Data Analysis of Spatial and Temporal Gene Expression in Developing Mouse Choroid Plexus

By

Fatima Subhani Khan

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Supervisor

Dr. Sumbul Khalid
Assistant Professor, DES.
International Islamic University
Islamabad

Co-Supervisor

Prof. Dr. Shahid Nadeem Chohan
Foreign Professor, DBS.
COMSATS Institute of Information Technology, Islamabad

Department of Environmental Sciences
Faculty of Basic and Applied Sciences
International Islamic University
Islamabad

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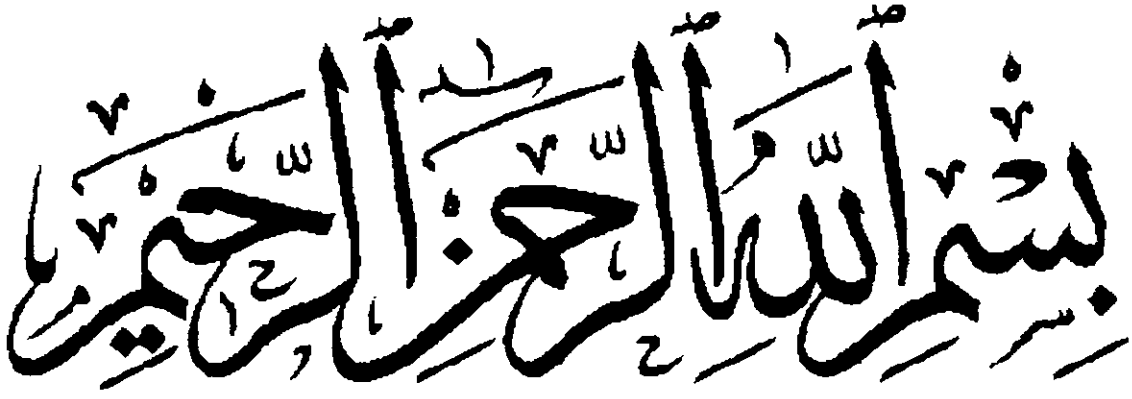

In the name of Allah Most Gracious and Most Beneficial

Department of Environmental Sciences

International Islamic University Islamabad

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FINAL APPROVAL

It is certified that we have read the thesis submitted by Ms. Fatima Subhani Khan and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the MS Degree in Bioinformatics

COMMITTEE

External Examiner

Dr. Muhammad Zeeshan Hyder
Assistant Professor
Department of Bio Science,
COMSATS Institute of Information technology,
Islamabad.

Internal Examiner

Dr. Sobia Tabbassum
Assistant professor
Department of Environmental Sciences,
International Islamic University, Islamabad.

Supervisor

Dr. Sumbul Khalid
Assistant professor
Department of Environmental Sciences,
International Islamic University, Islamabad.

Co-Supervisor

Dr. Shahid Nadeem Chohan
Foreign Professor
Department of Bio Science,
COMSATS Institute of Information technology,
Islamabad.

Shahid Chohan

Dean, FBAS

Prof. Dr. Muhammad Irfan Khan
International Islamic University, Islamabad.

A thesis submitted to Department of Environmental Sciences,
International Islamic University, Islamabad as a partial
fulfillment of requirement for the award of the
degree of MS Bioinformatics.

DECLARATION

I hereby declare that the work present in the following thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

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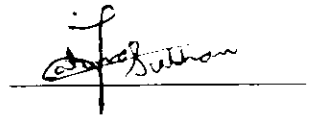

Fatima Subhani Khan.

DEDICATION

I dedicate this thesis to my parents. There is no doubt in my mind that without their continued support and counsel I could not have completed this research.

CONTENTS

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In the name of Allah, The most gracious and The merciful, who created me from a clot of blood, brought me up as a human being, bestowed me with a mind of understanding, taught me which I didn't know and chosen me for this research.

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I pray to Allah swt that may He bestow me with true success in all fields in both worlds and shower His blessed knowledge upon me for the betterment of Muslim Ummah and whole Mankind. Ameen.

Fatima Subhani Khan

LIST OF ABBREVIATIONS

A2m	Alpha-2-macroglobulin
ABA	Allen Brain Atlas
Ace	Angiotensin 1 converting enzyme
AGEA	Anatomic gene expression atlas
Aldh-2	Aldehyde dehydrogenase 2
Anxa11	Annexin A11
ApoE	Apolipoprotein E
Car2	Carbonic anhydrase 2
Clu	Clusterin
CP	Choroid plexus
CSF	Cerebrospinal fluid
E 11.5	Embryonic day 11.5
E 12.5	Embryonic day 12.5
E 13.5	Embryonic day 13.5

E 14.5	Embryonic day 14.5
E 15.5	Embryonic day 15.5
E 16.5	Embryonic day 16.5
E 17.5	Embryonic day 17.5
E 18.5	Embryonic day 18.5
E-CSF	Embryonic cerebrospinal fluid
Epas1	Endothelial PAS domain protein 1
Fgfr2	Fibroblast growth factor receptor 2
GEDDMCP	Gene Expression data of Developing Mouse Choroid Plexus
Hsbp8	Heat shock protein beta-8
Htr2C	5-hydroxytryptamine (serotonin) receptor 2C
IGF-2	Insulin like growth factor 2
Igfbps	Insulin like growth factor binding proteins
Igfbp-2	Insulin like growth factor binding protein 2
Igfbp-3	Insulin like growth factor binding protein 3
Igfbp-4	Insulin like growth factor binding protein 4

Igfbp-7	Insulin like growth factor binding protein 7
ISH	*In situ* hybridization
Itpr1	Inositol 1,4,5-trisphosphate receptor, type 1
NCBI	National Centre for Biotechnology Information
TCh	Telencephallic choroidal tissues
Tgfβ2	Transforming growth factor beta-2
Ttr	Transthyretin
P 1	Postnatal day 1
P 2	Postnatal day 2
P2Ch	Choroidal tissues of prosomere 2
P3Ch	Choroidal tissues of prosomere 3
P 4	Postnatal day 4
P 7	Postnatal day 7
P 14	Postnatal day 14
P 28	Postnatal day 28
PDB	Protein Data Bank

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ABSTRACT

Choroid plexus (CP) is a secretary epithelium inside the ventricular system of brain that serves to produce and secrete important factors into cerebrospinal fluid (CSF) and the fluid itself. CSF then acts as a transportation medium to deliver these factors to other parts of the brain. Throughout the development of brain, CP of all the three ventricles continues to secrete factors into CSF since very early stages of embryonic development. However, it is thought that all the three CPs contribute differentially, temporally and spatially, for the production and secretion of important factors required by the developing brain. This behaviour of the three CPs can be interpreted by means of comparative gene expression analysis of the CP genes. In this research, the gene expression data for developing brain of mouse (*Mus musculus*) has been analyzed for a total of 20 CP genes throughout four embryonic time points (E11.5, 13.5, E15.5 and E18.5) and three postnatal time points (P4, P14 and P 28). Whole brain gene expression data available in the database of Allen Institute of brain sciences were scanned. All the three CPs were then located individually from this extracted data, analyzed temporally and fed into a database created specifically for CP. The analysis revealed that the gene expression level and pattern of every CP gene is unique and there is some sort of differential switching on and off of different CP genes temporally and spatially. The findings of this study suggest that CP starts to produce some proteins at very early embryonic age even before E 15.5 when the CP is not even fully developed and it is in the form of choroidal tissues which secrets these factors into Embryonic CSF (ECSF). It has also been found that most of

these CP genes are produced by the ependymal cells that line the ventricles and thus they can be thought of contributing in the secretion of extra choroidal CSF. Furthermore, almost all of these CP genes have shown some relation with Alzheimer's disease. This study has resulted in the development of a user friendly tool named, "Gene Expression Data of Developing Mouse Choroid Plexus" which will facilitate future researchers to work in this area.

CHAPTER 1

INTRODUCTION

Inside the mammalian brain there is a system of fluid-filled cavities called ventricles. There are three ventricles,

(i) Two lateral ventricles,

(ii) the third ventricle and

(iii) the fourth ventricle.

The choroid plexus (CP) is a secretary epithelium inside these ventricles that serves to produce and secrete cerebrospinal fluid (CSF) (Speake *et al.*, 2001 and Fang *et al.*, 2009) and also regulate intraventricular pressure. Once secreted from choroid plexus, CSF leaves the ventricles and circulates around the outside of the brain before draining through the venous and lymphatic systems (Pollay, 2010). 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 little further contributed by the CP of the third and fourth ventricles and around 25% is contributed by extracellular fluid (ECF) across the ependymal layer (Veening, 2010). In the developing mouse brain, the amount of the protein content is high from that in the adult brain, in which the fluid has extremely low protein levels. The same is the case following trauma or degenerative changes to the brain. This concludes that the CSF is a vital fluid for the development of the brain and for

the normal function of the brain (Miyan *et al.*, 2003). During development the CSF can be used as a growth medium in which to grow neuronal progenitor cells without the addition of any other component (Lehtinen *et al.*, 2011). In order to provide evidence for one or other of these ideas it is necessary to identify the source of proteins found in the CSF throughout the brain development and following trauma or degenerative changes to the brain. One way to identify it is to interpret the gene expression data of different factors produced and secreted by CP into the CSF during different developmental stages.

1.1 Anatomy of brain and CSF system:

Anatomy of a vertebrate brain as described by Standring, 2008, has three major parts, the **forebrain**, the **midbrain**, and the **hindbrain.** Forebrain is called **Prosencephalon** and is subdivided into **Telencephalon** and **Diencephalon**, midbrain is called **Mesencephalon** and hind brain is **Rhombencephalon**. In adult brain, Telencephalon contains cerebral hemispheres, basal ganglia and hippocampus. Diencephalon contains thalamus, hypothalamus, pineal body and infundubulum. Mesencephalon contains tectum, tegmentum and cerebral peduncles (crus cerebri). Rhombencephalon contains pones, cerebellum and medulla oblongata (Fig 1.1).

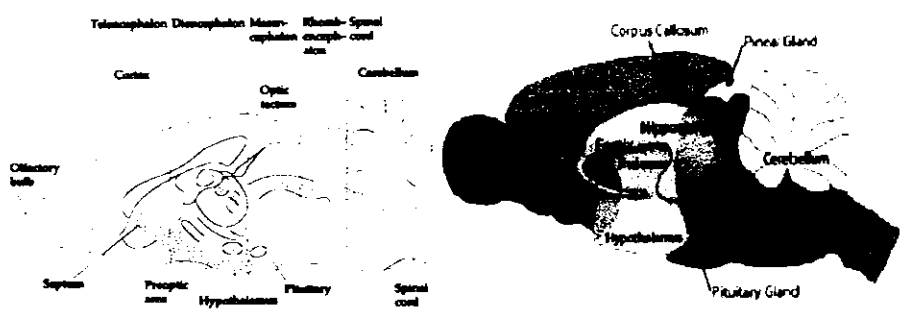


Fig 1.1: The mouse brain.

Ventricular system of the brain consists of three ventricles which are the two **lateral ventricles,** the **third ventricle** and the **fourth ventricle** respectively (Kalat, 2009). Lateral ventricles are in a form of C-shaped pair, one in each cerebral hemisphere. They both communicate via interventricular foramina (the opening that transmit nerve) with the third ventricle which is located between the two hemispheres and is slit like, surrounded by diencephalon. The third ventricle communicates via the cerebral aqueduct, to a triangular fourth ventricle. The forth ventricle is found within the hindbrain in the centre of the medulla, between the cerebellum and the brainstem (Fig 1.2). Specialized structure called choroid plexus is present in all of these ventricles.

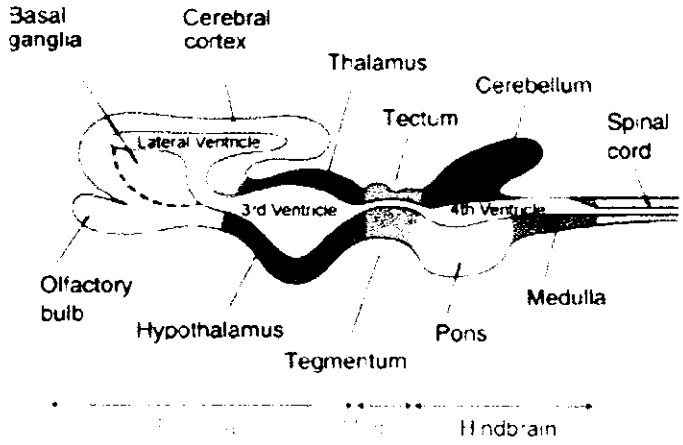


Fig 1.2: Basic Plane of the vertebrate Brain.

The brain is surrounded by protective layers called **meninges.** They are in a form of three continuous layers of connective tissues which are called dura mater, the arachnoid and the pia matter. The region between arachnoid and pia matter is called sub arachnoid space and is filled with cerebrospinal fluid (CSF) which is largely produced by

Choroid plexus present in ventricular system. Pores from the fourth ventricle open into the subarachnoid space that surrounds the brain, permitting cerebrospinal fluid (CSF) produced in the ventricles to surround the brainstem, cerebellum, and cerebral cortex, while some of it flows into the central canal of the spinal cord. In dura mater there is superior sagittal sinus (dural sinus) which is filled with venous blood. Arachnoid granulations (arachnoid villi) are present in the sagittal sinus which aids the absorption of CSF from subarachnoid space into the venous blood (Fig 1.3).

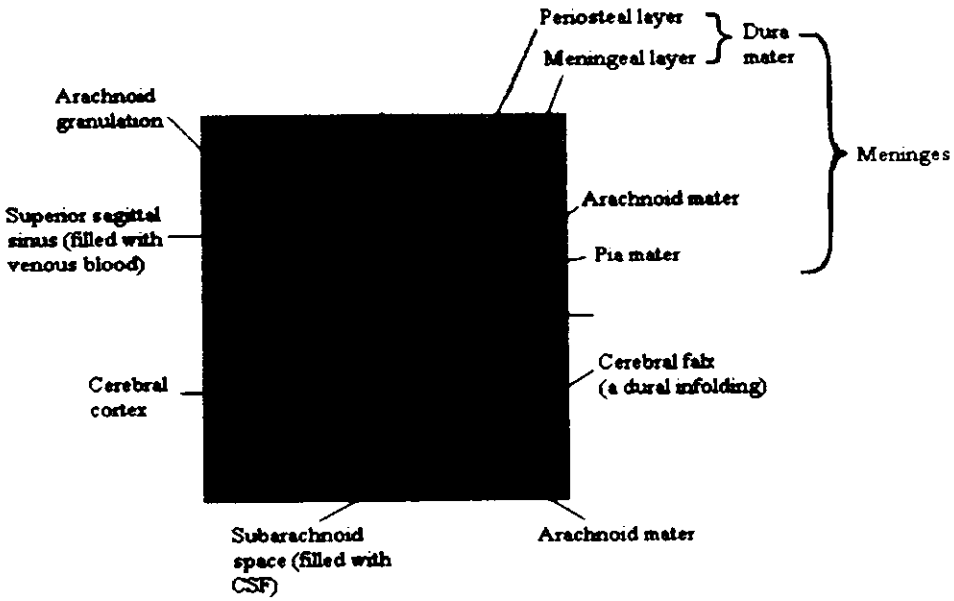


Fig 1.3: Meninges of the brain and CSF filling.

1.2 Choroid plexus composition and functions:

Choroid plexus is a vascular tissue which is present in all components of the ventricular system except for the occipital and frontal horns of the lateral ventricles and the cerebral aqueduct. The choroid plexus of lateral and third ventricle is a continuous

structure whereas the choroid plexus of fourth ventricle is a separate structure which is not physically connected to the CP of lateral and third ventricle (Siegel *et al.*, 2010).

The functional unit of choroid plexus, as described by Ranganathan, 2010, is comprised of a rich capillary bed which has fenestration (openings), pia mater and choroid epithelial cells (Fig 1.4).

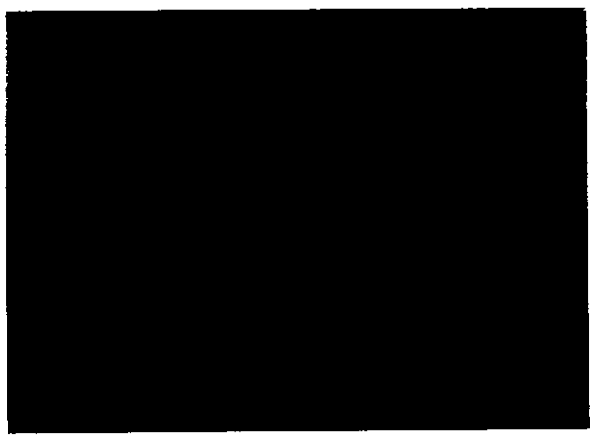


Fig 1.4: The arrangement of tissues forming the choroid plexus.

Capillaries inside the choroid plexus are highly specialized for their function and they are different from those in the brain because there is a free movement of molecules across the endothelial cell through fenestrations and intercellular gaps. Microvilli are present at the CSF facing surfacing of the choroidal epithelial cells which greatly increase the surface area of the membrane and may aid in fluid secretion (Siegel *et al.*, 1999).

The choroidal epithelial layer is continuous with the ependymal cell layer that lines the ventricles but it differ essentially from the ependymal layer despite of the

common embryological origin from the neuroepithelial cells that line the internal surface of the neural tube (Netsky *et al.*, 1975).

At the choroid plexus epithelial layer there are tight junctions between the cells which give rise to **blood-CSF barrier** It secretes cerebrospinal fluid (CSF), synthesizes numerous molecules, carries nutrients from the blood to CSF and participate in brain immunosurveillance (Emerich *et al.*, 2004 and Chodobska *et al.*, 2007).

1.2.1 Production of CSF:

As the first step of CSF production, the plasma is filtered across permeable and fenestrated choroidal capillaries. Then the coordinated transport of ions (Na^+, K^+, Cl^-, HCO_3^-) and water across the epithelial interface from basolateral membrane (blood-facing membrane) to cytoplasm and across the apical membrane (CSF-facing membrane) aids in the secretion of CSF across the ependymal cell layer of the choroid plexus epithelium into the respective ventricle (Brown *et al.*, 2004).

1.2.2 Other Functions:

Apart from secretion of CSF, it regulates the concentration of molecules inside CSF by synthesizing and secreting into CSF numerous factors including proteins and growth factors which are needed by the brain for its proper development in growth and differentiation in developing brain (Liddelow *et al.*, 2009).

1.3 Composition and functions of CSF:

The composition of CSF largely depends upon the activities of surrounding tissues and specific circumventricular (CVO) organs (Skipor *et al.*, 2008, Reiber *et al.*, 2003 and Vio *et al.*, 2008). CSF production and composition is under the influence of an extensive set of regulatory brain mechanisms because choroid plexus is itself controlled by autonomic nervous system and the specific circumventricular organ by serotonergic control (Jimenez *et al.*, 2001). Choroid plexus synthesize and secretes into CSF various proteins which are thought to influence distant parts of the brain such as growth factors, binding proteins and those which aids in transportation of other factors (Nilsson *et al.*, 1992). CSF carries these proteins to the other parts of the brain thus taking a vital part in brain growth and development by providing a transportation medium. It harbours peptides and proteins manufactured locally. Its protein composition is different from plasma protein composition due to inherent CSF functions, such as ongoing proteolytic processes involved in cell surface remodelling, protein shedding, and synthesis of regulatory peptides (Reiber, 2003, Yuan and Desiderio, 2005 and Zougman *et al.*, 2008,).

CSF also contains ions like Na^+, k^+, Ca^{2+}, HCO_3^-, Cl^- and Mg^{2+} and glucose too (Segal *et al.*, 2000).

1.4 Blood- CSF barrier during brain development:

Vertebral brain development takes place at the anterior end of the neural tube and ultimately physiologically sealed cavity is formed after the closure of neuropore and the

cavity encloses within it the embryonic CSF (E-CSF) which is a protein rich fluid. During the early stages of development, the blood vessels control E-CSF protein composition and homeostasis through blood-CSF barrier. This barrier dynamically controls E-CSF composition before the formation of functional CP (Parvas and Bueno, 2010 and Parvas *et al.*, 2008). In embryonic rats the CP tight junctions are impermeable to small molecules as early as E 15.5 which indicates that the blood-CSF barrier is functionally and morphologically becomes mature at this age. Different transfer processes for proteins and small molecules operates across the embryonic blood-CSF interface (Johansson *et al.*, 2006).

Until now not much work has been done on the gene expression data analysis for developing mouse choroid plexus.

1.5 Gene expression data for developing mouse Choroid Plexus:

In situ hybridization data (gene expression data) for developing mouse brain including CP has been generated by the Allen Institute for brain sciences and is made available online as a free resource. The Institute has spent the past few years generating gene expression data and carrying out a comprehensive expression study of the mouse brain and through development. But the data analysis task remains and there is still a need of bioinformaticians to organize the experimentally obtained data into databases for different specific areas of the brain including CP.

Enormous work has been reported related to gene expression analysis of mouse CP (Reid and Ferretti, 2003 and Hana *et al.*, 2009) and for various proteins of CSF (Rajaram *et al.*, 1997, Ramaswamy *et al.*, 2005, Sobanska *et al.*, 2009, Schultz *et al.*, 2010, Yerbury *et al.*, 2010, Leduc *et al* 2011., Wostyn *et al* 2011). However in those analyses the focus is either on adult CP only or on one gene or on a particular set of gene like Reid and Ferretti, in their 2003 study analyzed the differential expression of fibroblast growth factor receptors in the developing murine choroid plexus for embryonic CP only while considering the three time points E12.5, E15.5 and E18.5. Similarly, Nillson *et al.*, in their 1996 study analyzed the autocrine role of insulin-like growth factor II secretion by the rat choroid plexus. Ramaswamy *et al.*, in their 2005 study examined the effect of domain interaction on Apolipoprotein E levels in mouse brain. Zimatkin through his study in 1991 reported high activity of Aldh (Aldehyde dehydrogenase), as a brain metabolic barrier for Aldehydes, in the choroid plexus of rat brain. Stylianopoulou *et al.*, in their 1998 study analyzed the gene expression of the insulin-like growth factor II gene in the choroid plexus and the leptomeninges of the adult rat central nervous system.

There are databases available online for Mus Musculus gene expression data like MGI (Mouse genome database), ABA (Allen brain atlas) and GENSAT (Gene expression nervous system atlas) but none of them contains annotated gene expression data specifically for CP.

1.6 Aims and Objectives:

Therefore this study was designed with the following objectives:

1. What proteins are potentially being generated in the cells of the three different locations of the choroid plexus at different stages of development?

2. Are certain proteins switched 'on' at specific times in the three locations and/or is there a sequence in which locations they are switched 'on'?

3. Are these proteins produced by CP only and provided to the brain by CSF? Or there are other producing factories too for such proteins in the brain at different stages of development?

4. Where are the possible actions of the proteins produced by the choroid plexus?

5. Creation and development of a Database for Gene Expression data of Choroid plexus only at different stages of development of a mouse brain.

6. Creation and development of a website to provide an online resource of the above mentioned CP data.

CHAPTER 2

LITERATURE REVIEW

Approximately 75% of the CSF is produced by choroid plexus. The rest of 25% is called extra choroidal CSF and evidences suggest that it comes from brain interstitial fluid drainage and from the ependymal layer that line the ventricles (Figares *et al.*, 2001, Edsbagge *et al.*, 2004 and Veening, 2010). The adult rat brain contains a constant volume of about 200 µl of CSF and it takes about 2 hours to replace the CSF. In 30 month-old rats with 300 µl, it may take up to 8 hours for complete replacement (Preston, 2001). Choroid plexus has a rich blood supply. The rat lateral and fourth ventricle plexuses receive 3- 4ml.min^{-1}.g^{-1} which is almost 10 times greater than the flow to the cerebral cortex (Chodobska *et al.*, 1994 and Speake *et al.*, 2001).

Choroid plexus synthesize and secretes into CSF various proteins which are thought to influence distant parts of the brain such as growth factors (Riikonen *et al.*, 2006), binding proteins (Corbo *et al.*, 2010, Roghani *et al.*, 1991 and Pez-Bermejo *et al.*, 2003) and those which aids in transportation of other factor (Palha *et al.*, 2002, Zheng *et al.*, 2001 and Parada *et al.*, 2008). CSF carries these proteins to the other parts of the brain thus taking a vital part in brain growth and development by providing a transportation medium. It harbors peptides and proteins manufactured locally (Veening *et al.*, 2010). Its protein composition is different from plasma protein composition due to

inherent CSF functions, such as ongoing proteolytic processes involved in cell surface remodeling, protein shedding, and synthesis of regulatory peptides (Reiber *et al.*, 2003, Zougman *et al.*, 2008 and Yuan *et al.*, 2005).

CP specifically responds to many neurodegenerative diseases including Alzheimer's disease (Serot *et al.*, 2003, Carrette *et al.*, 2003, Conrad *et al.*, 2004 and Marques *et al.*, 2011).

Until now not much work has been done on the gene expression data analysis for developing mouse choroid plexus, although some researchers have worked on such analysis for adult choroid plexus and have found that the genes which are highly expressed in adult mouse transcriptome signature of CP are implicated in energy metabolism and ribosomal function. They also studied that gene expression of some genes which are known to be relevant to developing brain and these includes various axonal guidance factors, angiogenesis molecules and growth factors (Marques *et al.*, 2011). Apolipoprotein and low density lipoproteins in E-CSF play critical roles in embryonic development (Parada *et al.*, 2008). Choroidal epithelial cells have a high secretion capacity and it secretes many biologically active molecules mainly polypeptides which includes transport proteins, collagen subunits and other cell matrix proteins, proteases, protease inhibitors and neurotrophic factors and they are distributed globally (Thouvenot *et al.*, 2006).

2.1 Transthyretin (Ttr):

Transthyretin is a well known as CP specific transport protein in brain (Herbert *et al.*, 1986). The name Transthyretin indicates its function which is to **trans**port **thy**roxin and **retin**ol in serum and cerebrospinal fluid (Richardson, 2007). Transthyretin mediate the transfer of thyroid hormone into brain tissues by acting as a carrier protein across the blood-CSF barrier (Nicola *et al.*, 2003). During development, Transthyretin is produced, secreted and regulated only by choroid plexus in the brain which secrets it to CSF and other than brain it is produced by liver (Schreiber *et al.*, 1993 and Chanoine *et al.*, 1992). Studies show that as much as 25% of the total CSF proteins consist of Ttr (Aldred *et al.*, 1995). Thyroxin is produced by thyroid gland and is a major metabolic hormone that regulates the rate of oxygen used by cells and generation of body heat, thus influence directly the development and metabolism of brain and body. Retinol is an Animal form of vitamin A, which when converted into retinoic acid, works for skin health and bone growth and when converted into retinal, becomes a vision element. About 90-95 % of retinol circulates in blood in the form of a trimolecular complex comprising of retinol, Ttr and retinol binding protein which is also being produced and secreted by CP (Palha *et al.*, 2002 and Vogel *et al.*, 1999). Studies show that lower concentration of Ttr in CSF can alter thyroid hormone homeostasis in CNS that ultimately causes depression and might contribute to failure of standard antidepressant treatment (Sullivan *et al.*, 1999). Lower concentrations of Ttr in CSF can also cause Alzheimer's disease (Söderqvist *et al.*, 2010

and Schultz *et al.*, 2010). In spite of intensive study for Ttr, it is still believed that there are yet some undiscovered functions (Buxbaum and Reixach, 2009).

2.2 Transforming growth factor beta 2 (Tgfβ2):

Tgfβ2 (transforming growth factor beta 2) belongs to the family Tgfβ (transforming growth factor beta), which includes multi-functional cytokines that regulate cell growth and differentiation and plays a vital role during embryonic development (Heine *et al.*, 1991). It functions as a disulphide-linked homodimer. Its sequence is characterized by the presence of several C-terminal cysteine residues, which form interlocking disulphide links arranged in a knot-like topology (Kingsley, 1994). It is an extracellular glycosylated protein and is an important component of many vital processes including blood vessel development and remodelling, axon guidance, extracellular matrix organisation, cell growth and apoptosis, neuron development, neuron fate commitment, positive regulation of cell cycle and cell division, skeletal system development, pathway-restricted SMAD protein phosphorylation, cell migration, cell morphogenesis, etc (Pelton *et al.*, 1991). High concentration of this protein has been found in blood and CSF in Alzheimer's disease. (Swardfager *et al.*, 2010).

2.3 Insulin like Growth Factor 2 (IGF-2):

IGFs are the proteins which are structurally similar to insulin and act as potent growth factors and hormones. They bind to insulin like growth factor II receptor and can

also bind to insulin receptor and trigger an action (Morrione and Valentinis, 1997 and Dell and Day, 1998). The protein plays an essential role in growth and development before birth and It is a main component of many developmental processes including carbohydrate metabolic process, glucose metabolic process, positive regulation of MAPKKK cascade, positive regulation of cell division, positive regulation of insulin receptor signalling pathway, positive regulation of mitosis, positive regulation of protein kinase B signaling cascade, exocrine pancreas development, etc (Baker *et al.*, 1993 and Nillson *et al.*, 1996). Although many researchers have found IGF-2 in CSF but no serious diseased has been found to occur due to imbalance in the normal CSF level of IGF-2 except various cancers (Riikonen *et al.*, 2006, Lehtinen *et al.*, 2011). According to a dissertation of Harvard University, IGF-2 in CSF is needed for the proliferation of cortical neural progenitors (Zappaterra and Dylan, 2008).

