Data Analysis of Spatial Gene Expression of Adult Mouse Choroid Plexus

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In the name of Allah Most Gracious and Most Beneficial

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FINAL APPROVAL

It is certified that we have read the thesis submitted by Ms. Arshia Iram and it is our judgment that this thesis is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the M.S Degree in Bioinformatics

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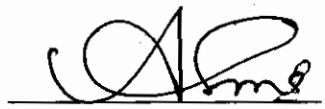

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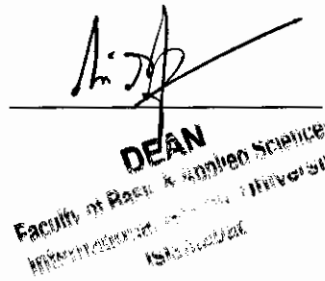

A thesis submitted to Department of Environmental Sciences,
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fulfillment of requirement for the award of the
degree of MS in Bioinformatics

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort; all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Arshia Iram

DADICATED

TO

MY SWEET PARENTS

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In the name of **Allah**, The most gracious and The merciful, The Creator, The most supreme Whose mercy enabled me to accomplish this task and bestowed me with success. Next to this entire messenger, **Hazrat Mohammad (Peace Be Upon Him)** Who is the internal touch of guidance and knowledge. For humanity as a whole and is an ever inspiration for all the learned means.

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Arshia Iram

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LIST OF ABBREVIATIONS

Acaa2	Acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)
Acadm	Acyl-Coenzyme A dehydrogenase, medium chain
Aco2	Aconitase 2, mitochondrial
Acox1	Acyl-Coenzyme A oxidase 1, palmitoyl
Apoe	Apolipoprotein E
B2m	Beta-2 microglobulin
Brd4	Bromodomain containing 4
Brrn1	Bromodomain containing 4Barren homolog (Drosophila)
CEC	Choroidal epithelial cells
Cfh	Complement component factor h
CNS	Central nervous system
Col1a1	Procollagen, type I, alpha 1
Col3a1	procollagen, type III, alpha 1
Col5a2	Procollagen, type V, alpha 2
Cp	Ceruloplasmin
CP	Choroid plexus
CSF	Cerebrospinal fluid

Cst3	Cystatin C
Ctsd	Cathepsin D
Cyb561d2	Cytochrome b-561 domain containing 2
DCN	Decorin
Efemp1	Epidermal growth factor-containing fibulin-like extracellular matrix protein 1
Efemp2	Epidermal growth factor-containing fibulin-like extracellular matrix protein 2
Efhc1	EF-hand domain (C-terminal) containing 1
Enpp2	Ectonucleotide pyrophosphatase/phosphodiesterase 2
Fbln1	Fibulin 1
Fbln2	Fibulin 2
Fn1	Fibronectin 1
GEDAMCP	Gene Expression Data Analysis of Adult Mouse Choroid Plexus
Gja7	Gap junction membrane channel protein alpha 7
Gpihbp1	GPI-anchored HDL-binding protein 1
Gpm6b	Glycoprotein m6b
Gsn	Gelsolin
Gzma	Granzyme A
Hdlbp	High density lipoprotein (HDL) binding protein
Igfbp2	Insulin-like growth factor binding protein 2
Igfbp5	Insulin-like growth factor binding protein 5

ISH	In Sito Hybridization
Itpr1	Inositol 1,4,5-triphosphate receptor 1
Maob	Monoamine oxidase B
Mmp2	Matrix metallopeptidase 2
Pomc1	Pro-opiomelanocortin-alpha
Serpinf1	Serine (or cysteine) peptidase inhibitor, clade F, member 1)
Slc13a4	Solute carrier family 13 (sodium/sulfate symporters), member 4
SPARC	Secreted acidic cysteine rich glycoprotein
Spint2	Serine protease inhibitor, Kunitz type 2
Srpk1	Serine/Arginine-rich protein specific kinase 1
Svep1	Sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1)
Thbs1	Thrombospondin 1
Timp2	Tissue inhibitor of metalloproteinase 2
TtR	Transthyretin
Uqcrc1	Ubiquinol-cytochrome c reductase core protein 1
Vcam1	Vascular cell adhesion molecule 1
VEC	Ventricle ependymal cells
Wfikkn2	WAP, follistatin/kazal, immunoglobulin, kunitz and netrin domain containing2

ABSTRACT

Choroid plexus exists in a cavity region of brain producing the cerebrospinal fluid (CSF) that surrounds the brain and spinal cord. CSF acts as the vehicle for delivering nutrients to the different areas of brain. Choroid plexus (CP) plays multiple vital roles in the central nervous system and supply a variety of transport proteins that are necessary for the homeostasis of the CNS microenvironment. In this study, expression analysis of 51 CP genes of adult mouse brain (*Mus musculus*) was carried out on the basis of spatial gene expression data. Although the gene expression was analyzed in the whole brain but the main focus was on choroid plexus at its three locations. Evaluation criteria of this spatial gene expression analysis included three tasks; gene expression pattern, gene expression level and gene expression specificity at all the three locations of CP. According to evaluation criteria 13 out of 51 genes were found as CP specific genes and 38 as non-CP specific genes. This analysis was done by using different tools of Allen brain institute and a secondary web linked database GEDAMCP (Gene Expression Data of Adult Mouse Choroid Plexus) was also created to provide a platform for the CP genes information. This software application provides the information according to the above evaluation criteria for CP specific and non-CP specific genes individually. This study also reveals some more aspects including; (i) presence of CP expressed proteins in the CSF flow that were not previously reported, (ii) some CP genes are not only expressed by

choroid plexus epithelial cells but also by ventricular ependymal cells and (iii) involvement of CP genes in different CNS diseases especially the Alzheimer's disease.

Hoping this study will provide the ease to work further in the CP projects in future.

CHAPTER 1

INTRODUCTION

The Choroid plexus (CP) of mammalian brain appears as a ribbon-like structure within the cerebral ventricles that is a secretory epithelial structure responsible for producing the colorless cerebrospinal fluid (CSF) (Speake, 2001 and Fang *et al.*, 2009). The ventricles are cavities that contain the CSF flow from the lateral ventricles through the third and fourth ventricles and then out of the brain into the subarachnoid space around the brain and spinal cord (Alcamo *et al.*, 2003). Various estimates are present in the published literature indicating that around 75% of the fluid volume is generated by the CP of the lateral ventricles with a further 10% and 5% contributed by the CP of the third and forth ventricles respectively (Veening and Barendreg, 2010). The CP controls the cellular and molecular traffic between the blood and the CSF. In the adult mouse brain CSF fluid has extremely low protein levels as compared with developing mouse brain. Recent research states that the CSF is a vital fluid for the development of the brain and for the normal function of the brain but little data is available on the origin of the vital proteins in the CSF.

1.1 Structure of choroid plexus

Choroid plexus is the convolutions of blood vessels protruding into the ventricles from the specific parts of the ventricular wall (Kahle and Frotscher, 2002). During the

early stage of development these vascular convolutions are covered by thin hemispheric wall but finally turn into epithelial cell layer which is called plexus epithelium (Kahle and Frotscher, 2002). Adult choroid plexus consists of two components (Fig 1.1); vascularized connective tissue of pia matter and plexus epithelium (Kahle and Frotscher, 2002).

Fig 1.1 The arrangement of tissues forming choroid plexus.

Tight junctions are present between the epithelial cells that separate the blood from the CSF, thus performing like barriers. This is called the blood brain barrier that normally hinders the free movement of molecules and cells from the blood into the CSF (Redzic and Segal, 2004). Origin of epithelial cells of CP and ventricular ependymal cells is same according to some extent and the epithelial cells of the CP are called modified form of ependymal cells (Matsumoto *et al.*, 2003). Ependymal cells of choroid plexus can increase the nerve regeneration in vivo (Ide *et al.*, 2001) and promote the neurite (projection of neuron) outgrowth in vitro (Chakrabortty *et al.*, 2000).

1.2 CSF Composition and flow

CSF fluid is a clear, watery liquid produced by CP of the lateral, third, and fourth ventricles. Even it is also originated from the ependymal cells lining the ventricles and contains a variety of proteins and molecules. CSF flows from lateral ventricle to third ventricle then into fourth ventricle and finely enters into the subarachnoid space. Once CSF enters into subarachnoid space it is free to move in the brain and spinal cord (Fig 1.2).

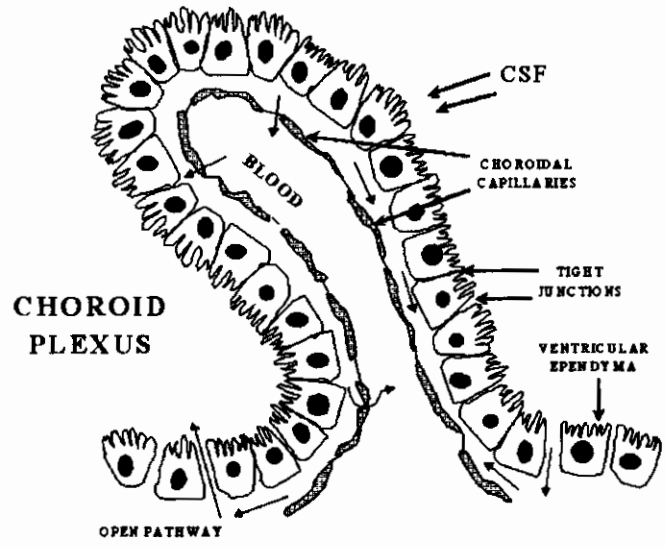


Fig 1.2 Structure of choroid plexus.

Absorption of CSF into the blood vessels is done through the arachnoid villi (Fig 1.3). Various molecules can pass the ependymal lining of ventricle and the pia–glial membrane in most areas of the brain to provide the communication between CSF and brain's extracellular fluid (Matsumoto *et al.*, 2003).

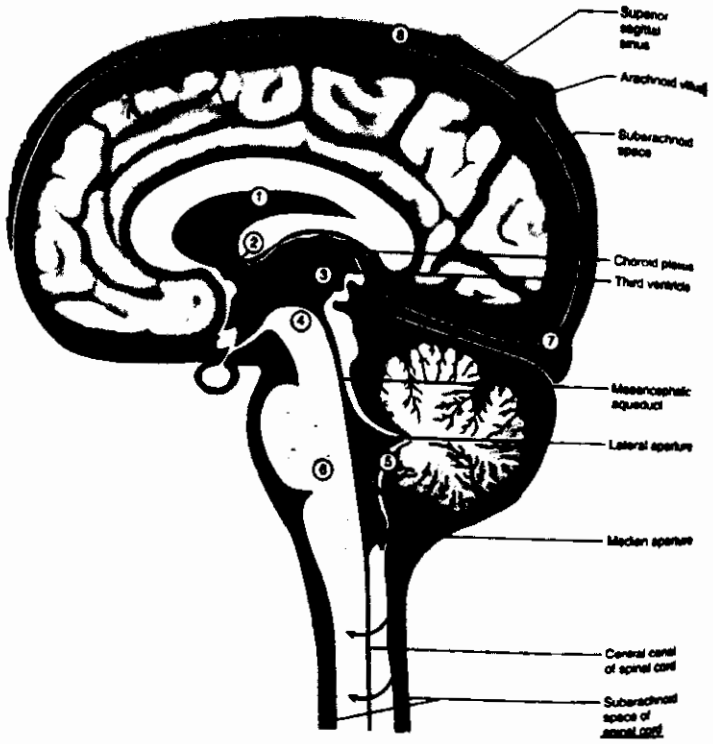


Fig 1.3 CSF flow (black arrows show the CSF flow).

1.3 Functions of CP

CP plays a number of vital roles in the CNS either in the context of its functioning or in perspective of CNS diseases. CP plays an important role to transmit signals into and out of the brain through CSF (Emerich *et al.*, 2005). Recently, it has been recommended that the CP may be the primary route of immune cells to enter into the CSF in the experimental allergic encephalomyelitis model of mouse (Brown and Sawchenko, 2007 and Reboldi *et al.*, 2009). The CP may also facilitate the entry of bacteria in a mouse model of infection with Streptococcus suis (Dominguez-Punaro *et al.*, 2007). The CP response kinetics proved to be similar to that of the liver (Ceciliani *et al.*, 2002 and Gabay and Kushner, 1999) in terms of response onset and shut-off; and in some of the

acute-phase proteins secreted (e.g., SAA, LCN2, IL-6, IL-1β) into the CSF and their acute responses also share common signaling pathways like NF-kB, JAK/STAT, and MAPK (Marques *et al.*, 2009). It was recently reported that the CP also responds to peripheral inflammation, by secreting several immuno modulators (Konsman *et al.*, 2004 and Marques *et al.*, 2009). Moreover CP seems to be important in the regulation of brain iron homeostasis during inflammation conditions (Marques *et al.*, 2008). Sometimes mutations in proteins secreted/produced by CP lead to a variety of CNS disorders (Strand *et al.*, 1984 and Davidsson *et al.*, 1997).

A vast number of studies related to CP mRNA/proteins of the mouse have been previously described in the literature by different people and sources through different gene expression projects. For example; Marques and colleagues (2011) found closest similarity of mouse CP transcriptome with endothelial cells of the blood-brain barrier by finding the gene expression profile of CP. Thouvenot and group in their 2006 study carried out proteomic analysis of mouse CP to reveal the high protein secretion capacity of choroidal epithelial cells. Role of CP cells for survival and growth during brain injuries was studied by Skinner and coworkers in 2009 while analysis of proteins in the CSF and blood brain barrier was carried out by Reiber (2003) etc. The expressions of several genes which are involved in molecular transport were also detected in CP by Girard and colleagues in 1999.

This study will make use of published Allen institute in situ hybridization data of gene expression in the choroid plexus (CP) of adult mouse brain. The Allen Brain Institute for Brain Sciences has spent the past few years carrying out a comprehensive

expression study of the adult mouse brain and also through developmental stages. The expression data for 2000 genes and all parts of the CNS are thus available. Although different projects are going on at Allen institute to extract and retrieve the data but no specific attention has been paid to the choroid plexus.

All the previous work/research does not fulfill the specific tasks which is explained here in this study, i-e. to find the gene expression of the choroid plexus (CP) in adult mouse brain as nobody has worked on the choroid plexus (CP) in such a way to explore the spatial gene expressions of the CP at its three locations (lateral, third and fourth ventricles) and this may be vital for a complete understanding of its function.

Different online resources are available for CP of mouse brain like MGI, GenePaint, GENSAT etc. All these sites have expression images of genes in the CP and also their introductory reports but they do not show the spatial expression of genes. They do not explain the gene expression at the CPs location level, their expression patterns and expression levels (high, medium or low). These missing informations lead to less understanding of the functioning of CP. While these gaps are tried to fill in the Gene Expression Data Analysis of Adult Mouse CP (GEDAMCP) by adding the spatial gene expressions with their expression levels and patterns. User/researcher will easily get the required information of CP genes even at the ventricular level with expression level and pattern information in the form of images. This repository will help to understand the functioning of the CP according to different aspects.

1.4 Objectives

In this study the main task was to analyze the spatial gene expression data of adult mouse CP and its significance in the CSF. The objectives of this study were as follows.

1. To find out what factors are being expressed/produced in the cells of the three different locations of the CP (lateral, third and fourth ventricles).
2. Analysis of pattern and level of gene expression in CP.
3. Categorizing the expression level and pattern of genes in CP depending on their ventricular locations.
4. Development of a database specifically of CP genes.
5. Development of a website containing the available information about CP genes along with their expression images.

CHAPTER 2

LITERATURE REWIEV

Choroid plexus (CP) plays multiple vital roles in the central nervous system due to its unique localization between the blood and cerebrospinal fluid compartments (Fig 2.1). Choroid plexus epithelial cells (CECs) have great capacity to secrete the proteins then other cells of the CP which make them potential neural precursor cells in the adult mammalian brain e.g. astrocytes release 12 mg proteins/mg of the total protein (Lafon-Cazal *et al.,* 2003; Li *et al.*, 2002 and Delcourt *et al.,* 2005). Choroid plexus epithelial cells (CECs) are major source of secretory proteins throughout the brain and also supply a variety of transporter proteins that are necessary for the homeostasis of the CNS microenvironment (Thouvenot *et al.*, 2006).

The choroid plexus plays a key role in supporting neuronal function and is involved in the regulation of many soluble factors by secreting CSF. This CSF is involved in various kinds of signaling activities in the brain. Previous studies have proved the existence of growth factors, enzymes, polypeptides and a number of key proteins in the CSF (Speake *et al.* 2001). Blood brain barrier controls the diffusion of macromolecules to the CSF and also stop the uncontrolled distribution of proteins in the CNS. CSF produced by choroid plexus plays model roles in central nervous system.

