients with Alzheimer's disease. High levels of FAC1 were detected in fetal brain and in patients with neurodegenerative diseases.
Hs00702141_s1	*GJB1*	The gap junction proteins are membrane-spanning proteins that assemble to form gap junction channels that facilitate the transfer of ions and small molecules between cells. According to sequence similarities at the nucleotide and amino acid levels, the gap junction proteins are divided into two categories, alpha and beta. Mutations in this gene cause X-linked Charcot-Marie-Tooth disease, an inherited peripheral neuropathy.
Hs00248163_m1	*GLS*	The encoded protein is a phosphate-activated amidohydrolase that catalyzes the hydrolysis of glutamine to glutamate and ammonia. This protein is primarily expressed in the brain and kidney plays an essential role in generating energy for metabolism, synthesizing the brain

		neurotransmitter glutamate and maintaining acid-base balance in the kidney.
Hs00168219_m1	*GRIN2A*	The encoded protein is an N-methyl-D-aspartate (NMDA) receptor subunit. NMDA receptors are both ligand-gated and voltage-dependent, and are involved in long-term potentiation, an activity-dependent increase in the efficiency of synaptic transmission thought to underlie certain kinds of memory and learning. Disruption of this gene is associated with focal epilepsy and speech disorder with or without cognitive disability.
Hs00168230_m1	*GRIN2B*	The NMDA receptors mediate a slow calcium-permeable component of excitatory synaptic transmission in the central nervous system. The early expression of this gene in development suggests a role in brain development, circuit formation, synaptic plasticity, and cellular migration and differentiation. Naturally occurring mutations within this gene are associated with neurodevelopmental disorders including autism spectrum disorder, attention deficit hyperactivity disorder, epilepsy, and schizophrenia.
Hs00181352_m1	*GRIN2D*	NMDA channel has been shown to be involved in long-term potentiation, an activity-dependent increase in the efficiency of synaptic transmission thought to underlie certain kinds of memory and learning.
Hs00275656_m1	*GSK3B*	The protein encoded by this gene is a serine-threonine kinase belonging to the glycogen synthase kinase subfamily. It is a negative regulator of glucose homeostasis and is involved in energy metabolism, inflammation, ER-stress, mitochondrial dysfunction, and apoptotic pathways. Defects in this gene have been associated with Parkinson's disease and Alzheimer's disease.
Hs00189576_m1	*HSD17B10*	This gene encodes 3-hydroxyacyl-CoA dehydrogenase type II, a member of the short-chain dehydrogenase/reductase superfamily. The gene product is a mitochondrial protein that catalyzes the oxidation of a wide variety of fatty acids and steroids, and is a subunit of mitochondrial ribonuclease P, which is involved in tRNA maturation. The protein has been implicated in the development of Alzheimer's disease.
Hs00610438_m1	*IDE*	This gene encodes a zinc metallopeptidase that degrades intracellular insulin, and thereby terminates insulins activity, as well as participating in intercellular peptide signaling by degrading diverse peptides such as glucagon, amylin, bradykinin, and kallidin. The preferential affinity of this enzyme for insulin results in insulin-mediated inhibition of the degradation of other peptides such as beta-amyloid. Deficiencies in this protein's function are associated with Alzheimer's disease and type 2 diabetes mellitus but mutations in this gene have not been shown to be causative for these diseases.
Hs00174143_m1	*IFNG*	The active protein is a homodimer that binds to the interferon gamma receptor which triggers a cellular response to viral and microbial infections. Mutations in this gene are associated with an increased susceptibility to viral, bacterial and parasitic infections and to several autoimmune diseases.
Hs00174092_m1	*IL1A*	This cytokine is a pleiotropic cytokine involved in various immune responses, inflammatory processes, and hematopoiesis. This cytokine