2.4 Insulin like Growth Factor Binding Protein 2 (Igfbp-2):

Insulin like growth factor binding protein 2 (Igfbp-2) plays an important role in the regulation of cellular growth and proliferation. It is an important component of cytoplasmic vesicle, extracellular region and apical plasma membrane of cell. It binds insulin-like growth factor I and II and modulates their biological actions by regulating their availability to target tissues and it can modulate the activity of IGF-2 in both the positive and negative manner (Hoeflich and Reisinger, 2001). In addition to functioning as simple carrier proteins, it functions to modulate IGF-2-induced cell growth and proliferation. Igfbp-2 is present in high concentrations in CSF. Igfbp-2 in serum serves to

regulate the endocrine action of IGF-2. Igfbps are known to prolong the half life of IGFs, control the bioavailability of IGFs, facilitates the storage of IGFs in extracellular matrix (Rajaram *et al.*, 1997). Higher levels of Igfbp-2 have been found in the CSF of Alzheimer's disease patients as compared to healthy individuals (Tham *et al.*, 1993).

2.5 Insulin like Growth Factor Binding Protein 3 (Igfbp-3):

Insulin like growth factor binding protein 3 (Igfbp-3) is the most abundant IGFBP in blood. The majority of the IGFs circulate as a ternary complex of 150-kDa that consists of IGF and Igfbp-3 (Li *et al.*, 2004). Due to this complex formation, the half lives of IGFs are prolonged to 12-15 hours in comparison with half life of unbound IGFs and they do not readily leave the vascular compartment (Firth and Baxter, 2002). In this way it serves as carrier protein for IGFs and prevents them from degradation. IGFs are released when needed by aproteolysis of Igfbp-3 (Lee and Rechler, 1996). Igfbp-3 binds with insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-2 (IGF-2). It plays a major role in P53 signalling pathway where it controls apoptosis by inhibiting IGF. It is important for regulation of catalytic activity and regulation of cell growth. All the mature Igfbps are found in extracellular region. In extracellular fluids, IGF-1 and IGF-2 form complex with Igfbp-3 (Baxter, 2000). Elevated levels of this protein in CSF have been found in the brain of Alzheimer's disease patients (Rensink *et al.*, 2002).

2.6 Insulin like Growth Factor Binding Protein 4 (Igfbp-4):

Igfbp-4 interacts with IGFs and intrinsic bioactivities. It binds with IGF-1 and IGF-2 (which is a CP protein) and modulates the availability and activity of the IGFs either by inhibiting or stimulating their growth promoting effects. It alters the interaction of IGFs with their respective receptors on cell surface. Igfbps are secretory proteins that are found in extracellular matrix and bound to cell surfaces and cell surface receptors (Clemmons, 1997). Studies show that Igfbp-4 has inhibitory effect for IGF-1. Binding of Igfbps also prolong the half life of IGFs (Baxter, 2000).

2.7 Insulin like Growth Factor Binding Protein 7 (Igfbp-7):

Igfbp-7 also belongs to the class of secreted binding proteins called 'Insulin-like growth factor binding protein'. All of them are soluble proteins that bind IGF-1 and IGF-2 with high affinity but Igfbp-7 is found to bind the IGFs with relatively low affinity (Kim *et al.*, 1997). Thus it regulates and modulates the availability and binding of IGFs to their receptors (Youngman *et al.*, 1996). The protein is involved in cell adhesion, negative regulation of proliferation and regulation of cell growth. Both up and down regulations of Igfbp-7 have been reported in cancers (Sprenger *et al.*, 2002, Wajapeyee *et al.*, 2008 and Ruan *et al.*, 2010). Some past researches have indicated the presence of Igfbp-7 in normal cerebrospinal fluid (Wilson *et al.*, 1997 and Pez-Bermejo *et al.*, 2003).

2.8 Alpha-2-macroglobulin (A2m):

The protein Alpha-2-macroglobulin (A2m) is also a CP protein which can inhibit all the classes of proteinases by trapping them with the help of a cleavage site of peptide stretch called 'bait region' which traps the proteinase. It is a secreted protein which is found in plasma. It also acts as cytokine transporter (Feldman *et al.*, 1985). The protein is also found to be associated with Alzheimer's disease (Rudrasingham *et al.*, 1999 and Korovaitseva and Premkumar, 1999). Some previous researchers have indicated the presence of Alpha-2-macroglobulin in cerebrospinal fluid and it is also found to serve as a parameter for the condition of blood-CSF barrier (Link *et al.*, 1972 and Schliep and Felgenhauer, 1978).

2.9 Aldehyde dehydrogenase 2 (Aldh-2):

Aldehyde dehydrogenase 2 belongs to the family of 19 genes which encodes $NAD(P)^+$-dependent enzymes called Aldehyde dehydrogenase which functions to catalyze the transformation of acetaldehyde to acetic acid, retinaldehyde to retinoic acid and other aldehydes to their corresponding carboxylic acids (Vasiliou and Nebert, 2005). In this way mitochondrial Aldh-2 degrades toxic aldehydes and thus works for the prevention of neurodegenerative diseases (Ohsawa *et al.*, 2008). At physiological concentrations, Aldh-2 catalyzes the process of acetaldehyde oxidation and the enzyme is typically found after alcoholic consumption by the body (Peng and Yin, 2009). Some previous researches showed that Aldh-2 is present in blood brain barriers including CP

(Zimatkin and Ostrovskiĭ, 1988). High activity of Aldh, as a brain metabolic barrier for Aldehydes, has been found in the choroid plexus of rat brain (Zimatkin, 1991). It is thought that this gene can be used as a potent therapeutic agent for the treatment of Alzheimer's disease and other neurodegenerative diseases (Bai *et al.*, 2011).

2.10 CD164 antigen:

CD164 antigen (cluster of differentiation 164 (antigen)) is a protein which is also named as Sialomucin and it is a musin domain containing molecule. Musins are heterogeneous group of secreted or membrane associated protein molecules which are produced by most epithelial cells and they are heavily glycosylated proteins with high molecular weight. They have the ability to form gel. Sialomucins are thought to be either anti-adhesive agents or cell surface adhesion receptors (transmembrane). CD164 is a Sialomucin that functions as a cell adhesion receptor on $CD34^+$ hemopoietic progenitor cells and regulates their proliferation and cell growth depending upon the interacting ligand. (Watt *et al.*, 1998, Zannettino *et al.*, 1998, Doyonnas *et al.*, 2000 and Zannettino *et al.*, 2001). CD164 is also found to be associated with myoblasts (embryonic progenitor cells that give rise to muscle cells) where they work for cell motility and myotube formation (Bae *et al.*, 2008).

2.11 Heat Shock protein beta-8 (Hsbp8):

Heat Shock protein beta-8 (Hspb8) is a small heat shock protein and it is one of 11 members of a sub family of heat shock proteins family called Hspb family of small heat shock proteins. Hspbs are often found as oligomeric complexes and are particularly abundant in nerve cells (Kampinga *et al.*, 2009). Heat shock proteins protect cell from thermal or other proteotoxic stresses and creates a stress response (De Maio *et al.*, 1999). They are named according to their molecular weight (Valdez-Cruz *et al.*, 2011). Heat shock proteins are both cellular and extracellular proteins and some of them, including Hspb8, act as molecular chaperons (Li *et al.*, 2004). Thus they play their role for removal of miss folded proteins and assist correct folding of denatured proteins (Walter and Buchner, 2002 and Crippa *et al.*, 2010). Gene expression of Hspb8 in lateral and dorsal ventricles of mouse brain has been recently reported in the literature (Quraishe *et al.*, 2008).

2.12 Angiotensin I converting enzyme (Ace):

Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1 (Ace) is an enzyme that catalysis the conversion of inactive angeotensin 1 to active angiotensin II while degrading bradykinin and thus regulates angiotensin. Angeotensin II is a potent vasopressor and aldosterone-stimulating peptide that controls blood pressure and fluid-electrolyte balance. Ace contains a signal peptide and is a cell membrane bounded protein (Welches *et al.*, 1993). It also acts as an endopeptidase and is a key component of

autonomous RAS (renin angiotensin system) that is involved in blood pressure and electrolyte homeostasis (Coates, 2003). Researchers have detected the presence of Ace in Cerebrospinal fluid (Erickson *et al.*, 2003). Some association between Ace and CSF monoamine metabolite concentrations of some acids have also been found (Annerbrink, 2010). Ace have found to effect thirst, blood pressure and vasopressin release, anxiety related behaviours, cognition, and memory functions and thus Ace level alteration in cerebrospinal fluid have been found in various neuro-psychiatric conditions and in schizophrenia (Schweisfurth and Schiegnitz, 1984 and Wahlbeck *et al.*, 2000). High level of Ace has been found in choroid plexus in rats and man which suggests its activity in CSF (Skidgel, 1993 and Rao, 2007). Decreased levels of Ace in CSF have been observed in diseases such as Alzheimer disease, Parkinson disease, and progressive supranuclear palsy (Zubenko *et al.*, 1985).

2.13 Clusterin (Clu):

Clusterin is a CP gene which is also known as apolipoprotein J and it is a glycosylated protein. It is a multifunctional protein which is normally associated with lipids in plasma and cerebrospinal fluid, and it is secreted as lipoparticles by hepatocytes and astrocytes (Miguel *et al.*, 1999). It is present in many biological fluids like semen, urine, breast milk, plasma and cerebrospinal fluid (Aronow *et al.*, 1993). This protein can form complex by binding with numerous factors including immunoglobulins, lipids, heparin, bacteria, complement components, beta amyloid and leptin etc. Its major function is to bind misfolded proteins (Leskov *et al.*, 2003). Clusterin is an extracellular

chaperon and acts as a global quality control of extracellular protein folding, that prevents the precipitation of proteins during stress such as elevated temperatures and oxidative stress and thus stabilize them by producing high molecular weight complexes with them (Wyatt, 2009). Clusterin is also involved in the differentiation of epithelia and in the regulation of epithelial cell phenotype (Jones and Jomary, 2002). Many studies show that Clusterin is contained in cerebrospinal fluid and the protein is thought to be associated with Alzheimer's disease (Nilselid *et al.*, 2006 and Yerbury and Wilson, 2010).

2.14 Inositol 1,4,5-trisphosphate receptor, type 1 (Itpr1):

Inositol 1,4,5-trisphosphate receptor (Itpr1) is a Ca2+ release channel and is located in the cytoplasm and mainly on the endoplasmic reticulum. Studies indicate that this is involved in early development and neuronal plasticity. It is a developmentally regulated protein which is missing in the cerebellar mutant mice and controls a variety of Ca2+-dependent cell functions like cell proliferation, differentiation, fertilization, embryonic development, secretion, muscular contraction, immune responses, brain functions, chemical senses, light transduction, etc (Monkawa *et al.*, 1998 and Mikoshiba, 2006). It plays an important role in many biological pathways including Salivary secretion, long-term depression, gastric acid secretion, Gap junction, Calcium signalling pathway, Alzheimer's disease, Huntington's disease, etc. CSF plays a major role in all of these processes/diseases.

2.15 Carbonic anhydrase 2 (Car2):

Carbonic anhydrase 2 (Car2) is an isozyme that belongs to the family of (at least 7) enzymes called carbonic anhydrase which catalyses the reversible reaction of carbon dioxide hydration and HCO_3^- dehydration. This process involves the conversion of H_2CO_3 into CO_2 and H_2O. In cerebrospinal fluid, the formation of HCO_3^- [(2)] and transport of Cl^- depend upon this process and thus the inhibition of CP carbonic anhydrase reduces the formation of CSF by 30-50 % (Maren and Broder, 1970 and Swenson, 2003). Carbonic anhyderase2 is one of the major contributors to CSF production and secretion by choroid plexus (Brown *et al.*, 2004 and Han *et al.*, 2009). In mammalian brain including rat, the protein Car 2 has been detected in normal CSF and it has been observed that its concentration in CSF increases in neurological diseases caused by brain damage (Parkkila *et al* 1997). Like most of the CP genes, this gene has also been found to be associated with Alzheimer's disease and this protein can be a potential drug target for Alzheimer's disease therapy (Sultana *et al.*, 2006 and Wostyn *et al.*, 2011).

2.16 Fibroblast growth factor receptor 2 (Fgfr2):

Fibroblast growth factor receptor 2 (Fgfr2) is a receptor protein for some growth factors including fibroblast growth factor and is a receptor tyrosine kinase. It is a cell membrane bounded protein which binds signalling proteins and triggers a cascade of chemical reactions inside of the cell in MAPK cell signalling process (Eswarakumar *et al.*, 2005 and Ezzat and Asa, 2005). It is an important protein for embryonic development

and stem cell proliferation (Takashi *et al.*, 1993 and Iseki *et al.*, 1999). Gene expression of fibroblast growth factor receptor 2 has been reported in several researches for choroid plexus which indicate that it might play a role in (a) the secretion of CSF and (b) CNS fluid homeostasis through Cp in adult brain (Yazaki *et al.*, 1994, Reid and Ferretti, 2003 and Conrad *et al.*, 2004).

2.17 5-hydroxytryptamine (serotonin) receptor 2C (Htr2C):

5-hydroxytryptamine (serotonin) receptor 2C (Htr2C) is a subtype receptor for serotonin (5-hydroxytryptamine) which functions as a neurotransmitter, a hormone and a mitogen. The receptor is located primarily in the choroid plexus, cortex, limbic system and basal ganglia and not outside the CNS and thus it is a brain specific gene (Hoyer *et al.*, 2002). The receptor exhibits signal transduction activity by association with G proteins that activate a phosphatidyl inositol-calcium second messenger system. Serotonin is degraded into its metabolite, 5-hydroxyindole acetic acid (5-HIAA), by an enzyme called monoamine oxidase (MAO). This metabolite is then secreted into CSF (Lappalainen *et al.*, 1999). The receptor HTR2C was first discovered in the choroid plexus where it is expressed in high concentrations (Pazos *et al.*, 1984). Htr2C null mutant mice display obesity, so the gene is thought to be closely associated with regulation of food intake and body weight (Reynolds *et al.*, 2002, Reynolds *et al.*, 2006, Hill and Reynolds, 2007 and Rösman *et al.*, 2008). Serotonin in the CSF can bind to Htr2c in CP and reduce CSF secretion (Perez-Figares *et al.*, 2001). This gene has also

been found to be associated with Alzheimer's disease (Holmes *et al.*, 1998 and Zarros *et al.*, 2005).

2.18 Apolipoprotein E (Apoe):

Apolipoprotein E (Apoe) is essential for lipoprotein metabolism and plays a role in catabolism of triglycerides rich lipoprotein constituents. Apolipoprotein E is a soluble protein and is the main lipid carrier protein in CNS which combines with lipids to form lipoproteins (Hatters *et al.*, 2006). As a carrier protein, it functions for the uptake and distribution of plasma lipids and thus it transports lipid from its site of synthesis to the other parts of the body where it is needed or stored (Reynolds *et al.*, 1997). Apoe has been found in CSF (Yamauchi *et al.*, 1999 and Riddell *et al.*, 2008). In embryonic CSF, this protein is thought to be required for neural differentiation (Parada *et al.*, 2008). Apoe 4 genotype is thought to be the strongest risk factor for Alzheimer's disease and in many researches varied levels of CSF Apoe in AD has been reported (Jacob and Yadong, 2004, Ramaswamy *et al.*, 2005, Sobanska *et al.*, 2009, Vance and Hayashi, 2010 and Leduc *et al.*, 2011).

2.19 Endothelial PAS domain protein 1 (Epas1):

Epas1 is a hypoxia – responsive transcription factor and is commonly referred to in the literature as Hypoxia Inducible Factor-2 *alpha* (HIF-2*alpha*). It contains a basic helix-loop-helix (bHLH)/ PAS domain which is a property of signaling proteins. Its PAS domain responds to oxygen and thus the transcription factor induces the genes regulated

by oxygen and the gene is expressed selectively in vascular endothelial cells (Tian *et al.*, 1997). The transcription factor binds directly to DNA and induces transcription of the target gene. VEGF (vascular endothelial growth factor) is one of the target genes (Marti *et al.*, 2000).

2.20 Annexin A11 (Anxa11):

Annexin A11 (Anxa11) is a widely expressed calcium- and phospholipid-binding protein that binds negatively charged phospholipids in a calcium dependant manner, and it resides in the nucleoplasm in many cultured cell lines. This protein interacts with S100A6 and PDCD6 in a calcium-dependent manner. It interacts with KIF23 during cytokinesis. A pair of Annexin repeats may form one binding site for calcium and phospholipid (Gerke and Moss, 2002). For now, in mammalian CSF, Anxa11 has not been found in the published literature.

2.21 Bioinformatics database application:

Building a bioinformatics database is not much different from building any other complex databases in other fields. For a good bioinformatics database application, the quality, quantity and originality of data as well as the quality of the web interface are the most important factors. One needs to:

- understand the information the database is going to store and present,
- translate that understanding into a rigid framework,

- write and debug programs using programming tools,
- and then finally actually run and use the database, usually with additional 'on-site' development and debugging.

Generally a team work is required, to accomplish all of the above mentioned steps, which includes, a bioinformatician who understands the biological data (information), a web developer who understands how to generate user friendly web pages, a programmer who actually understands and writes the database and supporting software, and a specific contact person in the systems group who can forewarn people of systems upgrades and provide support during specific systems problems (Birney and Clamp, 2004 and Batemen, 2007).

Ensemble is one of the largest bioinformatics project to organize biological information and around the sequences of large genomes. It is available as an interactive Web site, a set of flat files, and as a complete, portable open source software system for handling genomes. Ensemble has been built using relational database system (RDB) encoded by MySQL database engine. The RDB forms the core anchor point for the design and construction of the systems architecture. They have used multiple SQL select statements to carry out the gene-build process (Birney *et al.*, 2004, and Cuff *et al.*, 2004).

CHAPTER 3

MATERIALS AND METHODS

The present research has been conducted in the department of Bioinformatics in International Islamic University Islamabad and in the department of Biosciences in COMSATS Institute of Information Technology. The research has been conducted all *in silico*.

3.1 Data Sources:

3.1.1 Allen Developing Mouse Brain Atlas (ABA):

The Allen developing mouse brain atlas is an online public resource of gene expression data which is provided by Allen Institute of Brain Sciences and is accessible via the Allen Brain Atlas portal (www.brain-map.org). ISH data for developing Mus musculus brain for more than 2000 genes over embryonic and postnatal time points is available at ABA under their data module. Both sagittal and coronal ISH data have been generated at ABA mainly for four embryonic time points (E11.5, E13.5, E15.5, and E18.5) and three early postnatal time points (P4, P14 and P28). For one sagittal-sectioned brain they have generated 8 series of 5 slides, each containing four 25 µm thick sections per slide. At ABA there is not much data for gene expression for coronal slides yet so only sagittal data was used for this project.

3.1.2 Bioinformatics Resources for Protein Information:

Various protein databases and online journals have been used as source for obtaining functional information about the CP proteins which we were selected for this study. These include NCBI, Swiss Prot, PDB, PubMed, Biomed Central, Elsivier and Science Direct. Information relevant to CSF was mainly obtained from the journal of Fluids and barriers of CNS.

3.2 Data extraction:

In order to extract the gene expression data for choroid plexus in developing mouse brain, first of all developmental anatomy of ventricular system was fully understood to locate choroid plexus of the developing brain considering the seven time points i.e. E 11.5 (embryonic day 11.5), E 13.5, E 15.5, E 18.5, P 4 (postnatal day 4), P 14 and P 28. For such purpose initially the Allen developing mouse brain atlas ontology legend browser was used which contains many reference coloured images for each time point along with gene expression images (HP yellow stained images) (Table 3.1). However, there is not much information about choroid plexus over there but after understanding the anatomy of mammalian brain the location of ventricles and choroid plexus were traced by locating the surrounding brain structures such as hippocampal formation, corpus callosum, prosomere 2 and 3 in forebrain and mid brain, cerebellum and rhombomeres in hind brain etc. The major problem was to locate the choroid plexus of early embryonic ages because at E 11.5 and E 13.5, there is undifferentiated set of precursors and functioning organs and thus the ability to localize gene expression at

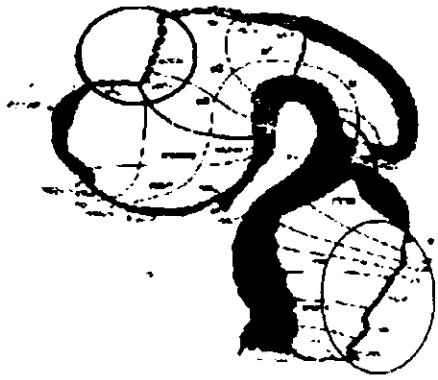
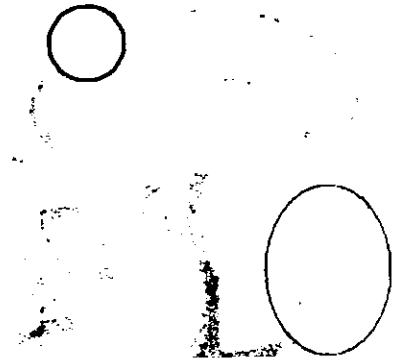
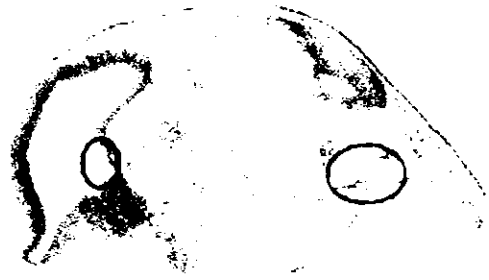
specific stages of development was highly desirable. Therefore for these time points ontology legend browser was not much helpful. After doing a lot of literature survey is was found that at the very early embryonic stages the choroid plexus of lateral and third ventricle (which is a continuous structure) is present in the form of choroidal tissues of prosomere 2 and 3 and the telencephallic choroidal tissues. The choroidal tissues of prosomere 2 and 3 develop into to the choroid plexus of third ventricle whereas telencephallic choroidal tissue grows into choroid plexus of lateral ventricles. These choroidal tissues can be visualized in the coloured images of reference atlas or by using the tool BrainExplorer 2. The choroid plexus of fourth ventricle is in the form of the tissues of rhombomeres in the hind brain. As the brain develops, the rhombomeres are differentiated into choroid plexus and the basal plates, alar plates and roof plates of rhombomeres from rhombomere 1 to rhombomere 11.

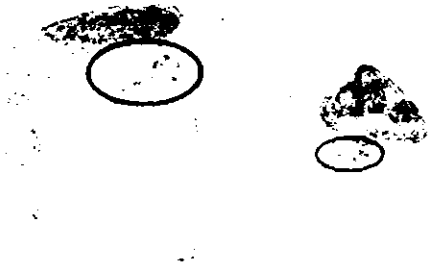
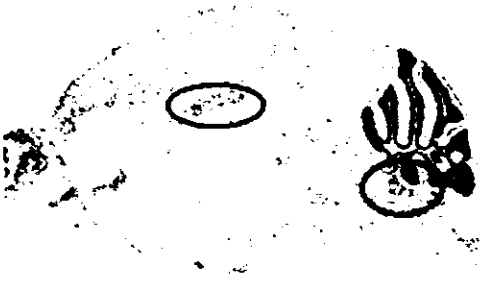
In this study data extraction part was comprised of two parts which were:

i. The *gene expression data extraction* for choroid plexus. Data was in the form of gene expression images. And

ii. *Extraction of Some introductory information* about the protein products of these genes to know about the possible roles these genes play during development of the mouse brain and in CSF that whether these factors are secreted into the fluid either to be delivered to other parts of the brain or to provide nourishment to the fluid itself.

Table 3.1: Sagittal sections of coloured reference images and HP-yellow stained images at different stages of development of Mus musculus brain. The black circle show the choroid plexus of lateral and third ventricle and red circle shows choroid plexus of fourth ventricle respectively.

Age	Coloured reference image	Hp-Yellow image
E 11.5		
E 13.5		
E 15.5		

E 18.5		
P 4		
P 14		
P 28	Nill	

3.2.1 Extraction of ISH data:

In order to extract the ISH data for only the choroid plexus of developing Mus musculus brain, the **gene finder** in **developmental AGEA** (developmental anatomic gene expression atlas) was used which enable the users to point out the genes whose expression is predominantly enriched within a particular brain region at a specific developmental age (Fig 3.1). By setting the voxel to the place where choroid plexus is present the top 100 genes which expresses in choroid plexus region were selected (Fig 3.2). By opening the individual gene from there, their detailed ISH images along with their 3D expression images were accessed for each of the seven time points and even for some more time points for some genes. From there, the image series of a gene for a particular time point was accessed and CP in individual images were analysed by zooming in. Structure unionizer is also available there which is a coloured expression summary for anatomic regions of the brain for each time point ranging from light yellow to dark red corresponding to the log of expression values between -1.5 (light yellow) and 3.5 (dark red). However there is no information for choroid plexus specifically in structure unionizer, therefore the expression levels were judged by looking at the anatomic regions where choroid plexus exists.

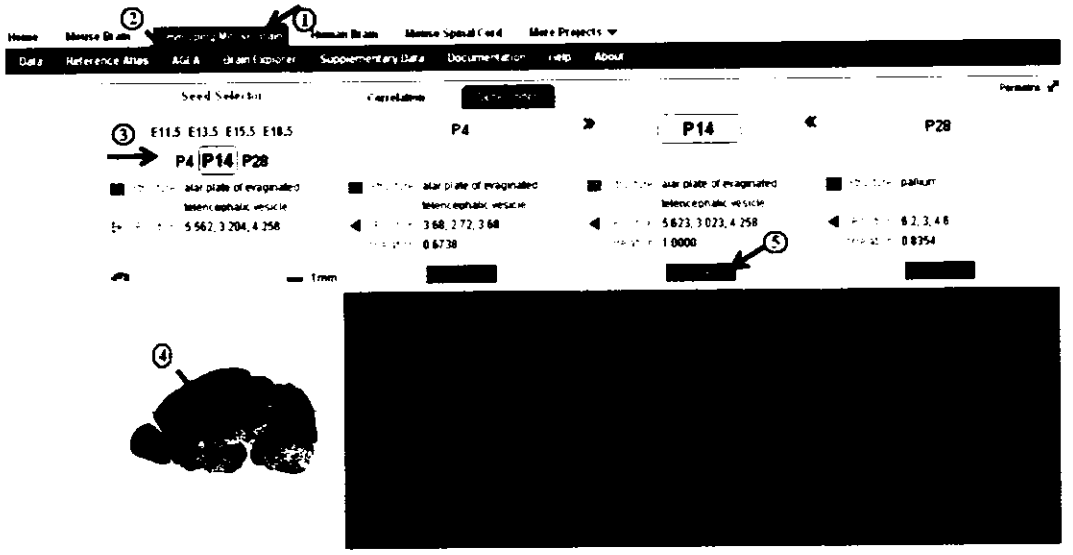


Fig 3.1: Developmental AGEA at ABA. The arrows with numbering show the sequence in which the data is retrieved. (1) developing mouse brain. (2) AGEA. (3) set the three consecutive ages for which data is to be retrieved. (4) move voxel to the place where CP is present.

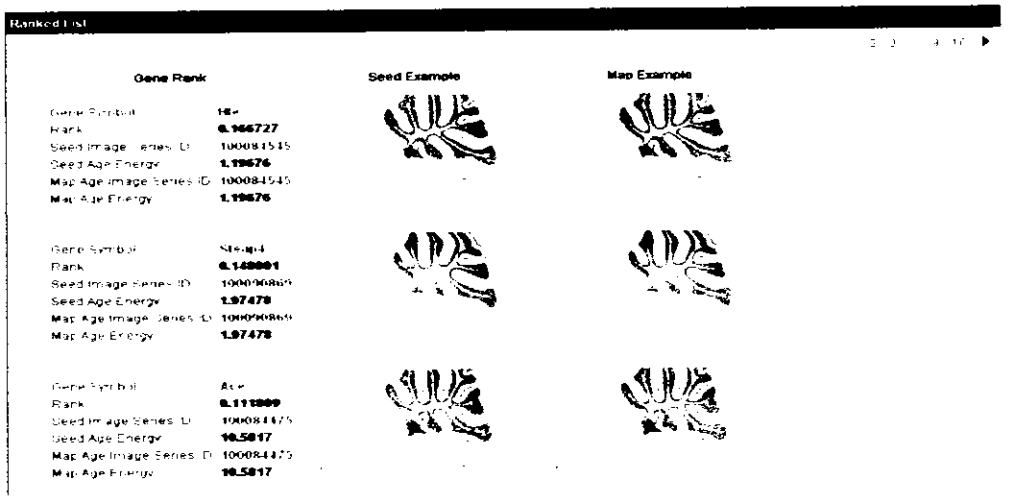


Fig 3.2: list of genes expression in the CP of fourth ventricle as provided by gene finder in AGEA

3.2.2 Extraction of Expression data:

To retrieve the gene expression data for the genes which were found for choroid plexus in Allen brain atlas, the expression masks for every ISH image of the gene were used. The expression masks are based upon the heat map colour. The light blue colour indicates lower level of gene expression and dark red colour indicates higher level. The gene expression levels were judged by using expression masks for ISH images already extracted (Fig 3.3).