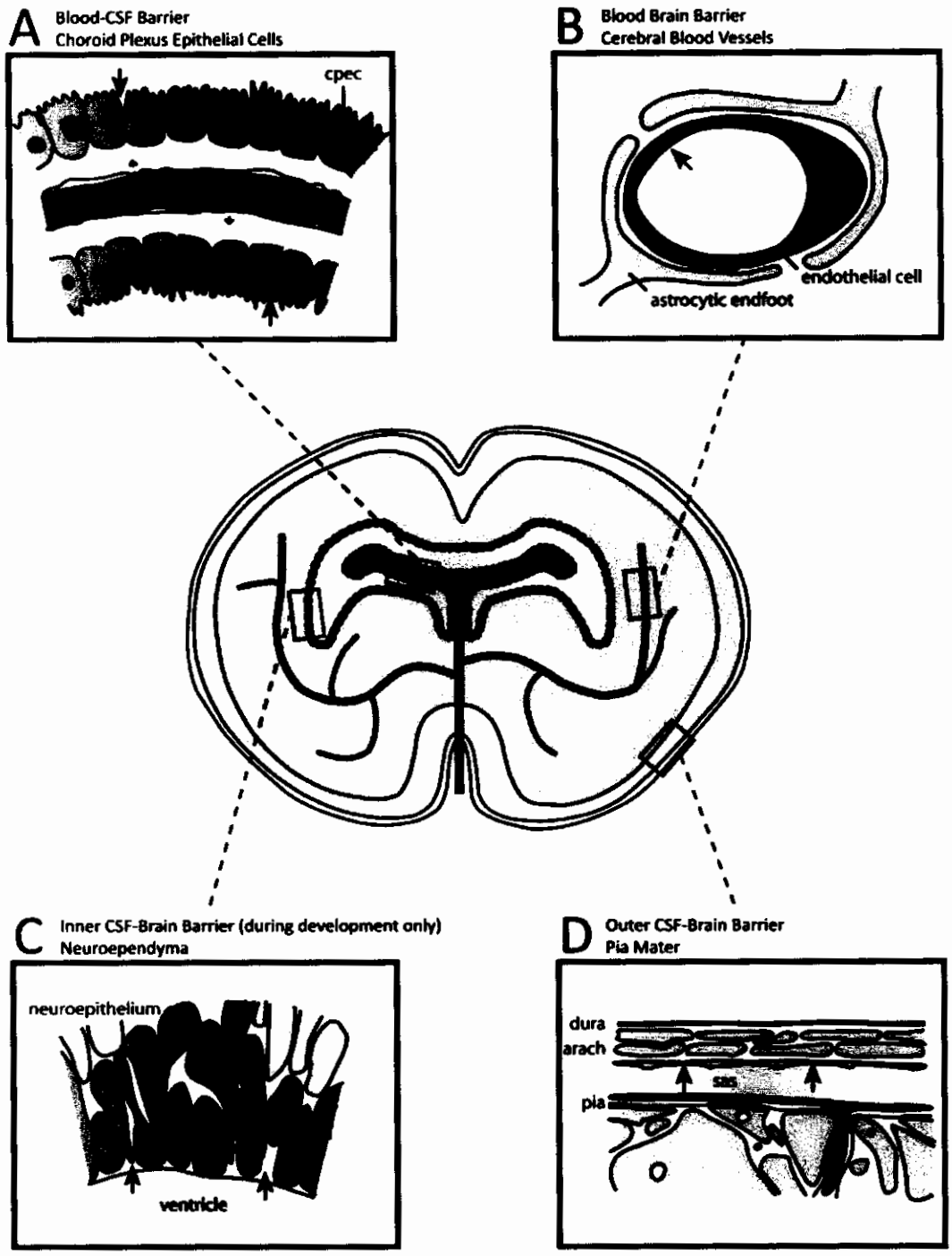


Fig 2.1 Inner structure of CP.

2.1 CP factors and their functions

There are a number of CSF secretory proteins/molecules from CP that are involved in different functions of CNS like amyloid β-protein (Aβ), transthyretin (Ttr), apolipoprotein E (ApoE), phosphodiesterase Iα, phospholipid transfer protein, ATP-binding cassette transporter sub-family A member 8, Secreted acidic cysteine rich glycoprotein (SPARC), cysteine, androgen-inducible aldehyde reductase and vascular cell adhesion molecule-1(Matsumoto *et al.*, 2003). In CSF, gelsolin (Gsn) functions as an anti-amyloidogenic protein, it binds to Amyloid beta (Aβ) and prevents Aβ fibrillization and helps to maintain Aβ in a soluble form (Ray *et al.*, 2000) just as Transthyretin (Ttr) does (Schwarzman *et al.*, 1994). Apolipoprotein E (Apoe) also interacts with Aβ (Naslund *et al.*, 1995) and play roles in neuronal protection, repair, and remodeling (Mahley and Huang, 1999). It is also suggested that CP may be involved in Aβ clearance (Matsumoto *et al.*, 2003). The CP also produces and secretes those molecules which are involved in growth and motility of neural cells in the brain. Secretory protein autotoxin (Enpp2) reported as a tumor-cell motility-stimulating factor, (Murata *et al.*, 1994 and Kawagoe *et al.*, 1995) while secretory glycoprotein SPARC interacts with cell surface and influences the migration, growth factors, and extracellular matrix proteins (Matsumoto *et al.*, 2003). Membrane glycoprotein PDIα is reported as a regulator of CSF production (Narita *et al.*, 1994). Although CP is an active site of protein synthesis (Marques *et al.*, 2009), but the nature of the proteins secreted by the CP may change in response to disease or specific stimuli and for various cytokines, carrier proteins, and iron-related proteins (Marques *et al.*, 2008; Hughes *et al.*, 2002 and Thibeault *et al.*,

2001). Cystatin c (Cst3) has been identified as an autocrine or paracrine trophic factor and is required for FGF-2 mitogenic activity on neural stem cells (Taupin *et al.*, 2002 and Dahl *et al.*, 2004). Complement component factor h (Cfh) is produced by the epithelial cells of the CP (CEC) not by the ependymal cells of the ventricle and secreted by the CECs through the vesicular pathway (Thouvenot *et al.* 2006). It is present in the endothelial cells (Maresh *et al.* 2005) but not in astrocytes (Thouvenot *et al.* 2006). Acetyl-Coenzyme A acyltransferase 2 (Acaa2) plays role for the linkage between fatty acid metabolism and apoptosis of cells; also acts as a functional binding partner for BNIP3 (unique pro-apoptotic protein) protein (Cao *et al.*, 2008).

2.1.1 CP Protiens

Transthyretin is about 25% of the total protein synthesized by the CP and secreted into the CSF and is reported for many vital roles in the CSF (Aldred *et al.*, 1995). It is a major plasma carrier of thyroid hormones (Palha *et al.*, 1994; Davis *et al.*, 1970 and Schreiber, 2002), main thyroid hormone-binding protein in CSF (Hagen and Solberg, 1974), involved with T4 transfer from the blood into the brain across the blood-choroid-plexus-CSF barrier (Dickson *et al.*, 1987 and Southwell *et al.*, 1993).

Autotaxin protein has been thought to regulate the nucleotide metabolism in the extracellular space (Clair *et al.*, 1997; Goding *et al.*, 1998 and Bollen *et al.*, 2000). While Ctsd encodes protein **cathepsin D** that is apparently involved in the processing of antigens, hormones and neuropeptides (Mohamadzadeh *et al.*, 2004).

Apolipoprotein E is essential for the normal catabolism of triglyceride-rich lipoprotein constituents (Singh *et al.*, 2002).

Cystatin C protein is involved in the promotion of sperm maturation (Björck *et al.*, 1989; Veerman and Nieuw-Amerongen, 1998), the formation of intercellular junctions (Kasprzykowski *et al.*, 2000), germ cell migration from basal to luminal regions (Cimerman *et al.*, 1996), adherence of germ cell to Sertoli cells (Björck *et al.*, 1990), it is also active against a variety of bacteria and viruses (Mruk *et al.*, 1997; Erickson-Lawrence *et al.*, 1991 and Peloille *et al.*, 1997). It is reported as a counter-adhesive protein, as a modulator of growth factor activity (Brekken *et al.* 2000).

SPARC (secreted acidic cysteine rich glycoprotein) is secreted acidic cysteine rich glycoprotein that mediates cell–matrix interactions (Lane and Sage, 1994) and performs function when tissues undergo changes in cell–matrix or cell–cell contact like tissue renewal, tissue remodeling, and embryonic development (Bornstein, 1995). SPARC also influences endothelial cell homeostasis, interact with some angiogenic factors (Brekken *et al.* 2000), regulates the activity of fibroblast growth factor (FGF)-2 (Hasselaar and Sage, 1992), interferes with effectors in the FGFR1 signaling pathway and prevents dimerization of FGFR1 (Brekken *et al.* 2000). SPARC also regulates TGF-β1 expression in renal mesangial cells (Francki *et al.*, 1999) and also can counteract the proliferative capacity of bFGF on smooth muscle cells (Brekken *et al.* 2000 and Yan *et al.*, 1999).

Inositol 1,4,5-triphosphate receptor type 1protein is involved in neuronal plasticity (Mikoshiba, 2006) and early development. It controls a variety of Ca^{2+}-

dependent cell functions like cell proliferation, differentiation, fertilization, embryonic development, secretion, muscular contraction, immune responses, brain functions, chemical senses, light transduction, etc. (Mikoshiba, 2006).

Decorin1 is a matrix proteoglycan. Main functions of Decorin1 include; regulation of both fibrillogenesis and phagocytic degradation that requires binding to collagen (Bhid *et al.*, 2005), wound healing (Hakkinen *et al.*, 2000), binding and neutralizing significant amounts of transforming growth factor, a potent, pro-fibrotic cytokine (Yamaguchi *et al.*, 1999 and Border *et al.*, 1992). Decorin modulates the interactions of matrix molecules such as fibronectin with cells (Lewandowska *et al.*, 1987 and Schmidt *et al.*, 1987). Decorin binds a variety of adhesive and non-adhesive proteins, including fibronectin, thrombospondin and various types of collagens (Comalada *et al.*, 2003).

Insulin-like growth factor-binding protein 5 promotes cell differentiation (Ren *et al.*, 2008). It is involved in apoptosis, protein-protein interaction, cell motility, cell survival, and cellular trafficking (Akkiprik *et al.*, 2008). Other reported functions include stimulation of cell migration through interaction with cell surface heparan sulfate proteoglycans (Hsieh *et al.*, 2003), activation of p38 MAP kinase and Erk 1/2 signal transduction pathways (Kuemmerle *et al.*, 2002), regulation of gene transcriptions (Xu et al. 2004) and a potential role in carcinogenesis (Mohan *et al.*, 2002 and Dufner-Beattie *et al.*, 2006).

2.2 Role of CP genes in CNS disorders

Timp2 gene is involved in the invasion and metastasis of colorectal cancer (Park *et al.*, 2011), important additional indicator to analyze the malignant potential of papillary thyroid cancer (Delektorskaia *et al.*, 2010) and play etiological role in multiple sclerosis (Lee *et al.*, 1999). **Ttr** performs neuroprotective role in Alzheimer disease (Stein *et al.*, 2004 and Buxbaum *et al.*, 2008). **Aco 2** gene is involved in the Alzheimer, Huntington and Parkinson diseases (Kim *et al.*, 2005). **Igfbp5** plays potential role in carcinogenesis (Mohan and Baylink, 2002 and Dufner-Beattie *et al.*, 2006). **SPARC** causes the pathogenesis like cancer metastasis (Reed and Sage, 1996). **Apoe** gene is involved in central nervous system inflammatory diseases, Alzheimer disease and its concentration varies in CSF during these diseases (Carlsson *et al.*, 1991 and Saito *et al.*, 1997). Mutations in the **Cfh** gene cause many diseases like hemolytic-uremic syndrome (single amino acid change in complement factor H) leading to the nonfunctional version of the protein or the blockage of protein production (Atkinson and Goodship, 2007) ; and in the severe case an uncontrolled activation of immune system. **Ceruloplasmin** (Cp) gene is used as a peripheral marker for the copper imbalance associated diseases (Capo *et al.*, 2008) and plays role in Alzheimer's disease (Loeffler et al., 1994).

CHAPTER 3

MATERIALS AND METHODS

The present study was conducted in the department of Bioinformatics, International Islamic University and the department of Biosciences, COMSATS Institute of Information Technology Islamabad.

3.1 Data source

Allen brain institution (institute for brain sciences) website http://www.alleninstitute.org/ was used to get the genes expression information in the choroid plexus of adult mouse brain by using the in situ hybridization (ISH) data of **Allen Mouse Brain Atlas** (ABA) http://mouse.brain-map.org/welcome.do . The Allen Brain Institute has spent the past few years carrying out a comprehensive expression study of the human brain, mouse brain and through development. Dr. Gregor Eichele's Laboratory at the Max Planck Institute and Baylor College of Medicine designed the procedures which are followed by the Allen Brain Atlas (ABA) project. 8-week old C57Bl/6J male mouse brains were used to get the data for Allen Mouse Brain Atlas and Reference Atlases. The Allen Mouse Brain Reference Atlas provides many facilities to users like direct comparison between gene expression patterns and neuroanatomical structures, 3D computer graphic models of the mouse brain to develop the informatics based annotation tools and detailed searchable gene expression database. In this way Allen Brain Institute

presents a comprehensive online platform to explore the brain information at the cellular and molecular level. One of the major goals of Allen brain institute is to create a detailed, cellular-resolution, genome-wide map of gene expression in the mouse brain. Although different projects are continued at Allen institute to extract and retrieve the data but somehow the CP region is untouched.

3.2 Data extraction

The genes from **AGEA** (Allen Gene Expression Atlus) tool of ABA site for CP genes were selected by using the gene finder tab. AGEA provides the gene lists for specific area of the brain by dragging the line at the required area (Fig 3.1).

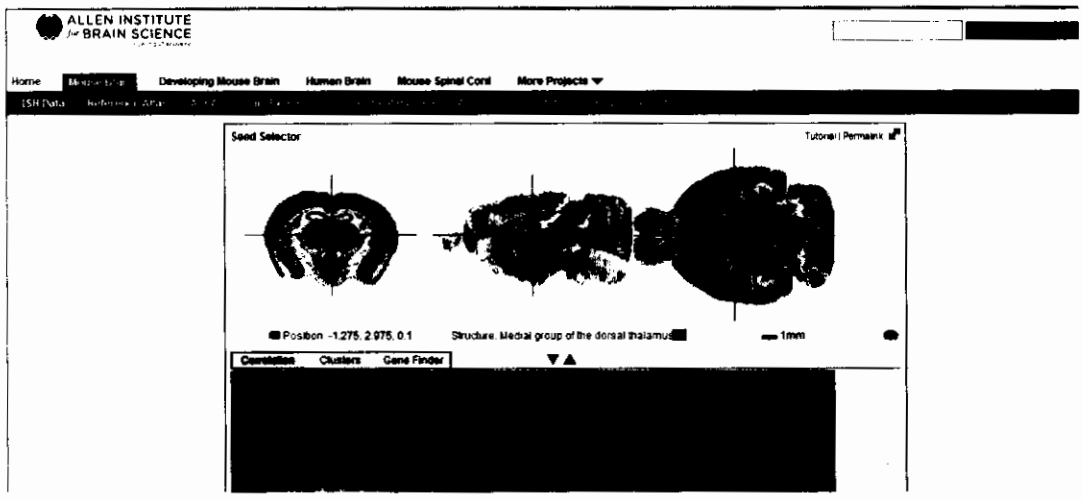


Fig 3.1 AGEA tool of ABA site.

3.2.1 Brain explorer

The brain explorer software of ABA was also used to check the gene expression in the whole brain of adult mouse. Brain Explorer is a desktop software application for

viewing the Allen Mouse Brain Atlas gene expression data in the framework of the Allen Reference Atlas (ARA). This software provides the 3D visualization of the gene expression in the adult mouse brain.

To understand the basic regions of the adult mouse brain in the images and the gene expression in those regions, one sagittal image of brain explorer is given below (Fig 3.2).

Fig 3.2 Brain explorer image shows the basic regions of adult mouse brain.

The abbreviations of these basic regions are given below.

- CTX: Cerebral cortex.
- CBX: Cerebellar cortex.
- OLF: Olfactory area.
- STR: Striatum.
- HP: Hippocampus.

MB: Midbrain.

MY: Medulla

VL/LV: Lateral ventricle.

VL/LV: Lateral ventricle.

V3: Third ventricle.

- TH: Thalamus. V4: Fourth ventricle.
- HY: Hypothalamus. Pons: Pons.

Different basic regions of muse brain appear as different colored areas and gene expression is indicated as yellow dots, as shown in figure 3.3.