		is produced by monocytes and macrophages as a proprotein, which is proteolytically processed and released in response to cell injury, and thus induces apoptosis. It has been suggested that the polymorphism of these genes is associated with rheumatoid arthritis and Alzheimer's disease.
Hs00174097_m1	*IL1B*	This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. The induction of cyclooxygenase-2 (PTGS2/COX2) by this cytokine in the central nervous system (CNS) is found to contribute to inflammatory pain hypersensitivity.
Hs00174131_m1	*IL6*	This gene encodes a cytokine that functions in inflammation and the maturation of B cells. In addition, the encoded protein has been shown to be an endogenous pyrogen capable of inducing fever in people with autoimmune diseases or infections. The protein is primarily produced at sites of acute and chronic inflammation.
Hs00169631_m1	*INSR*	Binding of insulin or other ligands to this receptor activates the insulin signaling pathway, which regulates glucose uptake and release, as well as the synthesis and storage of carbohydrates, lipids and protein. Mutations in this gene underlie the inherited severe insulin resistance syndromes including type A insulin resistance syndrome.
Hs00233856_m1	*LRP1*	This receptor is involved in several cellular processes, including intracellular signaling, lipid homeostasis, and clearance of apoptotic cells. In addition, the encoded protein is necessary for the alpha 2-macroglobulin-mediated clearance of secreted amyloid precursor protein and beta-amyloid, the main component of amyloid plaques found in Alzheimer patients. Expression of this gene decreases with age and has been found to be lower than controls in brain tissue from Alzheimer's disease patients.
Hs00158875_m1	*LRPAP1*	This gene encodes a protein that interacts with the low density lipoprotein (LDL) receptor-related protein and facilitates its proper folding and localization by preventing the binding of ligands. Mutations in this gene have been identified in individuals with myopia 23.
Hs00177066_m1	*MAPK1*	This gene encodes a member of the MAP kinase family. MAP kinases, also known as extracellular signal-regulated kinases (ERKs), act as an integration point for multiple biochemical signals, and are involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development.
Hs00385075_m1	*MAPK3*	The protein encoded by this gene is a member of the MAP kinase family. MAP kinases, also known as extracellular signal-regulated kinases (ERKs), act in a signaling cascade that regulates various cellular processes such as proliferation, differentiation, and cell cycle progression in response to a variety of extracellular signals.
Hs00213491_m1	*MAPT*	This gene encodes the microtubule-associated protein tau (MAPT) whose transcript undergoes complex, regulated alternative splicing, giving rise to several mRNA species. MAPT transcripts are differentially expressed in the nervous system, depending on stage of neuronal maturation and neuron type. MAPT gene mutations have been associated with several neurodegenerative disorders such as

		Alzheimer's disease, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy.
Hs00153519_m1	*MME*	The protein encoded by this gene is a type II transmembrane glycoprotein and a common acute lymphocytic leukemia antigen that is an important cell surface marker in the diagnosis of human acute lymphocytic leukemia (ALL). The encoded protein is present on leukemic cells of pre-B phenotype, which represent 85% of cases of ALL. This protein is not restricted to leukemic cells, however, and is found on a variety of normal tissues. The protein is a neutral endopeptidase that cleaves peptides at the amino side of hydrophobic residues and inactivates several peptide hormones including glucagon, enkephalins, substance P, neurotensin, oxytocin, and bradykinin.
Hs00299716_m1	*NCSTN*	The encoded protein cleaves integral membrane proteins, including Notch receptors and beta-amyloid precursor protein, and may be a stabilizing cofactor required for gamma-secretase complex assembly. The cleavage of beta-amyloid precursor protein yields amyloid beta peptide, the main component of the neuritic plaque and the hallmark lesion in the brains of patients with Alzheimer's disease; however, the nature of the encoded protein's role in Alzheimer's disease is not known for certain.
Hs00405493_m1	*PDE8B*	The protein encoded by this gene is a cyclic nucleotide phosphodiesterase (PDE) that catalyzes the hydrolysis of the second messenger cAMP. The encoded protein, which does not hydrolyze cGMP, is resistant to several PDE inhibitors. Defects in this gene are a cause of autosomal dominant striatal degeneration (ADSD).
Hs00708570_s1	*PSENEN*	Presenilins, which are components of the gamma-secretase protein complex, are required for intramembranous processing of some type I transmembrane proteins, such as the Notch proteins and the beta-amyloid precursor protein. Signaling by Notch receptors mediates a wide range of developmental cell fates. Processing of the beta-amyloid precursor protein generates neurotoxic amyloid beta peptides, the major component of senile plaques associated with Alzheimer's disease.
Hs00160118_m1	*PLD1*	This gene encodes a phosphatidylcholine-specific phospholipase which catalyzes the hydrolysis of phosphatidylcholine in order to yield phosphatidic acid and choline. The enzyme may play a role in signal transduction and subcellular trafficking.
Hs00427259_m1	*PPP2CA*	This gene encodes the phosphatase 2A catalytic subunit. Protein phosphatase 2A is one of the four major Ser/Thr phosphatases, and it is implicated in the negative control of cell growth and division. It consists of a common heteromeric core enzyme, which is composed of a catalytic subunit and a constant regulatory subunit, that associates with a variety of regulatory subunits.
Hs00176944_m1	*PRKACB*	The protein encoded by this gene is a member of the serine/threonine protein kinase family. The encoded protein is a catalytic subunit of cAMP (cyclic AMP)-dependent protein kinase, which mediates signalling though cAMP. cAMP signaling is important to a number of processes, including cell proliferaton and differentiation. Multiple alternatively spliced transcript variants encoding distinct isoforms have been observed.