Secondly the expression data was retrieved through ABA by using **Brain Explorer2**. It is a useful tool developed only for gene expression analysis of developing mouse brain which has direct link to ABA and retrieve information for gene expression through that atlas. It gives a quite useful 3D visualization of expression of a particular gene along with the intensity and density of gene expression at the particular point being selected using the selection tool.

Fig 3.3: ISH image (right) and its corresponding expression mask image (left) for gene expression in Mus Musculus brain. This particular image is of gene expression of Igfbp-2.

3.2.2.1 For early embryonic ages of E11.5, E13.5 and E15.5:

In early embryonic time points during development of Mus musculus brain, the structure of CP is not fully developed and it is in the form of choroidal tissues. Thus for the very early embryonic stage of E 11.5, it is very difficult to distinguish between the choroidal tissues and the other brain tissues. For such purpose, BrainExplorer2 was used to understand the brain anatomy of developing mouse brain. By using it the choroidal tissues can be located and distinguished between choroidal tissues of prosomeres and rhombomeres and telencephallic choroidal tissues (Fig 3.4). These terms are explained in the Table 3.2.

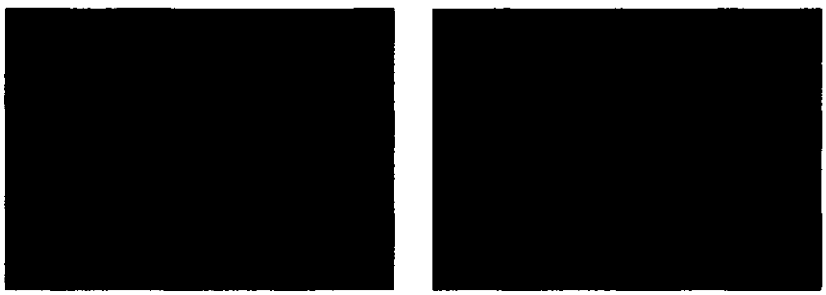


Fig 3.4: Neural tube of mouse at E11.5 (left) and E13.5 (right) as viewed by Brain Explorer2. The brown structures are choroidal tissues of prosomere 2 and 3 and the red structure is telencephallic choroidal tissues. The blue and purple structures are rhombomeres which differentiate into choroid plexus in later ages.

Table 3.2: Terminologies used in BrainExplorer 2 for the three CPs and other brain structures for a developing embryonic brain.

Abbreviation	Anatomic location/ Brain structure	Corresponds to/ differentiate into
TCh	Telencephallic choroidal tissues	Choroid plexus of lateral ventricles
P2Ch	Choroidal tissues of prosomere 2	Choroid plexus of third ventricle
P3Ch	Choroidal tissues of prosomere 3	Choroid plexus of third ventricle
rR	Roof plates of rhombomeres	Choroid plexus of fourth ventricle
R	Rhombomeres	Segments of the developing neural tube in the rhombencephalon. There are 11 rhombomeres in a developing mouse embryo.
--	Rhombencephalon	That part of the brainstem constituting the medulla oblongata (myelencephalon) and pons (metencephalon)

Pal	Pallium	Layers of gray and white matter that cover the upper surface of the cerebrum in vertebrates, and it becomes cerebral cortex.
SubPal	Subpallium	A layer of cerebrum bellows the pallium
Rsp	Rostral secondary prosencephalon	A part of forebrain
Tel	Telencephallic vesicle	Subdivision of forebrain. CP of lateral ventricle exisst here.
PedHy	Perpendicular hypothalamus	Hypothalamus
P 2	Prosomere 2	Early developing forebrain.
P 3	Prosomere 3	Early developing forebrain
P 1	Prosomere 1	Early developing forebrain
M	Mid brain	Mid brain
PPH	Prepontine hind brain	Pones of hind brain. CP of fourth ventricle exists here.
PH	Pontine hindbrain	Pones of hind brain. CP of fourth

		ventricle exists here.
PMH	Pontine medullary hindbrain	Pones of medulla in hind brain. CP of fourth ventricle exists here.
MH	Medullary hind brain	Medulla in hind brain. CP of fourth ventricle exists here.

For the later embryonic ages of E 13.5 and E 15.5, the structure of choroid plexus is apparent, though not fully developed, but can be recognized and distinguished from other brain organs. But still during these ages it is difficult to distinguish between the choroidal tissues of lateral and third ventricles. So for this purpose again BrainExplorer2 was used to extract data.

3.2.2.2 For E 18.5, P 4, P 14 and P 28:

During the late embryonic time point of E 18.5 and during postnatal ages, the structure of CP is fully developed and CP can easily be located. So for these time points, instead of using BrainExplorer2, ISH data and its corresponding expression data provided by Allen Brain Atlas at their web site were used directly.

3.2.2.3 Extraction of whole brain gene Expression data and summary image:

Brain explorer2 has also been used to locate the gene expression in areas/organs of the brain other than CP. Whole brain expression images were then analyzed and highlighted to locate these areas (Fig 3.5) (Fig 3.6).

Gene expression summary image for whole Mus musculus brain has also been taken from ABA to know about the brain areas other than CP where the specific gene is being expressed temporally. The image is in the form of colours ranging from light yellow to dark red which corresponds to log value from – 1.5 to 3.5 respectively. The abbreviations which are used in expression summary image are listed in the Table 3.2.

Fig 3.5: Expression image of IGF2 in the mouse brain at age E11.5. The red circles point to the choroid plexus in the brain.

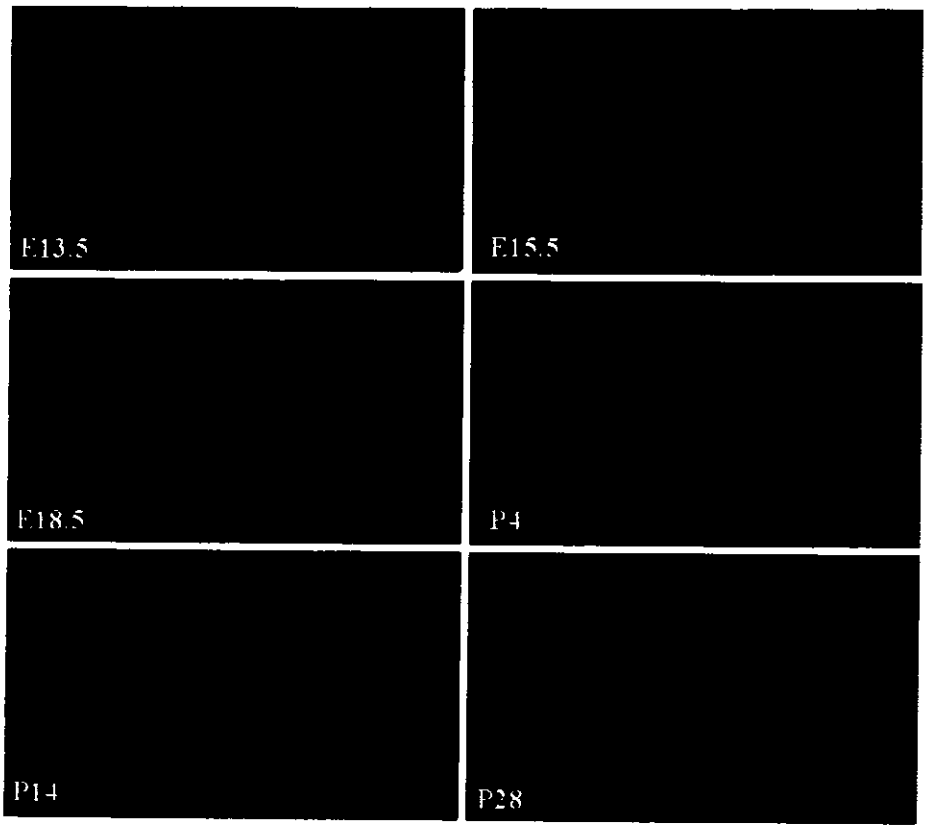


Fig 3.6: whole brain gene expression image throughout the six stages of development. The red circles point to the choroid plexus in the brain. The purple structures point to the pallium (basal ganglia) and blue circles are showing expression in alar plate of prosomere 2.

3.2.2.4 Protein information:

For the data analysis which was carried out, it was necessary to know about the presence of each of the gene product in CSF and to get its functional information to know about its role in CSF. Such information was obtained from various bioinformatics resources for protein information which are already explained under the section 3.1. For

each gene it was highly preferred to learn about its function and target from numerous research papers which defined its role especially in CSF and brain.

3.3 Spatial and temporal Gene expression data analysis for CP:

Spatial and temporal gene expression data for a total of 20 genes (Table 3.3) was extracted and analyzed based upon the evaluation criteria as explained under section 3.3.1.

Table 3.3: List of CP genes.

Sr no.	Gene Symbol	Gene Name	Gene Category
1	A2m	Alpha 2 microglubullin.	Secretory protein
2	Ace	Angiotensin I converting enzyme.	Enzyme
3	Aldh2	Aldehyde dehydrogenase 2 (mitochondrial).	Enzyme
4	Anxa 11	Annexin A11	Binding protein
5	Apoe	Apolipoprotein E	Carrier protein
6	Car 2	Carbonic anhydrase 2	Isozyme (enzyme)

7	Clu	Clusterin.	Chaperon
8	CD 164 (antigen)	CD 164 (antigen), Sialomucin, Endollyn.	Receptor
9	Epas 1	Endothelial PAS domain protein 1.	Transcription factor
10	Fgfr 2	Fibroblast growth factor receptor 2.	Receptor protein
11	Htr2c	5-hydroxytryptamine (serotonin) receptor 2C.	Receptor protein
12	Hspb8	Heat shock protein 8.	Heat shock protein
13	Itpr 1	inositol 1,4,5-triphosphate receptor, type 1.	Receptor protein
14	Igf-2	Insulin like growth factor 2.	Growth factor
15	Igfbp-2	Insulin like growth factor binding protein 2.	Binding protein
16	Igfbp-3	Insulin like growth factor binding protein 3.	Binding protein

17	Igfbp-4	Insulin like growth factor binding protein 4.	Binding protein
18	Igfbp-7	Insulin like growth factor binding protein 7.	Binding protein
19	Tgfb-2	Transforming growth factor beta 2.	Growth factor
20	Ttr	Transthyretin	Carrier protein

The gene expression data has been extracted and analyzed for seven time points of development which includes four embryonic time points of E 11.5, E 13.5, E 15.5 and E 18.5, and three post natal time points of P 4, P 14 and P28 respectively, with the exception of only one genes 'Transthyretin' whose expression has been analyzed for a total of fourteen time points which are E 11.5, E 12.5, E 13.5, E 14.5, E 15.5, E 16.5, E 17.5, E 18.5, P 1, P 2, P 4, P 7, P 14 and P 28.

3.3.1 Evaluation Criteria:

For these twenty genes, different types of gene expression patterns have been observed. To carry out the gene expression analyses, two evaluation criteria were formulated on the bases of these expression patterns. The two criteria were named as gene expression level and gene expression specificity.

3.3.1.1 Gene Expression Level:

Gene expression level has been analyzed based upon the expression colour and the expression pattern. The expression colour varies from light blue to dark red. For some genes, the expression level is composed of two colours. The key to evaluate the expression level according to colours is shown in Fig 3.7.

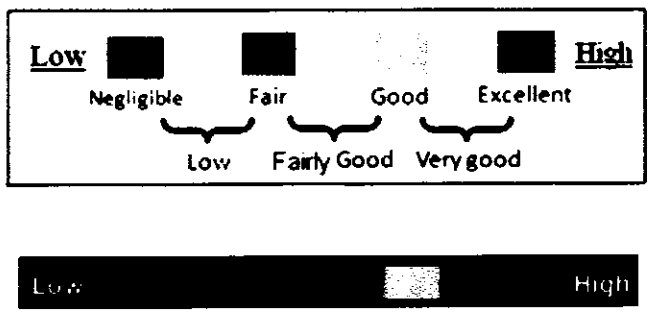


Fig 3.7: Key of gene expression level

Two types of expression patterns have been observed which were named as dense and scattered.

- Scattered: Expression is not indicated in all of the cells but is scattered throughout the structure of choroid plexus. In other words the expression is low (Fig 3.8).
- Dense: Expression is indicated in almost all of the cells of choroid plexus (Fig 3.9).

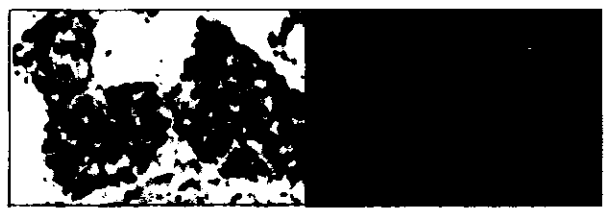


Fig 3.8: Scattered gene expression. Right: ISH image. Left: coloured expression image.

Fig 3.9: Dense gene expression. Right: ISH image. Left: coloured expression image.

3.3.1.2 Gene expression specificity:

For some genes, there is a case when the gene is found to be expressed only in choroid plexus and not in any other part of the brain or glial cells. For such cases the gene expression is said to be specific to choroid plexus only and the gene is called as CP-specific.

For each of the 20 genes, the gene expression is either confined to the outer epithelial layer of CP or to the inner layer of CP (which is the choroidal capillary) or

throughout the structure of CP. It was also observed for some genes that they show their expression only in some and not in all of the seven time points of development being considered for analysis. Therefore according to the gene expression specificity, the genes were categorized under following three categories:

- CP specific genes and non CP specific genes.
- Temporal specificity (Specificity to a developmental stage or otherwise).
- Spatial specificity (Epithelial or inner).
  - **Epithelial**: Expression is indicated only in the choroid epithelium (Fig 3.10).
  - **Inner**: Expression is indicated only in the inner side of the choroid plexus (most probably in the choroid capillary) and not in the choroidal epithelium (Fig 3.11).

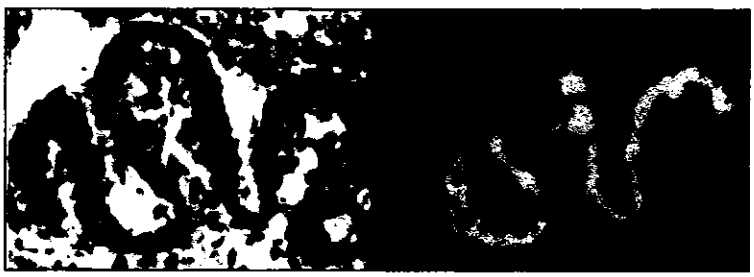


Fig 3.10: Epithelial Specific gene expression. Right: ISH image. Left: coloured expression image.

Fig 3.11: Inner Specific gene expression. Right: ISH image. Left: coloured expression image.

The basic keywords used throughout 'Results and Discussion', to describe the type of gene expression for every gene are described in Table 3.4.

Table 3.4: List of keywords used to describe gene expression.

Criteria		**Key words**
Gene expression level	Level	Negligible, low, fair, fairly good, good, very good, excellent.
	Pattern	Scattered, dense.
Gene expression specificity	Ventricular specificity	Lateral ventricles, third ventricle, fourth ventricle
	Structural specificity	Epithelial, inner,

	Anatomical specificity	CP specific, non CP specific.

3.4 Database application development:

What is meant by database application is a database with a user web interface to access the database. After completing the data extraction and analysis task, the plan was to organize the results in such a way that they may provide the future researchers with a repository of gene expression data specifically for choroid plexus of developing Mus musculus brain. Thus a database was developed and a website has been created. The database contains necessary information about the genes of CP, functional annotation of each gene in the context of CP/CSF in the form of gene reports and direct access to their gene expression images. For every gene there is a gene report and total of 21 gene expression images for three CPs across the seven time points. The tools which were used for this task were as follows:

- Visual web developer (VWD) 2010 express to develop the website.
- Sql server 2008 to develop the database.
- SmartDraw to draw UML diagrams.

The languages used for developing the website were Asp.NET, C# and Sql commands for database.

CHAPTER 4

Database Application: Design and Implementation

Here database application means software which provides a user web interface to a database. There was a need to create a database which contains both types, temporal and spatial gene expression data only for choroid plexus of all the three ventricles of mouse brain. The software is to provide gene expression information to different researchers working for proteins of cerebrospinal fluid and choroid plexus throughout the development of mouse brain. For this purpose, the database should contain data for both gene expression images and their annotation for the genes found to be expressed spatially and temporally in choroid plexus of Mus musculus brain. The database was created and populated with gene reports and 21 gene expression images for each gene. The data is made available to the users/researchers by creating a website and linking the database with it.

Gene expression analysis was carried out for a total of 20 genes. A Bioinformatics Relational database was created on SQL server 2008, which contains detailed temporal and spatial gene expression images and their annotation. This database was named as

'*Gene Expression Data of Developing mouse Choroid Plexus*' (**GEDDMCP**). Then a web application was developed using Microsoft visual web developer 2010 express.

4.1 Database Scheme:

For every database, it is always good to keep it simple. When relational database is created, the most important issue is to select an appropriate scheme. Information must be fed in different groups and relations should be created among them in such a way to eliminate or reduce redundancy. For GEDDMCP, two groups of records (tables/relations) have been created which holds temporal and spatial gene expression data.

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

First a conceptual design of database was prepared in which the entity types, their attributes, the key-attributes, the relationships, their cardinalities and constraints were described. The underlined attributes are primary keys.

- There are two entity types which are described below:
  - **Gene:** Gene ID, Gene symbol, Gene name, Spatial Expression (ventricle), Temporal Expression, Type, Description, Images.
  - **Temporal Expression Images:** Gene ID, Gene name, E11.5 VL, E11.5 V3, E11.5 V4, E12.5 VL, E12.5 V3, E12.5 V4, E13.5 VL, E 13.5 V3, E 13.5 V4, E14.5 VL, E14.5 V3, E14.5 V4, E15.5 VL, E 15.5 V3, E 15.5 V4,

E16.5 VL, E16.5 V3, E16.5 V4, E17.5 VL, E17.5 V3, E17.5 V4, E18.5 VL, E18.5 V3, E18.5 V4, P1 VL, P1 V3, P1 V4, P2 VL, P2 V3, P1 V4, P4 VL, P4 V3, P4 V4, P7 VL, P7 V3, P7 V4, P14 VL, P14 V3, P14 V4, P28 VL, P28 V3, P28 V4.

- Relationship types with cardinality ratios:
  - Gene HAS Temporal Expression Images:
    - Cardinality ratio (1:N) as:
      - 1 Gene can have N Expression images.
      - 1 image belongs to one Gene.

4.1.2 Conceptual Scheme: ERD (entity relationship diagram):

In ERD, the entity types are represented as rectangles and their attributes as ovals. The underlined attribute is the primary key of that entity. ERD has been drawn as shown in figure Fig 4.1.

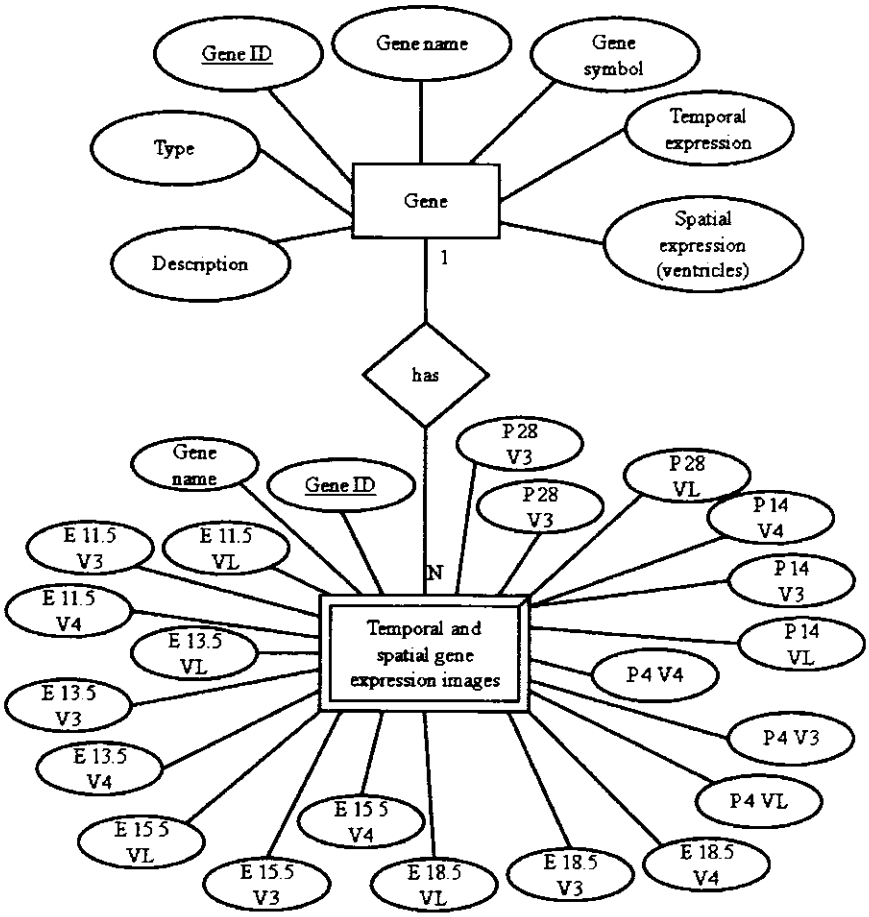


Fig 4.1: Entity relationship diagram (ERD)

4.1.3 Relational Data Model:

Relational Database in fact is a collection of integrated records in the form of 'relations' which are called 'tables' in physical design. GEDDMCP is consists of two tables which are mapped as entities in ERD. These are 'Genes' and 'Temporal and spatial gene expression images'. The columns of these tables are mapped as 'attributes' in ERD. They are described as:

- **Gene:**

  - Gene ID: It is the primary key column of the table 'Genes'. It contains Entrez Gene IDs of all the genes contained in the database.

  - Gene symbol: contains gene symbol of the corresponding gene.

  - Gene name: name of the gene.

  - Type: this column contains information about the type of gene and it can contain only two values which are 'CP specific' and 'non CP specific'. This gives information about the gene that whether it is CP specific or not.

  - Description: it contains URLs of gene information with general expression summary image of temporal and spatial gene expression in whole mouse brain.

  - Spatial expression (ventricles): is the gene specific to any ventricle or it shows its expression in all of the ventricles. This column contains values as 'all', 'lateral ventricle', 'third ventricle', 'fourth ventricle', 'lateral and third ventricle', 'lateral and fourth ventricle' and 'third and fourth ventricle'. Each tuple (row/record) contains one of these values depending upon the corresponding gene.

- Temporal expression: contain information about at which time point of development the particular gene shows its expression.

The relation 'Gene' is described in table 4.1.

- **Temporal and spatial gene expression images:**

  - Gene ID: It is the primary key column of the table 'Genes'. It contains Enters Gene IDs of all the genes contained in the database.

  - Gene name: name of the gene.

  - E 11.5: contains URL of the Gene expression image for the corresponding gene in the tuple at embryonic age 11.5.

  - E 13.5: contains URL of the Gene expression image for the corresponding gene in the tuple at embryonic age 13.5.

  - E 15.5: contains URL of the Gene expression image for the corresponding gene in the tuple at embryonic age 15.5.

  - E 18.5: contains URL of the Gene expression image for the corresponding gene in the tuple at embryonic age 18.5.

  - P 4: contains URL of the Gene expression image for the corresponding gene in the tuple at embryonic age P 4.

- P 14: contains URL of the Gene expression image for the corresponding gene in the tuple at embryonic age P 14.

- P 28: contains URL of the Gene expression image for the corresponding gene in the tuple at embryonic age P 28.

Similar data is contained in the rest of the 21 columns which are E 12.5 VL, E12.5 V3, E12.5 V4, E14.5 VL, E14.5 V3, E14.5 V4, E16.5 VL, E16.5 V3, E16.5 V4, E17.5 VL, E17.5 V3, E17.5 V4, P1 VL, P1 V3, P1 V4, P2 VL, P2 V3, P1 V4, P7 VL, P7 V3 and P7 V4.

The relation 'Temporal and spatial gene expression images' is described in table 4.2.

Table 4.1: The relation 'Gene' in GEDDMCP.

Gene ID	**Gene Symbol**	**Gene Name**	**Type**	**Description**	**Temporal expression**	**Spatial expression (ventricles)**
11421	Ace	Angiotensin I converting enzyme ...	Non Cp specific	ACE.aspx	E15.5, E18.5, P4, P14, P28	All
11669	Aldh2	Aldehyde dehydrogenase	Non Cp specific	ALDH2.aspx	E13.5, E15.5,	All

		2, …			E18.5, P4, P14, P28	
16002	Igf2	Insulin-like growth factor 2	Cp specific	IGF2.aspx	All	All
21808	Tgfb2	Transforming growth factor beta 2	Non Cp specific	TGFB2.aspx	E18.5, P14, P28	All
22139	Ttr	Transthyretin	Cp specific	TTR.aspx	All	All
16008	Igfbp2	Insulin-like growth factor …	Cp specific	IGFBP2.aspx	All	All
. . .	. . .	. . .	. . .	. . .	. . .	. . .

Table 4.2: The relation 'Temporal and spatial gene expression images' in GEDDMCP.

Gene ID	**Gene Name**	**E 11.5 VL**	**E 11.5 V3**	**E 11.5 V4**	**E 12.5 VL**	**E12.5 V3**	.....
11421	Angiotensin I converting enzyme ...	Ace/ E11.5 VL.bmp	Ace/ E11.5 V3.bmp	Ace/ E11.5 V4.bmp	Ace/ E12.5 VL.bmp	Ace/ E12.5 V3.bmp	.....
11669	Aldehyde dehydrogenase 2, ...	Aldh2/ E11.5 VL.bmp	Aldh2/ E11.5 V3.bmp	Aldh2/ E11.5 V4.bmp	Aldh2/ E12.5 VL.bmp	Aldh2/ E12.5 V3.bmp	.....
16002	Insulin-like growth factor 2	Igf2/ E11.5 VL.bmp	Igf2/ E11.5 V3.bmp	Igf2/ E11.5 V4.bmp	Igf2/ E12.5 VL.bmp	Igf2/ E12.5 V3.bmp	.....
21808	Transforming growth factor beta 2	Tgfb2/ E11.5 VL.bmp	Tgfb2/ E11.5 V3.bmp	Tgfb2/ E11.5 V4.bmp	Tgfb2/ E12.5 VL.bmp	Tgfb2/ E12.5 V3.bmp	.....
22139	Transthyretin	Ttr/ E11.5 VL.bmp	Ttr/ E11.5 V3.bmp	Ttr/ E11.5 V4.bmp	Ttr/ E12.5 VL.bmp	Ttr/ E12.5 V3.bmp	.....
16008	Insulin-like growth factor	Igfbp2/ E11.5	Igfbp2/ E11.5	Igfbp2/ E11.5	Igfbp2/ E12.5	Igfbp2/ E12.5	.....

	...	VL.bmp	V3.bmp	V4.bmp	VL.bmp	V3.bmp	
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.

Out of these two tables, three views were also created to retrieve information for the three ventricles separately. Each of these views contains six columns from the table 'Gene' which are Gene ID, Gene Name, Gene Symbol, Description, Temporal expression and Spatial expression (Ventricles) and fourteen columns from the table 'Temporal and spatial gene expression images' which are either of lateral or third or fourth ventricle.

4.1.4 Querying the Relational Data Model:

Using the ER model, a physical database was created and queries were designed according to the need of the database application. Queries were written is SQL (Structured query language). Relational DBMS is the basis for SQL. GEDDMCP is fed with the research data of this study and the aim of this software application is to act like a repository that provides information about the gene expression data for only choroid plexus of developing mouse. Therefore this application has no complicated process to be executed. It only requires **retrieving** the appropriate data which user wants. For this purpose some SELECT queries were designed depending upon the appropriate web page.

With further research progress related to CP of Mus musculus, GEDDMCP can be further updated and new queries may be inserted as per requirement.