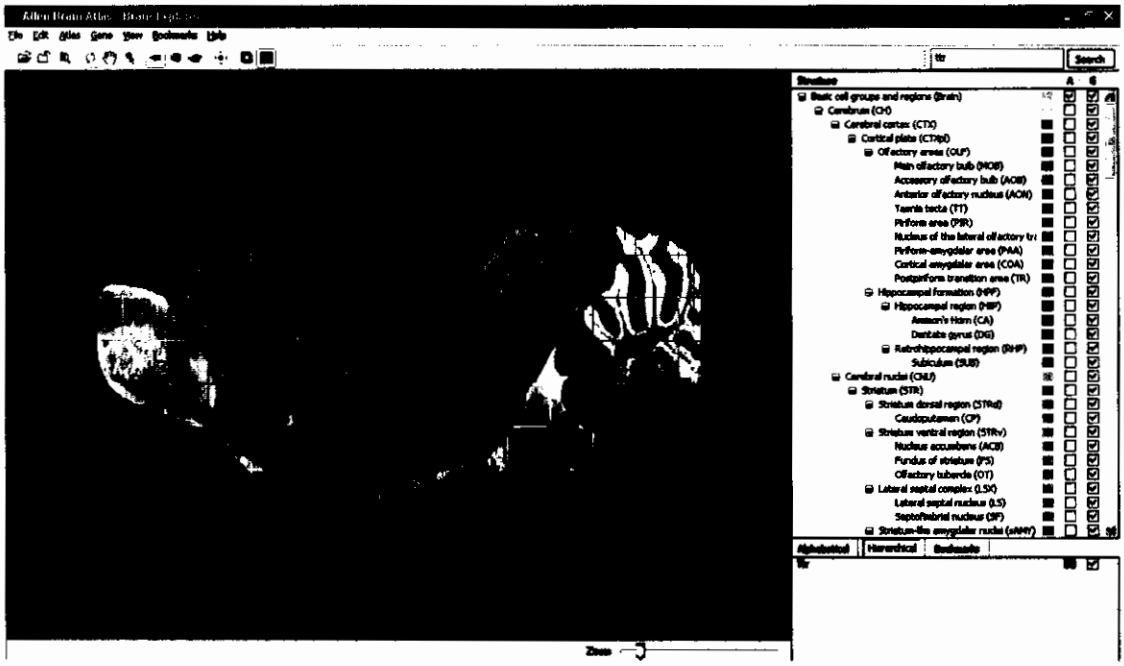


Fig 3.3 Brain explorer screen short for gene expression image of adult muse brain.

Brain explorer gives the facility to check the gene expression at different planes (Fig 3.4).

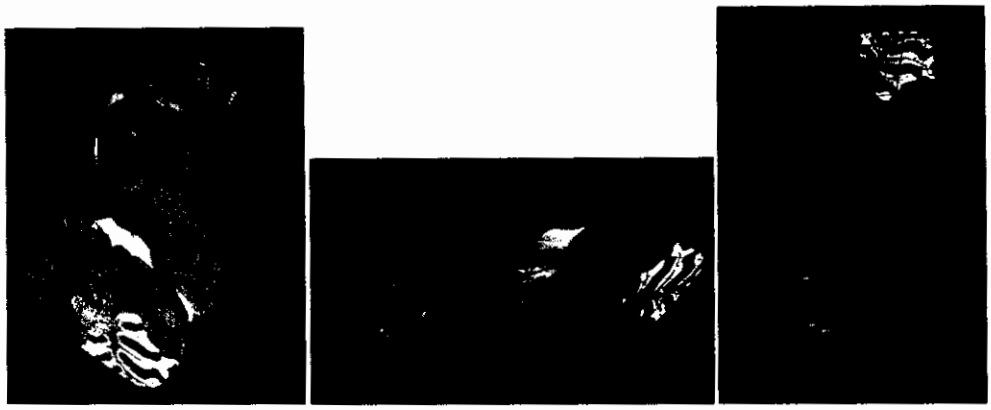


Fig 3.4 3D visualization of gene expression by brain explorer software.

3.2.2 Allen reference atlas (ARA)

The expressions of selected genes were checked in the choroid plexus and in basic regions of the brain by using both reference images given at **ARA** and brain explorer images.

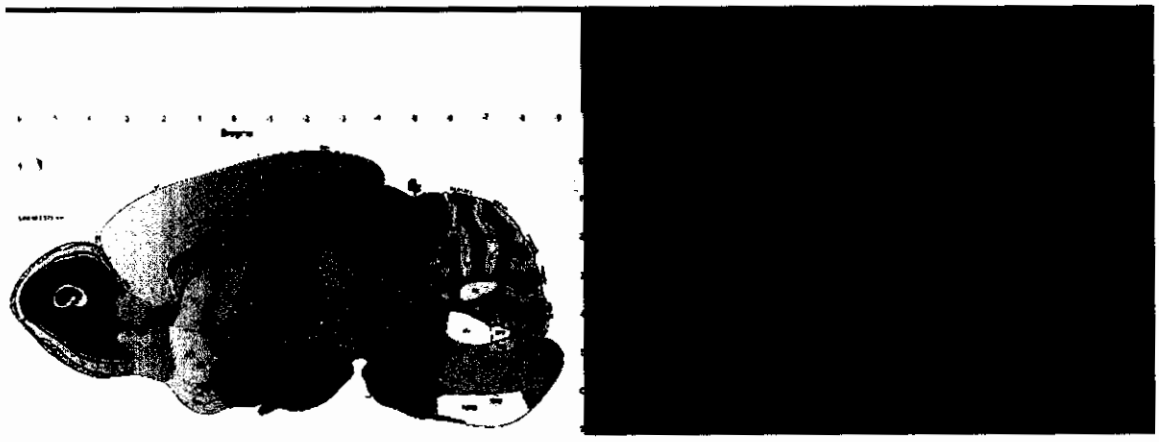


Fig 3.5 ARA sagittal image and its gene expression image.

Left side (Fig 3.5) reference colored image shows the different regions of the adult mouse brain and right side image shows the gene expression in those colored areas

of the brain. Colored bar at the top of right side image shows the levels of gene expression. Red color shows the highest expression level and blue color shows the lowest expression level according to Allen brain institute criteria.

3.3 Spatial gene expression analysis

3.3.1 Analysis of gene expression

First of all the gene expression in the whole brain of the adult mouse was observed but main focus was on the CP. The gene expressions in the CP of all the three locations (lateral, third and fourth ventricles) were analyzed the complete strategy for gene expression analysis is explained by using the Ttr gene expression images. By understanding the gene expression images of Ttr in the whole brain and in CPs would make easy to understand the remaining expression images of genes.

Fig 3.6 ARA sagittal image of adult mouse brain.

In the above image (Fig 3.6), red circled black area shows the fourth ventricle at the right side and third ventricle at the top middle side of the figure. Gene expression in CP of third and fourth ventricles is shown below (Fig 3.7).

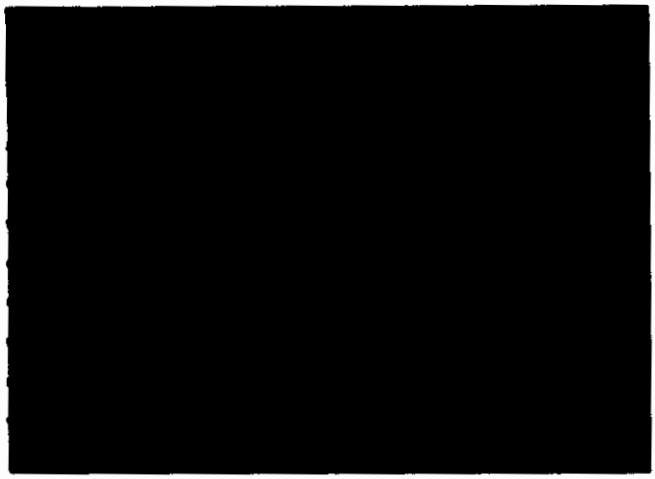


Fig 3.7 Gene expression in the CP of third and fourth ventricles.

Above image (Fig 3.7) shows the filtered gene expression image of Ttr in the adult mouse brain. At the top left side colored line shows the intensity level of gene expression. Blue color shows the low expression level and red color shows high expression level and the circled red color shows the high gene expression of Ttr in CP of third and fourth ventricles. Ttr is also expressed in other parts of brain but its expression level is low.

Zoomed images of CP of the fourth (Fig 3.8), lateral (Fig 3.9) and third (Fig 3.10) ventricles with the zoomed expression of Ttr gene in the CPs are shown below.

Fig 3.8 ISH image and Expression image of Ttr in CP of fourth ventricle.

Fig 3.9 ISH image and Expression image of Ttr in CP of lateral ventricle.

Fig 3.10 ISH image and Expression image of Ttr in CP of third ventricle.

By following the above procedure, only those genes were selected that are highly expressed in CP as compared with other regions of the brain or those that have missing expression in one or two ventricle choroid plexus in the adult mouse brain. The list of these genes is given below (table 3.1).

Table 3.1 List of CP genes.

Sr.No	Gene Symbol	Gene Name
1	TtR	Transthyretin
2	Enpp2	Ectonucleotide pyrophosphatase/phosphodiesterase 2
3	DCN	Decorin
4	Igfbp5	Insulin-like growth factor binding protein 5
5	Uqcrc1	Ubiquinol-cytochrome c reductase core protein 1
6	Itpr1	Inositol 1,4,5-triphosphate receptor 1
7	SPARC	Secreted acidic cysteine rich glycoprotein
8	Gja7	Gap junction membrane channel protein alpha 7
9	Gsn	Gelsolin
10	Cfh	Complement component factor h
11	Ctsd	Cathepsin D
12	Igfbp2	Insulin-like growth factor binding protein 2
13	Efhc1	EF-hand domain (C-terminal) containing 1
14	Gpm6b	Glycoprotein m6b
15	Apoe	Apolipoprotein E
16	Cp	Ceruloplasmin
17	Col1a1	Procollagen, type I, alpha 1
18	Col3a1	procollagen, type III, alpha 1
19	Col5a2	Procollagen, type V, alpha 2
20	Fn1	Fibronectin 1
21	Fbln1	Fibulin 1
22	Fbln2	Fibulin 2

23	Efemp1	Epidermal growth factor-containing fibulin-like extracellular matrix protein 1
24	Efemp2	Epidermal growth factor-containing fibulin-like extracellular matrix protein 2
25	Cyb561d2	Cytochrome b-561 domain containing 2
26	Gzma	Granzyme A
27	Thbs1	Thrombospondin 1
28	Mmp2	Matrix metallopeptidase 2
29	Cst3	Cystatin C
30	Timp2	Tissue inhibitor of metalloproteinase 2
31	Spint2	Serine protease inhibitor, Kunitz type 2
32	Srpk1	Serine/Arginine-rich protein specific kinase 1
33	Wfikkn2	WAP, follistatin/kazal, immunoglobulin, kunitz and netrin domain containing2
34	Svep1	Sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1)
35	Serpinf1	Serine (or cysteine) peptidase inhibitor, clade F, member 1)
36	B2m	Beta-2 microglobulin
37	Brd4	Bromodomain containing 4
38	Brrn1	Barren homolog (Drosophila)
39	Gpihbp1	GPI-anchored HDL-binding protein 1
40	Hdlbp	High density lipoprotein (HDL) binding protein
41	Slc13a4	Solute carrier family 13 (sodium/sulfate symporters), member 4
42	Vcam1	Vascular cell adhesion molecule 1
43	Acaa2	Acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)
44	Acadm	Acyl-Coenzyme A dehydrogenase, medium chain
45	Aco2	Aconitase 2, mitochondrial
46	Acox1	Acyl-Coenzyme A oxidase 1, palmitoyl
47	Maob	Monoamine oxidase B

48	Pomc1	Pro-opiomelanocortin-alpha
49	Sntb1	Syntrophin, basic 1
50	Gja4	Gap junction membrane channel protein alpha 4
51	Ccnd2	Cyclin D2

3.3.2 Evaluation Criteria

For these 51 genes, different types of gene expression patterns have been observed. To carry out the gene expression analyses, the evaluation criteria considered the following three factors.

- Gene expression level
- Gene expression pattern
- Gene expression specificity.

3.3.2.1 Expression level

To explain the expression level difference between the CP of different ventricles; three standards are made according to Allen brain institute color bar (Fig 3.11) i-e red, yellow and blue colored boxes.

Fig 3.11 ABA color bar to explain the gene expression levels.

Expression level of genes is shown by different colors, blue color shows the lowest level of expression and red color shows the highest expression. First three colors were taken as low expression level and shown it by blue colored box. Middle colors from green to dark yellow took as medium expression level and shown it by yellow box while the remaining red shaded colors took as high expression level and shown it by red box (Table 5.1 & 5.2). If gene expression contains first four colors, it considered as medium color due to the presence of green color which comes in the medium category. In the

same way if any red shade was present in the medium level of gene expression, it considered as high expression level. Because according to Allen brain institute, there is slight difference between color shades which show even little differences. To classify the expression levels between genes and among their own CPs becomes too much difficult according to ABA criteria. To sort out this problem above standers were made to show these differences clearly. If a gene expressed in few number of cells in the CP that it can be taken as negligible; it would low expression level although the expression level fall into low, medium or high expression levels according to Allen brain institute.

3.3.2.2 Expression pattern

To find out the production specificity by the choroid plexus in brain; gene expression pattern was classified into two categories.

- Dense
- Scattered

Following images make easy to understand this criteria (Fig 3.12; Fig 3.13).

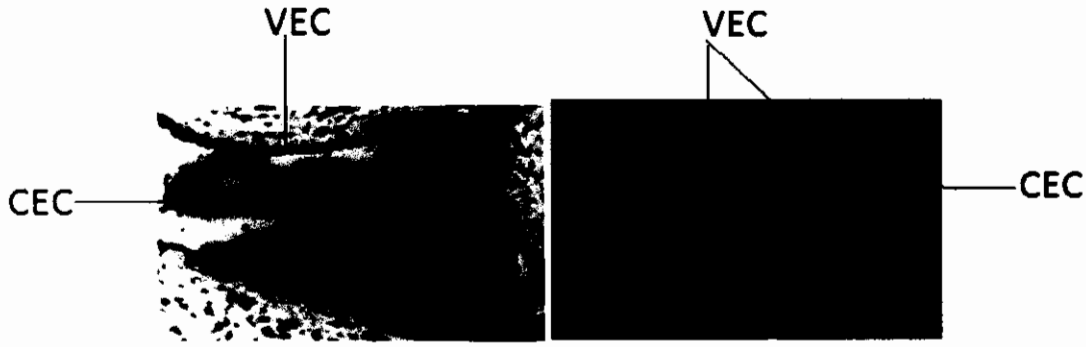


Fig 3.12 Dense pattern of gene expression in the CP.

Dense expression means that the main production area is CP. It also shows that brain needs this gene in more quantity to perform different functions. VEC and CEC terms define the ventricular ependymal cells and CP epithelial cells. If the gene is expressed in both VEC and CEC; it means it is secreted into CSF in large quantity in brain regions.

Fig 3.13 Scattered pattern of gene expression in the CP.

If the gene expression is in scattered pattern then gene may be a CP specific but that gene performed limited functions in the brain. Because it produces in less quantity as the brain cells require.

3.3.2.3 Gene expression specificity

Gene expression specificity includes the information either gene is present only in the CPs or in other regions of the brain also. By following this criteria genes were classified into two groups.

- CP specific genes.
- Non-CP specific genes.

If the gene is present only in CPs then took it as CP specific gene. If a gene is present in CPs but also in the other regions of the brain, took it as CP non-specific gene.

3.4 Software application

To preserve the spatial gene expression data of adult mouse brain, SQL database was made. To show and retrieve the data, a user interface was also required. To solve this issue, a web site is also developed. In this way software application was made that is a web linked database. This software application provides the information about CP gene list, CP specific gene list, non-CP specific gene list, spatial expression images and gene reports. Visual studio 2008 express edition was used to make this software. To create the website by using visual studio 2008, asp.net, HTML and C sharp languages were used.

3.4.1 Smart draw software

Smart draw is simple and easily useable software to draw the system diagrams, flow charts etc. To understand the work flow of software application by users/researchers; DBMS (database management system) presentation of the system have to made. This presentation required the entity relationship diagrams (ERD), use case diagram, activity diagram etc. Smart draw software was used to make the ERD, use case and activity diagrams to represent the work flow of the software.

CHAPTER 4

SOFTWARE APPLICATION: SYSTEM DESIGN AND SPECIFICATIONS

This software application provides user friendly web interface with a database. The software will give a platform to researchers/users interested in the choroid plexus projects of adult mouse brain. The name of this software application is '*Gene Expression Data of Adult Mouse Choroid Plexus*' (GEDAMCP). This software is a web linked SQL database. This Relational database is created to store the choroid plexus (CP) gene data with the specifications of CP specific and non-CP specific gene lists. GEDAMCP website was created by using the Microsoft visual web developer 2008 express. It contains the reports for CP genes their spatial gene expression images according to three locations of CP in the adult mouse brain. It also gives the gene expression pattern and level in all the CPs.

4.1 Database scheme

Database scheme is its structural description in a formal language supported by the database management system (DBMS) to show how a database is constructed. A relational database always requires an appropriate scheme that includes different groups containing relation with each other to remove the redundancy. There is one group in

database containing different records (tables) of spatial gene expression data of adult mouse brain.