Hs00176973_m1	*PRKCA*	The protein encoded by this gene is one of the PKC family members. This kinase has been reported to play roles in many different cellular processes, such as cell adhesion, cell transformation, cell cycle checkpoint, and cell volume control. Knockout studies in mice suggest that this kinase may be a fundamental regulator of cardiac contractility and Ca(2+) handling in myocytes.
Hs00176998_m1	*PRKCB1*	The protein encoded by this gene is one of the PKC family members. This protein kinase has been reported to be involved in many different cellular functions, such as B cell activation, apoptosis induction, endothelial cell proliferation, and intestinal sugar absorption. Studies in mice also suggest that this kinase may also regulate neuronal functions and correlate fear-induced conflict behavior after stress.
Hs00178455_m1	*PRKCE*	The protein encoded by this gene is one of the PKC family members. This kinase has been shown to be involved in many different cellular functions, such as neuron channel activation, apoptosis, cardioprotection from ischemia, heat shock response, as well as insulin exocytosis. Knockout studies in mice suggest that this kinase is important for lipopolysaccharide (LPS)-mediated signaling in activated macrophages and may also play a role in controlling anxiety-like behavior.
Hs00177010_m1	*PRKCG*	The protein encoded by this gene is one of the PKC family members. This protein kinase is expressed solely in the brain and spinal cord and its localization is restricted to neurons. It has been demonstrated that several neuronal functions, including long term potentiation (LTP) and long term depression (LTD), specifically require this kinase.
Hs00177028_m1	*PKN1*	This kinase is activated by Rho family of small G proteins and may mediate the Rho-dependent signaling pathway. The 3-phosphoinositide dependent protein kinase-1 (PDPK1/PDK1) is reported to phosphorylate this kinase, which may mediate insulin signals to the actin cytoskeleton. The proteolytic activation of this kinase by caspase-3 or related proteases during apoptosis suggests its role in signal transduction related to apoptosis.
Hs00997789_m1	*PSEN1*	Alzheimer's disease patients with an inherited form of the disease carry mutations in the presenilin proteins (PSEN1; PSEN2) or in the amyloid precursor protein (APP). These disease-linked mutations result in increased production of the longer form of amyloid-beta (main component of amyloid deposits found in AD brains). Presenilins are postulated to regulate APP processing through their effects on gamma-secretase, an enzyme that cleaves APP. Also, it is thought that the presenilins are involved in the cleavage of the Notch receptor
Hs01577197_m1	*PSEN2*	Alzheimer's disease patients with an inherited form of the disease carry mutations in the presenilin proteins (PSEN1 or PSEN2) or the amyloid precursor protein (APP). These disease-linked mutations result in increased production of the longer form of amyloid-beta (main component of amyloid deposits found in AD brains). Presenilins are postulated to regulate APP processing through their effects on gamma-secretase, an enzyme that cleaves APP. Also, it is thought that the presenilins are involved in the cleavage of the Notch receptor
Hs00240906_m1	*SNCA*	Alpha-synuclein is a member of the synuclein family, which also includes beta- and gamma-synuclein. Synucleins are abundantly

		expressed in the brain and alpha- and beta-synuclein inhibit phospholipase D2 selectively. SNCA may serve to integrate presynaptic signaling and membrane trafficking. Defects in SNCA have been implicated in the pathogenesis of Parkinson's disease. SNCA peptides are a major component of amyloid plaques in the brains of patients with Alzheimer's disease.
Hs00162077_m1	*SOAT1*	This gene has been implicated in the formation of beta-amyloid and atherosclerotic plaques by controlling the equilibrium between free cholesterol and cytoplasmic cholesteryl esters.
Hs00167309_m1	*SOD2*	This gene is a member of the iron/manganese superoxide dismutase family. It encodes a mitochondrial protein that forms a homotetramer and binds one manganese ion per subunit. This protein binds to the superoxide byproducts of oxidative phosphorylation and converts them to hydrogen peroxide and diatomic oxygen. Mutations in this gene have been associated with idiopathic cardiomyopathy (IDC), premature aging, sporadic motor neuron disease, and cancer.
Hs00260517_s1	*CAPNS2*	CAPNS2 (Calpain Small Subunit 2) is a Protein Coding gene. Diseases associated with CAPNS2 include Coffin-Siris Syndrome 2 and Coffin-Siris Syndrome 1. Among its related pathways are Degradation of the extracellular matrix and HIV Life Cycle.
Hs00174128_m1	*TNF*	This gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. This cytokine is mainly secreted by macrophages. It can bind to, and thus functions through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. This cytokine has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, psoriasis, rheumatoid arthritis ankylosing spondylitis, tuberculosis, autosomal dominant polycystic kidney disease, and cancer. Mutations in this gene affect susceptibility to cerebral malaria, septic shock, and Alzheimer's disease.
Hs00188233_m1	*UCHL1*	The protein encoded by this gene belongs to the peptidase C12 family. This enzyme is a thiol protease that hydrolyzes a peptide bond at the C-terminal glycine of ubiquitin. This gene is specifically expressed in the neurons and in cells of the diffuse neuroendocrine system. Mutations in this gene may be associated with Parkinson's disease.
Hs00374305_m1	*VSNL1*	This gene is a member of the visinin/recoverin subfamily of neuronal calcium sensor proteins. The encoded protein is strongly expressed in granule cells of the cerebellum where it associates with membranes in a calcium-dependent manner and modulates intracellular signaling pathways of the central nervous system by directly or indirectly regulating the activity of adenylyl cyclase.
Hs00544355_m1	*GAL*	This gene encodes a neuroendocrine peptide that is widely expressed in the central and peripheral nervous systems and also the gastrointestinal tract, pancreas, adrenal gland and urogenital tract. The encoded protein is a precursor that is proteolytically processed to generate two mature peptides: galanin and galanin message-associated peptide (GMAP).