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

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

These queries are designed as:

- To retrieve information only about the CP specific genes:

SELECT [Gene ID] AS Gene_ID, [Gene Symbol] AS Gene_Symbol, [Gene Name] AS Gene_Name, [All Images] AS All_Images, [Description] FROM [Gene] WHERE ([Type] = @Type) (Fig 4.2)

In this query the value of '@Type' is 'Cp Specific'

- To display all genes which are expressed in CP:

SELECT [Gene ID] AS Gene_ID, [Gene Symbol] AS Gene_Symbol, [Gene Name] AS Gene_Name, [Type], [Description] FROM [Gene] (Fig 4.3)

- To retrieve information only about the CP non specific genes:

SELECT [Gene ID] AS Gene_ID, [Gene Symbol] AS Gene_Symbol, [Gene Name] AS Gene_Name, [Description], [All Images] AS All_Images, [Type] FROM [Gene] WHERE ([Type] = @Type) (Fig 4.4)

In this query the value of '@Type' is 'Cp non Specific'

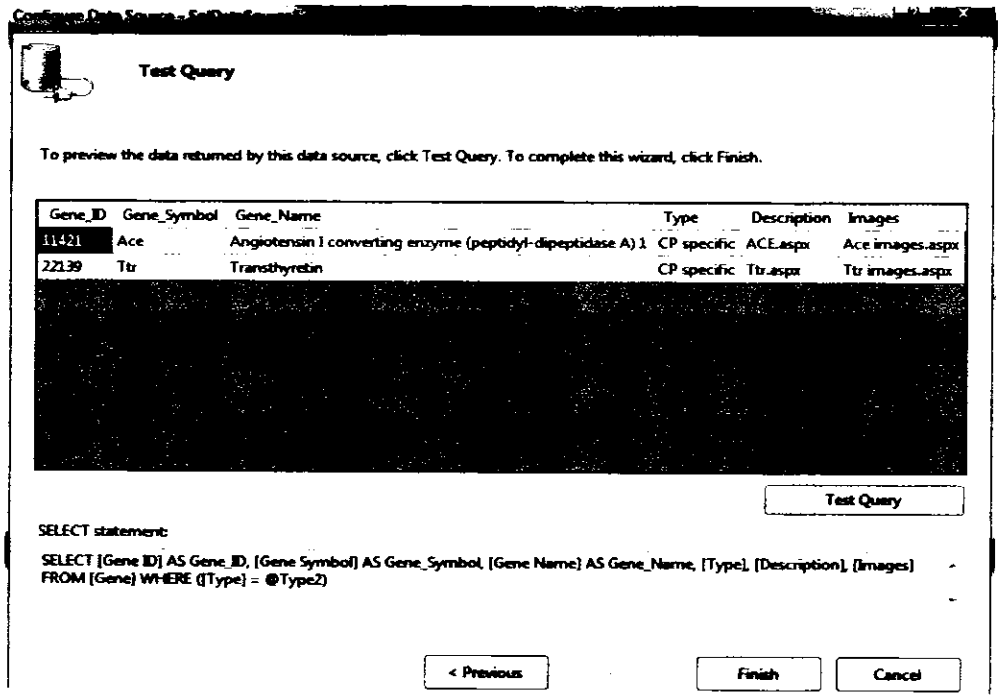


Fig 4.2: Test query for CP Specific genes.

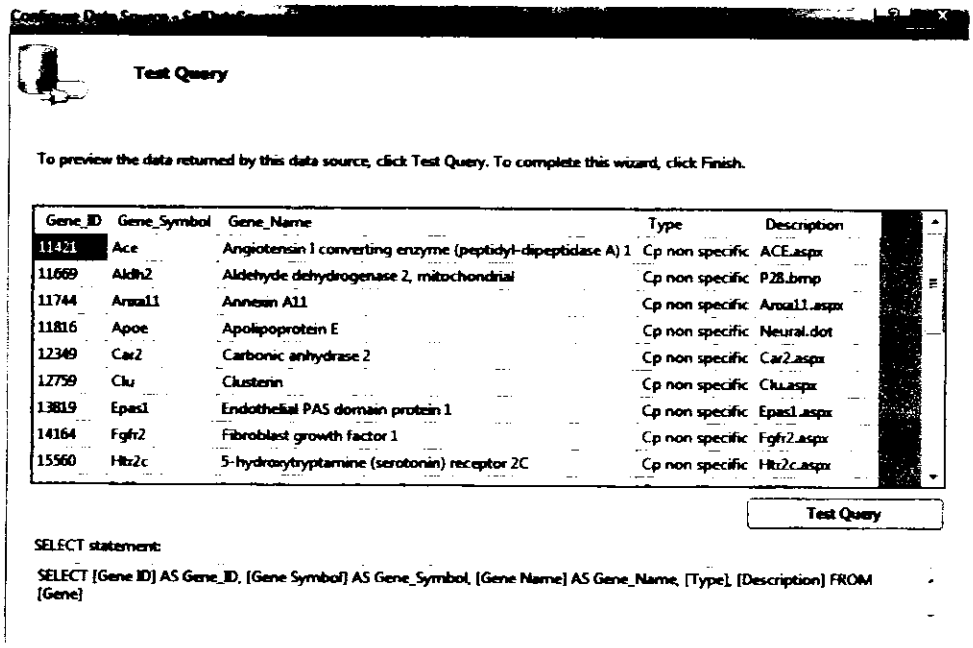


Fig 4.3: Test query for all genes

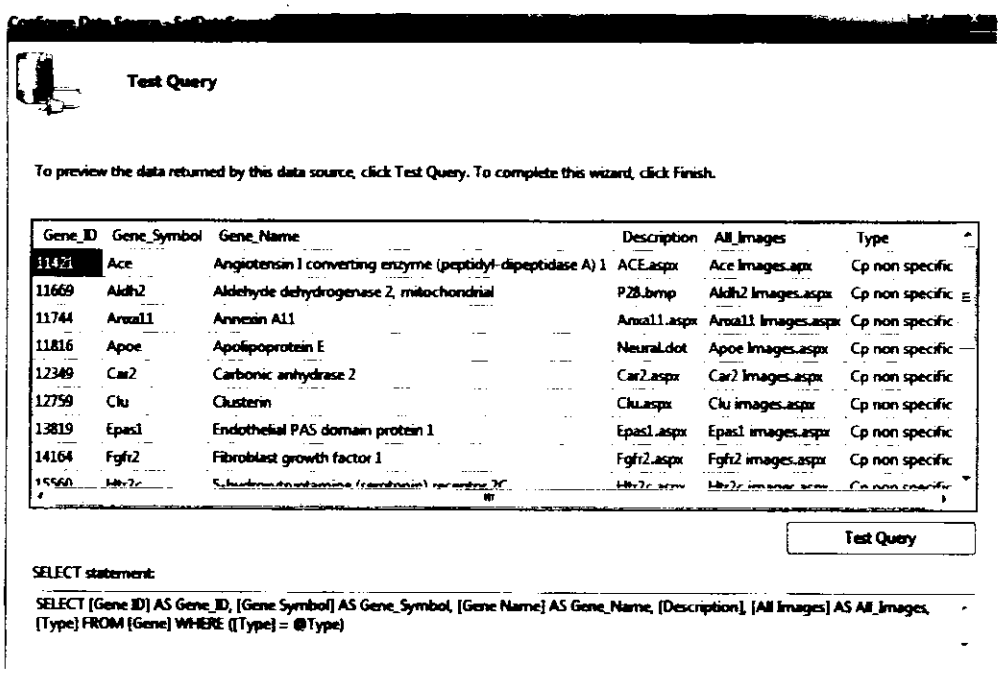


Fig 4.4: Test query for non CP Specific genes

- To retrieve spatial gene expression information about the genes according to ventricles:

  Several SELECT queries are designed for this task depending upon the respective ventricle, for which the gene expression data is required. Query for Lateral ventricle is:

  SELECT [Gene ID] AS Gene_ID, [Gene Symbol] AS Gene_Symbol, [Gene Name] AS Gene_Name, [Description], [All Images] AS All_Images, [Type], [Ventricle] FROM [Gene] WHERE (Ventricle = "All") OR (Ventricle = "1") OR (Ventricle = "1, 3") OR (Ventricle = "1, 4") (Fig 4.5).

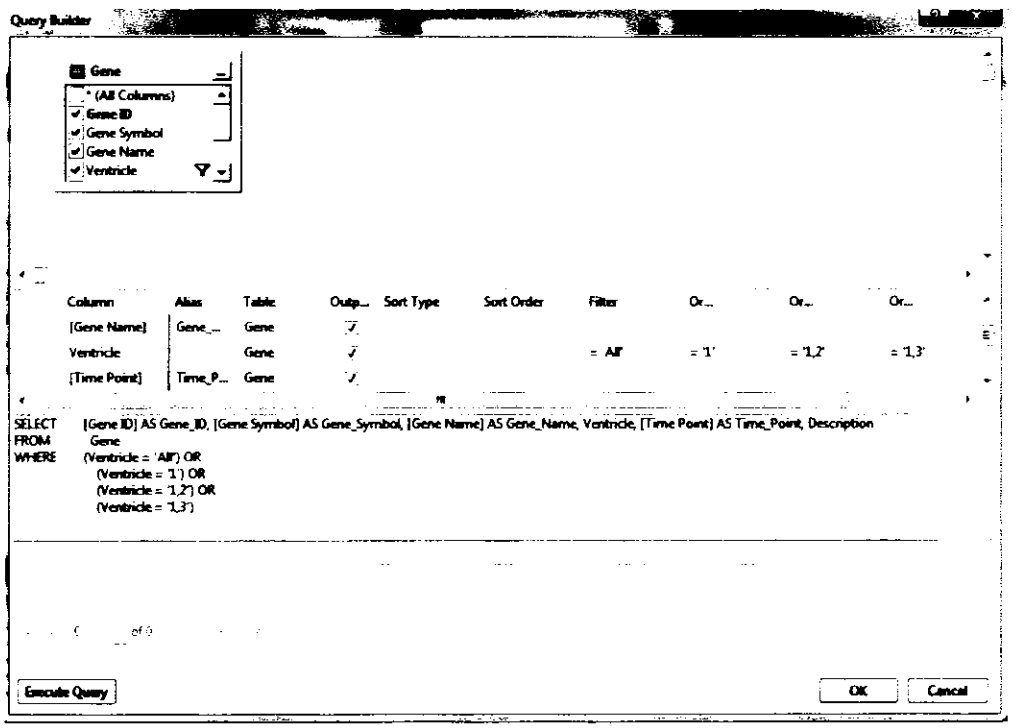


Fig 4.5: Query in query builder to retrieve genes of lateral ventricles.

- To create view:

Views are created to retrieve information about one of the three ventricles from both of the tables simultaneously. The query for lateral ventricle is:

SELECT Gene.[Gene ID] AS Gene_ID, Gene.[Gene Symbol] AS Gene_Symbol, Gene.[Gene Name] AS Gene_Name, Gene.Ventricle, Gene.[Time Point] AS Time_Point, Gene.Description, [Temporal and spatial gene expression images].Gene_name AS name, [Temporal and spatial gene expression images].[E11.5 VL] AS cloumn1, [Temporal and spatial gene expression images].[E12.5 VL] AS cloumn2, [Temporal and spatial gene expression images].[E13.5 VL] AS cloumn3, [Temporal and spatial gene

expression images].[E14.5 VL] AS cloumn4, [Temporal and spatial gene expression images].[E15.5 VL] AS cloumn5, [Temporal and spatial gene expression images].[E16.5 VL] AS cloumn6, [Temporal and spatial gene expression images].[E17.5 VL] AS cloumn7, [Temporal and spatial gene expression images].[E18.5 VL] AS cloumn8, [Temporal and spatial gene expression images].[P1 VL] AS P1_VL, [Temporal and spatial gene expression images].[P2 VL] AS P2_VL, [Temporal and spatial gene expression images].[P4 VL] AS P4_VL, [Temporal and spatial gene expression images].[P7 VL] AS P7_VL, [Temporal and spatial gene expression images].[P14 VL] AS P14_VL, [Temporal and spatial gene expression images].[P28 VL] AS P28_VL FROM Gene CROSS JOIN , [Temporal and spatial gene expression images] WHERE (Gene.Ventricle = "All") OR (Gene.Ventricle = "1") OR (Gene.Ventricle = "1, 3") OR (Gene.Ventricle = "1, 4") (Fig 4.6)

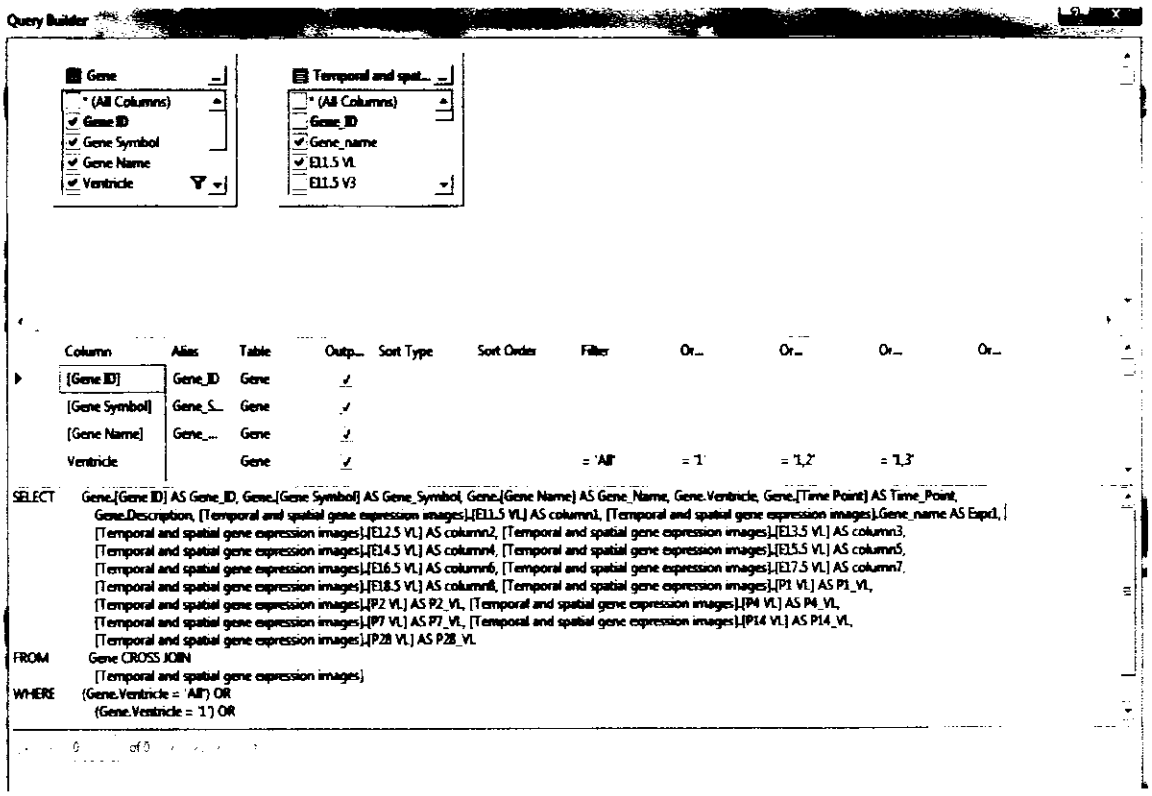


Fig 4.6: Query to create view

4.2 Use Case Diagram:

The aim of this software application is to design a repository which holds temporal and spatial gene expression data in form of images and its annotation in the form of reports. This repository will provide important information and gene expression data to different users/researches seeking information about gene expression data of choroid plexus and different factors of cerebrospinal fluid which are thought to be secreted into the fluid by choroid plexus during different embryonic and postnatal stages of the development of mouse brain. Thus the application does not contain any complex

processes to be executed when user interacts with the application. Rather it just retrieves the data which user wants by making selection operations.

The following useful terms and entities were identified for this system:

- **Users/researcher** who want to retrieve Gene expression information for Choroid plexus.
- Gene expression **data** for Cp genes.

4.2.1 Actors:

Actors in a use case diagram are those entities which performs certain role or business process. In this system the actors are:

- Users who make selection for retrieving data.

4.2.2 Use cases:

Use cases in a use case diagram define the processes or the basic functionality of a system. The primary business flow in GEDDMCP which can be a use case is

- To retrieve gene expression data.

But, some discrete processes in this primary business flow can be determined. To retrieve specific information user might want to look at complete list of existing genes of Choroid plexus, view temporal and spatial gene expression images of the gene of their interest, introductory information about the gene (other than gene expression

information) or retrieve information about our research project. Therefore within 'retrieve gene expression data' use case the following sub processes can be identified:

- View complete gene list.
- Retrieve temporal or spatial gene expression or ISH images.
- Retrieve introduction about genes.

Therefore the use case diagram is as follows (fig 4.7).

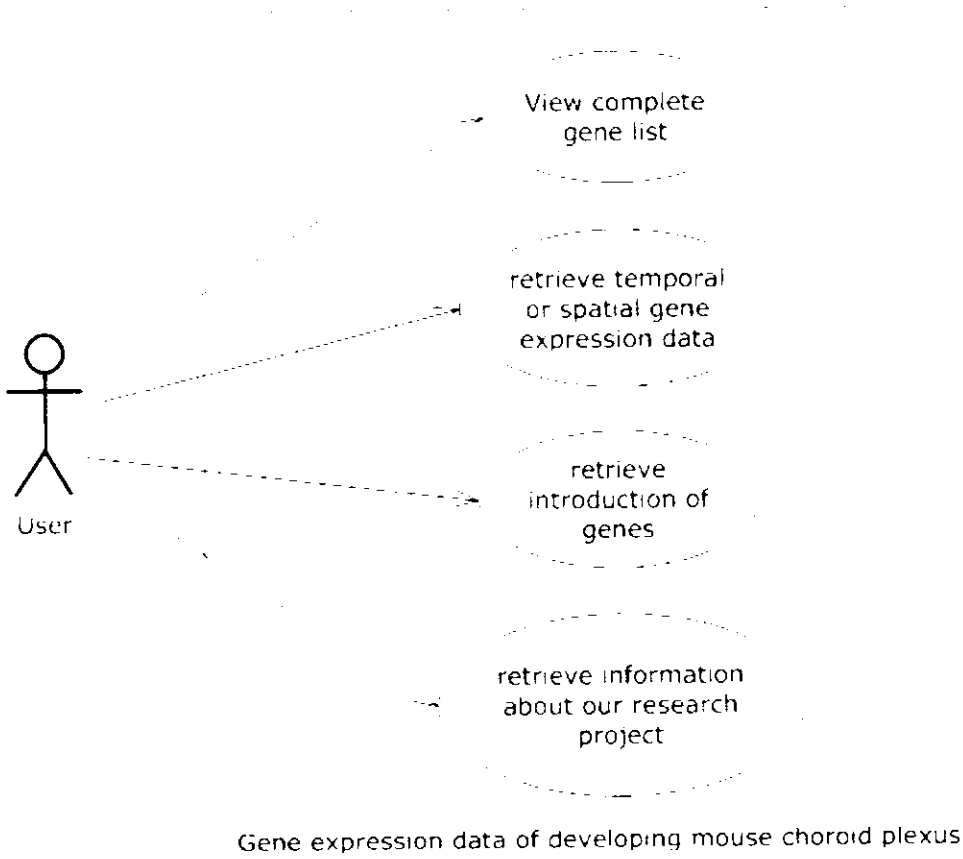


Fig 4.7: Use case diagram

4.3 Activity diagram:

Activity diagram was drawn to represent operational workflow the system (fig 4.8). Whenever a user interacts with GEDDMCP, he/she has to go through certain selection processes to reach to the data of interest. In this type of data retrieval system, the operational workflow varies with different users. Some might want to know about all of the genes which are found to be expressed in choroid plexus, some nay need to know about the gene expression with respects to ventricles only others might be interested in the data for a certain age or for only embryonic or post-natal ages, some are only interested in images others are interested to get data for both the expression images and the introductory literature about genes etc. For all the users, their activity ends up getting the data of their interest.

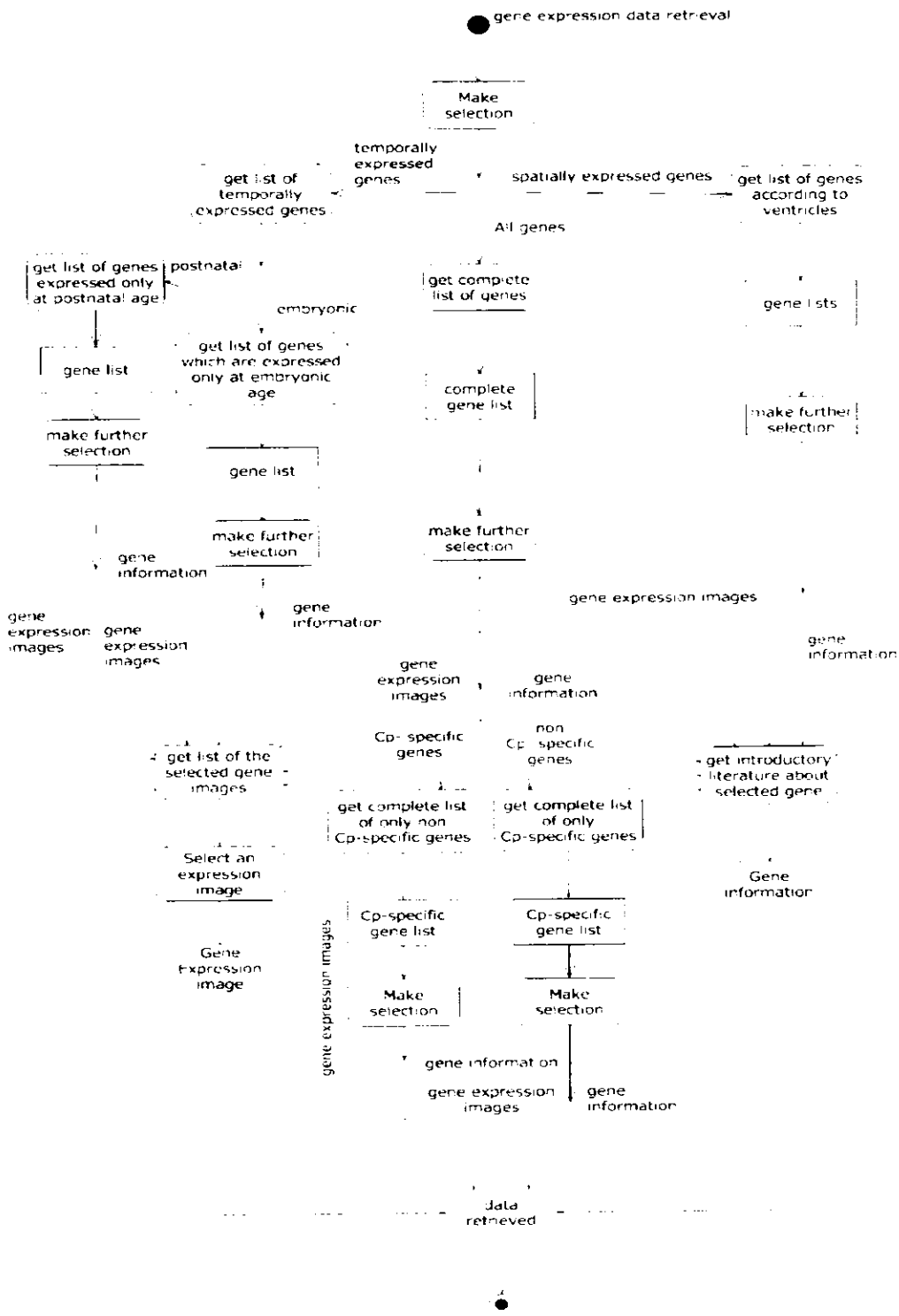


Fig 4.8: Activity diagram

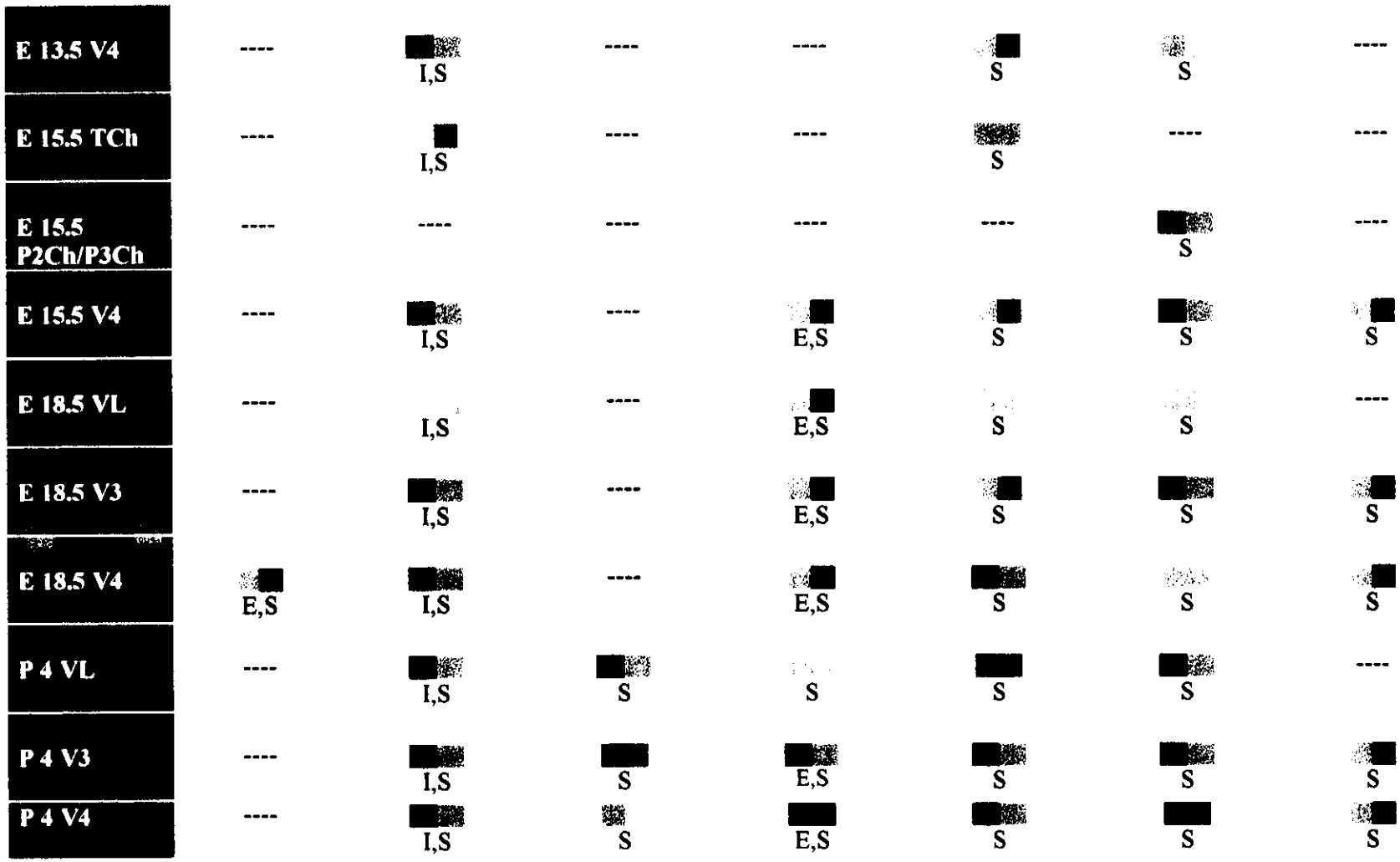
CHAPTER 5

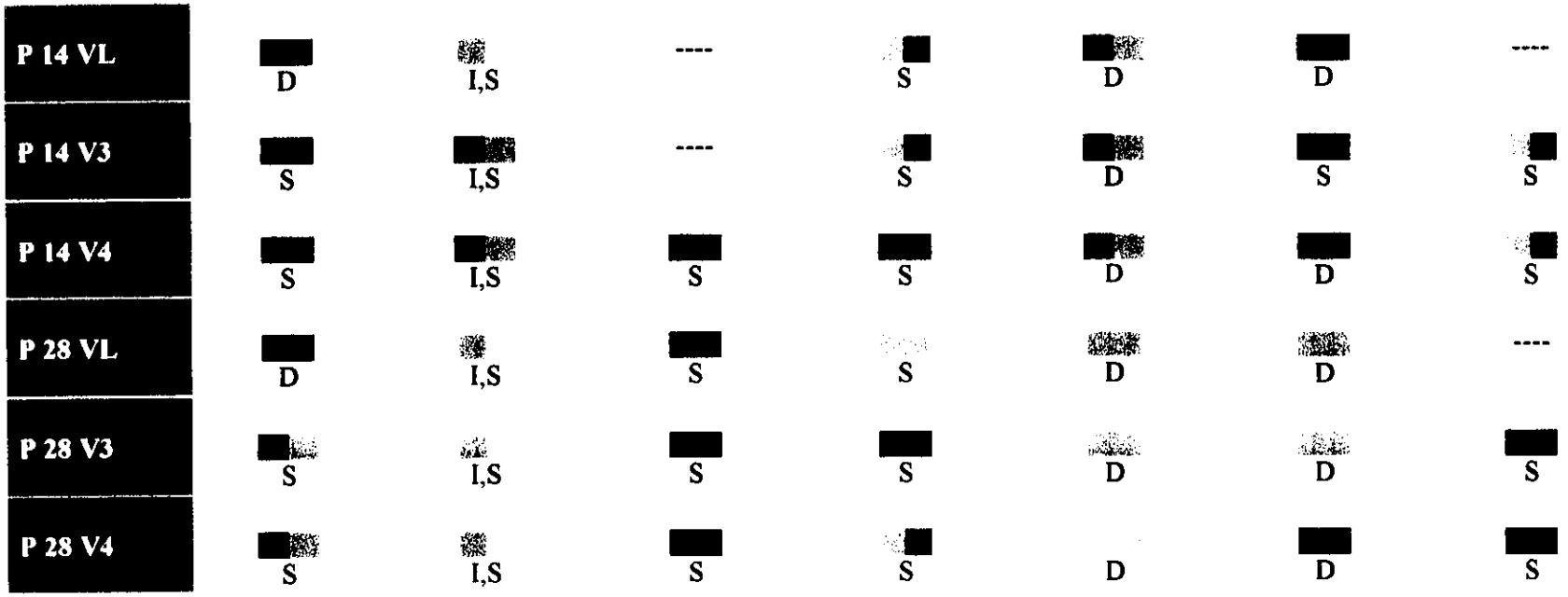
RESULTS

Results of gene expression data analysis for 20 CP genes are described under their respective headings. The results are categorized into four categories which are CP-specific genes, genes specific to certain time points, CP-specific genes specific to certain time points and other genes. These 20 genes long with their temporal and spatial gene expression level and pattern are listed in the following tables (Table 5.1 to Table 5.8). These tables are created according to the previously mentioned key of expression level described in Chapter 3 table 3.4. Same key is used to describe the results throughout this chapter. Description of these genes and with relation to CSF is described in Chapter 2.