4.1.1 Conceptual Design of database with Entity-Relationship Model (ERM)

First of all a conceptual design of the system was made that explains the entity types, their attributes, the key-attributes, the relationships their cardinalities and constraints. Primary keys are shown by the underline attribute.

- Entity types:

Gene: Gene ID, Gene symbol, Gene name, Spatial Expression data, type, Description, Images.

4.1.2 Conceptual scheme: ERD (entity relationship diagram)

Entity relationship diagram of the system is given below (Fig 4.1). Rectangle represents entities, oval represents attribute and underline attributes represents primary keys.

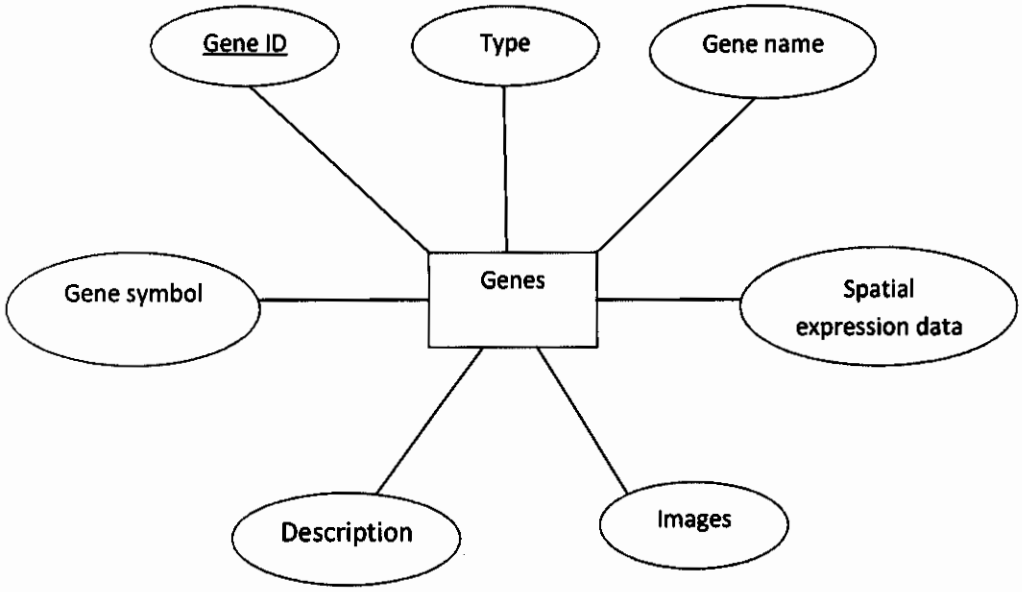


Fig 4.1 Entity relationship diagram (ERD) of GEDAMCP.

4.1.3 Relational Data model

A relational database is a collection of data items ordered as a set of tables. These tables provide the opportunity to retrieve data in many ways without rearranging the database tables. Database consisted of one big table (Genes) that was taken as entity and its columns as attributes in ERD diagram.

- **Genes**
  - Gene ID: Primary key column of the table 'Genes'. It contains Entrez Gene IDs of all the genes present in the database.
  - Gene name: Name of the gene.
  - Gene symbol: It contains gene symbol of the corresponding gene.

- Type: This column contains information about the type of gene and contains only two values which are 'CP specific' and 'CP non- specific'. This gives the information about CP specific or CP non-specific genes.
- Description: It contains URLs of gene reports that contain introductory information of CP gene.
- Spatial gene expression: It contains the spatial gene expression information according to ventricles. This column contains values as 'all', 'lateral ventricle', 'third ventricle', 'fourth ventricle', 'lateral and fourth ventricle', 'third and fourth ventricle. Each tuple (row/record) contains one of these values depending upon the corresponding gene.
- Images: contains URLs of the gene expression images and ISH (in situ hybridization) images of CP gene.

Summarized detail of the genes entity according to its attributes is given in the form of following table (Table 4.1).

Table 4.1 Information of the attributes of Gene entity.

Gene ID	Gene Symbol	Gene Name	Type	Description	Spatial expression (ventricles)	Images
11364	Acaa2	Acyl-Coenzyme A dehydrogenase, medium chain	CP specific	Acaa2.htm	All	Acaa2.bmp
11429	Aco2	Aconitase 2, mitochondrial	CP non-specific	Aco2.htm	All	Aco2.bmp
11430	Acadm	Acyl-Coenzyme A oxidase 1, palmitoyl	CP specific	Acadm.htm	All	Acadm.bmp
11816	Apoe	Apolipoprotein E	CP non-specific	Apoe.htm	All	Apoe.bmp
12010	B2m	Beta-2 microglobulin	CP non-specific	B2m.htm	All	B2m.bmp
12444	Ccnd2	Cyclin D2	CP specific	Ccnd2.htm	All	Ccnd2.bmp
12628	Cfh	Complement component factor h	CP specific	Cfh.htm	All	Cfh.bmp
12825	Col3a1	Procollagen, type III, alpha 1	CP non-specific	Col3a1.htm	All	Col3a1.bmp
.	.	.	.	.	.	.
.	.	.	.	.	.	.

4.1.4 Querying Relational data Model

The physical database according to ERD model was developed and designed the SQL queries according the requirements of database. The main purpose to design this database was to provide the information about spatial expression of CP genes just like a repository. Simplicity was maintained to design this database for the users to easily access the data. SELECT queries were created to retrieve the required data as user wants. Further amendments may be possible in this database by adding more useful features.

The select query was made up of three clauses: SELECT, FROM and WHERE.

SELECT < list of attribute> FROM <table> WHERE <condition>

Our queries are designed as:

To get the complete list of CP genes following query was used (Fig 4.2).

SELECT [Gene ID] AS Gene_ID, [Gene Symbol] AS Gene_Symbol, [Gene Name] AS Gene_Name FROM [genes]

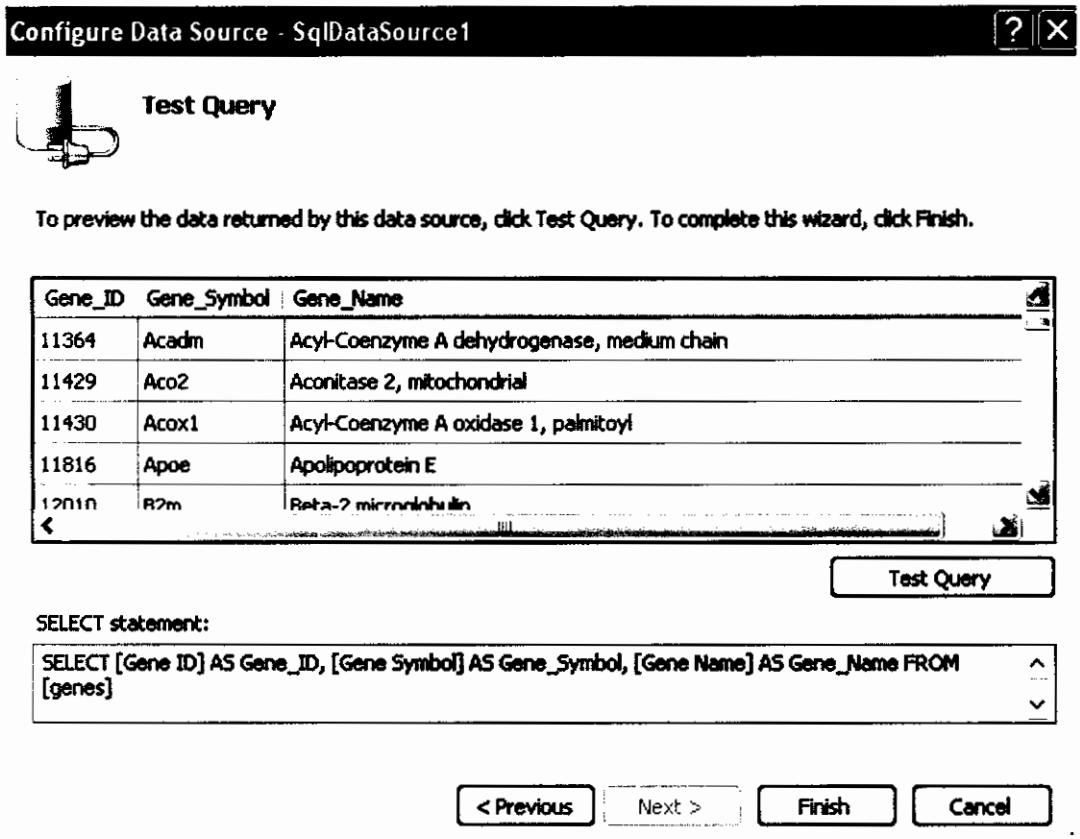


Fig 4.2 Test query for all CP genes.

To retrieve the list of CP specific genes following query was used (Fig 4.3).

SELECT [Gene ID] AS Gene_ID, [Gene Symbol] AS Gene_Symbol, [Gene Name] AS Gene_Name FROM [genes] WHERE ([CP spesific genes] = @CP_spesific_genes)

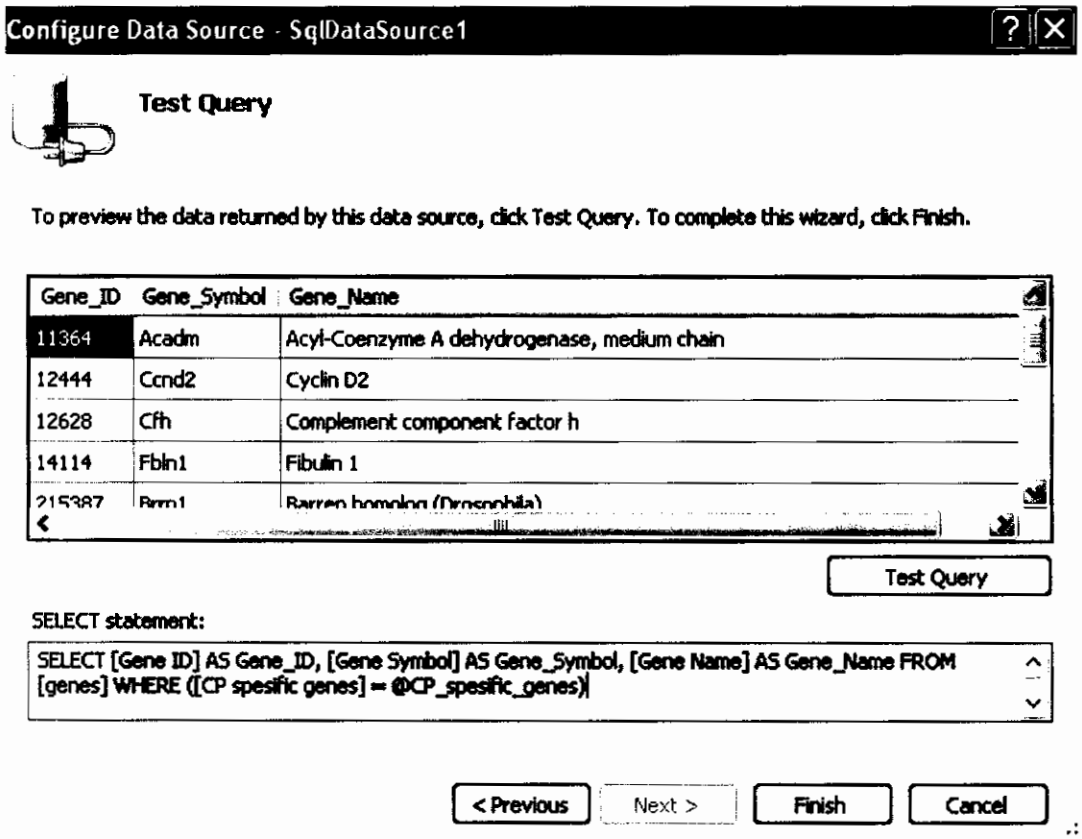


Fig 4.3 Test query for CP Specific genes.

To retrieve list of non-CP specific genes following query was used (Fig 4.4).

SELECT [Gene ID] AS Gene_ID, [Gene Symbol] AS Gene_Symbol, [Gene Name] AS Gene_Name FROM [genes] WHERE ([CP spesific genes] = @CP_spesific_genes)

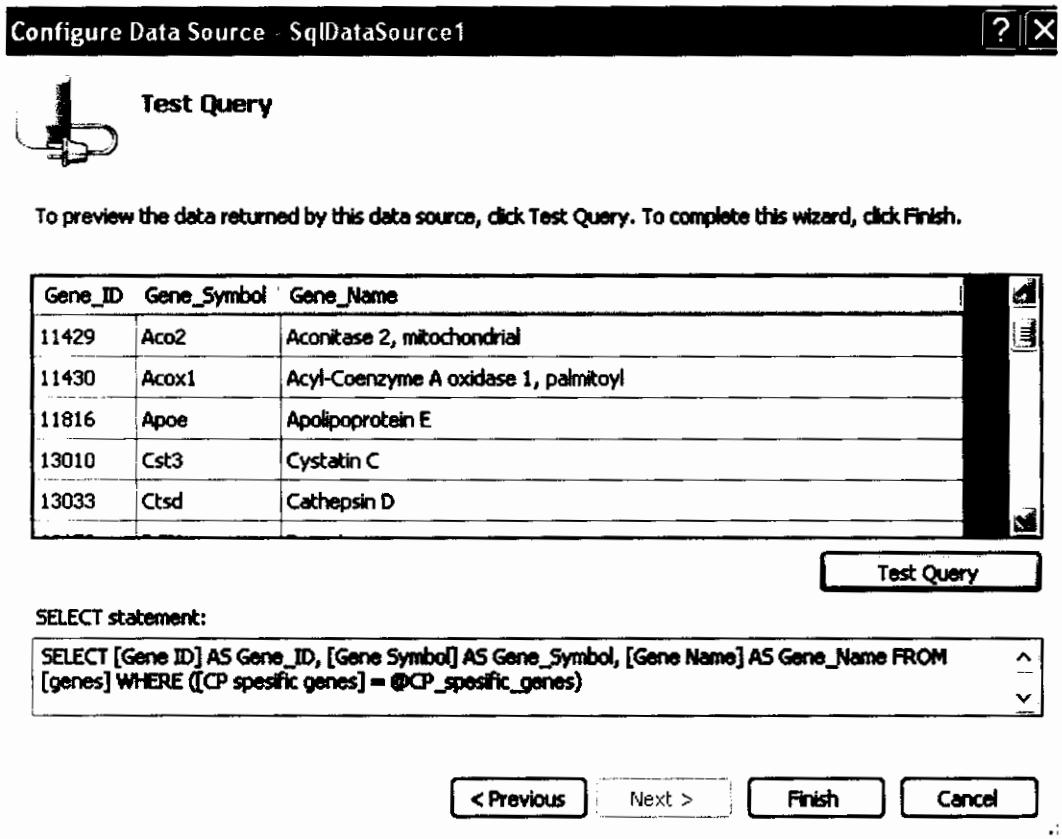


Fig 4.4 Test query for non-CP Specific genes.

To get information about the spatial gene expression according to their ventricles like either one gene is present in lateral, third or fourth ventricle, following query was used.

SELECT [Gene ID] AS Gene_ID, [Gene Symbol] AS Gene_Symbol, [Gene Name] AS Gene_Name FROM [genes] WHERE ([Fourth Ventricle] = @Fourth_Ventricle)

Above query is specific to get the list of genes that have expression in the fourth ventricle. But the same queries are used for lateral and third ventricles by removing the word fourth from the "WHERE" clause and putting the required ventricle name.

4.2 Use case diagram

Use case diagram was used to identify the primary elements and processes that make the system. The primary elements were 'actors' and processes were 'use casesy'. The key purpose of a use case diagram was to illustrate what system functions are performed for which actor. One of the basic goals of software was to fulfill the need of spatial gene expression data of adult mouse choroid plexus that provides the images of spatial gene expression data (SGED) and their reports. This repository will be useful for those researchers/users who are interested in the spatial gene expression data of adult mouse brain. This repository not only provides the images and information about the SGED but also some overview for the proteins which are secreted by the choroid plexus and then secreted into the CSF. This is a user friendly application without any complexity.

- **User/researcher:** The person who want to retrieve the information about SGED.

4.2.1 Actors

Actors are the entities performing certain roles or processes and these entities may be a person or a machine that interact with system to retrieve the required information. In this application actors are users.

4.2.2 Use cases

Use cases are the steps or actions between an actor and a system leading to get some useful information. Basically use case is a visual representation of distinct business functionality in a system.

The primary business flow in this system which can be a use case is given below.

- To retrieve gene expression data.

But user can also find some discrete processes in this primary business flow. For example, user can retrieve the full list of choroid plexus (CP) genes, images of the CP genes, reports of the genes and information about the project etc. So within this primary use case, it can also determine the following sub processes.

- ➢ To retrieve gene expression data.
- View complete CP gene list.
- Retrieve CP specific gene list.
- Retrieve non-CP specific gene list.
- Retrieve spatial gene expression/ISH images.
- Retrieve gene reports.
- Retrieve information about the research project.

Use case diagram of this software application is given below (Fig 4.5).