Hs01085739_g1	*ACHE*	Acetylcholinesterase hydrolyzes the neurotransmitter, acetylcholine at neuromuscular junctions and brain cholinergic synapses, and thus terminates signal transmission. The major form of acetylcholinesterase found in brain, muscle and other tissues is the hydrophilic species, which forms disulfide-linked oligomers with collagenous, or lipid-containing structural subunits.
Hs00542592_g1	*AGER*	The advanced glycosylation end product (AGE) receptor encoded by this gene is a member of the immunoglobulin superfamily of cell surface receptors. It is a multiligand receptor, and besides AGE, interacts with other molecules implicated in homeostasis, development, and inflammation, and certain diseases, such as diabetes and Alzheimer's disease.
Hs01000370_m1	*NAE1*	The protein encoded by this gene binds to the beta-amyloid precursor protein. Beta-amyloid precursor protein is a cell surface protein with signal-transducing properties, and it is thought to play a role in the pathogenesis of Alzheimer's disease. In addition, the encoded protein can form a heterodimer with UBE1C and bind and activate NEDD8, a ubiquitin-like protein. This protein is required for cell cycle progression through the S/M checkpoint.
Hs00992319_m1	*BCHE*	This gene encodes a cholinesterase enzyme and member of the type-B carboxylesterase/lipase family of proteins. The encoded enzyme exhibits broad substrate specificity and is involved in the detoxification of poisons including organophosphate nerve agents and pesticides, and the metabolism of drugs including cocaine, heroin and aspirin.
Hs00998426_m1	*CAPNS1*	This encoded protein is essential for the stability and function of both calpain heterodimers, whose proteolytic activities influence various cellular functions including apoptosis, proliferation, migration, adhesion, and autophagy. Calpains have been implicated in neurodegenerative processes, such as myotonic dystrophy.
Hs01063373_m1	*CHRNA7*	The protein encoded by this gene forms a homo-oligomeric channel, displays marked permeability to calcium ions and is a major component of brain nicotinic receptors that are blocked by, and highly sensitive to, alpha-bungarotoxin. Once this receptor binds acetylcholine, it undergoes an extensive change in conformation that affects all subunits and leads to opening of an ion-conducting channel across the plasma membrane.
Hs01017895_m1	*CSNK1D*	This gene is a member of the casein kinase I (CKI) gene family whose members have been implicated in the control of cytoplasmic and nuclear processes, including DNA replication and repair. The encoded protein may also be involved in the regulation of apoptosis, circadian rhythm, microtubule dynamics, chromosome segregation, and p53-mediated effects on growth.
Hs01042347_m1	*CYP46A1*	The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This endoplasmic reticulum protein is expressed in the brain, where it converts cholesterol to 24S-hydroxycholesterol. While cholesterol cannot pass the blood-brain barrier, 24S-hydroxycholesterol can be secreted in the brain into the circulation to be returned to the liver for catabolism.