Table 5.1: Spatial and temporal gene expression of genes specific to certain time points. 'S' is used for scattered expression pattern, 'D' is used for dense expression pattern, 'E' is used for choroidal epithelial specificity and 'I' is used for inner choroidal specificity. Cell with dotted line indicates that gene expression is absent.

Time point ↓ / Gene →	Tgfβ2 (Transforming growth factor beta-2)	Igfbp-3 (Insulin-like growth factor-binding protein 3)	Anxa 11 (Annexin A11)	A2m (alpha-2-macroglobulin)	Aldh-2 (Aldehyde dehydrogenase 2)	CD 164 (antigen)	Hspb8 (Heat shock protein 8)
E 11.5 TCh	----	----	----	----	----	----	----
E 11.5 P2Ch/P3Ch	----	----	----	----	----	----	----
E 11.5 V4	----	----	----	----	----	----	S
E 13.5 TCh	----	I,S	----	----	S	S	----
E 13.5 P2Ch/P3Ch	----	I,S	----	----	S	S	----


E 13.5 V4
E 15.5 TCh
E 15.5 P2Ch/P3Ch
E 15.5 V4
E 18.5 VL
E 18.5 V3
E 18.5 V4
P 4 VL
P 4 V3
P 4 V4
I,S
E,S
S


P 14 VL
D
I,S

S
D
D

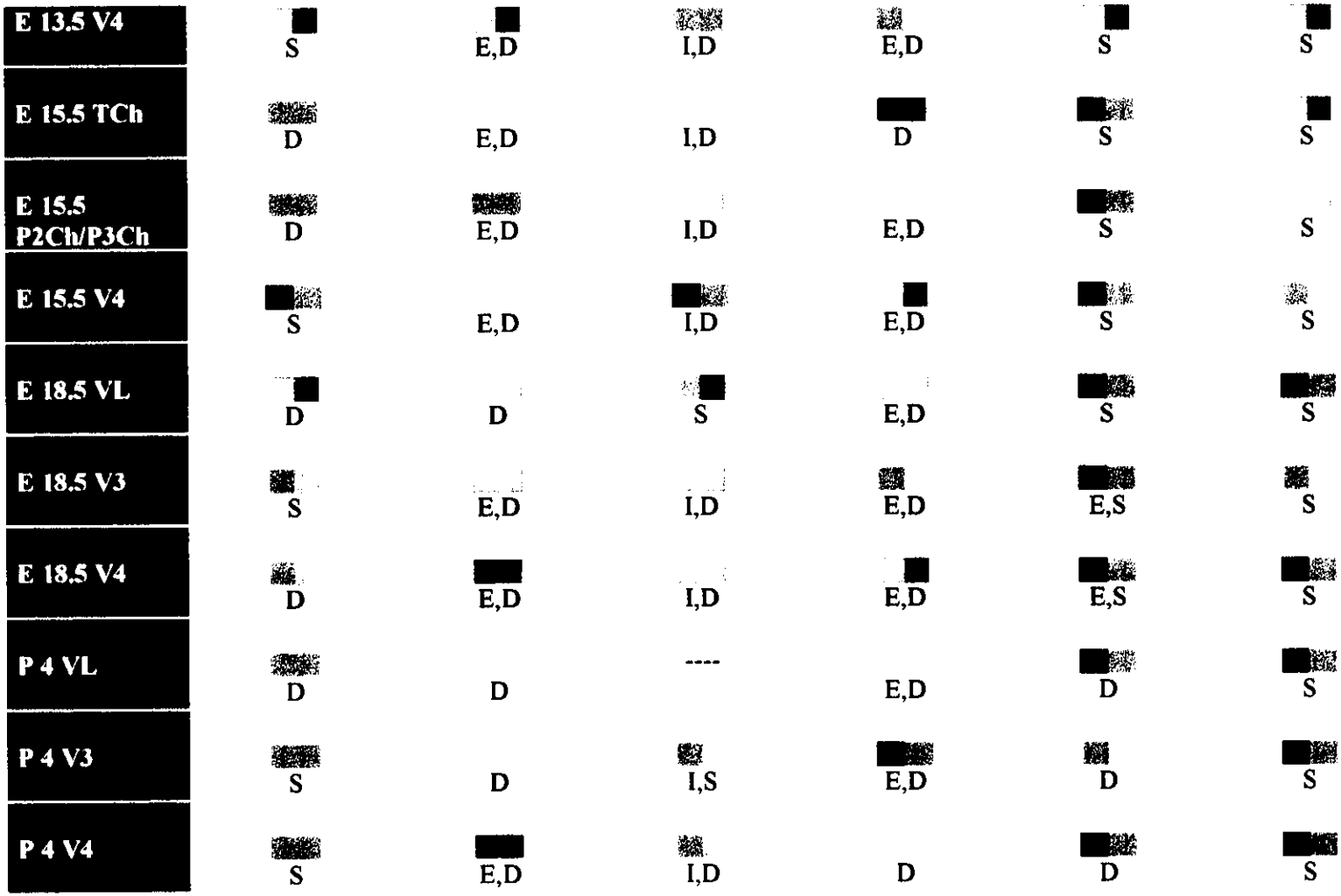
P 14 V3
S
I,S

S
D
S
S
P 14 V4
S
I,S
S
S
D
D
S
P 28 VL
D
I,S
S
S
D
D

P 28 V3
S
I,S
S
S
D
D
S
P 28 V4
S
I,S
S
S
D
D
S

Table 5.2: Spatial and temporal gene expression of genes which are expressed in all of the time points. 'S' is used for scattered expression pattern, 'D' is used for dense expression pattern, 'E' is used for choroidal epithelial specificity and 'I' is used for inner choroidal specificity. Cell with dotted line indicates that gene expression is absent.

Gene / Time point	Igf-2 (Insulin-like growth factor-2)	Igfbp-2 (Insulin-like growth factor-binding protein 2)	Igfbp-4 (Insulin-like growth factor-binding protein 4)	Clu (Clusterin)	Car-2 (Carbonic anhydrase 2)	Apoe (Apolipoprotein E)
E 11.5 TCh	S	D	----	S	S	S
E 11.5 P2Ch/P3Ch	D	D	D	S	S	S
E 11.5 V4	S	D	D	D	S	S
E 13.5 TCh	D	E,D	I,D	E,D	S	S
E 13.5 P2Ch/P3Ch	D	E,D	I,D	E,D	S	S


E 13.5 V4
S
E,D
I,D
E,D
S
S
E 15.5 TCh
D
E,D
I,D
D
S
S
E 15.5 P2Ch/P3Ch
D
E,D
I,D
E,D
S
S
E 15.5 V4
S
E,D
I,D
E,D
S
S
E 18.5 VL
D
D
S
E,D
S
S
E 18.5 V3
S
E,D
I,D
E,D
E,S
S
E 18.5 V4
D
E,D
I,D
E,D
E,S
S
P 4 VL
D
D

E,D
D
S
P 4 V3
S
D
I,S
E,D
D
S
P 4 V4
S
E,D
I,D
D
D
S

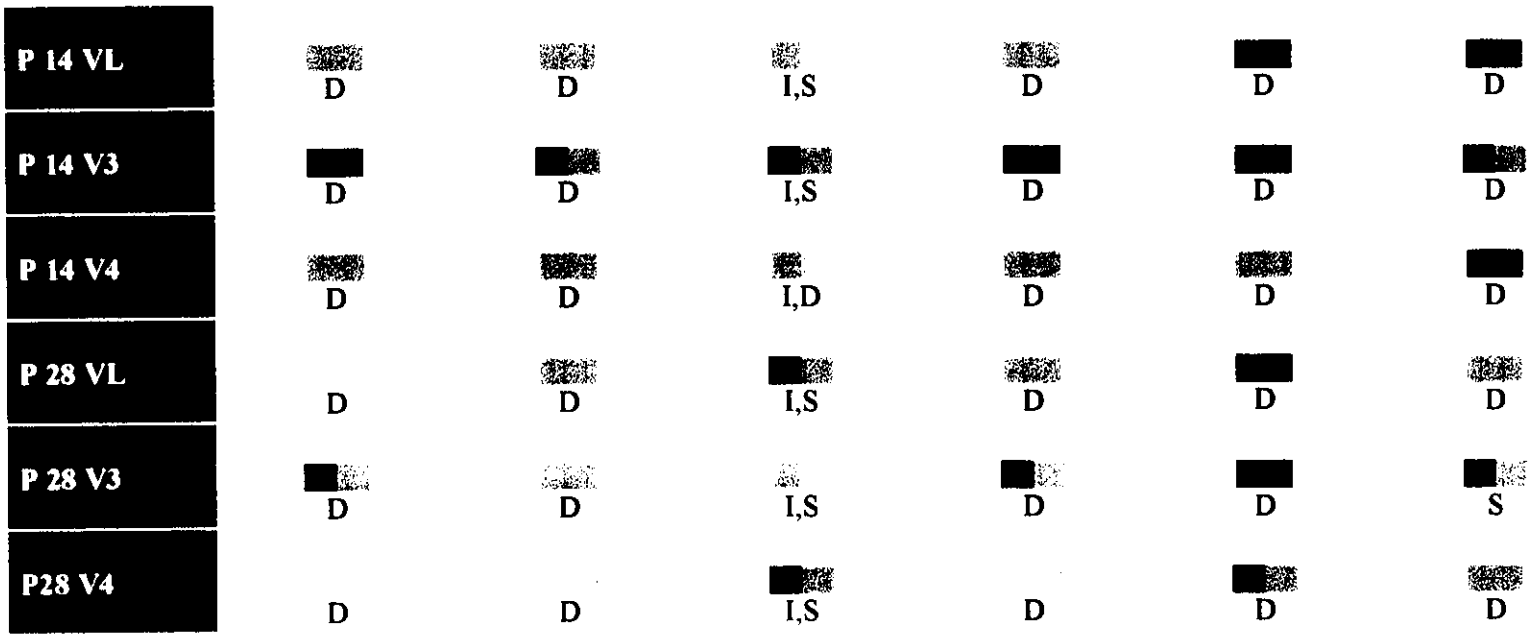
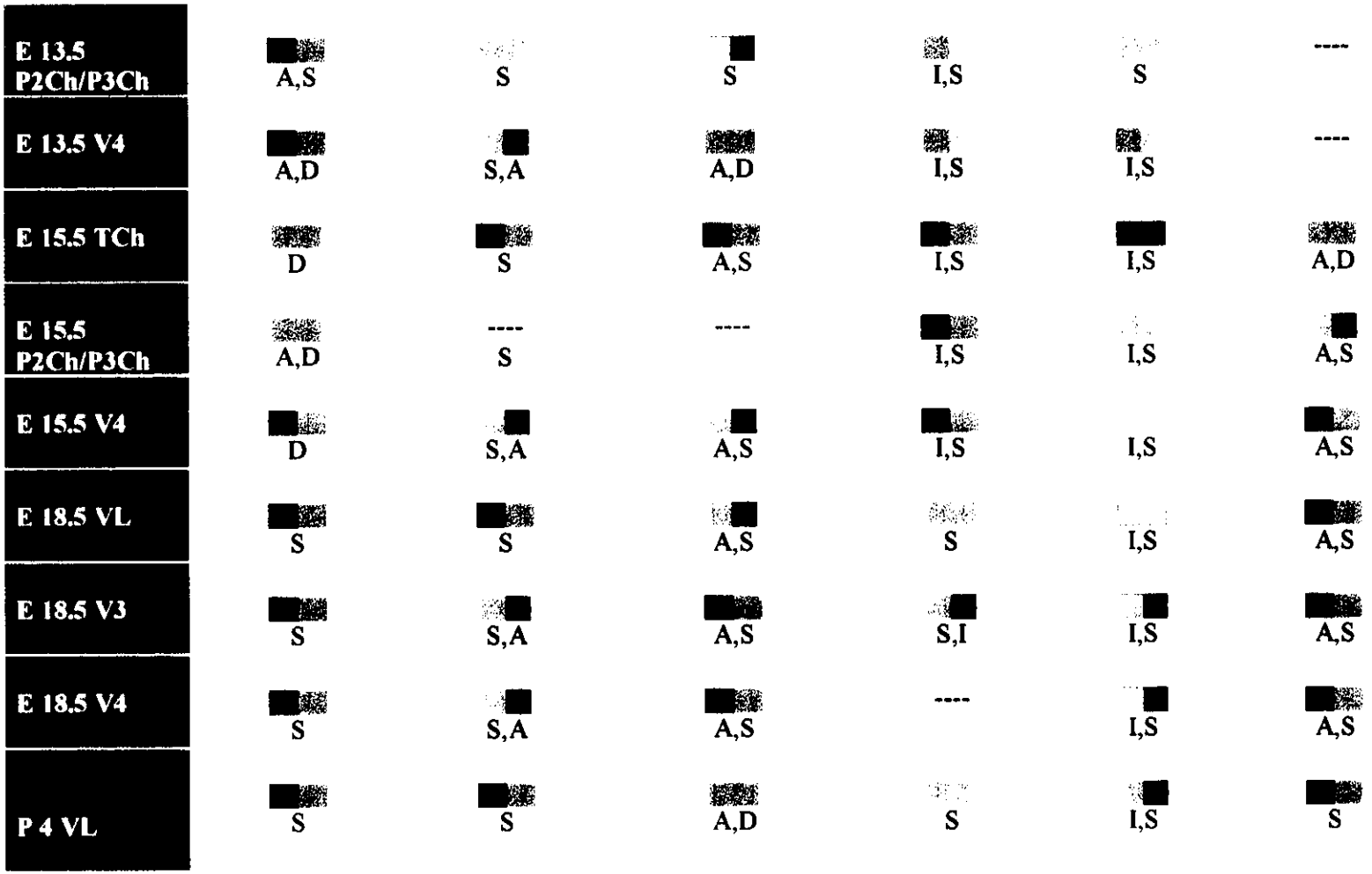

P 14 VL
D
D
I,S
D
D
D
P 14 V3
D
D
I,S
D
D
D
P 14 V4
D
D
I,D
D
D
D
P 28 VL
D
D
I,S
D
D
D
P 28 V3
D
D
I,S
D
D
S
P28 V4
D
D
I,S
D
D
D

Table 5.3: Spatial and temporal gene expression of genes which are present in all of the time points and one CP-Specific gene (ACE) which is temporally specific to some of the seven time points. 'S' is used for scattered expression pattern, 'D' is used for dense expression pattern, 'E' is used for choroidal epithelial specificity and 'I' is used for inner choroidal specificity. Cell with dotted line indicates that gene expression is absent.

Gene / Time point	Fgfr2 (Fibroblastic growth factor receptor-2)	Itpr-1 (inositol 1,4,5-triphosphate receptor, type 1)	Htr2c (5-hydroxytryptamine (serotonin) receptor 2C)	Epas1 (Endothelial PAS domain protein 1)	Igfbp-7 (Insulin-like growth factor-binding protein 7)	Ace (Angiotensin I converting enzyme)
E 11.5 TCh	----	S	----	----	----	----
E 11.5 P2Ch/P3Ch	----	S	----	S	----	----
E 11.5 V4	D	S	S	S	S	----
E 13.5 TCh	A,D	S	A,S	I,S	I,S	----


E 13.5 P2Ch/P3Ch
A,S
S
S
I,S
S

E 13.5 V4
A,D
S,A
A,D
I,S
I,S

E 15.5 TCh
D
S
A,S
I,S
I,S
A,D
E 15.5 P2Ch/P3Ch
A,D

S

I,S
I,S
A,S
E 15.5 V4
D
S,A
A,S
I,S
I,S
A,S
E 18.5 VL
S
S
A,S
S
I,S
A,S
E 18.5 V3
S
S,A
A,S
S,I
I,S
A,S
E 18.5 V4
S
S,A
A,S

I,S
A,S
P 4 VL
S
S
A,D
S
I,S
S

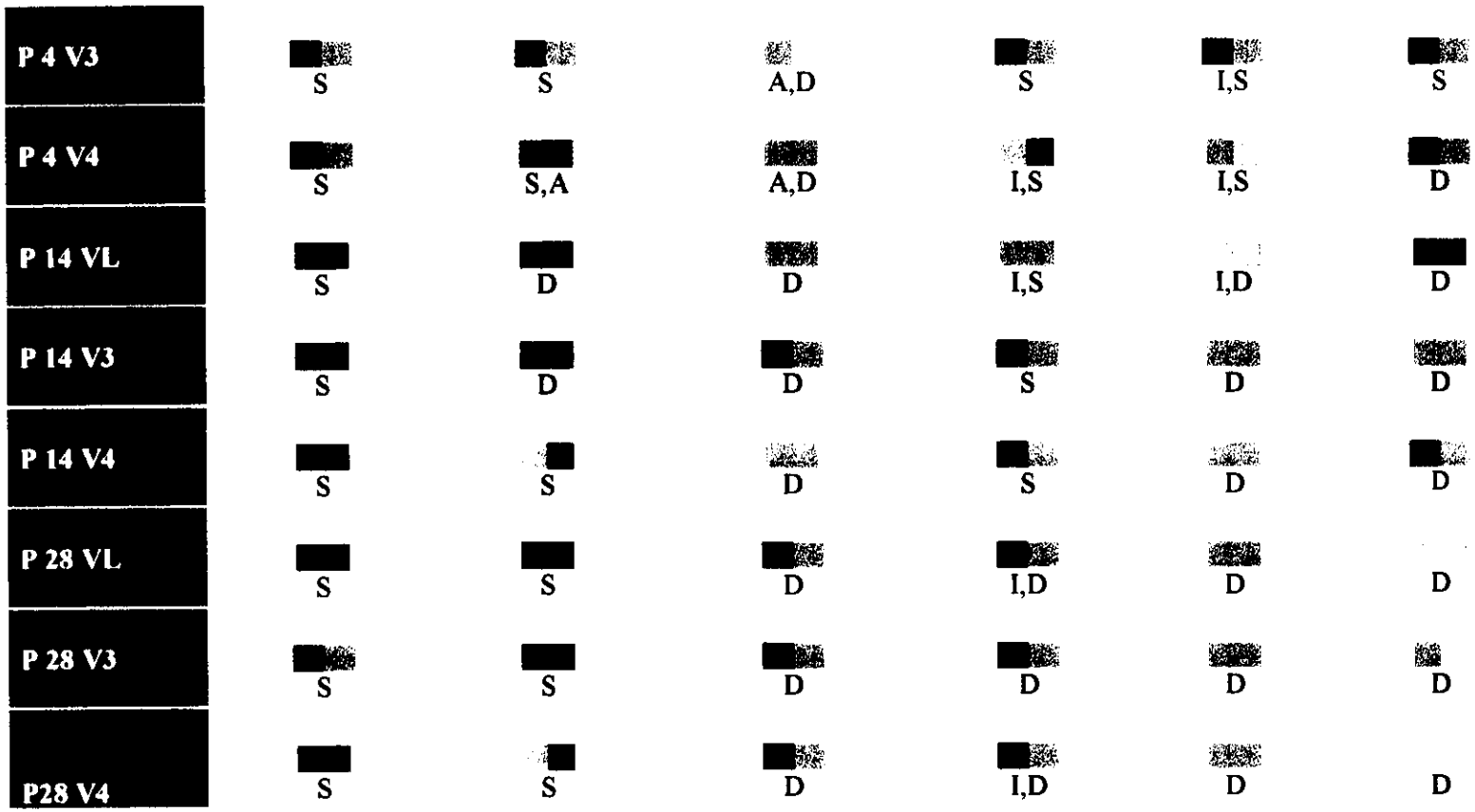

P 4 V3
S
S
A,D
S
I,S
S
P 4 V4
S
S,A
A,D
I,S
I,S
D
P 14 VL
S
D
D
I,S
I,D
D
P 14 V3
S
D
D
S
D
D
P 14 V4
S
S
D
S
D
D
P 28 VL
S
S
D
I,D
D
D
P 28 V3
S
S
D
D
D
D
P28 V4
S
S
D
I,D
D
D

Table 5.4: Spatial and temporal gene expression of CP-Specific gene Ttr across three early embryonic time points. 'D' is used for dense expression pattern.

Gene \ Time point	E 11.5 TCh	E 11.5 P2Ch/P3Ch	E 11.5 V4	E 12.5 TCh	E 12.5 P2Ch/P3Ch	E 12.5 V4	E 13.5 TCh	E 13.5 P2Ch/P3Ch	E 13.5 V4
Ttr (Transthyretin)	D	D	D	D	D	D	D	D	D

Table 5.5: Spatial and temporal gene expression of CP-Specific gene Ttr across three embryonic time points. 'D' is used for dense expression pattern.

Gene \ Time point	E 14.5 TCh	E 14.5 P2Ch/P3Ch	E 14.5 V4	E 15.5 TCh	E 15.5 P2Ch/P3Ch	E 15.5 V4	E 16.5 VL	E 16.5 V3	E 16.5 V4
Ttr (Transthyretin)	D	D	D	D	D	D	D	D	D

Table 5.6: Spatial and temporal gene expression of CP-Specific gene Ttr across two embryonic time points and one postnatal time point. 'D' is used for dense expression pattern.

Gene \ Time point	E 17.5 VL	E 17.5 V3	E 17.5 V4	E 18.5 VL	E 18.5 V3	E 18.5 V4	P 1 VL	P 1 V3	P 1 V4
Ttr (Transthyretin)	D	D	D	D	D	D	D	D	D

Table 5.7: Spatial and temporal gene expression of CP-Specific gene Ttr across two later embryonic and one early postnatal time point. 'D' is used for dense expression pattern.

Gene \ Time point	P 2 VL	P 2 V3	P 2 V4	P 4 VL	P 4 V3	P 4 V4	P 7 VL	P 7 V3	P 7 V4
Ttr (Transthyretin)	D	D	D	D	D	D	D	D	D

Table 5.8: Spatial and temporal gene expression of CP-Specific gene Ttr across two later postnatal time points. 'D' is used for dense expression pattern.

Gene \ Time point	P 14 VL	P 14 V3	P 14 V4	P 28 VL	P 28 V3	P 28 V4
Ttr (Transthyretin)	D	D	D	D	D	D

5.1 CP- Specific genes:

Expression of a gene Transthyretin has been found to be confined to choroid plexus only and is not present in any other part of the brain or glial cells. This gene is named as 'CP- Specific gene'. There is no spatial and temporal specificity for its gene expression and the expression has been found in all of the three ventricles and throughout the seven time points. The detailed temporal and spatial gene expression results are as follows:

5.1.1 Ttr (Transthyretin):

The gene Ttr is found to be highly expressed CP- Specific gene. Gene expression data for this gene has been analyzed for a total of fourteen instead of seven time points. The expression pattern is 'dense' spatially and temporally throughout the fourteen time points in all of the ventricles. No spatial specificity with regards to internal structure of CP has been found which means that the expression of Ttr is not confined to the outer epithelial or the inner layer of CP but the gene is expressed by the complete CP including all of the three CPs. The overall expression is dense and excellent which shows that the protein Transthyretin is actively produced by choroid plexus throughout the development and in good amount.

5.1.1.1 E 11.5 and E 12.5:

An 'excellent' level of expression for Ttr is detected in choroidal tissues. This means that the choroidal tissues start to produce Ttr even during very early stages of

development when the choroidal tissues are not completely differentiated into functional CP.

The observed gene expression pattern was 'dense' in all of the three CPs in both of the time points. The expression was of 'excellent' level in telencephallic choroidal tissues and choroidal tissues of prosomeres and is of 'very good' level in choroidal tissues of fourth ventricle (Fig 5.1).

5.1.1.2 E 13.5, E 14.5 E 15.5, E 16.5, E 17.5 and E 18.5:

At E 13.5 and E 14.5 the structure of choroid plexus is apparent, though not fully developed, but can be distinguished easily. The expression pattern was found to be 'dense' spatially and temporally with no spatial specificity in CP. But the expression level varies a little. Likewise the previous two time points of E 11.5 and E 12.5, the level of gene expression during E 13.5, E14.5 and E16.5 is 'excellent' in telencephallic choroidal tissues and choroidal tissues of prosomeres and is 'good' in choroidal tissues of fourth ventricle (Fig 5.2).

During E 15.5 the expression level was 'good' in choroidal tissues of all of the three ventricles and during E 18.5 the expression level was 'excellent' in all of the three CPs. During E17.5, the expression level was 'excellent' in CP of third ventricle and 'good' in CP of lateral and fourth ventricles.

Fig 5.1: ISH image (white) and its corresponding expression image (black) of Ttr. A: Choroidal tissues of lateral and third ventricle at E12.5. B: Choroidal tissues of fourth ventricle at E12.5.

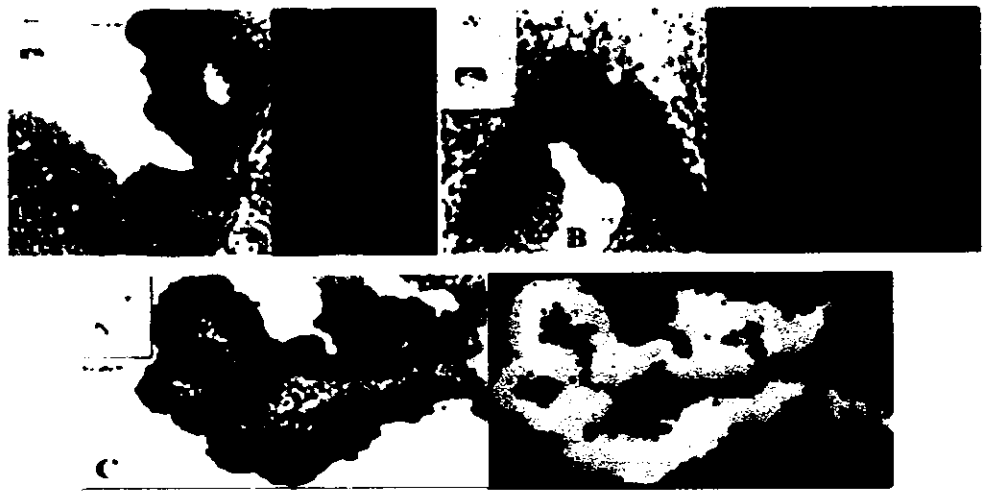


Fig 5.2: ISH image (white) and its corresponding expression images (black) of Ttr. A: CP of lateral ventricle at E13.5. B: CP of third ventricle at E13.5. C: CP of fourth ventricle at E13.5.

5.1.1.3 P 1, P 2, P 4, P 7, P 14 and P 28:

Similar to the embryonic time points Ttr shows an excellent expression in the postnatal time points too. The observed expression pattern was dense with no spatial specificity with regards to the internal structure of CP.

During the early postnatal time points of P1, P2 and P4, the observed level of gene expression was 'excellent' spatially and temporally (Fig 5.3).

During P7, the observed expression was of 'excellent' level in CP of third ventricle and 'good' in the CP of lateral and fourth ventricles.

During the late postnatal ages of P 14 and P 28, a decline was observed in the expression level (Fig 5.4). The expression was not of 'excellent' level but was good in all of the three CPs during the both postnatal ages.

5.1.1.4 Expression of Ttr in Brain other than Choroid Plexus:

Other than choroid plexus, there was no place in the developing mouse brain where the expression of the gene Transthyretin was detected. Thus gene is CP-specific gene and is expressed only in CP throughout the fourteen time points of development which were examined above (Fig 5.5).