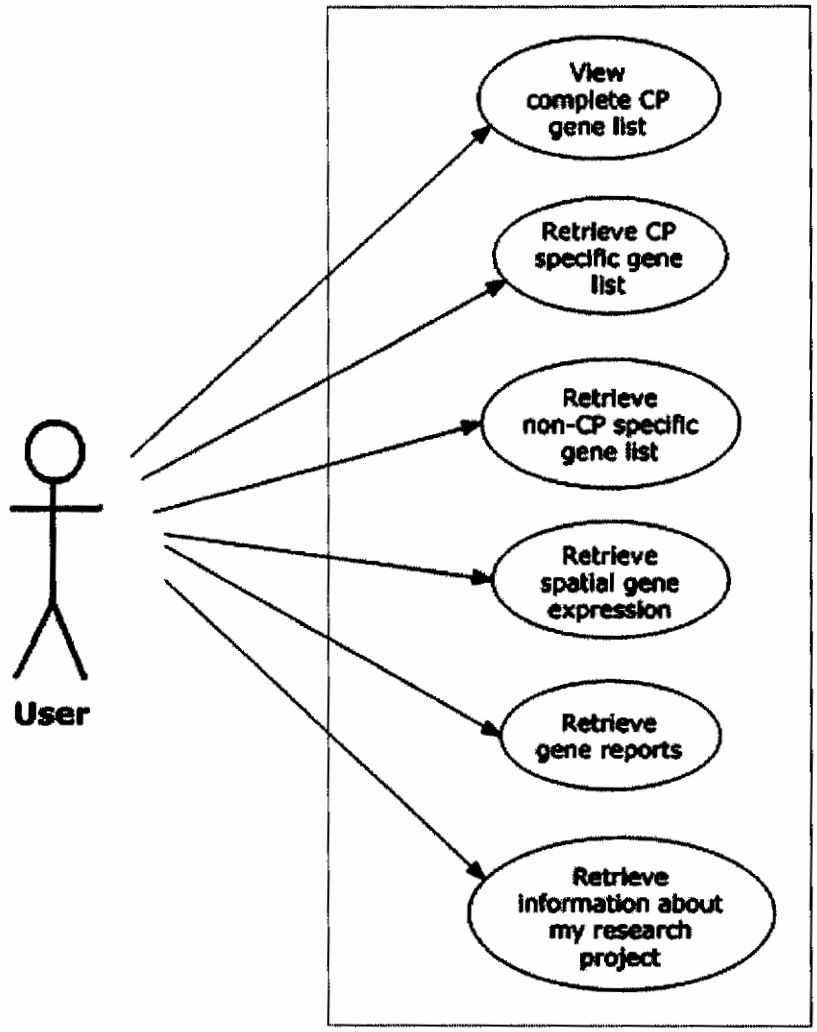


Fig 4.5 Use case diagram of GEDAMCP.

4.3. Activity diagram

According to software application different users can get different information about CP genes as they need. Every user has different interests in the choroid plexus project e.g some want to retrieve only the CP gene list, CP specific gene list, non-CP specific gene list, spatial gene expression data with respect to ventricles, expression images etc. All this information can be access by using this web linked database

application. If a user interacts with this software application, he/she will have to go through certain selection processes to reach to the data of his interest. To make this selection process easy, an activity diagram is also created to present the operational workflow of this system (Fig 4.6). According to activity diagram, a user can easily understand the operational workflow of his interest and can retrieve the required data.

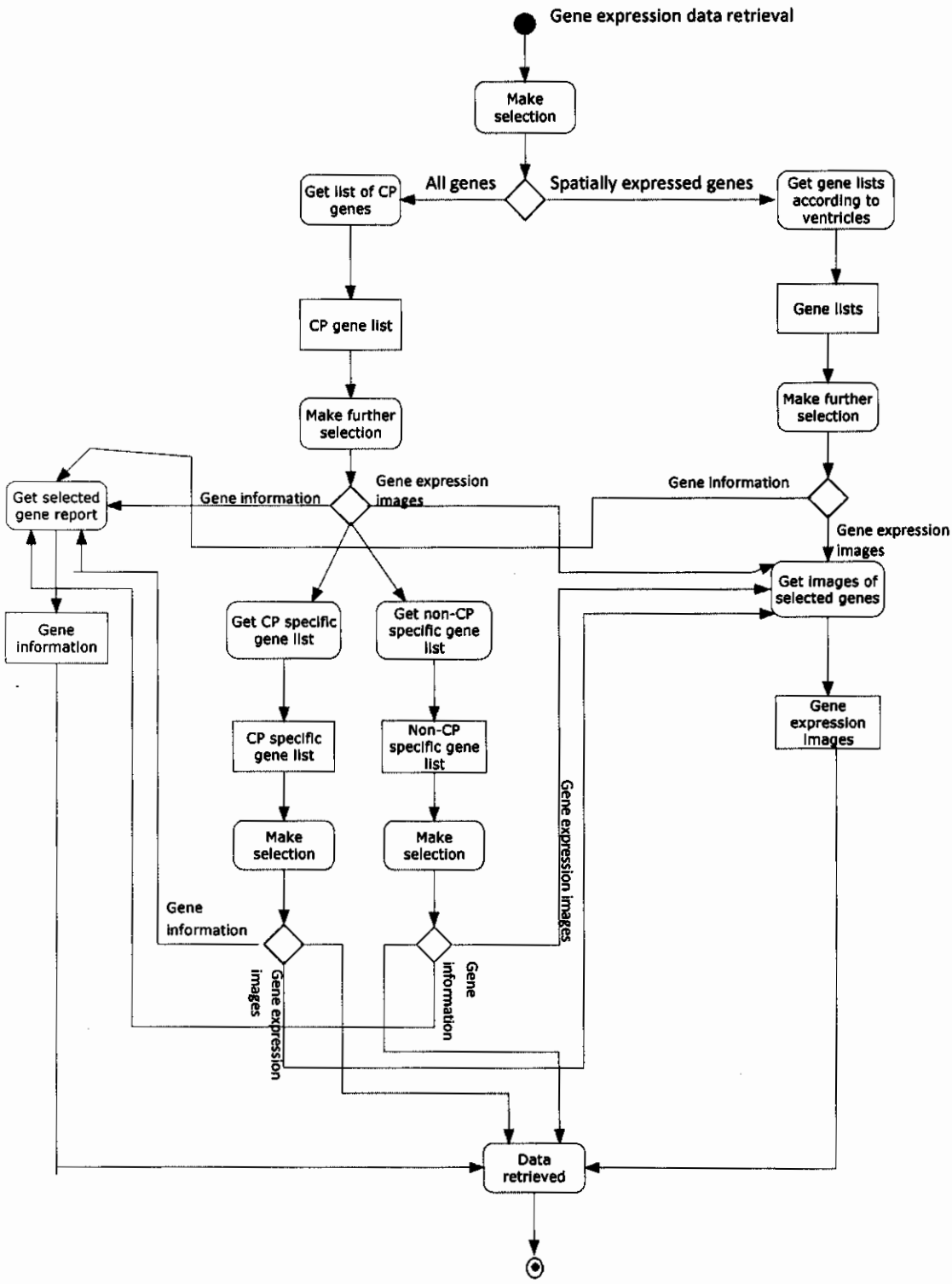


Fig 4.6 Activity diagram of GEDAMCP.

CHAPTER 5

RESULTS

The expression analysis of the 51 CP genes of adult mouse brain (Mus musculus) was carried out. Gene expression level and pattern varied from gene to gene even within the CPs of the same gene.

5.1 CP specific genes

Out of 51 genes, 13 were found as CP specific genes i-e Ttr, Acaa2, Acadm, Brd4, Brrn1, Cfh, Ccnd2, Efhc1, Fbln1, Gpihbp1, Gzma, Mmp2 and Wfikkn2 while remaining were found to be non-CP specific (Fig 5.1). Among the CP specific gene expression analysis, scattered pattern was found in all the genes excluding Ttr and Acaa2 showed dense pattern (Fig 5.2, 3, 4). In the case of expression level all these 13 genes vary even among their own ventricle CPs (table 5.1). Ttr and Acaa2 showed high expression level in all the CPs and also in some ventricular ependymal cells. Ttr, Brrn1, Efhc1 and Fbln1 gene expression was also observed in the ventricular ependymal cells while the other CP specific genes did not show expression in those cells. Although all the CP specific genes are responsible for the functioning of different proteins but not even a single same protein was found that handles by more than one gene.

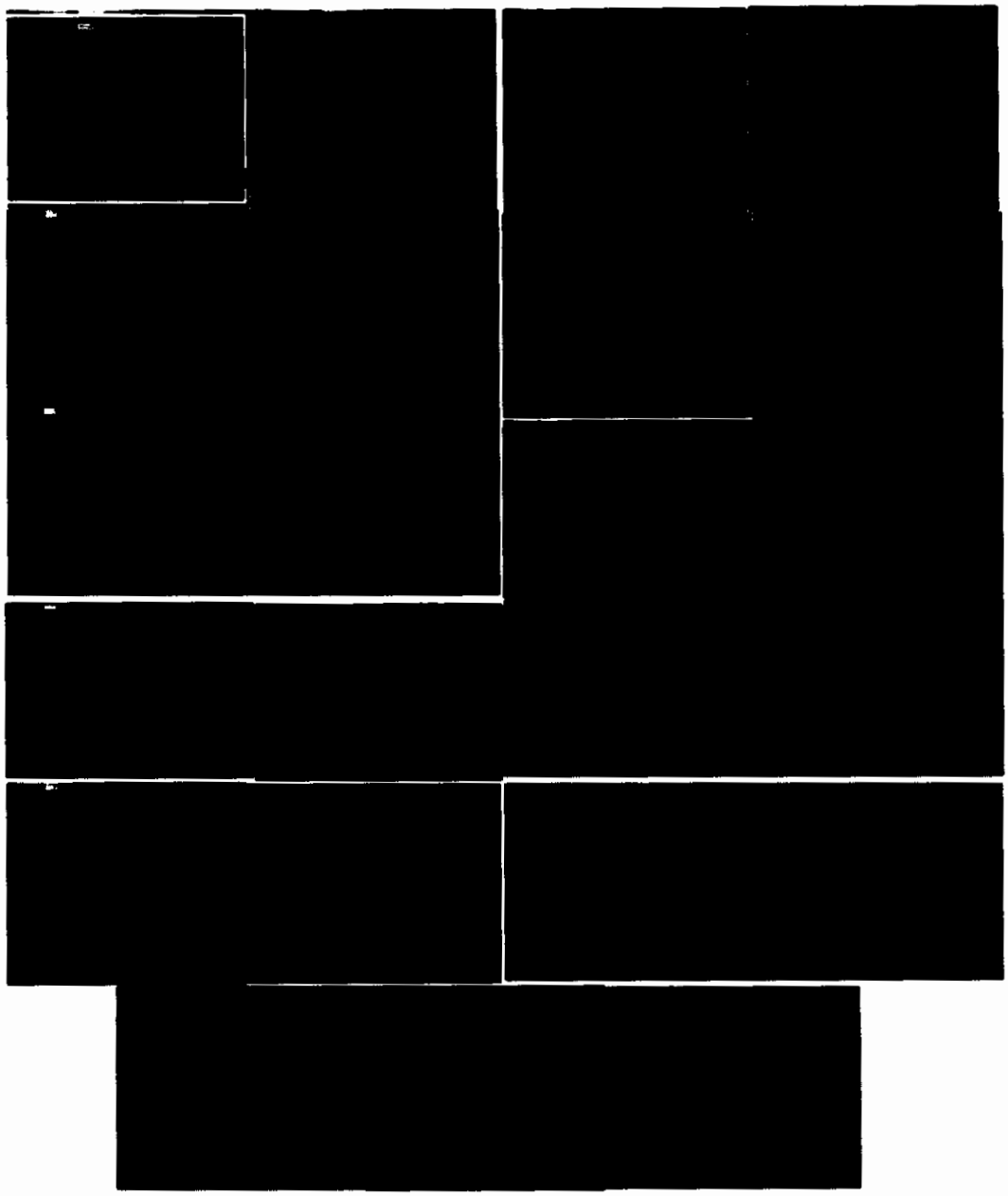


Fig 5.1 Expression images of CP specific genes in adult mouse brain. Circled areas show gene expression in ventricles.

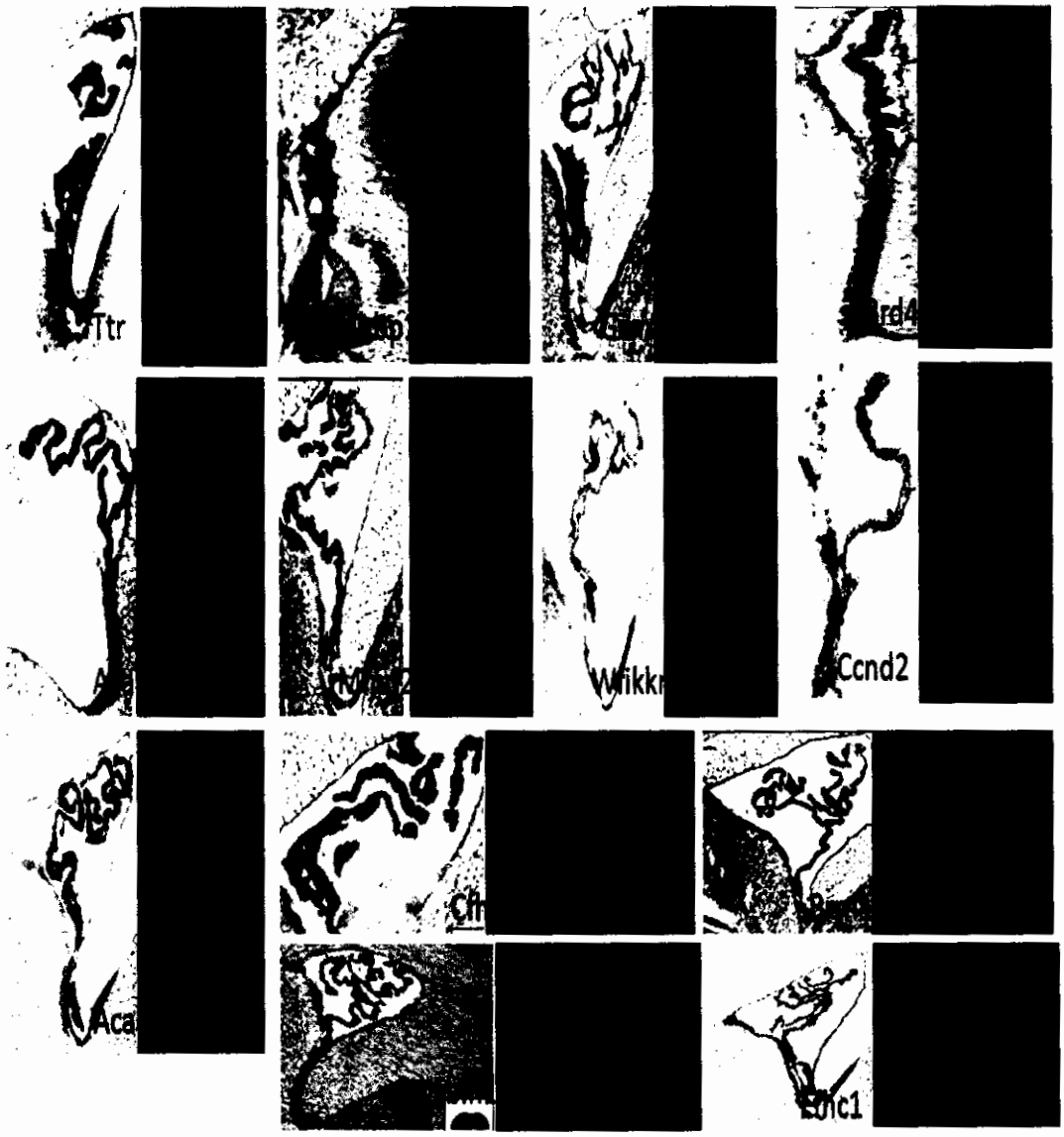


Fig 5.2 Expression images of CP specific genes in lateral ventricle of adult mouse brain.

Fig 5.3 Expression images of CP specific genes in third ventricle of adult mouse brain.