Hs00967138_m1	*GAP43*	The protein encoded by this gene has been termed a 'growth' or 'plasticity' protein because it is expressed at high levels in neuronal growth cones during development and axonal regeneration. This protein is considered a crucial component of an effective regenerative response in the nervous system.
Hs01016626_m1	*GRIN2C*	This gene encodes a subunit of the N-methyl-D-aspartate (NMDA) receptor, which is a subtype of ionotropic glutamate receptor. NMDA receptors are found in the central nervous system, are permeable to cations and have an important role in physiological processes such as learning, memory, and synaptic development. Alterations in the subunit composition of the receptor are associated with pathophysiological conditions such as Parkinson's disease, Alzheimer's disease, depression, and schizophrenia.
Hs00949382_m1	*ST6GAL1*	The encoded protein is a type II membrane protein that catalyzes the transfer of sialic acid from CMP-sialic acid to galactose-containing substrates. The protein, which is normally found in the Golgi but can be proteolytically processed to a soluble form, is involved in the generation of the cell-surface carbohydrate determinants and differentiation antigens HB-6, CD75, and CD76.
Hs00923840_m1	*UBQLN1*	This gene encodes an ubiquitin-like protein (ubiquilin) that shares a high degree of similarity with related products in yeast, rat and frog. Ubiquilins contain an N-terminal ubiquitin-like domain and a C-terminal ubiquitin-associated domain. They physically associate with both proteasomes and ubiquitin ligases, and thus are thought to functionally link the ubiquitination machinery to the proteasome to affect in vivo protein degradation. This ubiquilin has also been shown to modulate accumulation of presenilin proteins, and it is found in lesions associated with Alzheimer's and Parkinson's diseases.

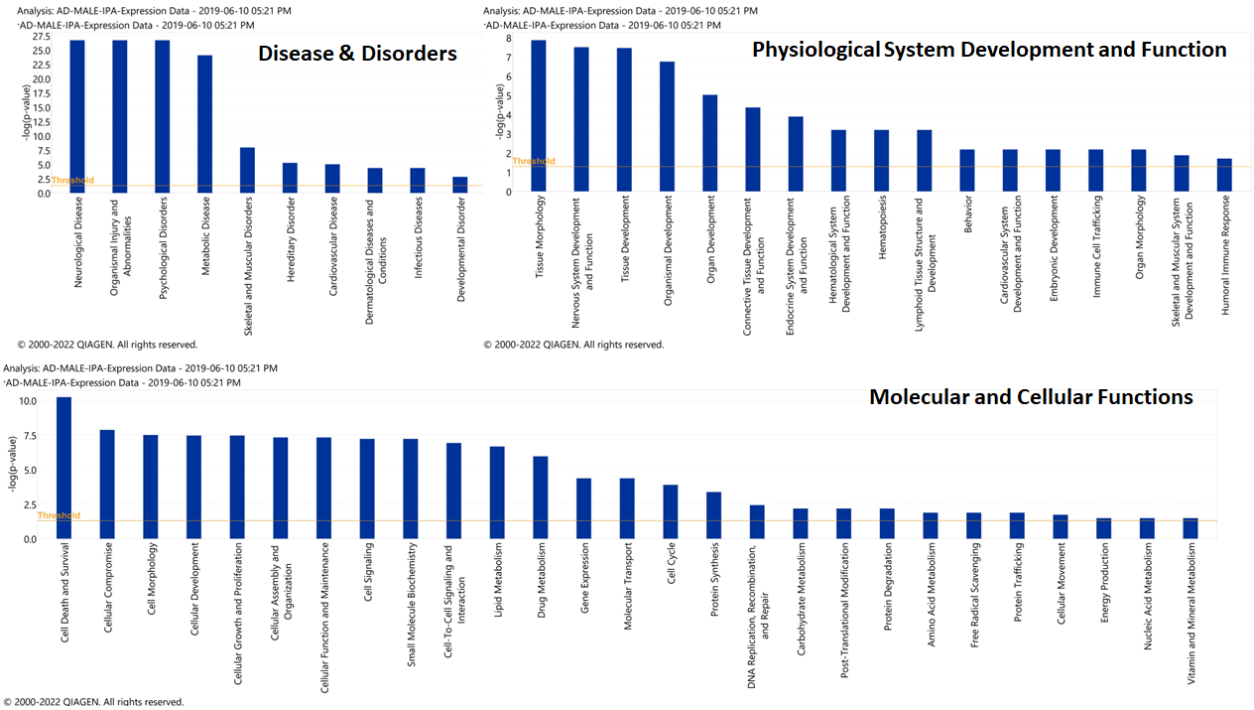
Supplementary Table 3. Global expression and TLDA cohort

Characteristics	Global gene expression study cohort (n=8)		TLDA study cohort (n=33)	
	AD Participants (n=4)	Healthy Control (n=4)	AD Participants (n=15)	Healthy Control (n=18)
Age (Y)	72.25±10.66	65±2.45	78.80±9.56	71.05 ±7.58
Gender (M/F)	2/2	2/2	6/9	7/11
BMI (kg/m^2)	20.87±1.43	22.5±1.77	21.18±2.36	21.56±2.12
HbA1c (mmol/mol)	5.4±0.24	4.95±0.42	5.39±0.43	4.93±0.68
Mini-Mental State Examination score	8.62±0.47		8.8±0.67	
Family History of AD (%)	50	-	46	-