In telencephallic vesicle and prosomeres there exists CP of lateral and third ventricles and in pontine hind brain and medulary hind brain region, there exist CP of fourth ventricle and the expression of Ttr was only detected in these regions.

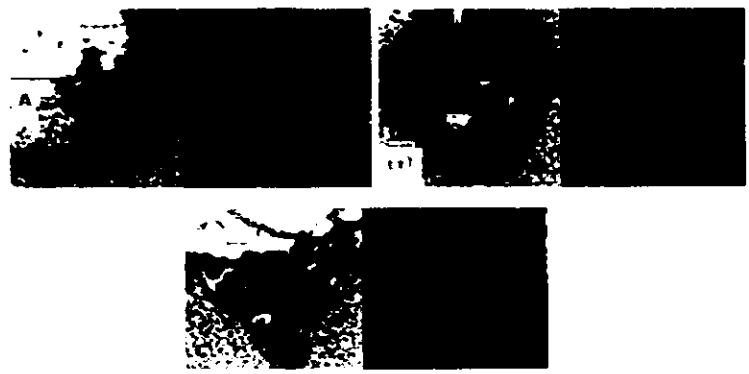


Fig 5.3: ISH image (white) and its corresponding expression images (black) of Ttr in CP. A: CP of lateral ventricle at P 2. B: CP of third ventricle at P 2. C: CP of fourth ventricle at P 2.

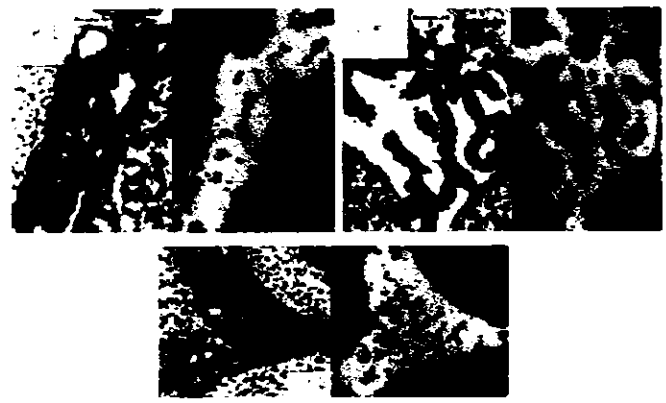


Fig 5.4: ISH image (white) and its corresponding expression images (black) of Ttr in CP. A: CP of lateral ventricle at P 28. B: CP of third ventricle at P 28. C: CP of fourth ventricle at P 28.

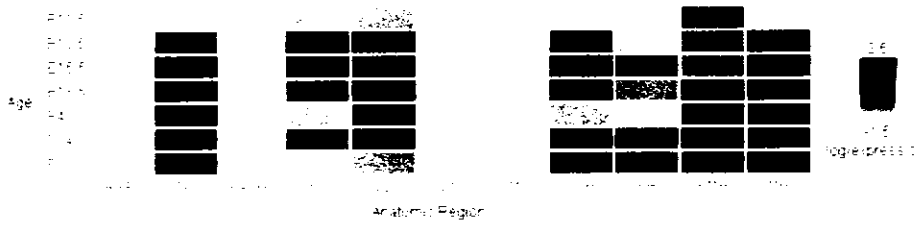


Fig 5.5: Expression summary of Ttr in Mus musculus brain.

5.2 Genes Specific to certain time points:

Gene expression of some genes has been found to be temporally specific to one or more but not all of the seven time point. But this temporal specificity is not the same for all of these genes and it varies with every individual gene. Out of the twenty genes whose gene expression data was analyzed, seven genes have been found to show such temporal specificity for their expression in choroid plexus. Out of these seven none of the genes showed any kind of similarity or interconnection with another gene in terms of expression pattern, specificity or level. Results of these seven genes are as follows:

5.2.1 Tgfβ2 (Transforming growth factor beta-2):

The gene expression of Tgfβ2 is specific only to two postnatal time points which are P14 and P28, and to the fourth ventricle of late embryonic time point of E 18.5. Other than these time points, no expression of this gene was observed in any of the CPs. The overall expression pattern of this gene has been found to be 'scattered' throughout the structure of CP.

5.2.1.1 E18.5:

At age E18.5, no expression was detected in the CP of lateral and third ventricle but a very little expression was present in the CP of fourth ventricle. This showed that among all of the choroid plexus, only the CP of fourth ventricle was producing Tgfβ2 at E18.5. Although the expression was rare. The expression was of 'low' level and was found to be expressed in a small portion of CP and not in the

whole CP of fourth ventricle. The expression was found to be confined to the outer epithelial layer of CP and not throughout the structure of CP (Fig 5.6).

5.2.1.2 P 14 and P 28:

During P 14 and P 28, considerable expression of Tgfβ2 was detected in all of the CPs. The expression pattern was 'dense' in case of CP of lateral ventricle during both of the postnatal ages but was 'scattered' in rest of the two CPs. The expression level was green which is 'fair' but not a good one (Fig 5.7).

5.2.1.3 Expression of Tgfβ2 in Brain other than Choroid Plexus:

Other than choroid plexus, a good expression of Tgfβ2 was detected in hippocampal formation at P14 and P28 only. Overall, Tgfβ2 did not show a very strong expression in any area of the brain at any time point (Fig 5.8). Yet there was some considerable expression in some regions at age E 15.5, E 18.5, P 14 and P 28 respectively. At P 14 and P 28, its expression in telencephallic vesicle was observed.

5.2.2 Igfbp-3 (Insulin-like growth factor binding protein 3):

Gene expression of Igfbp-3 has been observed during six out of the seven time point which are E 13.5 to P 28. This suggests that the gene does not show any expression in CP during the early stage of E 11.5. The overall gene expression was found to be very interesting in case of this protein as the observed gene expression was 'inner' specific while gene expression pattern was 'scattered'. This means that the expression pattern

Fig 5.6: ISH images (white) and expression images (black) of Tgfβ-2 in CP of fourth ventricle at E 18.5

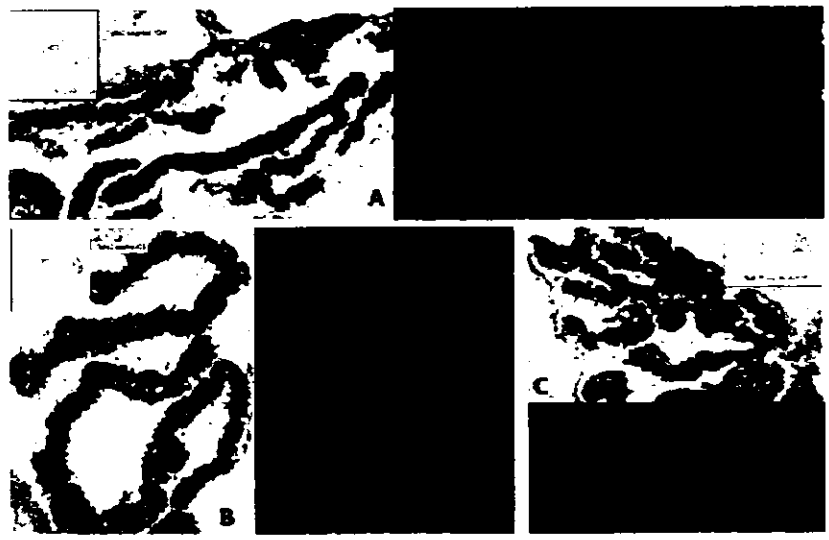


Fig 5.7: ISH images (white) and expression images (black) of Tgfβ2 in CP at P 14. A: CP of lateral ventricle. B: CP of third ventricle. C: CP of fourth ventricle.

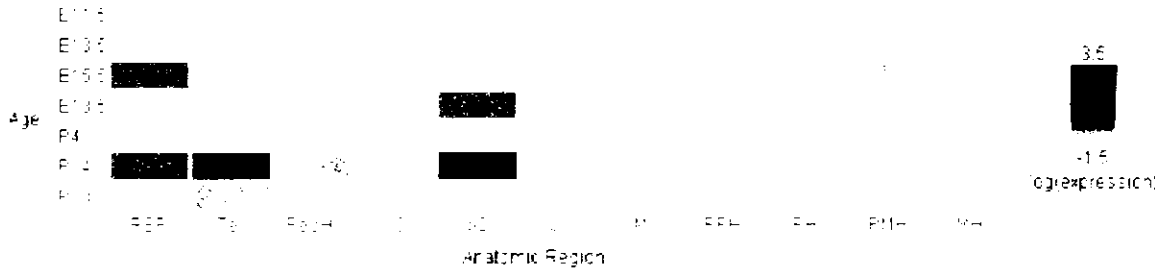


Fig 5.8: Expression summary of Tgfβ2 in Mus musculus brain.

was scattered but was confined to the inner layer of CP only. The expression level varies temporally but in general it was good.

5.2.2.1 E 13.5:

At E13.5, gene expression was observed in all of the CPs of the brain. The expression level was 'fairly good' in all of the three CPs. The expression pattern was 'scattered' and expression specificity was 'inner'. The expression specificity indicated that there is no production of Igfbp-3 by the choroidal epithelium (Fig 5.9).

5.2.2.2 E 15.5 and E 18.5:

During E 15.5, and E 18.5 the observed gene expression pattern was scattered and expression specificity was inner. CP of lateral ventricle was producing the protein with a 'very good' level of expression. The level of expression in CP of fourth ventricle was same as observed in the previous time point of E 13.5 and it was 'fairly good' (Fig 5.10). Expression was absent in the CP of third ventricle at E 15.5 but it is 'fairly good' expression was observed at E 18.5.

Thus during both of these time points, the CP of lateral ventricles is producing more protein as compared to the other two CPs, and in case of E 15.5 the CP of third ventricle was not producing the protein at all.

5.2.2.3 P 4, P 14 and P 28:

Expression of Igfbp-3 during all of these postnatal time points was not observed in the choroidal epithelium. The expression pattern was 'scattered' and was confined to the 'inner' of the CP.

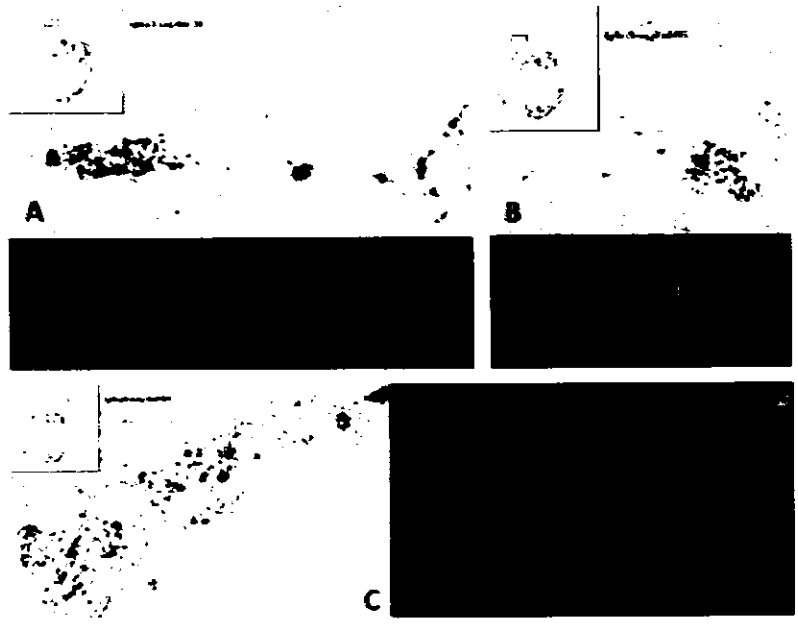


Fig 5.9: ISH images (white) and expression images (black) of Igfbp-3 in CP of lateral, third and fourth ventricles during E 13.5. A: CP of lateral ventricle. B: cp of third ventricle. C: CP of fourth ventricle.

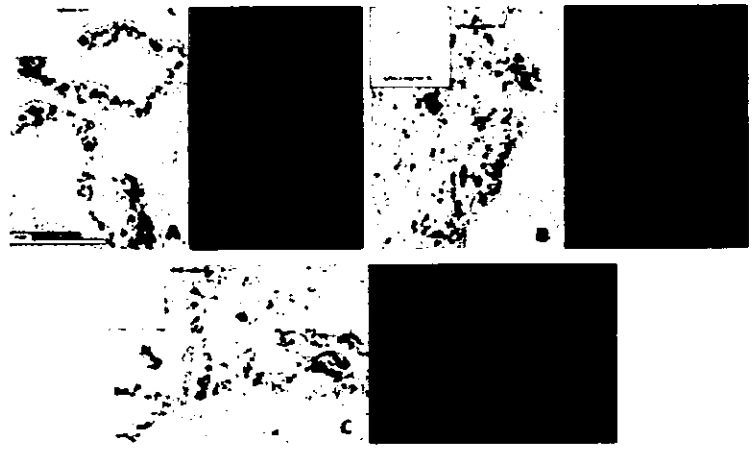


Fig 5.10: ISH images (white) and their corresponding expression mask images (black) for Igfbp-3. A: CP of lateral ventricle at E18.5. B: CP of fourth ventricle at E15.5. C: CP of fourth ventricle at E18.5.

Gene expression during the early postnatal time point of P 4 was observed to be similar to the gene expression during early embryonic time point of E 13.5. Expression level was 'fairly good' while expression pattern was 'scattered' with 'inner' specificity. During P 14, the observed gene expression in CP of lateral ventricles was higher than the gene expression in the other two CPs. In the CP of lateral ventricle the expression level was 'good' whereas it was 'fairly good' in the other two CPs. The observed expression level during P 28 was 'good' for all of the three CPs (Fig 5.11).

Thus while considering only the postnatal development, it has been observed that the CP production of Igfbp-3 was increased with the growing of age from early postnatal age of P 4 to the late postnatal age of P 28.

5.2.2.4 Expression of Igfbp-3 in Brain other than Choroid Plexus:

At E 11.5 there was no observation for expression of Igfbp-3 in the choroidal tissues very little expression was observed in the perpendicular hypothalamus and even lesser in roof plate of evaginated telencephallic vesicle. At E 13.5, a high expression was observed in perpendicular hypothalamus. A very little expression was observed in subpallium and also in the alar part of rhombomeres. Some expression in dorsal pallium was also observed at E 15.5, E 18.5 and P 4 but was negligible in P 14 and P 28. At P 4 and P14 only, a strong expression was observed in alar plate of evaginated telencephallic vesicle. In a general way, it can be assumed that extremely low level expression was present throughout the pallium in all of the seven time Points (Fig 5.12).

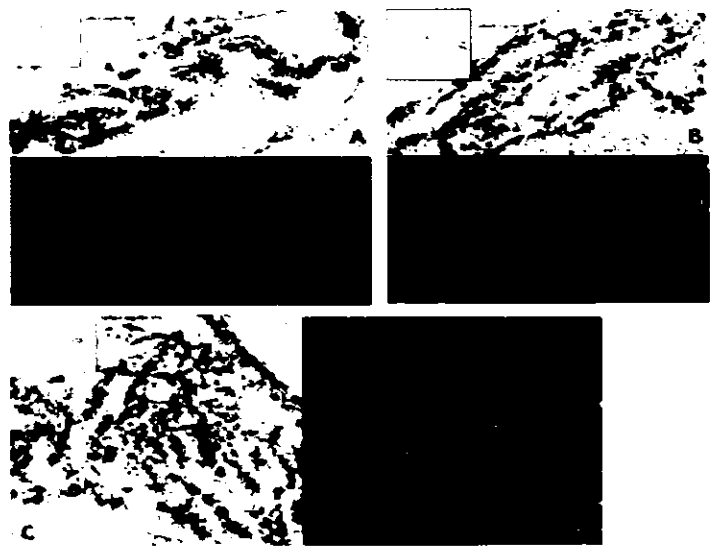


Fig 5.11: ISH image (white) and its corresponding expression image (black) of Igfbp-3. A: CP of lateral ventricle at P 28. B: CP of third ventricle at P 28.C: CP of fourth ventricle at P 28.

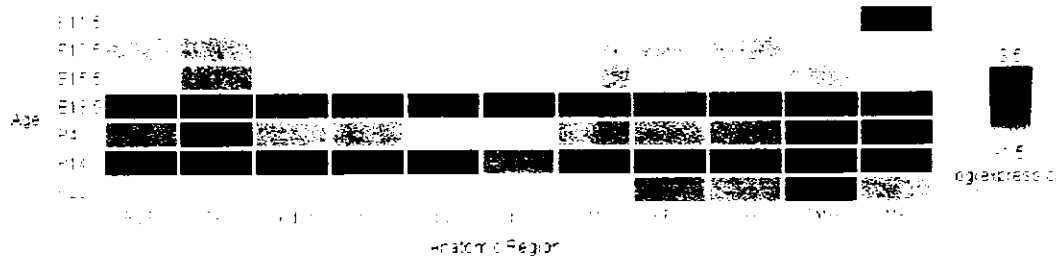


Fig 5.12: Expression summary of Igfbp-3 in Mus musculus brain.

But, except E 18.5, P 4 and P 14, there was no other place inside the brain other than CP which showed good expression of Igfbp-3.

5.2.3 Anxa 11 (Annexin A11):

The gene expression of Anxa11 has been found to be temporally specific to only postnatal time points. No gene expression was observed during any stage of the embryonic development in Mus musculus brain. In general there was no spatial specificity of Anxa11 gene expression and the expression pattern observed was 'scattered'. Overall the gene expression level was not good.

5.2.3.1 P 4, P 14 and P 28:

Gene expression of anxa11 has been observed in postnatal CP with varied level of expression. The expression pattern was 'scattered' and there was no expression specificity with regards to the structure of CP. Spatial specificity with respect to ventricles was observed in case of P 14 only but this specificity was not common throughout the postnatal development.

During P 4, the expression level of CP of fourth ventricle was highest among the three CPs and it was of 'good' level. Whereas the gene expression level in CP of lateral ventricles was 'fairly good' and only 'fair' in case of CP of third ventricle. This suggests that among the three CPs, highest production of Anxa11 was through the CP of fourth ventricle while lesser production was through the CP of third ventricle. The observed gene expression pattern was 'scattered' throughout the structure of CP. The observations in case of P 14 were special when it comes to gene expression of Anxa11. During P 14, no expression was detected in the CP of lateral and third

ventricle but gene expression was observed in the CP of fourth ventricle only. Thus for this time point, the gene expression appeared is specific to the CP of fourth ventricle only. Even there the expression level was only 'fair' the expression pattern was 'scattered'. During P 28, gene expression was detected in all of the three CPs. Again the gene expression level was only 'fair' and gene expression pattern was 'scattered'. The three CPs showed same type of expression for Anxa11 during this time point (Fig 5.13).

5.2.3.2 Expression of Anxa11 in Brain other than Choroid Plexus:

Other than choroid plexus in mouse brain, the expression of Anxa11 has been detected in hippocampal formation, where the observed expression level was higher than that of CP and expression pattern was very dense. Therefore this expression was observed only in postnatal brain and not in the embryonic brain. During P 4, whole brain showed the expression of Anxa11. This observation suggests that temporally the highest production of Anxa11 was during P 4 and spatially the highest production was by hippocampus (Fig 5.14). At P 28, Anxa11 showed its expression only in CP.

5.2.3.3 Important finding:

Literature does not report Anxa11 in CSF but this study has observed its gene expression in developing mouse brain which suggests the production of this protein by brain. The suggested reason is that as Anxa11 is a Ca^{2+} binding protein (Gerke and Moss, 2002) and the mechanism of CSF secretion involves movement of Ca^{2+} (Brown *et al.*, 2004) therefore this protein might be involved with CSF secretion from CP.

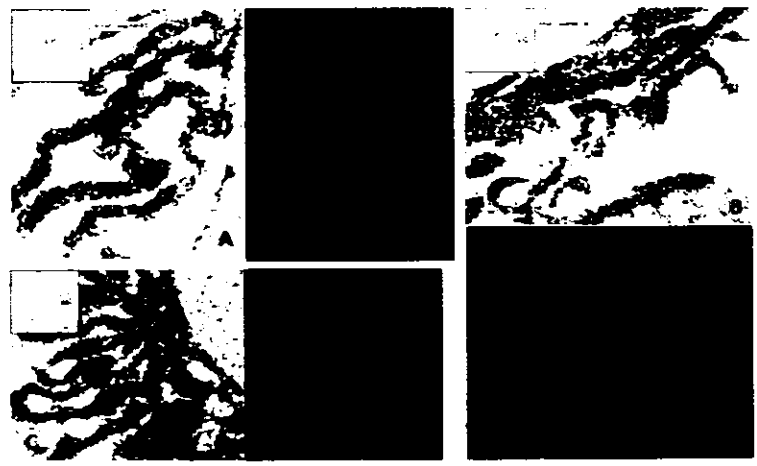


Fig 5.13: ISH image (white) and its corresponding expression image (black) of Anxa11. A: CP of lateral ventricle at P 28. B: CP of third ventricle at P 28.C: CP of fourth ventricle at P 28.

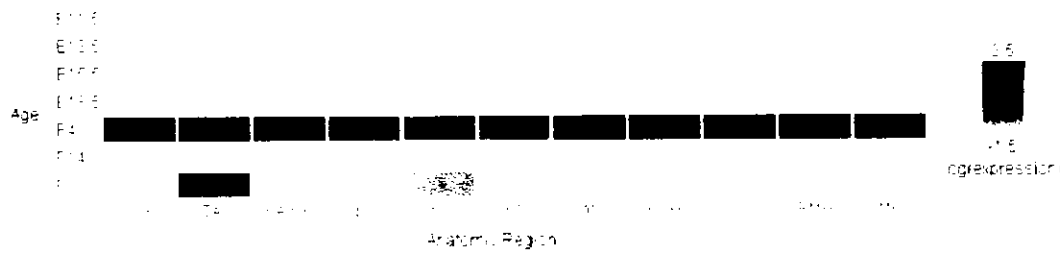


Fig 5.14: Expression summary of Anxa11 in Mus musculus brain.

5.2.4 A2m (Alpha 2 macroglobulin):

Gene expression of A2m was found to be temporally specific to the time points E 15.5, E18.5, P4, P14 and P28 only. Gene expression was not observed for A2m during the two early time points of E 11.5 and E 13.5 respectively. The overall gene expression level for A2m in choroid plexus was 'scattered'. Some sort of spatial specificity with regards to CP structure and with regards to ventricle also, was observed for certain developmental time points. For some time points the gene expression was specific only to the choroidal epithelium. The overall gene expression level was 'low' or 'fair'.

5.2.4.1 E 15.5 and E 18.5:

During E 15.5, no expression was observed in the two telencephallic choroidal tissues and choroidal tissues of prosomeres. However at the same time point, the choroidal tissues of fourth ventricle showed a little expression of A2m. The expression was of 'low' level and 'scattered' and was specific to CP epithelium. The gene expression of A2m also showed spatial specificity with regards to ventricles. During E 15.5, the gene expression was detected only in the CP of fourth ventricle.

Similar was the case regarding expression level and pattern during E 18.5. But at this stage of development, the expression of A2m has been detected in all of the choroidal tissues of the mouse brain.

5.2.4.2 P 4, P 14 and P 28:

During P 4, CP of lateral ventricle did not show any significant level of expression suggesting that the protein was not produced by the CP of lateral ventricle at P 4. But the CP of third and fourth ventricles did show the expression of A2m. The expression level was 'fairly good' in the CP of third ventricle and 'low' in fourth ventricle. The expression pattern was 'scattered' and was observed only in the 'outer epithelial' cells choroid plexus (Fig 5.15). Similar was the case with P 28 where the expression level was 'negligible' in lateral ventricle and was highest in the CP of third ventricle but still the expression level was 'fair' in the third ventricle. In the CP of fourth ventricle, the expression level was 'low'. The expression pattern in all of the CPs during this time point was observed as 'scattered' with no spatial specificity with regards to the structure of CP. At P 14, the gene expression was observed in the CPs of all of the three ventricles but the level of gene expression was high in fourth ventricle as compared to the other two ventricles where it appeared to be 'low'. In CP of fourth ventricle the observed expression level was 'fair'. Thus during P 14 and P 28, the gene expression was scattered with no regional specificity but during the early postnatal age P 4, the gene expression showed spatial specificity with regards to the anatomy of CP and was present only in the choroid plexus epithelium.

5.2.4.3 Expression of A2m in Brain other than Choroid Plexus:

Other than choroid plexus, no good expression for the gene A2m was observed but in cerebellum of post natal brain. The expression in cerebellum was even higher than CP (Fig 5.16). In the early two embryonic time points of E 11.5 and E 13.5, there was no significant expression in any part of the brain including CP.

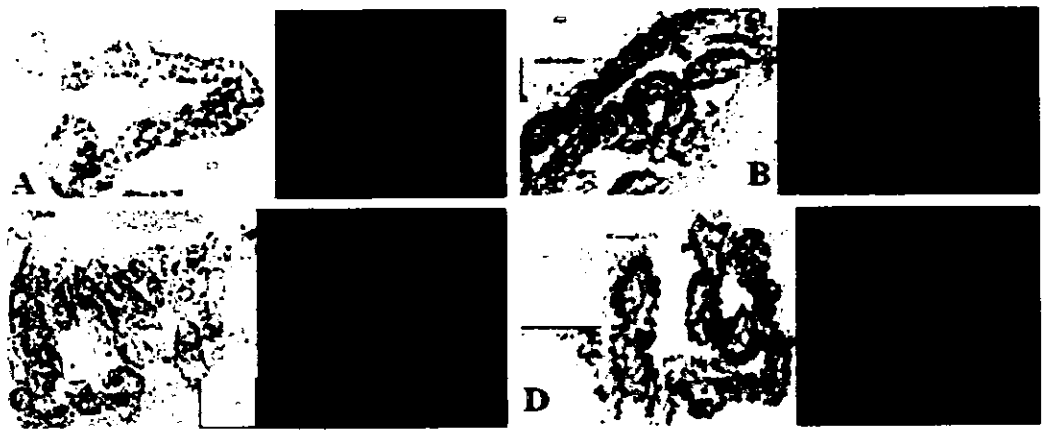


Fig 5.15: ISH image (white) and its corresponding expression image (black) of A2m in CP of mouse brain. A: CP of lateral ventricle at P 4 (negligible). B: CP of third ventricle at P 4. C: CP of fourth ventricle at P 4. D: CP of lateral ventricle at P 14.

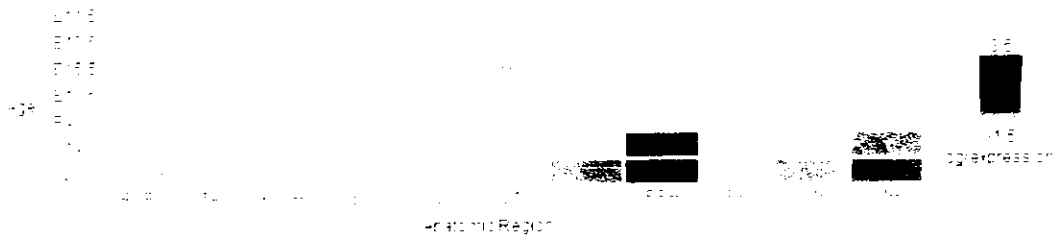


Fig 5.16: Expression summary of A2m in Mus musculus brain.

5.2.5 Aldh-2 (Aldehyde dehydrogenase 2):

Gene expression of Aldh-2 was found in choroid plexus during all the time points except E 11.5. The overall gene expression level increased with the increase in developmental age but still it never reached to excellent level. The expression pattern was 'scattered' during early development but ultimately became 'dense' in later ages. Thus the production of the enzyme was found to be increasing with the age during development of the Mus musculus brain.

5.2.5.1 E 13.5:

At this stage of development, the telencephallic choroidal tissues (TCh) showed expression of Aldh-2 which was higher in its level of expression than the other two choroidal tissues. But still the expression level was 'fair' in TCh. The choroidal tissues of prosomere showed a 'negligible' level of expression of Aldh2 (Fig 5.17). Choroidal tissues of fourth ventricle also showed a 'low' level of expression. The gene expression pattern was 'scattered' in all of the three CPs. This suggests that only the telencephallic choroidal tissues and choroidal tissues of fourth ventricle at E13.5 produced the protein Aldh2. Secondly, for this time point, gene expression shows spatial specificity with regards to ventricles.

5.2.5.2 E 15.5 and E 18.5:

During E 15.5, 'fairly good' expression of Aldh2 was observed in telencephallic choroidal tissues and no expression in choroidal tissues of prosomeres.