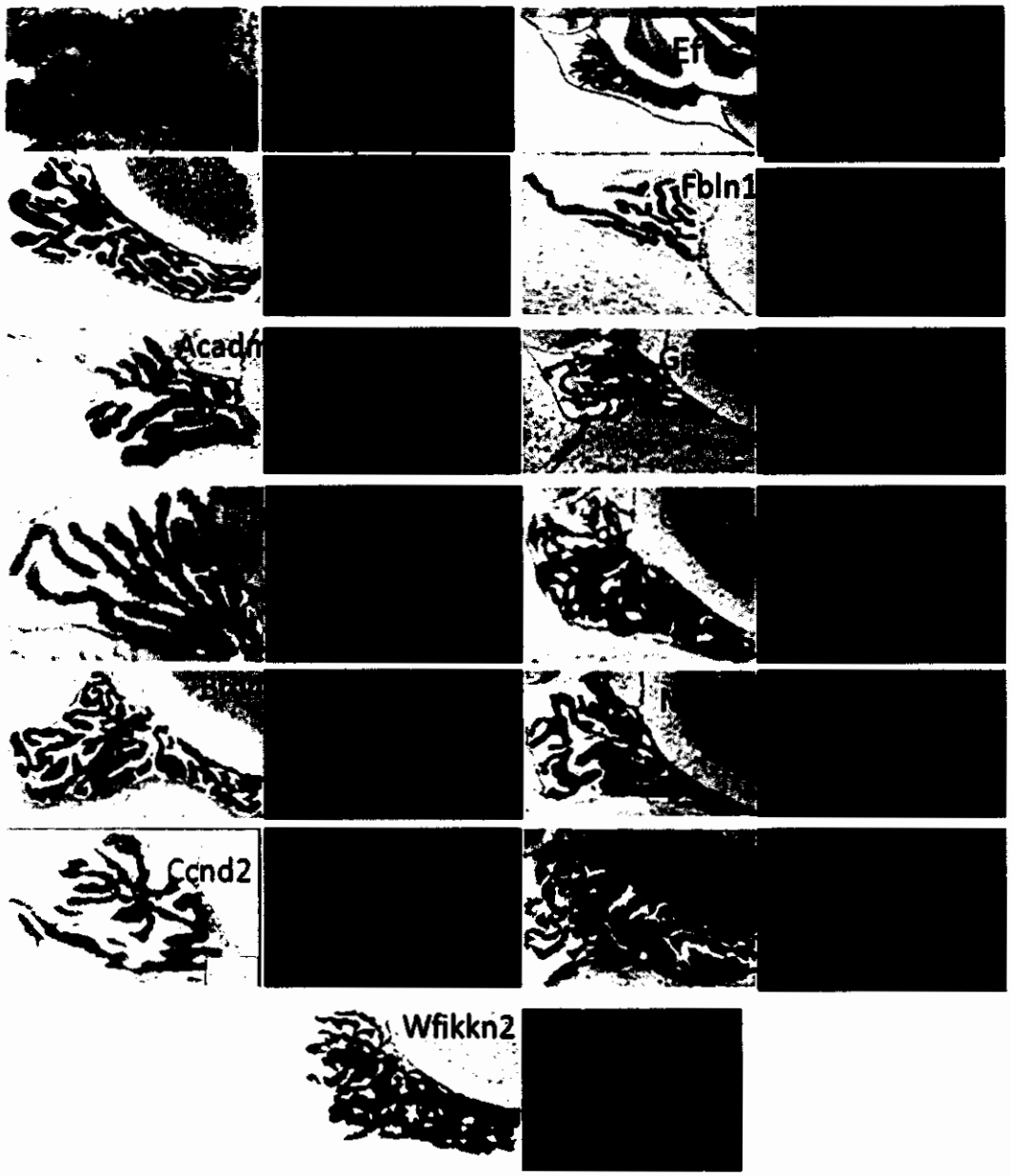


Fig 5.4 Expression images of CP specific genes in fourth ventricle of adult mouse brain.

Table 5.1 CP specific gene expression levels according to their CPs of different ventricles.

Sr.No	Gene ID	Lateral ventricle	Third ventricle	Fourth ventricle
1	Ttr			
2	Acaa2			
3	Brd4			
4	Acadm			
5	Ccnd2			
6	Cfh			
7	Efhc1			
8	Fbln1			
9	Gpihbp1			
10	Gzma			
11	Mmp2			
12	Brrn1			
13	Wfikkn2			

5.2 Non-CP specific genes

Now come to the second group that contains non-CP specific genes. These genes were mainly expressed in the CPs as compared with the other regions of the brain. Gene expression level in other regions of the brain is low or negligible in some cases. While in some cases the expression level is not low or negligible; but exceptions also exist there and may be those genes are housekeeping genes.

Enpp2, Ctsd , Igfbp2, Cst3, Uqcrc1, Timp2, Hdlbp, Slc13a4, Acox1, Aco2, Itpr1 and Spint2 genes have dense expression pattern in all the CPs (Fig 5.5). Apoe and Srpk1 also showed dense pattern of gene expression but not in all ventricular CPs. In case of Sparc, Vcam1 and Gsn genes lateral and third ventricle CPs show dense pattern of expression level while fourth ventricle CP showed scattered pattern. Apoe and Srpk1 genes have dense pattern of expression level only in third ventricle CP while lateral and fourth ventricle CPs have scattered pattern of gene expression.

Sntb1, Gja7, Efemp2, Serpinf1, B2m, Maob, DCN, Igfbp5, Col1a1, Col3a1, Col5a2, Fn1, Fbln2, Cp, Efemp1, Cyb561d2, Gja4, Thbs1, Svep1 and Pomc1 genes have scattered pattern of expression in all the CPs (Fig 5.6). In case of Gpm6b gene third ventricle CP has dense pattern while other two CPs have scattered pattern. Expression pattern of Sparc, Gsn, Vcam1, Srpk1, Apoe and Gpm6b genes that vary among their own CPs is shown in fig 5.7. Enpp2, Uqcrc1, Sparc, Apoe, Cp, Ctsd, Igfbp2, Cst3, Timp2, Spint2, and Srpk1, genes have high expression level in all CPs. Enpp2, Apoe, Ctsd, Igfbp2, Cst3 and Timp2 genes have their expression in the ventricular ependymal cells

but remaining genes did not show. Sntb1, Gja7, Efemp2, Serpinf1, B2m, Aco2, Maob and Itpr1 genes have medium level of expression in all the CPs and they do not have their expression in the ventricular ependymal cells. Detail of the expression level in all the CPs of the non-CP specific genes is given in Table 5.2.

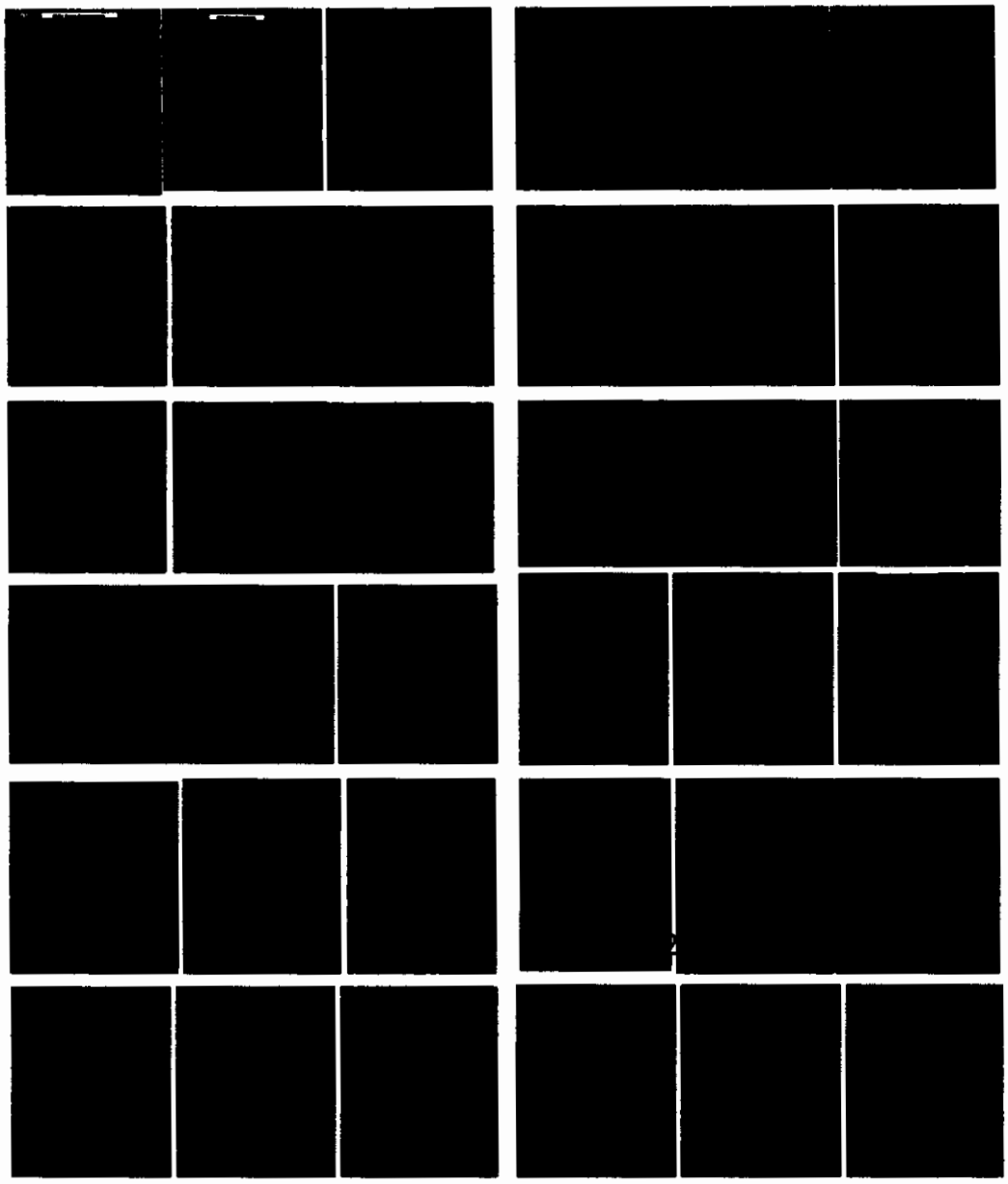


Fig 5.5 Expression images with dense pattern of non-CP specific genes in lateral, third and fourth ventricles respectively.

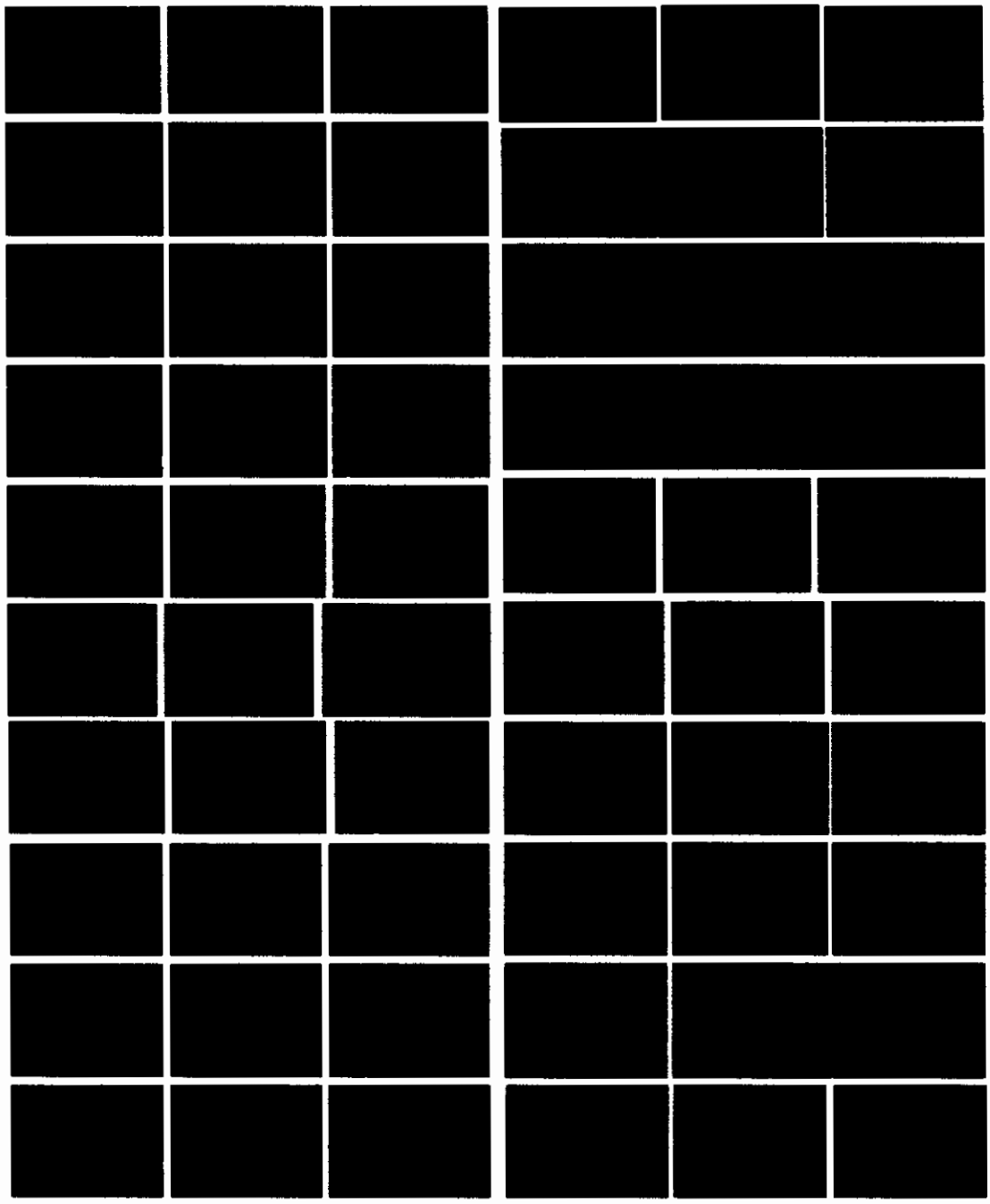


Fig 5.6 Expression images with scattered pattern of non-CP specific genes in lateral, third and fourth ventricles respectively.