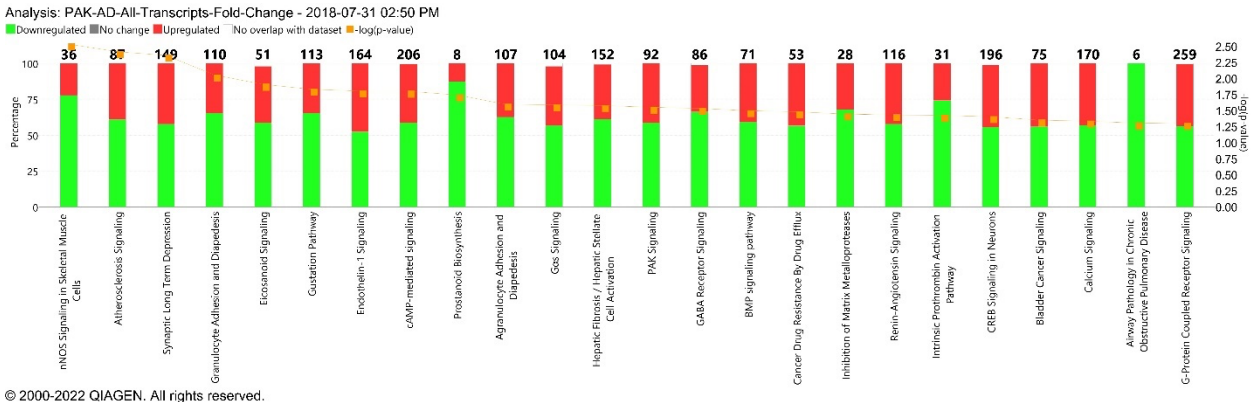
Supplementary Table 4. Top Canonical Pathways (TLDA) from IPA Results with their p-value in the AD population

Top Canonical Pathways	**p**	**Overlap (%)**
Amyloid Processing	1.80E-37	42.9
nNOS Signaling in Neurons	5.36E-15	22.7
ErbB4 Signaling	2.61E-18	19.7
Neuroinflammation Signaling Pathway	5. 36E-30	9.7
Reelin Signaling in Neurons	5.82E-14	9.4
Top Diseases and Bio functions		
Diseases and Disorders	**p Range**	**#Molecules**
Organismal Injury and Abnormalities	3.63E-02 - 1.08E-31	41
Neurological Disease	3.63E-02 - 1.08E-31	39
Psychological Disorders	2.74E-02 - 1.08E-31	35
Metabolic Disease	1.83E-02 - 6.99E-29	27
Skeletal and Muscular Disorders	2.74E-02 - 8.67E-09	15
Molecular and Cellular Functions		
	p Range	**#Molecules**
Cell Morphology	4.08E-02 - 7.75E-09	15
Cell Death and Survival	3.63E-02 – 2.24E-11	14
Cellular Compromise	1.83E-02 - 3.87E-11	8
Small Molecule Biochemistry	9.21E-03 - 6.27E-09	7
Lipid Metabolism	4.62E-03 – 6.27E-09	5
Physiological System Development and Function		
	p	**#Molecules**
Nervous System Development and Function	4.96E-02 – 2.31E-09	21
Tissue Development	3.19E-02 - 1.16E-08	17
Organismal Development	1.38E-02 - 2.92E-07	13
Tissue Morphology	4.08E-02 - 3.87E-11	9
Top Upstream Regulators		
	p	
APP	5.18E-06	
IL1B	5.76E-04	
SRF	4.61E-03	
NFATC4	4.61E-03	
MEOX2	4.61E-03	