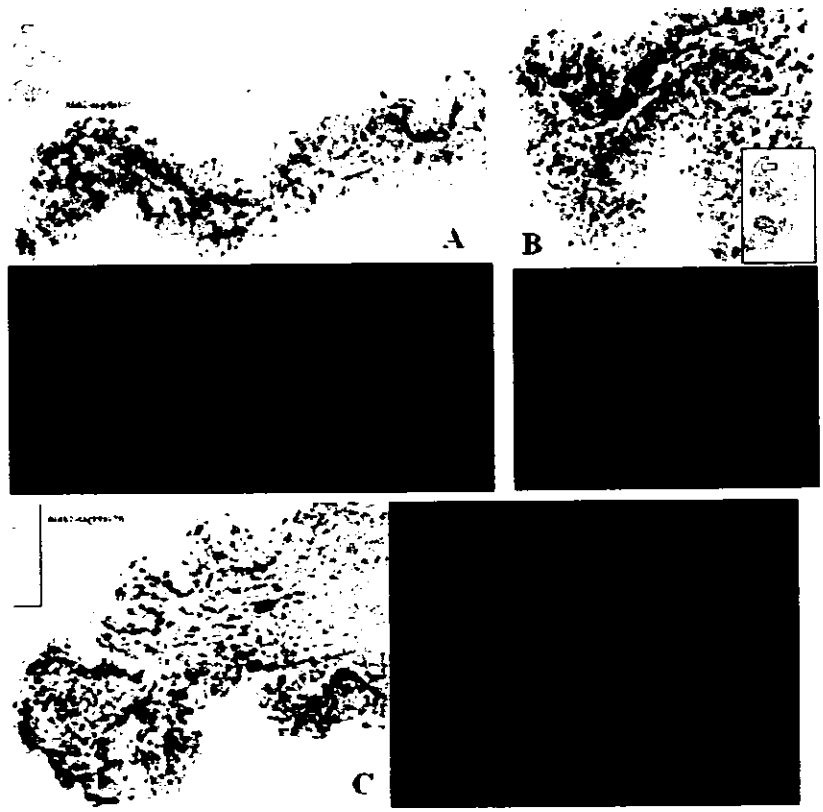


Fig 5.17: ISH image (white) and its corresponding expression image (black) of Aldh2 at E 13.5. A: Choroidal tissues of lateral ventricle. B: Choroidal tissues of third ventricle. C: Choroidal tissues of fourth ventricle.

The choroidal tissues of fourth ventricle showed a 'low' level of gene expression. Thus, like the previous time point, gene expression during E 15.5 also showed spatial specificity with regards to ventricles. The gene expression pattern in all of the choroidal tissues was 'scattered' and there was no gene expression specificity with regards to the internal structure of CP. Therefore is assumed that the protein Aldehyde dehydrogenase 2 was produced by the choroidal tissues of only lateral and fourth ventricle and not by the third ventricle at E 15.5.

During E 18.5, the CP of fourth ventricle showed 'fairly good' level of gene expression of Aldh2. The expression level was 'negligible' in the CP of lateral ventricle and 'low' in the CP of third ventricle. Therefore, at E 18.5 Aldh-2 is produced by CP of third and fourth ventricles only. Thus a spatial specificity has been observed during this time point too but this time the expression of CP is specific to third and fourth ventricles. Previously it was specific to the CP of lateral and fourth ventricles and in this case to the CP of third and fourth ventricles. The gene expression pattern was 'scattered'.

5.2.5.3 P 4, P 14 and P 28:

In the embryonic mouse brain the observed overall gene expression level of Aldh2 in CP was low. However, 'fairly good' level of gene expression has been detected in the choroid plexus of postnatal mouse brain. For all of the three postnatal time points, no spatial specificity was observed for the gene expression of Aldh-2.

During P4, CP of lateral ventricle showed lower level of gene expression as compared to the other two CPs during the same time point and the level was 'fair' in

the CP of lateral ventricle whereas it was found to be 'fairly good' in the other two CPs. But the observed expression pattern was 'scattered' for all of the CPs. During P 14, the gene expression was detected in all of the three CPs with 'fairly good' level of expression in all. But contrary to the previous time points, the expression pattern was not scattered but was apparently 'dense'. This suggests that in postnatal brain almost all of the cells of CP produce the protein in a fairly good amount. During P8, the observed gene expression for Aldh2 in CP was highest among all of the previous time points. The gene expression level was good in all of the three CPs and gene expression pattern was 'dense'.

This suggests that among all of the time points, the largest production of the enzyme Aldh2 is by all of the three CPs during P 28 where the expression level was good and expression pattern was dense (Fig 5.18).

5.2.5.4 Important finding:

Literature does not report Aldh-2 in CSF but this study has observed its gene expression in developing mouse CP which suggests the production of this protein by CP. The suggested reason is that as Aldh-2 degrades toxic aldehydes and works for the prevention of neurodegenerative diseases (Ohsawa *et al.*, 2008) and both CP and CSF are thought to have close relations with neurodegenerative diseases specially Alzheimer's disease (Serot *et al.*, 2003 and Carrette *et al.*, 2003) therefore this protein is may be secreted into CSF from CP.

Fig 5.18: ISH image (white) and its corresponding expression image (black) for Aldh-2 at P 28 A: CP of lateral ventricle. B: CP of third ventricle. C: CP of fourth ventricle.

5.2.5.5 Gene Expression of Aldh2 in Brain other than Choroid Plexus:

Other than choroid plexus, the gene expression of Aldh2 was observed almost throughout the pallium. The observed gene expression was highest during P4 and P14 in the pallium and was negligible during the embryonic development (Fig 5.19). Thus the production of Aldh2 has been found to be carried out by cerebral cortex too and the overall expression was higher in postnatal brain and was lower in embryonic brain.

5.2.6 CD 164 (antigen):

Just like the previous gene Aldh-2, gene expression of CD 164 was also found to be temporally specific to six out of seven time points which are E 13.5, E 15.5, E 18.5, P4, P 14 and P 28. No gene expression was observed for E 11.5. The observed overall gene expression pattern was 'scattered' during early stages of development but was 'dense' during the later ages. The observed overall gene expression level of CD 164 in CP is 'fair'.

5.2.6.1 E 13.5, E 15.3 and E 18.5:

During the three embryonic time points of development of mouse, the observed gene expression of CD164 was lowest in the CP of lateral ventricle as compared to the other two ventricles.

Fig 5.19: Expression summary of ALDH-2 in Mus musculus brain.

Expression pattern was 'scattered' during all of the three time points in all of the CPs but more scattered pattern was observed for the CP of lateral ventricle. The expression level was 'fairly good', though it varied among the time points and among the CPs. At E 13.5 the observed gene expression level was 'fairly good' in the CP of lateral ventricle and 'good' in CP of third and fourth ventricles. However the observed gene expression pattern for the CP of third ventricle is denser than the CP of lateral ventricle which suggests higher production of the protein by CP of third ventricle. The observed gene expression was completely absent or negligible in the CP of lateral ventricle during E 15.5 and E 18.5. The gene expression level was 'fairly good' in the choroidal tissues of third and fourth ventricle during E 15.5 and in the CP of third ventricle only during E 18.5. At E 18.5, the gene expression level of CD 164 in CP of fourth ventricle is 'negligible'.

Thus these results showed that during embryonic development, a significant gene expression was observed in all of the CPs at E 13.5 (Fig 5.20), only choroidal tissues of third and fourth ventricles at E 15.5, and only in CP of third ventricle at E 18.5.

5.2.6.2 P 4, P 14 and P 28:

The case of P 4 is somewhat similar to that of the previous time point in which the gene expression pattern is scattered and the expression is higher in the CP of third ventricle and is negligible or low in the CP of the other two ventricles. During P4, the level of gene expression in the CP of lateral and third ventricle is 'fairly good' and in the cp of fourth ventricle is just 'fair'.

Fig 5.20: ISH image (white) and its corresponding expression image (black) of CD 164. A: CP of lateral ventricle at E 13.5. B: CP of third ventricle at E 13.5. C: CP of fourth ventricle at E 13.5.

Expression pattern is 'scattered' for all but in the CP of third and fourth ventricle it is denser as compared to CP of lateral ventricle.

In the two later time points, P 14 and P 28, the observed gene expression pattern was 'dense' for all CPs except in the CP of third ventricle during P 14 in which the expression pattern was observed to be scattered. The gene expression level was 'fair' for all CPs during both ages except the CPs of lateral and third ventricle during P 28 in which the level of expression was 'good'. Thus during postnatal development, the highest CP production of CD 164 is by the CPs of lateral and third ventricles during P 28 and the expression pattern and level was 'dense' and 'good' (Fig 5.21).

5.2.6.3 Expression of CD 164 in Brain other than Choroid Plexus:

Other than choroid plexus, the gene expression of CD 164 was observed in pallium and in rhombomeres. But gene expression was significantly observed in the outer brain layer which can be meninges and in the ependymal cell layer that lines the ventricles (Fig 5.22). However the level of expression was not better than CP. During embryonic development, significant gene expression level was observed only during E 13.5 and E 15.5, and it varied spatially. During the early time point of E 11.5 and during the late embryonic age of E18.5 the observed gene expression was of very low level and was only present in medullary hind brain and in prosomeres and prepontine hind brain during E 18.5 only. However, these are all the locations where choroid plexus exists which suggest that CP produced the protein when there was no production of CD 164 in other areas of the brain during development. In postnatal

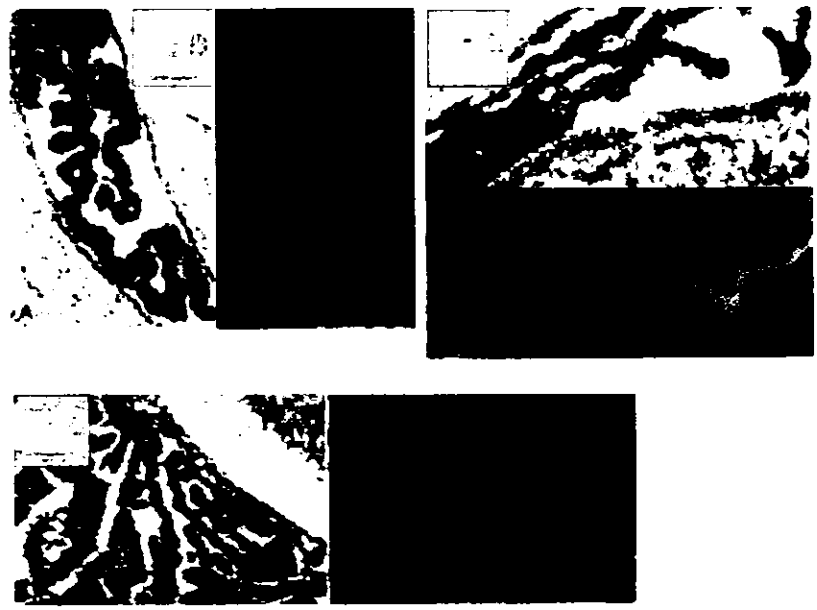


Fig 5.21: ISH image (white) and its corresponding expression image (black) of CD164. A: CP of lateral ventricle at P 28. B: CP of third ventricle at P 28. C: CP of fourth ventricle at P 28.

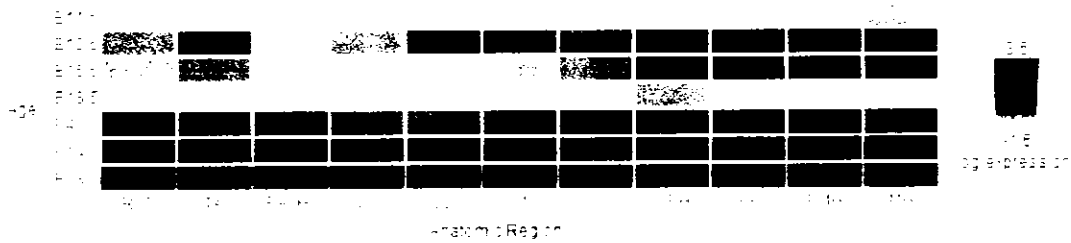


Fig 5.22: Expression of CD164 in Mus musculus brain.

brain, a good level of gene expression was observed throughout the brain with the highest level of expression in hind brain during P 28.

5.2.7 Hspb8 (Heat shock protein 8):

The observed gene expression for HSPB8 was not only temporally specific but it was spatially specific too with regards to the three CPs. It was observed to be spatially specific to the CP of third and fourth ventricle and was absent in the CP of lateral ventricle throughout the development. For embryonic CP, the observed gene expression was spatially specific to the CP of fourth ventricle only. The observed gene expression pattern was 'scattered' throughout the development and gene expression level was mostly 'low'. Therefore it is assumed that the gene did not produce this protein in a good amount. The observed gene expression was temporally specific to E 11.5, E 15.5, E 18.5, P 4, P 14 and P 28. The expression was absent at E 13.5.

5.2.7.1 E 11.5, E 13.5, E 15.5 and E 18.5:

The observed gene expression for embryonic CP is temporally specific to E 11.5, E 15.5 and E 18.5 only. Gene expression pattern was 'scattered' and expression level was 'low'.

No gene expression was observed during E 13.5 (Fig 5.23). During E 11.5 and E 15.5, the gene expression appeared only in the CPs of fourth ventricle (Fig 5.24).No gene expression was observed in the CPs of lateral and third ventricle for both of the time points.

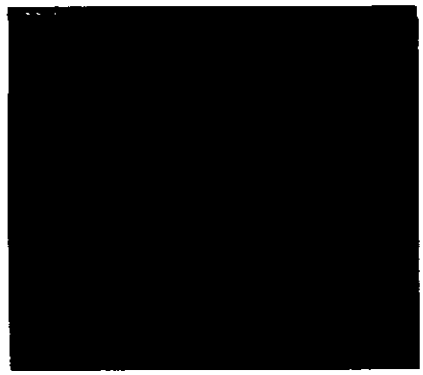


Fig 5.23: Neural tube (green), telencephallic choroidal tissues (pink), choroidal tissues of prosomeres (brown) and roof plates of rhombomeres (blue and purple) of Mus Musculus brain at E 13.5 (left). No gene expression was observed.

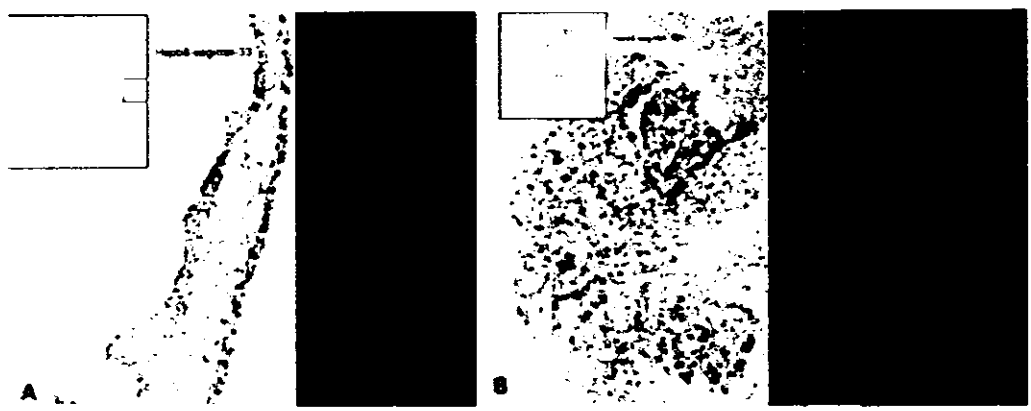


Fig 5.24: ISH image (white) and its corresponding expression mask image (black) of Hsbp8 in choroidal tissues of fourth ventricle. A: E 11.5, B: E 15.5.

At E 18.5, the gene expression also appeared in the CP of third ventricle along with the CP of fourth ventricle. Due to scattered, low level and spatial specificity of gene expression, the production of the heat shock protein is assumed to be very less by the CPs during embryonic development in normal Mus musculus brain.

5.2.7.2 P 4, P 14 and P 28:

Unlike the gene expression of Hspb8 in early embryonic CP, the observed gene expression in postnatal CP was not specific only to fourth ventricle. The gene expression pattern was 'scattered' in both of the CPs during all of the three postnatal time points. However, the gene expression level varied little. During P 4 and P 14, the observed gene expression level was 'low' in all of the CPs but during P 28 it was 'fair' (Fig 5.25). The observed gene expression pattern was scattered throughout development and the gene expression level was highest during P 28 thus the larger production of the protein was during the last analyzed postnatal time point.

5.2.7.3 Gene Expression of Hspb8 in Brain other than Choroid Plexus:

Other than choroid plexus, no good expression for Hspb8 was observed in the developing brain. The main areas where the gene expression was detected were the cells which line the ventricles most probably these cells are the ependymal cells. Other then these cells, some expression was also observed in the outer layer of mesomere pallium, subpallium and hypothalamic region and rhombomeres from E 13.5 onwards. But the expression was very low (Fig 5.26).

Fig 5.25: ISH image (white) and its corresponding expression image (black) of A: CP of third ventricle at P 28. B: CP of fourth ventricle at P 28.

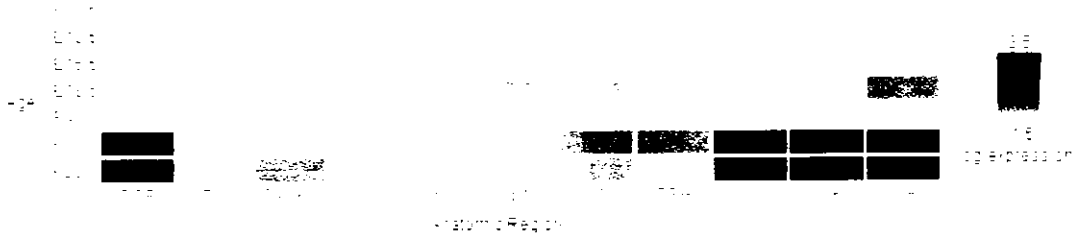


Fig 5.26: Expression summary of Hspb8 in Mus musculus brain.

5.2.7.4 Important finding:

Literature does not report Hspb8 in CSF but this study has observed its gene expression in developing mouse CP which suggests the production of this protein by CP. The suggested reason is that as Hspbs are particularly abundant in nerve cells (Kampinga *et al.*, 2009) and Marques *et al.*, in their 2011 study showed that the genes encoding for molecules that modulate adult neural stem cell proliferation and fate are transcribed in the CP and distributed via CSF therefore this protein might be secreted into CSF from CP.

5.3 CP-Specific Genes Specific to certain time points:

One CP-Specific gene ACE have been observed to be temporally-specific. The gene expression was observed only in CP in the Mus musculus brain and not in any other part of the brain. The gene expression was observed in some but not all of the seven time points. Detailed result for gene expression data analysis is as follows:

5.3.1 Ace (Angiotensin I converting enzyme):

The observed gene expression for Ace in CP was temporally specific to the time points E 15.5, E 18.5, P4, P 14 and P 28. No gene expression was observed during the two early time points of E 11.5 and E 13.5. The observed expression pattern was 'dense' during certain time points whereas it was 'scattered' for the others. During embryonic development, the observed gene expression was anatomically specific to the CP epithelium but this was not the case with postnatal

developing brain. The observed overall gene expression level was not very high but was 'good' for some time points while it was 'fair' for the others.

5.3.1.1 E 14.5, E 15.5 and E 18.5:

During embryonic time point of E 14.5, a good expression was observed in the choroidal tissues of all of the ventricles. At E15.5 and E18.5 the expression level varied spatially with regards to ventricles. For all of three embryonic time points the pattern was 'scattered' but was specific to ependymal cells of CP. This suggests that although all of the cells were not producing the protein Ace but level of expression was significant and CP is assumed to be secreting the protein into CSF during these embryonic ages.

During E 15.5, the observed gene expression was highest in the CP of lateral ventricle where it was of 'good' level while it was lowest in the third ventricle where it was 'low'. The level of observed gene expression in fourth ventricle was 'fairly good'. An interesting observation is that the expression pattern was scattered in the CP of third and the fourth ventricle but was 'dense' in the CP of lateral ventricle. During E 18.5, the observed expression level and pattern was somewhat similar to that of E 15.5 but it was scattered for all of the CPs. The observed expression specificity was also same as the previous time point, that is, it is specific to the epithelial layer of CP. The expression level was 'fairly good' in all of the three CPs.

Thus, during embryonic development, the production of Ace by CP was lowest by the CP of third ventricle during E 15.5 and was highest by the CP of lateral

ventricle at the same age (Fig 5.27) and the protein was produced by the choroidal epithelium only.

5.3.1.2 P 4, P 14 and P 28:

The observed expression level was almost the same or somewhat higher as compared to the embryonic CP but the observed expression pattern was not specific to the CP epithelium. This indicates that no spatial specificity has been found within the CP in case of postnatal brain.

The observed gene expression level during P 4 was 'fairly good' in all CPs. The observed gene expression pattern was 'scattered' for the CP of lateral and third ventricles but was 'dense' for the CP of fourth ventricle. This suggests that during this time point the highest production of Ace was by the CP of fourth ventricle. During the later postnatal time points of P 14 and P 28, the observed gene expression appeared to be 'dense' in all of the CPs in both of the time points. The observed gene expression level was 'fair' in the CP of lateral ventricle during P 14 and was 'fairly good' in the CP of third and fourth ventricles. During P 28, the observed gene expression level and pattern was better than all of the previous time points. Expression pattern was 'dense' and the expression level was 'good' for all of the CPs (Fig 5.28).

Therefore the overall production of the protein Ace by CP was observed to be increased as development proceeded from early embryonic to late postnatal age because the observed expression level increased with the increase in development and observed expression pattern changed from scattered to dense.

Fig 5.27: ISH image (white) and its corresponding expression image (black) of Ace

A: Choroidal tissues of lateral ventricle at E 15.5. B: Choroidal tissues of third ventricle at E 15.5.

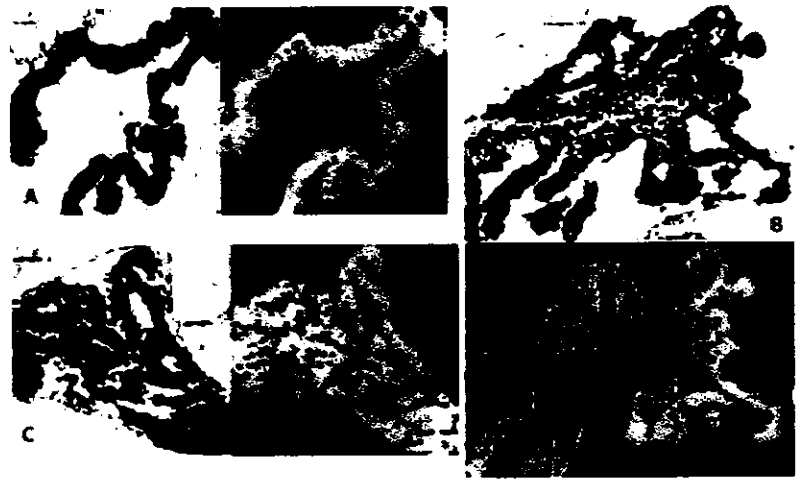


Fig 5.28: ISH image (white) and its corresponding expression images (black) of ACE.

A: CP of lateral ventricle at P 28. B: CP of third ventricle at P 28 C: CP of fourth ventricle at P 28.

5.3.1.3 Expression of Ace in Brain other than Choroid Plexus:

During E 11.5 no gene expression for Ace was observed in the mouse brain (Fig 5.29). At E13.5 a negligible level of expression was observed in all of the ventricular zones. This expression was not observed exactly in choroidal tissues but the location suggests that the observed expression is present in ependymal cells that line the ventricles or in those ependymal cells which will be differentiated into CP in later time points. At E15.5 gene expression for ACE was observed only in all CPs. Similar is the case with E 18.5 with the exception that gene expression for Ace was also observed in the outermost layer in some places of the brain which is the meningial layer of the brain. Also during all of three postnatal time points of P 4, P 14 and P 28, the expression of ACE was observed in some parts of the meningial layer, but not in the brain cells. Thus Ace was assumed to be CP specific gene.

5.4 Other Genes:

There are some genes for which the observed gene expression was not CP specific and was temporally specific to all of the seven time points. However differential spatial specificities with regard to the three CPs have been observed for these genes. Results for detailed data analysis for these genes are as follows:

5.4.1 IGF-2 (Insulin-like growth factor 2):

The gene for this growth factor was observed to be expressed in CP with a high level of gene expression throughout the development. The observed gene expression for IGF-2 was not spatial specific and was 'dense' with a few exceptions.

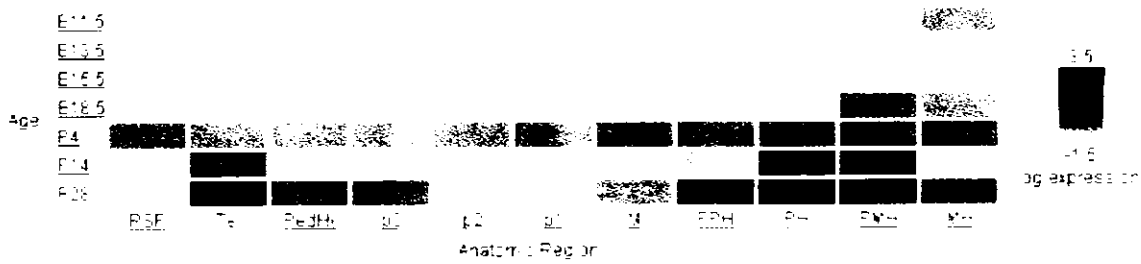


Fig 5.29: Expression summary of ACE in Mouse choroid plexus

5.4.1.1 E 11.5 and E 13.5:

In all of these choroidal tissues, a strong expression of IGF-2 was observed. During E 11.5, the observed expression pattern was 'scattered' in telencephallic choroidal tissues and choroidal tissues of fourth ventricle but was 'dense' in the choroidal tissues of prosomeres. The observed level of expression was 'very good' in telencephallic choroidal tissues and choroidal tissues of prosomeres and was 'excellent' in choroidal tissues of fourth ventricle.

During E 13.5, the observed expression pattern was 'scattered' in choroidal tissues of fourth ventricle and 'dense' in the telencephallic choroidal tissues and choroidal tissues of prosomeres. The level of expression was 'very good' in choroidal tissues of fourth ventricle and 'excellent' in choroidal tissues of prosomeres and telencephallic choroidal tissues

5.4.1.2 E 15.5, E 18.5, P 4, P 14 and P 28:

The observed gene expression level was decreased at E 15.5. The observed gene expression level of IGF-2 was 'good' in telencephallic choroidal tissues and in choroidal tissues of third ventricle and 'fairly good' in the choroidal tissues of fourth ventricle. The observed gene expression pattern was 'dense' in choroidal tissues of lateral and third ventricles and 'scattered' in choroidal tissues of fourth ventricle. Thus during E 15.5, the lower production of the growth factor was by choroidal tissues of fourth ventricle.

At E 18.5, the observed gene expression level was 'very good' in the CP of lateral ventricle and 'good' in the rest of the two CPs. The gene expression pattern

was 'scattered' in the CP of third ventricle and 'dense' in the rest of the two CPs. Thus at E 18.5 the lowest production of the protein was by the CP of third ventricle where the expression is good and scattered. The higher production was by the CP of lateral ventricle where expression was observed to be very good and dense. During postnatal time points, the gene expression level seemed to be decreased but the observed expression pattern was good. At P 4 the observed gene expression level was 'good' in the all of the three CPs and it was 'scattered' in the CP of third and fourth ventricle and 'dense' in the CP of lateral ventricle. Thus during P 4, the higher production of this protein was by the CP of lateral ventricle where the expression is good and dense. At P 14, the observed gene expression level was 'good' in the CP of lateral and fourth ventricle and 'fair' in the CP of third ventricle. The observed gene expression pattern was 'dense' in all of the CPs. Therefore at P 14 the lowest production was by the CP of third ventricle where the expression level was 'fair'. At P 28, likewise the previous time point, the observed gene expression level was 'good' in the CP of lateral and fourth ventricle but it was better as compared to the previous time point. The observed gene expression level was 'fairly good' in the CP of third ventricle. Gene expression pattern was 'dense' in all of the three CPs. Thus at P 28 the lowest production of this protein was by the CP of third ventricle where the gene expression level was fairly good. Thus during postnatal development, the overall gene expression pattern is scattered in the initial time point and it became dense when the development reached to higher postnatal ages. The expression level is almost same for all of the three time points thus the greater production of this protein during postnatal development is at P 14 and P 28 (Fig 5.30).

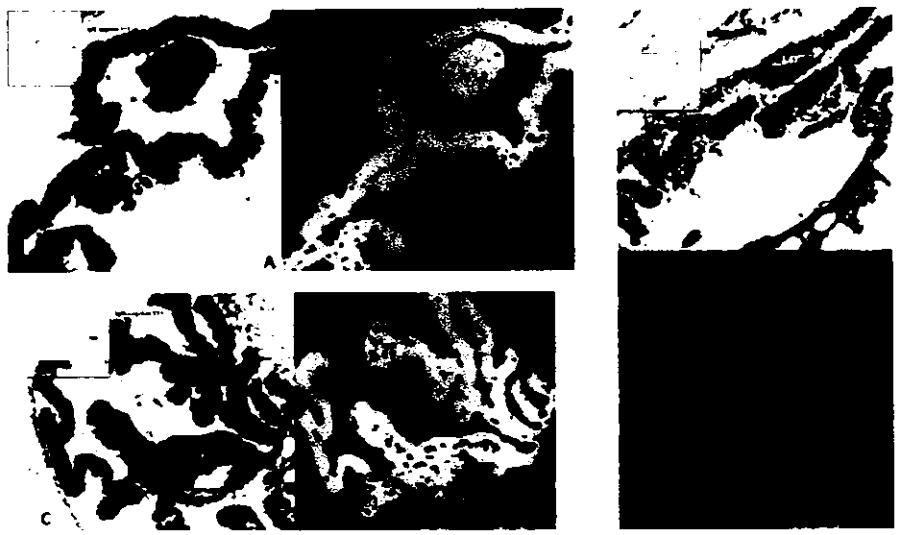


Fig 5.30: ISH and expression images of IGF-2. A: CP of lateral ventricles at P 28. B: CP of third ventricle at P 28. C: CP of fourth ventricle at P 28.