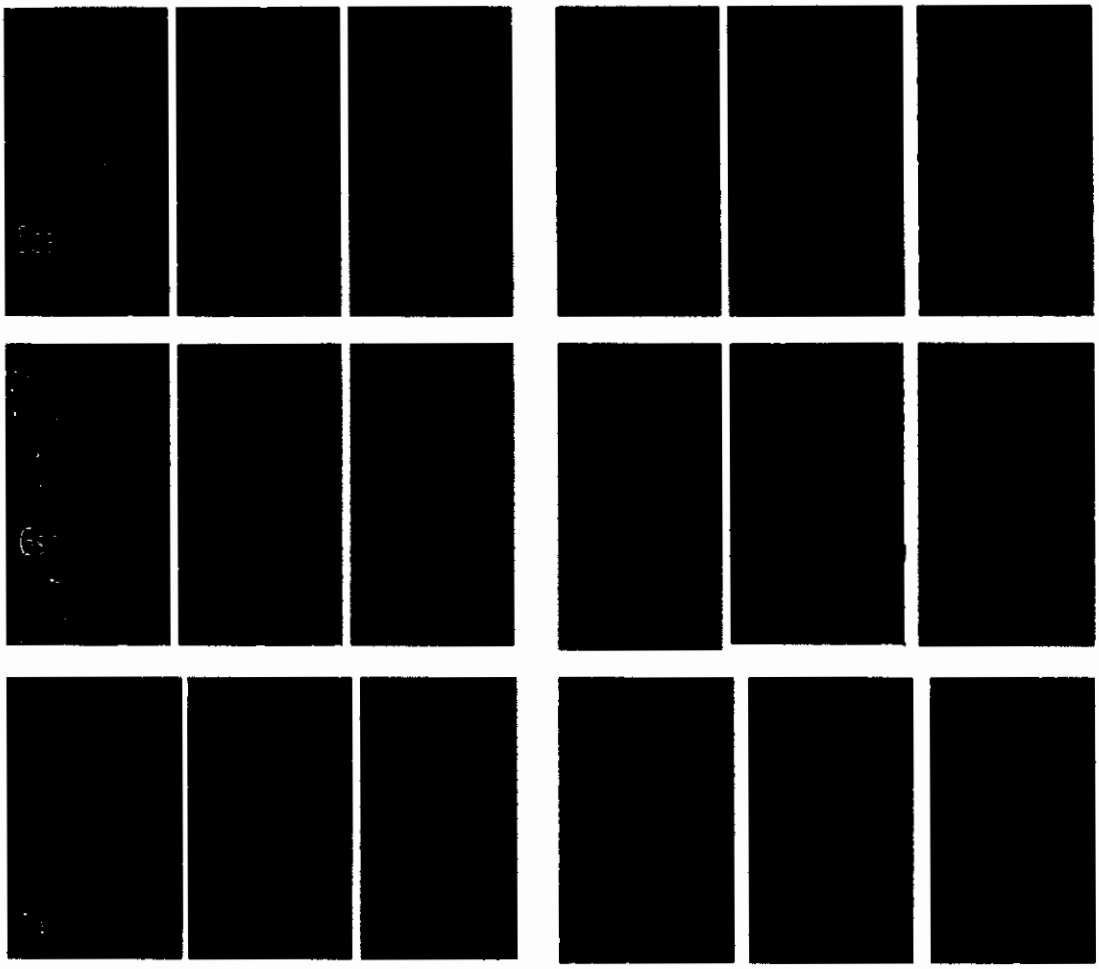
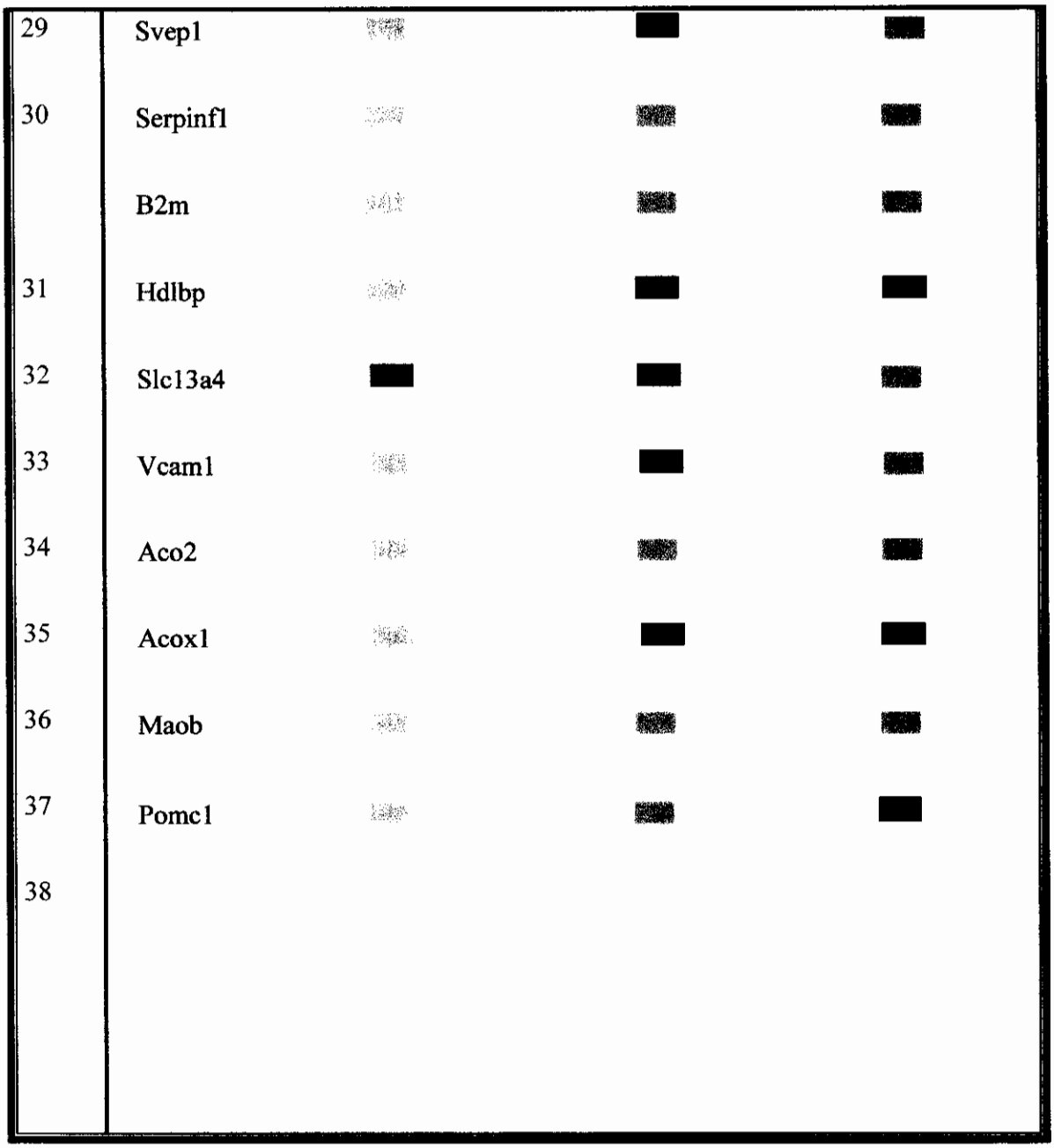


Fig 5.7 Expression images of non-CP specific genes that contain both dense and scattered pattern in lateral, third and fourth ventricles respectively.

Table 5.2 Non-CP specific gene expression levels according to their CPs of different ventricles.

Sr. No	Gene ID	Lateral Ventricle	Third Ventricle	Fourth Ventricle
1	Enpp2			
2	DCN			
3	Igfbp5			
4	Uqcrc1			
5	Itpr1			
6	SPARC			
7	Gja7			
8	Gsn			
9	Ctsd			
10	Igfbp2			
11	Gpm6b			
12	Apoe			

13	Cp			
14	Col1a1			
15	Col3a1			
16	Col5a2			
17	Fn1			
18	Fbln2			
19	Efemp1			
20	Efemp2			
21	Cyb561d2	----		
22	Gja4			
23	Thbs1			
24	Sntb1			
25	Cst3			
26	Timp2			
27	Spint2			
28	Srpk1			


29
Svep1
30
Serpinf1
B2m
31
Hdlbp
32
Slc13a4
33
Vcam1
34
Aco2
35
Acox1
36
Maob
37
Pomc1
38

According to evaluation criteria; the proteins or factors encoded by Ttr, Enpp2, Apoe, Ctsd, Igfbp2, Cst3 and Timp2 genes are secreted in large quantity from CPs into CSF that transfer them to the required regions of the brain. These genes not only have high expression level in CPs but also have expression in ventricular ependymal cells indicating that these genes play many important functions in the brain. Functions of these genes are also declared in the literature.

Here one thing is important and interesting that all CP genes either CP specific or non-Cp specific genes also contain gene expression in any part of cerebrum especially in the olfactory region. Because cerebrum is the main area of the brain for all variety of tasks like learning, memory, personality, behavior, emotions, judgment, planning, problem solving, speech: speaking and writing, body movement (motor strip), intelligence, concentration, self awareness etc. In case of non-CP specificgenes; the genes which were highly expressed in CP were also highly expressed in the cerebrum while genes which were less or weakly expressed in CP, they were also weakly expressed in the cerebrum. While in case of CP specific genes expression level is low in cerebrum or in some cases even negligible. It means these genes mainly interact between choroid plexus and cerebrum regions through CSF. The transport of such molecules may be in the signal form from CP to cerebrum regions or to the other parts of the brain through CSF. Both these CP specific and non-CP specific genes are also involved in many CNS disorders. Many diseases related to these genes have been reported in the literature.

Among these 51 genes; some genes belong to major protein categories (Table 5.3). These major categories are Cell-matrix proteins, Carrier proteins, Proteases and

Protease inhibitors. These categories are interlinked to play different roles in the CNS system. In some case they play very useful roles to stop CNS disorders but in some cases when mutations occur or some damage disturb the functioning of one category that may disturb the functioning of remaining categories also. These changes cause severe mal functioning and cause the dangerous neurodegenerative diseases and memory related disorders, especially the Alzheimer' s disease.

Table 5.3 Major protein categories.

Cell-matrix proteins	Carrier proteins	Proteases	Protease inhibitors
Efemp1	Ttr	Mmp 2	Timp2
Efemp2	APOE	Ctsd	Cfh
Fn1	IGFBP-2		Cst3
Col1a1	Igfbp5		Spint2
Col5a2	Cp		
Col3a1			
Fbln1			
Fbln2			

5.3 GEDAMCP

GEDAMCP (Gene Expression Data Analysis of Adult Mouse Choroid Plexus) is user friendly software that contains CP gene information. This information includes the gene lists that are expressed in the choroid plexus having their spatial expression images and introductory reports. User/researcher can easily find the required information from the home page of the GEDAMCP (Fig 5.8). Home page of the GEDAMCP contains the links for the detail of this CP project in the form of background, introduction, sources and FAQs hyperlinks. There is a dropdown list to choose the further information either about the all CP genes (Fig 5.8) or about the spatial gene expression data (Fig 5.9).

Spatial gene expression data page contains three links by using them user/researcher select the list of genes according to their expression levels with respect to their CPs like lists of genes with high expression level, with medium expression level and with low expression level in all the CPs. User/researcher can easily choose the option which he/she requires.

All genes page has list of all the CP genes present in the GEDAMCP with their introductory reports and two options either to select the list of CP specific genes or the list of non-CP specific genes (Fig 5.10). CP specific page has list of CP specific genes with their spatial images and their reports (Fig 5.11). Spatial images not only contain the gene expression images from the whole brain of adult mouse brain but also CP images according to its three locations. Non-CP specific page contains the list of non-CP specific genes with their spatial expression images and their introductory reports (Fig 5.12).

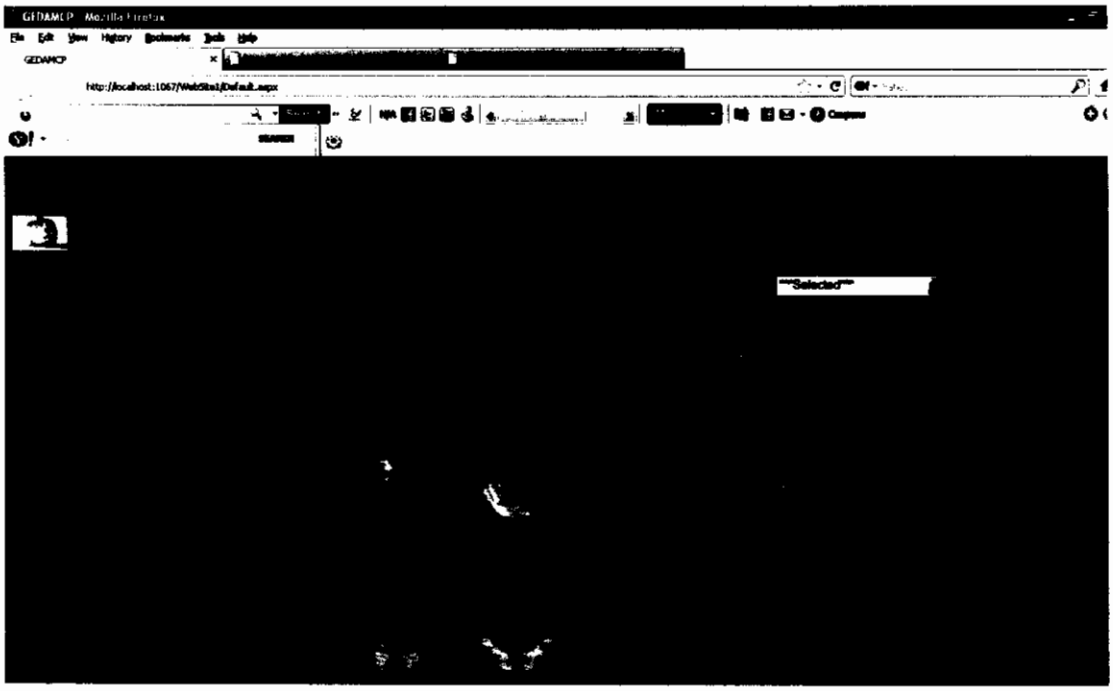


Fig 5.8 Home page of GEDAMCP.

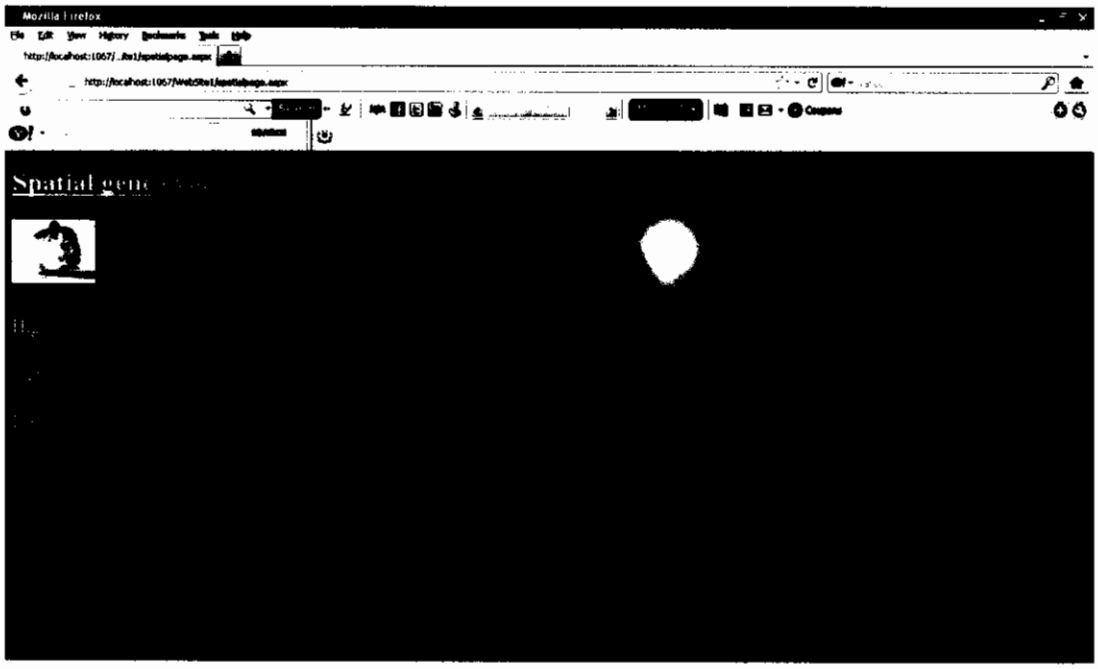


Fig 5.9 Spatial gene expression data page of GEDAMCP.

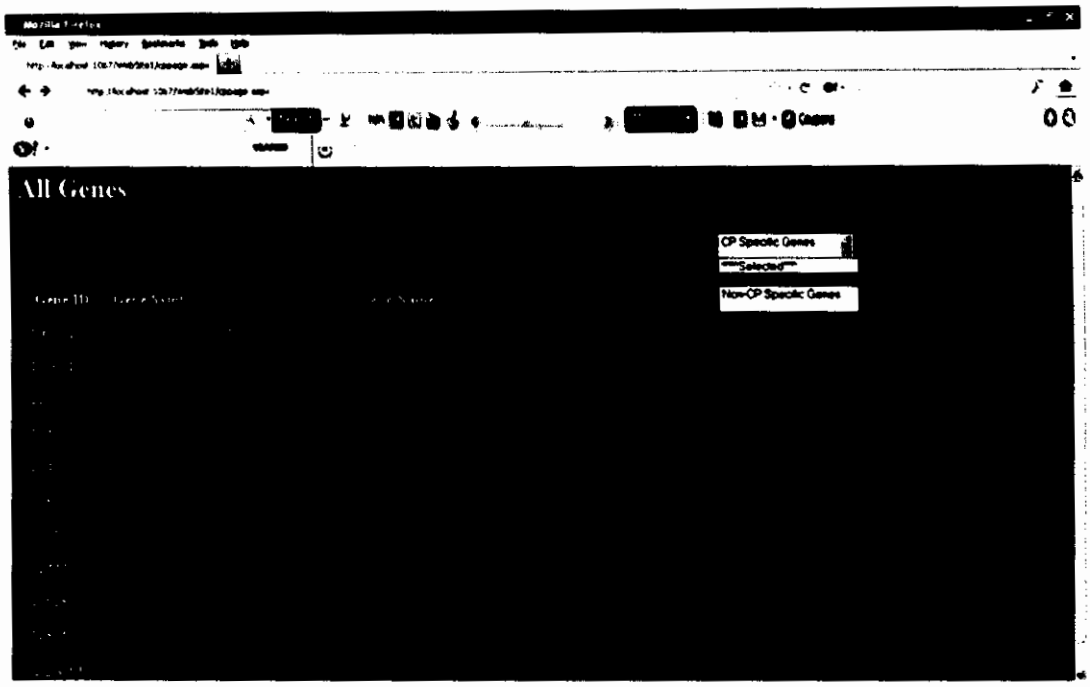


Fig 5.10 All genes page of GEDAMCP.

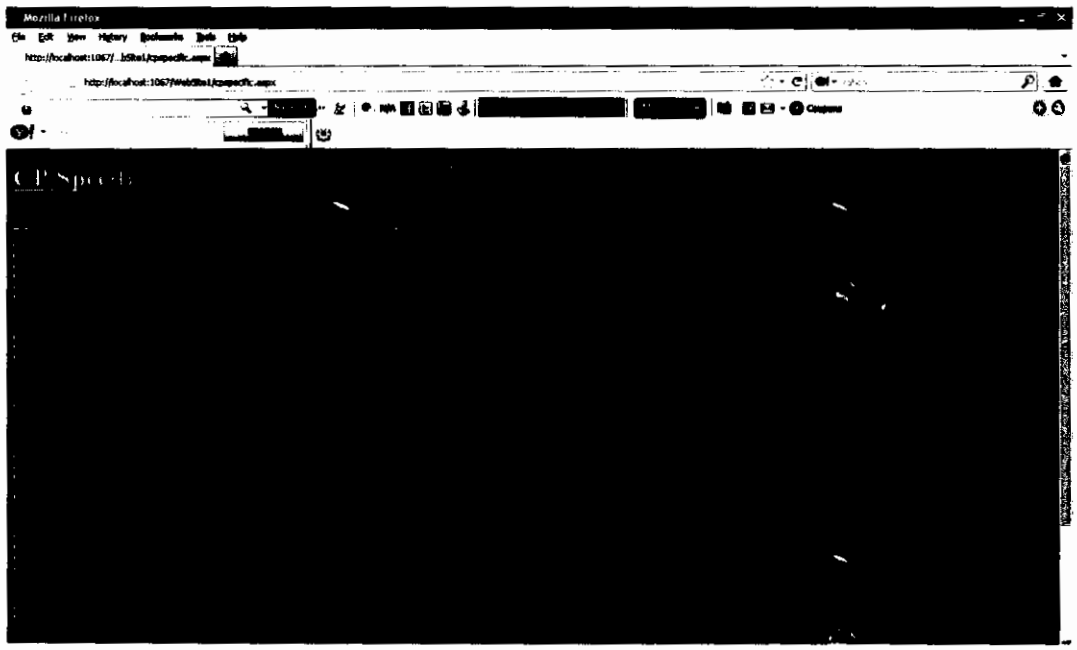


Fig 5.11 CP specific page of GEDAMCP.