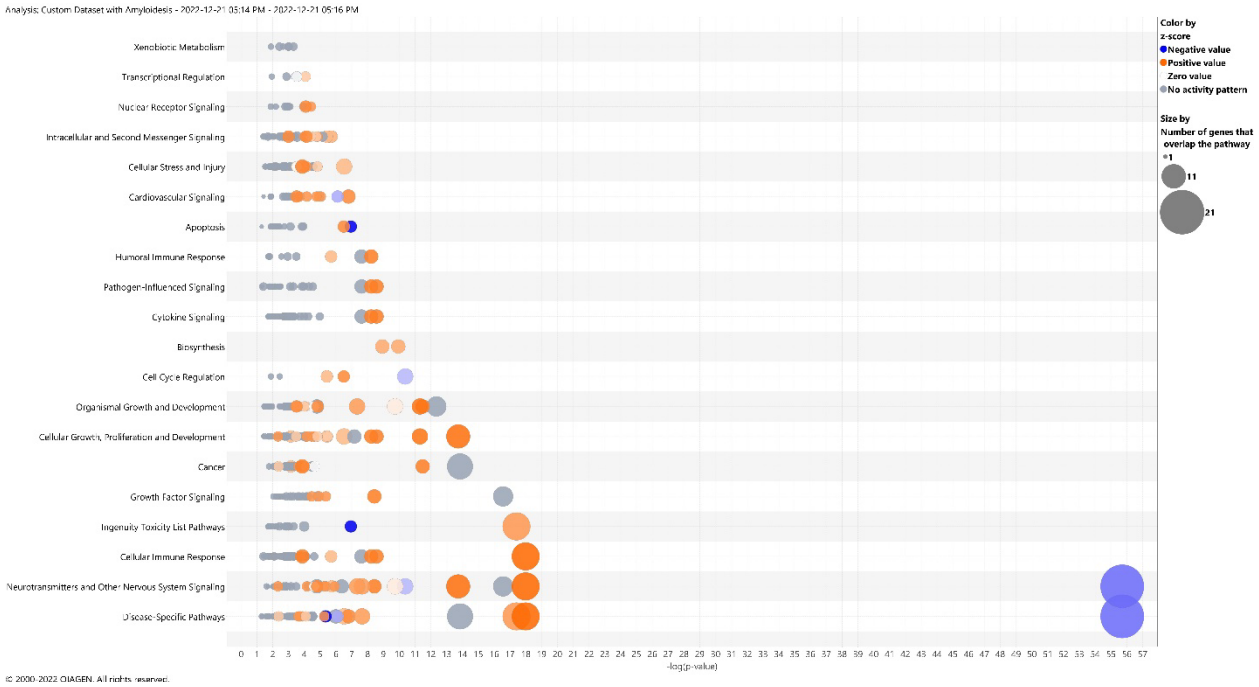
* The data was generated using Ingenuity Pathway Analysis (IPA) from QIAGEN, USA, January 2023 Release, and incorporated the relative gene expression levels measured in the patients' group. The percentage of overlap was calculated as a statistical measure to evaluate the enrichment of network-regulated genes in the input dataset. This measure quantifies the likelihood of observing the resulting overlap between the network-regulated genes (Ingenuity Knowledge Base) and the genes present in the dataset (observed result). The p-value was measured to understand the likelihood that the association between a set of molecules in our dataset and a related disease or function is due to random association. The smaller the p-value (which means a larger -log of that value), the less likely that the association is random and the more significant the association. In general, p-values ≤ 0.05 (-log = 1.3) indicate a statistically significant, non-random association. The p-value was calculated using a right-tailed Fisher's Exact Test. Molecules: Number of significant molecules that are associated with each function.



Supplementary Figure 1. The key bio-functions associated with AD subjects including Disease and disorder development (A), Physiological system development and functions (B), and Molecular and cellular functions (C). IPA core analysis identified the most statistically significant biofunctions which are listed according to their p-value (-log). The threshold line corresponds to a p-value of <0.05.



Supplementary Figure 2. The stacked bar chart represents a summary of total upregulated (**red**) and downregulated (**green**) genes representing 25 important signaling and disease pathways in AD subjects compared to controls in the core analysis, reflecting the differential gene expressions obtained from the microarrays (1657 gene sets with ≥ 2-fold change, t-test, $p < 0.05$). The threshold line corresponds to a *p*-value of 0.05. IPA analysis carried out depicting the core comparison, representing major cellular and molecular mechanism, and their associated signaling pathways.



Supplementary Figure 3. Ingenuity Pathway Analysis (IPA)-derived bubble chart of identified disease and biofunctions corresponds to -log(p-value).

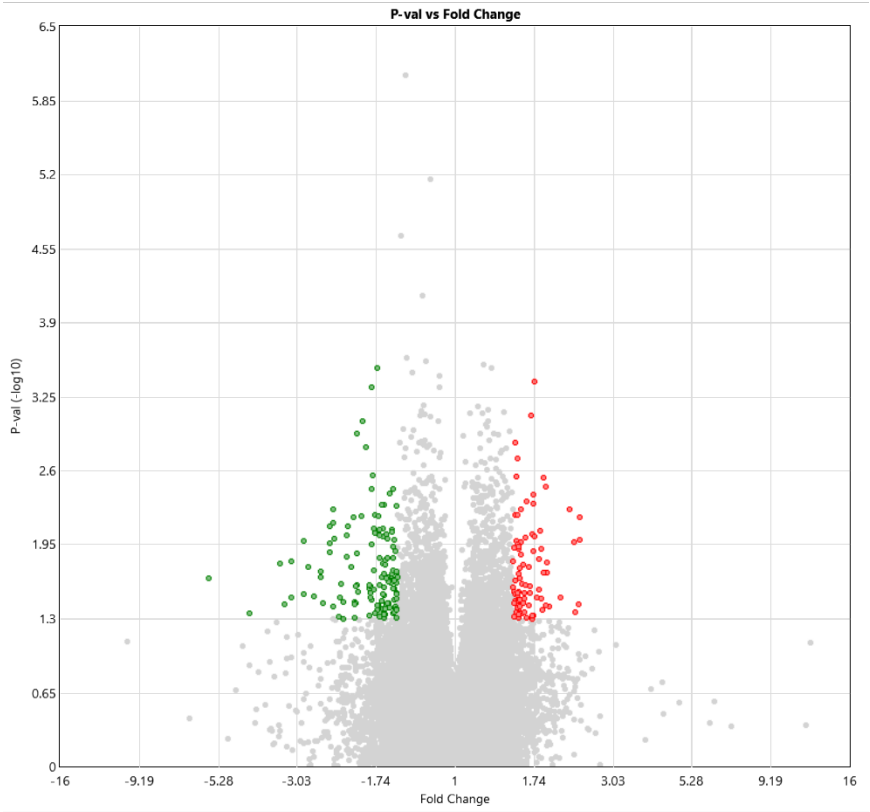
Supplementary Table 5. Average fold change of 16 differentially expressed genes identified in TLDA analysis.

Genes	*Average Fold Change*	*p*
ACHE	5.98	0.01
AGER	4.62	0.023
APP	2.43	0.014
BPTF	2.13	0.046
CAPN1	-5.52	0.042
CAPNS2	7.29	0.016
CDK5R1	4.87	0.021
CHRM1	5.45	0.001
GAL	2.08	0.048
GAP43	-2.98	0.041
GJB1	3.24	0.046
GRIN2A	-3.81	0.041
GRIN2B	-2.06	0.037
PSENEN	4.63	0.039
SLC18A3	6.50	0.001
SNCA	2.09	0.047

We applied t-test to assess the significance of differential expressions in between control and patients group samples. These tests take into account not only the fold change but also the variability in the data. The analysis yielded the level of p-values, which indicate the probability of observing the level of differential expression in the population. We considered a significance threshold ($p < 0.05$) to determine which genes are significantly differentially expressed. Based on TLDA data analysis, in the Manuscript Figure 1A we identified 16 genes *(ACHE, AGER, APP, BPTF, CAPNS2, CD5R1, CHRM1, GAL, GJB1, PSENEN, SLC18A3, SNCA, CAPN1, GAP43, GRIN2A*, and *GRIN2B*) that showed significant up- or downregulation compared to the control group.

Supplementary Table 6. The table displays the individual participants' records of comorbidities and history of AD, which were gathered during the recruitment process.

Disease Group	Hypertension	Years of Hypertension	Cardiac issue	Years of CVD	AD years	Family History of AD
AD1	No	0	No	0	6	No
AD2	No	0	No	0	2	No
AD3	Yes	3	Yes	14	2	No
AD4	Yes	16	No	0	2	Yes
AD5	Yes	10	Yes	5	5	No
AD6	Yes	8	No	0	7	Yes
AD7	Yes	6	Yes	15	6	Yes
AD8	Yes	4	Yes	21	5	No
AD9	Yes	11	Yes	26	4	Yes
AD10	Yes	10	No	0	5	Yes
AD11	Yes	4	No	0	3	No
AD12	Yes	15	Yes	30	10	No
AD13	Yes	10	No	0	5	Yes
AD14	Yes	10	No	0	2	Yes
AD15	Yes	7	No	0	24	Yes
AD16	No	0	No	0	6	No
AD17	Yes	15	Yes	30	8	Yes
AD18	No	0	Yes	18	6	No
AD19	Yes	7	No	0	7	Yes
Control1	No	0	No	0	-	-
Control2	No	0	No	0	-	-
Control3	No	0	No	0	-	-
Control4	No	0	No	0	-	-
Control5	No	0	No	0	-	-
Control6	No	0	No	0	-	-
Control7	No	0	No	0	-	-
Control8	No	0	No	0	-	-
Control9	No	0	No	0	-	-
Control10	No	0	No	0	-	-
Control11	No	0	No	0	-	-
Control12	No	0	Yes	13	-	-
Control13	No	0	No	0	-	-
Control14	No	0	Yes	12	-	-
Control15	Yes	21	Yes	21	-	-
Control16	Yes	25	No	0	-	-
Control17	No	0	Yes	15	-	-
Control18	Yes	24	No	0	-	-
Control19	Yes	22	No	0	-	-
Control20	Yes	20	No	0	-	-
Control21	No	0	No	0	-	-
Control22	No	0	No	0	-	-



Supplementary Figure 4. Volcano plot of the distribution of all differentially expressed genes captured by global expression.