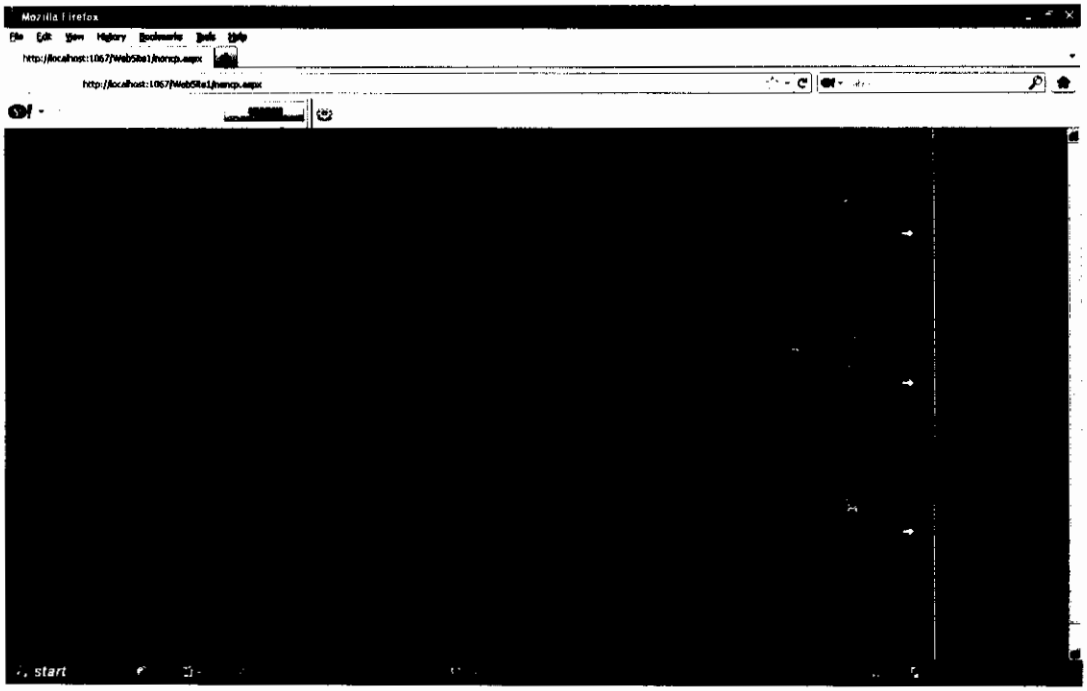


Fig 5.12 Non-CP specific page of GEDAMCP.

CONCLUSION AND FUTURE WORK

The present work reveals that the CP is a site of active expression of several significant molecules. It produces a vast number of important secretory proteins/molecules at its three locations that perform novel functions for the brain homeostatis. Those genes have high expression level in the CP secrete proteins/factors in large quantity into the CSF to perform many important functions in the brain. As this study proves the presence of gene expression in cerebrum region either CP specific or non-CP specific; confirms the CSF proteins/molecules relation with Alzheimer disease as previous evidences are present in the literature. There is need to more work on the CP project to understand the involvement of CP in various physiological and disease states. That work may provide novel clues for disease mechanisms and unravel novel targets for therapies against diseases of the central nervous system.

For the further implications in the CP project, wet experiments are required for these genes to know the answers of the following unknown mysteries.

- Why some genes are not expressed in all CPs locations either in one or two locations?
- Can we determine the signaling pathway of a key molecule within the adult mouse brain?
- Can we check the complete pathway of CP proteins from CP to the different regions of the brain through CSF?

- Is it possible to distinguish CSF-specific proteins from those produced by the brain?
- What is the source of control molecules in the adult mouse brain?
- If CP genes/proteins are also produced in other regions of the brains; what's their locations and targets?

GEDAMCP (Gene Expression Data Analysis of Adult Mouse Choroid Plexus) provides information about the CP genes including CP specific and non- CP specific genes. Anybody who takes interest in the CP genes can easily get information from this site and easily interpret the results by seeing the images. This site will open new horizons to the researchers to work forward for CP genes. Further amendments can be done in this software by making it annotated. 3D visualization of spatial expressions make easier to analyze them.

DISCUSSION

The CP is the major production area of CSF. It transmits signals into and out of the brain due to its unique architecture and localization at the interface between the blood and cerebrospinal fluid compartments. Many polypeptides and/or proteins are also synthesized by CECs and released through the vesicular secretory pathway (Chodobski *et al.*, 2001). It also plays multiple roles for the proper functioning of CNS.

In the case of CSF proteins; 51 genes encode different proteins which are secreted by the CP into the CSF and then transfer throughout the brain regions where these proteins are required. But all these proteins have different functions. Literature survey evidences also proving these results. Some proteins are already discussed in the previous work but some proteins have no such type of previous work. Secretory products of the choroidal epithelial cells into the CSF are ceruloplasmin (Thouvenot *et al.*, 2006) Ttr (Aldred *et al.*,1995), Enpps (Koike *et al.*, 2006), IGFBP5 (1. Lafon-Cazal *et al.*, 2003), MMPs (Rajaram *et al.*, 1997), Timp2 (Narita *et al.*, 1994), Cfh (Fuss *et al.*, 1997) and IGFBP-2 (Bächner *et al.*, 1999) that is main IGFBP found in the CSF binds with IGF II (Thouvenot *et al.*, 2006). Astrocytes also secrete IGFBP-2, Timp2 and ceruloplasmin in large quantity (Lafon-Cazal *et al.*, 2003). The genes that have CSF related literature are divided into four categories to discuss their functions here.

Cell-matrix protein category contains Efemp1, Efemp2, Dcn, Sparc, Fn1, Col1a1, Col5a2 Col3a1, Fbln1 and Fbln2 as non-CP specific genes. Cell matrix proteins are the

important part of the cellular environment and play vital roles by making association with growth factors and other proteins. The observed expression pattern for all these genes was scattered with different expression level in all the CPs. These expression levels were ranging from low to medium levels for all the gene except Efemp1, Dcn and Sparc genes which showed medium to high expression levels. These genes were also not expressed in all the regions of brain suggesting the limited expression. Therefore it can be said that these genes are not continuously expressing in whole brain, indicating that they are required at specific locations of the brain for specific purposes. Sparc is the only gene that differs from all the other genes of this category. It has shown high expression level with dense pattern in all the CPs and expressed in many regions of the brain. Some of the important features of cell matrix proteins are the regulation of differentiation (Adams and Watt, 1993), embryonic morphogenesis (Eddman and Crossin, 1991 and Elices *et al.*, 1991), regulation of gene expression at the transcriptional level and in cells of the adult organism (Juliano and Haskill, 1993). The genes which got matrix stimulated transcription of differentiation have been verified in the hepatocytes and mammary epithelial cells (Dipersio *et al.,* 1991). Serum albumin in hepatocytes and b-casein in mammary epithelial cells are the two genes getting activated by the response of cell matrix proteins (Adams and Watt, 1993). Therefore if the cell matrix proteins fail to fulfill their role; they may fail to activate these two genes resulting in abnormal functioning of memory cells that leading to memory disorders. Hence this category of proteins can be related to the memory functioning of the mouse brain.

Protease category includes Mmp2 as CP specific and Ctsd as non-CP specific genes. Mmp2 gene belongs to matrix metalloproteinases (MMPs) family. MMPs family contains the genes of neutral proteases that plays important roles in many biological processes e.g wound healing, pathological processes, normal development, spread of metastatic cancer cells, atherosclerosis, arthritic destruction of joints (Rosenberg, 2002); and the important roles include genesis of inflammatory demyelination, cell migration, blood–brain/nerve barrier breakdown, demyelination in many neuronal diseases, and cytokine activation (Hartung and Kieseier, 2000). When MMPs are responsible for the inflammatory mononuclear cells influx in the CNS, they contribute to the invasiveness of malignant glioma cells, myelin destruction, disrupt the integrity of the blood–brain barrier and sometimes regulate their angiogenic capacity (Yonga *et al.*, 1998). Ctsd gene has dense expression pattern with high expression level in all the CPs. This shows that the main production area of the cathepsin D is CP and transfer to the required regions of brain through CSF as Schwagerl *et al.*, (1995) have also been proved this. Proteases are also involved in many CNS diseases, tumors and especially in memory related diseases (Yonga *et al.*, 1998).

In the case of protease inhibitor category includes Cfh as CP specific, Timp2, Cst3 and Spint2 as CP non-specific genes. All these genes have dense expression pattern and high expression level in all the CPs except Cfh gene. Spatiality of this category is that all the genes are mainly produced by CP cells and also from ventricular ependymal cells. These evidences confirm the secretion of proteins in large quantity into the CSF and transfer to different regions of the brain. As systatin C (encoded by Cst3) synthesized

and secreted at high levels only after birth and taken as an original protein of CSF (Mannes *et al.*, 2003). Secretion of cystatin C concentration becomes increased in the CSF during pain states (Mannes *et al.*, 2003). Serine proteases including their related inhibitors (Spint2) quantity becomes higher in neural parenchyma and cerebrospinal fluid when injuries are occurred in the blood brain barrier (Turgeon and Houenou, 1997). Only the Cfh differs from the other genes of this category; having medium expression level with scattered pattern in all the CPs. Cfh encodes the complement component factor h that is produced by the epithelial cells of the CP and secreted into CSF through the vesicular pathway regulates the immune system of the body (Alexander and Quigg, 2007 and Thouvenot *et al.*, 2006). These proteins and factors are also involved in many neurodegenerative disorders of CNS.

Carrier protein category includes Ttr as CP specific Apoe, Igfbp-2, Igfbp5 and Cp as non-CP specific genes. All these genes have high expression level in all the CPs. So, the main production area for these genes is Cp. One thing more interesting all these genes are not only produced by the CP cells but also from ventricular ependymal cells. So, it is clear that the proteins of these genes are screted into the CSF and transfer to the required regions of the brain. The proteins encoded by these genes play many vital roles in the CSF. Transthyretin (Ttr) is a main thyroid hormone-binding protein in CSF (Hagen and Solberg, 1974) and T4 transfer from the blood into the brain across the blood-choroid-plexus-CSF barrier (Dickson *et al.*, 1987 and Southwell *et al.*, 1993). It is about 25% of the total protein synthesized by the choroid plexus which is secreted into the CSF (Aldred *et al.*, 1995).While the concentration of Apoe in CSF is one-tenth to one-twentieth of the

serum apoE concentration (Pirttilä *et al.,* 1996; Rösler *et al.,* 1996 and Hahne *et al,.* 1997). Because blood-brain barrier maintains the apolipoprotein E concentration lowers in cerebrospinal fluid (CSF) as compared with serum (Egleton and Davis, 1997 and Reiber, 1995). IGFBPs of the carrier proteins present in the CSF are produced by glial cells and neurons not from the plasma by crossing the blood-brain barrier (Ocrant *et al.*, 1990). IGFBPs also make easy the IGF-II secretion in the CSF by modulating its biological action at distant sites within the brain (Tseng *et al.*, 1989).

Although the genes of the above categories perform different functions but they are also involved in many CNS disorders and are interlinked to play different roles in the CNS system. In some cases they play very useful roles to stop CNS disorders but in some cases when mutations occur or some damages disturb the functioning of one category, leading to disturb the functioning of remaining categories also. These changes cause severe mall functioning and causing the dangerous neurodegenerative diseases and memory related disorders, especially the Alzheimer disease. Cell matrix proteins and proteases are involved in Alzheimer disease (Narindrasorasak *et al.*, 1995 and Yonga *et al.*, 1998). Transthyretin (Schwagerl *et al.*, 1995), cathepsin D (Rösler *et al.*, 1996), Apoe (Lefranc *et al.*, 1996), are used as biological markers for Alzheimer disease (Hahne *et al.*, 1997 and Thouvenot *et al.*, 2006).

By looking at the results of this study one thing is interesting that those genes contain high expression level in the CPs are mainly involved in Alzheimer disease. Main features of Alzheimer disease are memory failure, personality changes, problems carrying out daily activities, affects learning etc. All these functions are normally controlled by

cerebrum. Now it is proved that CSF levels significantly increase in Alzheimer's disease (Vandermeeren *et al.*, 1993). Proteins associated with CP genes performed different functions in the brain and many of these proteins are secreted by CSF into other regions of brain e.g Carrier protein (related to Ttr gene), Glycoprotein (related to Sparc gene) etc. If the functioning or concentration of these proteins becomes wrong they can also become a cause of Alzheimer disease e.g Apolipoprotein E (Hahne *et al.*, 1997 and Lefranc *et al.*, 1996). Main cause of the Alzheimer disease is failing of brain cells functioning especially in cerebrum and cells lose their ability to do their jobs and, eventually die, causing irreversible changes in the brain. Plaques and Tangles play major roles in the damaging and killing of nerve cells. Plaquesis also called as $A\beta_{42}$ amyloid that are deposits of a protein fragment, build up in the spaces present between the nerve cells. CP proteins are involved to prevent the functiong of $A\beta_{42}$ amyloid. As in case of Ttr protein, it performs a neuroprotective role in Alzheimer disease by preventing $A\beta_{42}$ aggregation (Schwarzman *et al.*, 1994). While tangles build up inside the cells and are twisted fibers of another protein. Tangles are also called as tau. CSF $A\beta_{42}$ levels were found to be significantly lower in AD patients (Motter *et al.*, 1995). CSF tau and CSF $A\beta_{42}$ are used as diagnostic markers for Alzheimer disease (Andreasen *et al.*, 2001).

MMPs break down the extracellular matrix (ECM) to allow the cell growth and facilitate the remodeling. If the normal balance between the proteases and their inhibitors, tissue inhibitors to metalloproteinases (TIMPs) is vanished, proteolysis becomes pathological that causes the neurological disorders e.g multiple sclerosis, Alzheimer's disease and brain tumors leading to the matrix-degrading proteases (Lukes *et al.*, 1999).

Cell matrix proteins start remodeling (fibrosis of blood vessels and gliosis) to cope with the proteolytic injury. In case of acute injury Timps starts to increase and in some cases they may add fibrotic buildup of extracellular matrix complex components (Lukes *et al.*, 1999). Proteases mediate the deposition of amyloid β-proteins in Alzheimer disease (Yonga *et al.* 1998). Basement membrane type cell matrix proteins are found to concern with the genesis of amyloids (Narindrasorasak *et al.*, 1995). Sometimes during the Alzheimer disease, binding of extracellular matrix proteins and beta PP (Alzheimer amyloid precursor proteins) may become a pathologic part of the amyloidogenic process (Narindrasorasak *et al.*, 1995). Research work proved that amyloid forms, collagen, and fibronectin are also involved in Alzheimer deposits (Narindrasorasak *et al.*, 1995).

In the present study, it has been found that almost all CP genes are expressed in the cerebrum region. Because cerebrum is the main area of brain for all types of works like learning, memory, Personality, behavior, emotions, Judgment, planning, problem solving, Speech: speaking and writing, Body movement (motor strip), Intelligence, concentration, self awareness etc. So these results show a strong relationship among CSF proteins and Alzheimer disease. Although scientists don't know from where the trouble starts but now it is proved that Alzheimer disease is mainly related by CSF proteins. One thing which is more interesting is that gene expression level is different for the adult and developing mouse brains. For example, in the case of Ttr gene expression only CP cells contain the gene expression in developing mouse brain while in case of adult mouse brain although CP cells have its expression but ventricular ependymal cells also show the gene expression. Some genes which are CP specific in developing mouse brain are not Cp

specific in the adult mouse brain e.g Ace gene and same case in the adult mouse brain e.g Cfh, Dcn, Acaa2 genes etc.

Software application GEDAMCP was developed to provide the ease for researchers/users that interested in the choroid plexus project. This software covers the main needs and tasks for the choroid plexus project like spatial gene expression data. Spatial gene expression data covers the gene expression pattern, expression level i-e high, medium and low and analysis of gene expression at all ventricular choroid plexus. Lack of this information has been creating difficulty for researchers/users working on choroid plexus projects. To fulfill these needs, this web linked database software application was developed.

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