5.4.1.3 Expression of IGF2 in Brain other than Choroid Plexus:

According to the gene expression summary explained in Fig 5.31, IGF2 showed a very strong expression in whole brain throughout the seven stages of development. But studies show that this expression was only in meninges, brain microvasculature and choroid plexus epithelium (Nillson, 1996 and Stylianopoulou *et al.*, 1988). Still this gene was not assumed to be CP-Specific gene because the gene expression was also detected in Pineal gland and pituitary only during development. However, this gene is CP-Specific in adult brain (Stylianopoulou *et al.* 1988).

5.4.2 Igfbp-2 (Insulin-like growth factor binding protein 2):

The observed gene expression of this growth factor binding protein also does not show any temporal or special specificity. A very good level of gene expression was observed in CP throughout the development of Mus musculus brain.

5.4.2.1 E 11.5, E 13.5, E 15.5 and E 18.5:

During embryonic development the gene expression for Igfbp-2 was observed throughout the four embryonic time points and in all of the CPs. The observed gene expression of was specific to choroidal epithelium.During all of the four time points, the observed gene expression level was 'good' in all of the choroidal tissues except the choroidal tissues of fourth ventricle at E 13.5 (Fig 5.32) in which it 'very good' and the CP of fourth ventricle at E 18.5 in which it was 'excellent' (Fig 5.33).

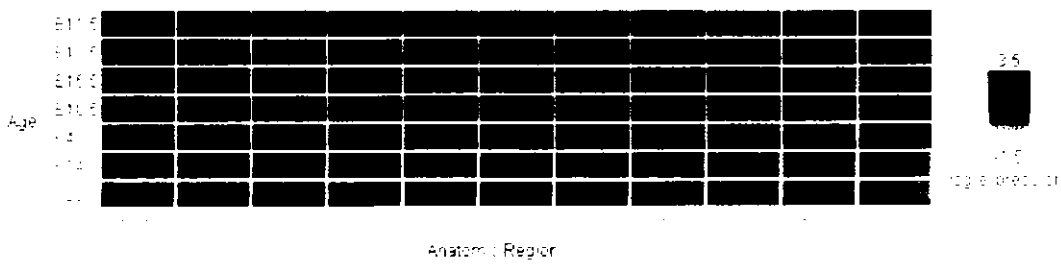


Fig 5.31: Expression summary of IGF-2 in Mus musculus brain.

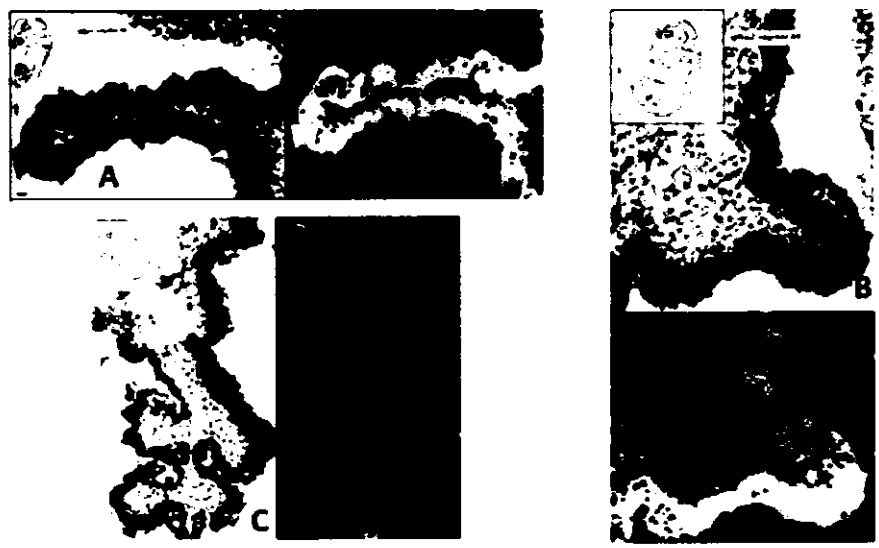


Fig 5.32: ISH image (white) and their corresponding expression images (black) of Igfbp-2. A: choroidal tissue of lateral ventricle at E 13.5. B: choroidal tissues of third ventricle at E13.5. C: choroidal tissues of fourth ventricle at E13.5.

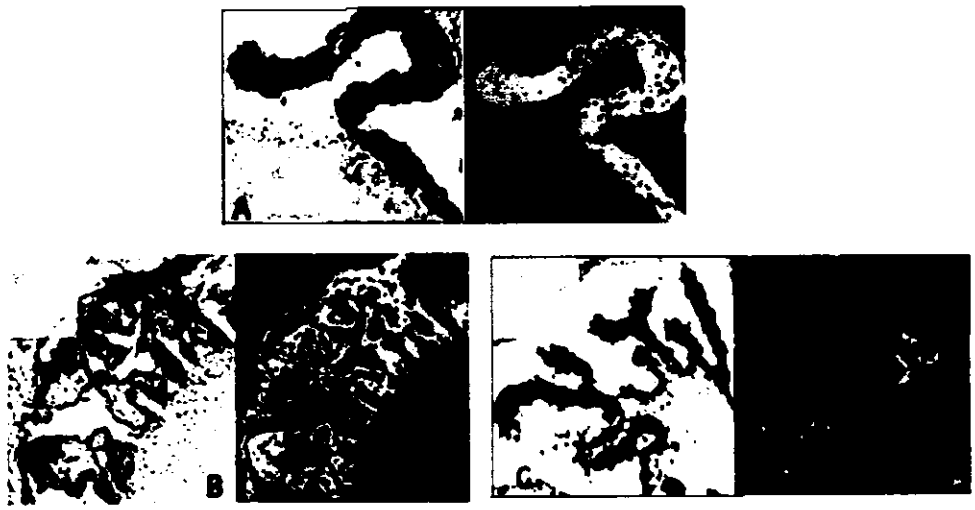


Fig 5.33: ISH image (white) and their corresponding expression images (black) of Igfbp-2 A: CP of lateral ventricles at E 18.5. B: CP of third ventricle at E18.5. C: CP of fourth ventricle at E18.5.

The observed gene expression pattern was 'dense' in all of the choroidal tissues in all of the four embryonic time points. This suggests that CP was producing the protein in a good amount throughout the embryonic development.

5.4.2.2 P 4, P 14 and P 28:

With proceed of the development to postnatal stage, no big change was observed in the gene expression for Igfbp-2 but the observed expression was no more confined to the choroidal epithelium rather it was present throughout the CP. With a further proceed in development there appeared a little decrease in the gene expression level but the pattern was dense and was not specific to any part of the CP.

At P 4, there was not much change in the observed gene expression level as compared to the previous time point. Thus the observed gene expression level was 'good' in the CP of lateral and third ventricle and 'excellent' in the CP of fourth ventricle. The observed gene expression pattern was 'dense' and for all of the three CPs it was observed that the gene expression was absent in the innermost layer of the CP which can be choroidal blood capillary.

At P 14 and P 28, the observed gene expression level decreased little and it was not epithelial or inner specific. The observed gene expression level was 'good' in all of the CPs during both the time points except in the CP of third ventricle at P 14 where it was 'fairly good' and in the CP of fourth ventricle at P 28 it was 'good' but slightly better than the other CPs. Thus among postnatal time points, the CP of fourth ventricle has been observed to be producing the protein in higher amount because the

gene expression was 'good', 'dense' and was present in almost every cell of CP (Fig 5.34).

5.4.2.3 Expression of Igfbp-2 in Brain other than Choroid Plexus:

Other than Choroid plexus, the gene expression of Igfbp-2 was detected in almost every region of the brain (Fig 5.35). But there was no particular region other than CP and meninges which can be specified either for higher or lower expression level. Even during embryonic development, a significant level of gene expression (in the places other than choroid plexus) appeared only in the late embryonic time point which is E 18.5 but still the observed expression was higher in CP. The gene expression for Igfbp-2 in the three postnatal time points was also observed in almost every region of the brain but with a higher level in meninges and CP.

5.4.3 Clu (Clusterin):

The observed gene expression of Clu varied temporally and spatially. The gene expression of Clu was observed in all CPs throughout the seven time points.

5.4.3.1 E 11.5, E 13.5 E 15.5 and E 18.5:

During E 11.5, the observed gene expression level for Clu was 'fairly good' in the choroidal tissues of lateral and third ventricles and 'good' in the choroidal tissue of fourth ventricle. The observed gene expression pattern in the choroidal tissues of lateral and third ventricles was 'scattered' and 'dense' for the choroidal tissues of fourth ventricle. Thus during this time point the CP of fourth ventricle is assumed to be producing the protein in higher amount.

Fig 5.34: ISH image (white) and their corresponding expression images (black) of Igfbp-2 in CP of fourth ventricle at P 28.

Fig 5.35: Expression summary of Igfbp-2 in Mus Musculus brain.

At E 13.5, the observed gene expression for Clu in all of the CPs was better than E 11.5. In all of the three choroidal tissues, the observed gene expression level was 'good' and the expression pattern was 'dense'. The observed gene expression level was little higher in choroidal tissues of fourth ventricle. Thus during this time point also, the CP of fourth ventricle is assumed to be contributing more for the production of this protein. During this time point the observed gene expression was specific to the outer choroidal epithelium. This suggests that during this embryonic time point, the protein was only produced by the outer layer of CP and not by all of the cells of CP.

During the two later time points of E 15.5 and E 18.5, the observed gene expression for Clu was highest as compared to the other five time points. Likewise E 13.5, the observed gene expression in all of the CPs show epithelial specificity except CP of lateral ventricle at E 15.5. The observed gene expression pattern was 'dense'. During E 15.5, the observed level of gene expression for Clu was highest in telencephallic choroidal tissues where it was 'excellent'. Expression level in choroidal tissues of third ventricle was 'good' and 'very good' in the choroidal tissues of fourth ventricle. This suggests that the protein was produced by the CPs in a good amount during E 15.5. But the highest amount of the protein was produced by the CP of lateral ventricle because the observed expression did not show epithelial specificity and it was dense throughout the CP (Fig 5.36).

During the late embryonic time point of E 18.5, the observed level of expression was 'good' in both CPs of lateral and third ventricle and 'very good' in the CP of fourth ventricle.

Fig 5.36: ISH image (white) and its corresponding expression image (black) of Clu. A: Choroidal tissues of lateral ventricle at E 15.5. B: Choroidal tissues of third ventricle at E 15.5. C: Choroidal tissues of fourth ventricle at E 15.5.

5.4.3.2 P 4, P 14 and P 28:

During postnatal development the observed expression pattern for Clu was dense throughout the three postnatal time points. During P 4 the observed gene expression level in the CP of lateral and fourth ventricle was 'good' and 'fairly good' in the CP of third ventricle. This indicates that the CP of third ventricle contributed less than the other two CPs for the production of this protein. The observed expression level was good in CP of fourth ventricle as compared to that of in the CP of lateral ventricle. The expression pattern in the CP of lateral and third ventricles was epithelial specific but there was no such specificity for the CP of fourth ventricle.

Thus the CP of fourth ventricle is assumed to be contributing more for the production of this protein than the other two CPs during P 4.

At P 14 and P 28, no choroidal epithelial specificity was observed for the gene expression (Fig 5.37). The observed gene expression pattern was 'dense' in all of the three CPs during both time points. The gene expression level was observed to be 'good' in the CP of lateral and fourth ventricle during P 14 and P 28 and 'fair' in the CP of third ventricle at P 14 while it was 'fairly good' in the CP of third ventricle at P 28. Thus during postnatal development, the CP of third ventricle was assumed to be contributing lesser than the other two CPs for the production of Clu. Among all of the CPs for all the three time points, the CP of fourth ventricle during P 28 has been found to be contributing more for the production of Clu.

Fig 5.37: ISH image (white) and its corresponding expression image (black) of A: CP of lateral ventricle at P 28. B: CP of third ventricle at P 28. C: CP of fourth ventricle at P 28.

5.4.3.3 Expression of Clu in the Brain other than Choroid Plexus:

The gene expression for Clu was observed at many other locations in the developing mouse brain other than CP (Fig 5.38). During the initial stages of development, there observed a little gene expression for Clu in the brain at only a few places. But with the proceed of the development the expression of Clu became stronger and it was observed to be appeared in the other parts of the brain too ultimately lead to a stage in which the gene was expressed in almost all of the regions of the brain. At the very early stage, E11.5, when the structures of brain were merely developed a significant level of expression was observed in the mesomere. At E13.5, the gene expression was observed in alar plate of mesomere1 and ventricular part of prosomere 1 and 2 (which developed into CP in the later ages of development). Similar is the case with E15.5, but the observed gene expression was stronger. From E18.5 to P28 a high level of gene expression was observed in the whole brain and the highest expression was observed during the last three time points P4, P14 and P28. However, in these ages too there were some places no gene expression was detected like preoptic area in forebrain and roof plates of rhombomeres in hind brain etc but they were not of interest for this study because the aim was to find out the places where the gene expression for Clu was observed along with the CP.

5.4.4 Igfbp-4 (Insulin-like growth factor- binding protein 4):

The observed gene expression for Igfbp-4 was specific to the inner layer of CP which can be the choroidal capillary. Gene expression pattern and level varied with age.

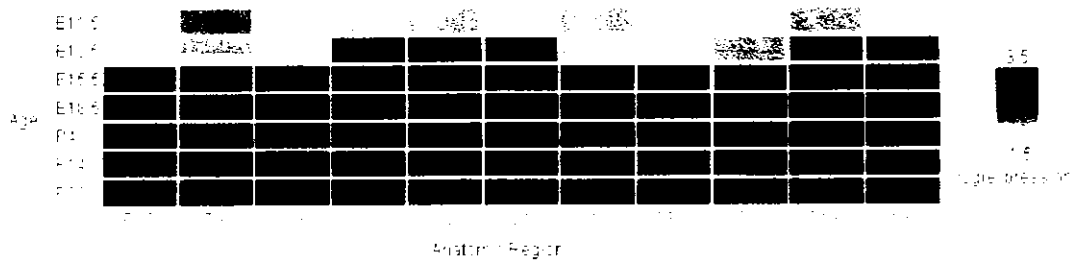


Fig 5.38: Expression summary of Clu in Mus musculus brain.

5.4.4.1 E 11.5, E 13.5, E 15.5 and E 18.5 :

At E 11.5, the gene expression was observed only in the choroidal tissues of prosomeres and fourth ventricles. The observed gene expression level was 'good' in the choroidal tissues of third ventricle and 'fairly good' in the choroidal tissues of fourth ventricle. Thus during this early embryonic age it is assumed that the choroidal tissues of third ventricle produced the protein in higher amount and choroidal tissues of lateral ventricle did not produce the protein.

During E 13.5, the observed gene expression pattern was 'inner specific' because it was observed only in the inner most layer of the CP. The observed gene expression level was 'good' in the choroidal tissues of lateral and fourth ventricles and 'excellent' in the choroidal tissues of third ventricle (Fig 5.39). During E 15.5 and E 18.5, the observed gene expression for Igfbp-4 was also specific to the inner layer of CP the pattern was 'dense' in all of the CPs except the CP of lateral ventricle in which the gene expression pattern was 'scattered'. Gene expression level was observed to be 'good' in the choroidal tissues of lateral and third ventricles during E 15.5 and in the CP of third and fourth ventricles during E 18.5.The observed gene expression level was 'fairly good' in the choroidal tissues of fourth ventricle and 'low' in the CP of lateral ventricle.

Thus it is assumed that the lowest production of Igfbp-4 by the embryonic CP was by CP of fourth ventricle at E 18.5.

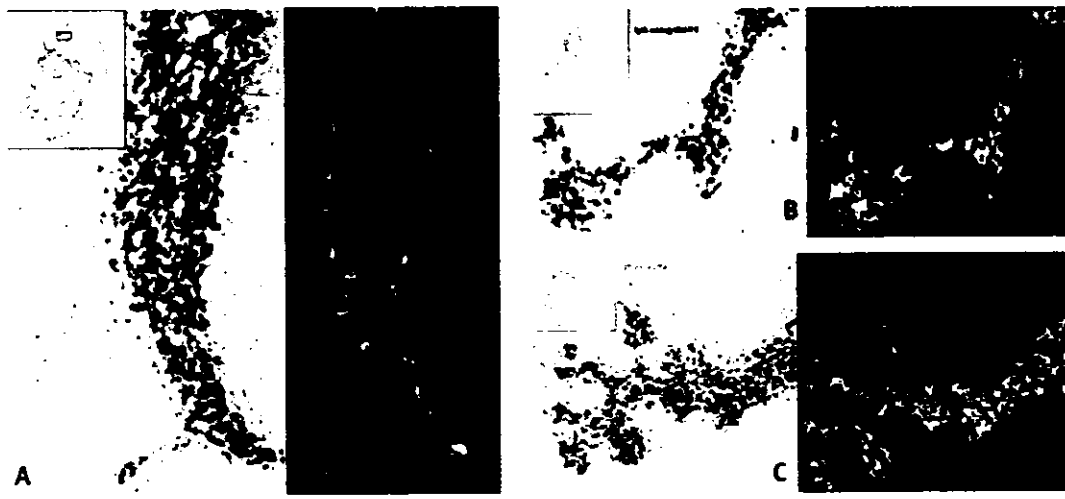


Fig 5.39: ISH image (white) and its expression mask image (black) of Igfbp-4 in choroidal tissues of mouse brain at E 11.5. A: Choroidal tissues of third ventricle. B: Choroidal tissues of lateral ventricle. C: Choroidal tissues of fourth ventricle.

5.4.4.2 P 4, P 14 and P 28:

Like the previous time points, the observed gene expression was 'inner specific' in all of the three postnatal time points. The observed gene expression pattern was 'dense' only in CP of fourth ventricle during P 4 and P 14. In all of the other CPs the gene expression pattern was 'scattered'. The observed gene expression level in the CP of lateral ventricle at P 4 was 'negligible'. Expression level was 'good' in the CP of third and fourth ventricles at P 4, in the CP of lateral and fourth ventricles at P 14 and in the CP of third ventricle at P 28. Expression level was 'fairly good' in the CP of third ventricle at P 14 and in the CP of lateral and fourth ventricles at P28.

Thus it is assumed that the highest production of Igfbp-4 is by the CP of fourth ventricle at P 4 and P 14 for which the observed expression level was 'good' and expression pattern was 'dense' (Fig 5.40).

5.4.4.3 Expression of Igfbp-4 in Brain other than Choroid Plexus:

Other than CP, the expression of Igfbp-4 was observed in basal ganglia and in alar part of prosomere 2 in certain time points (Fig 5.41). At E11.5 and E13.5, no gene expression was observed in brain other than CP. At E15.5 little expression was detected in alar plate of prosomere 2. At E 18.5 and P 4, a very good expression of Igfbp-4 was observed in basal ganglia in the pallium of cerebrum and lower expression in alar plate of prosomere 2 was again detected. At P 14 and P 28 too, like E 18.5 and P 4, there a very good expression for Igfbp-4 was observed in basal ganglia but no expression was detected in alar plate of prosomere 2.

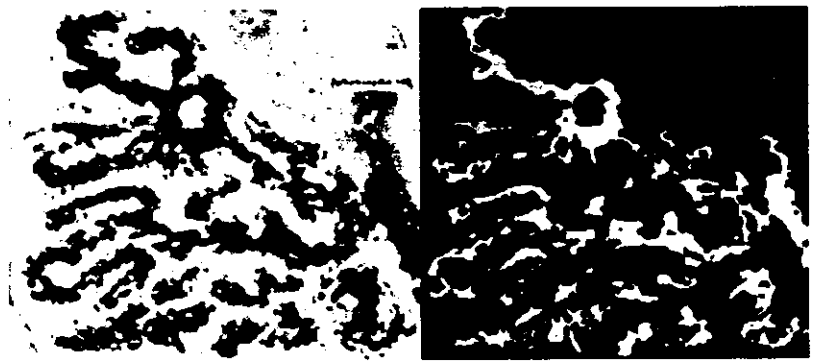


Fig 5.40: ISH image (white) and its corresponding expression images (black) of Igfbp-4 in CP fourth ventricle at P 14.

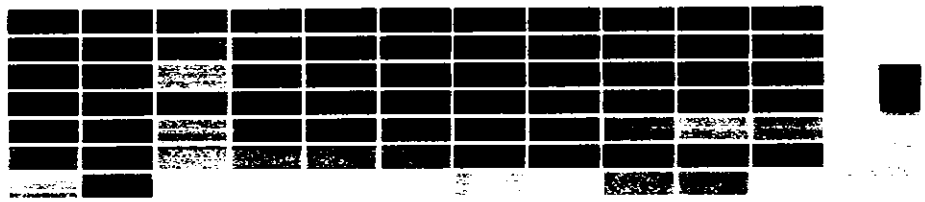


Fig 5.41: Expression summary of Igfbp-4 in Mus musculus brain.

This analysis suggests that along with the CP, the pallium of cerebrum also produced the protein especially during later ages of development i.e. after E15.5. Previous studies suggested that, that region in the pallium is basal ganglia (Brar *et al.*, 1993 and Stenvers *et al.*, 1994). A good expression was also observed in the ependymal layer that lines the ventricles and in brain meninges.

5.4.5 Itpr-1 (Inositol 1,4,5-triphosphate receptor, type 1):

The observed overall gene expression for Itpr1 was not good in CP during all of the seven time points. The gene expression pattern in all of the CPs was scattered with few exceptions. Detailed gene expression analysis of Itpr1 is as follows:

5.4.5.1 E 11.5, E 13.5, E 15.5 and E 18.5:

During E 11.5, the observed gene expression for Itpr1 was of 'low' level and 'scattered'. At E 13.5, the observed gene expression in choroidal tissues of lateral and third ventricles was 'negligible'. Gene expression level in CP of fourth ventricle was 'low'.

At E 15.5, the observed gene expression was spatially specific to the choroidal tissues of lateral and fourth ventricle and was completely absent in the choroidal tissues of third ventricle. Gene expression level was 'fairly good' in telencephallic choroidal tissues and 'low' in choroidal tissues of fourth ventricles. Gene expression pattern wan 'scattered'. The observed gene expression pattern of choroidal tissues of fourth ventricle show 'epithelial specificity' during E 11.5 and E 15.5.

At the embryonic time point of E 18.5, gene expression was observed in all of the CPs with varied levels. Gene expression level in CP of lateral ventricle was 'fairly good' while in the CPs of third and fourth ventricle it was 'low'. Epithelial specificity was observed in case of CP of third and fourth ventricle during E 18.5 but not for CP of lateral ventricle. Thus during the embryonic development, CP of lateral ventricle at E 18.5 has been found to produce the maximum amount of the protein as compared to all of the other CPs during (Fig 5.42).

5.4.5.2 P 4, P 14 and P 28:

During P 4 the observed gene expression level was 'fairly good' in the CP of lateral and third ventricle and 'fair' in the CP of fourth ventricle. The gene expression pattern was 'scattered' in all of the three CPs and it showed choroidal epithelial specificity for CP of all ventricles.

At P 14, the observed gene expression level was 'fair' in the CP of lateral and third ventricle but the expression pattern was 'dense' as compared to the previous time point and now no epithelial specificity was observed. The observed gene expression level in the CP of fourth ventricle was 'low' and 'scattered'. Thus it is assumed that during P 14, the CP of fourth ventricle did not produce the protein in a good amount (Fig 5.43). The observed gene expression level in P 28 was just the same as the previous time point but now the expression pattern did not appear dense and it was 'scattered' in the CP of all of the three ventricles.

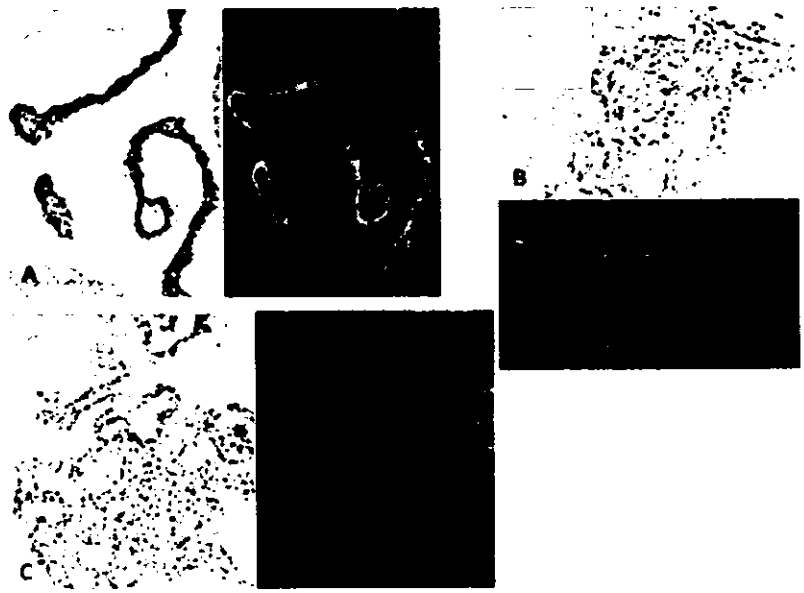


Fig 5.42: ISH image (white) and its corresponding expression images (black) of Itpr1. A: CP of lateral ventricles at E 18.5. B: CP of third ventricle. C: CP of fourth ventricle.

Fig 5.43: ISH image (white) and its corresponding expression images (black) of ITPR1 at P 14. A: CP of lateral ventricle. B: CP of third ventricle. C: CP of fourth ventricle.

5.4.5.3 Expression of Itpr1 in Brain other than Choroid Plexus:

Other than choroid plexus, the expression of Itpr1 was also observed in cerebral cortex, hippocampal formation and in cerebellum, throughout the five time points; E 15.5, E 18.5, P 4, P 14 and P 28 respectively. However, a strong expression in hippocampus was observed only at P 14 and P 28, whereas in cerebellum, a strong gene expression was observed in all of the four time point from E 18.5 to P 28. The observed expression in cerebral cortex, hippocampus and in cerebellum is even stronger then in choroid plexus at the same time point. No significant expression was observed at E 11.5 and E 13.5 and even not in choroid plexus (Fig 5.44).

5.4.5.4 Important finding:

Literature does not report Itpr-1 in CSF but this study has observed its gene expression in developing mouse brain which suggests the production of this protein by brain. The suggested reason is that as Itpr-1 is a Ca^{2+} release channel which is involved in early development and controls Ca^{2+} dependant cell functions (Monkawa *et al.*, 1998 and Mikoshiba, 2006) and the mechanism of CSF secretion involves movement of Ca^{2+} (Brown *et al.*, 2004) therefore this protein might be involved with CSF secretion from CP.

5.4.6 Car-2 (Carbonic anhydrase 2):

The observed gene expression for Car2 was higher than the other genes discussed under this category. The observed overall gene expression during development was somewhat different as compared to many other genes because it was higher in the choroidal tissues of early embryonic brain and expression level

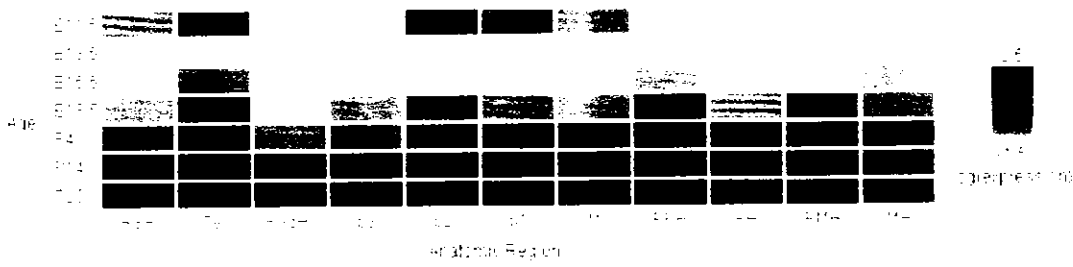


Fig 5.44: Expression summary of ITPR-1 in Mus musculus brain.

decreased as the development proceeded. The observed gene expression pattern was 'scattered' for embryonic CP but 'dense' for postnatal CP. Gene expression for Car2 did not show any spatial or temporal specificity.

5.4.6.1 E 11.5, E 13.5, E 14.5, E 15.5 and E 18.5:

During E 11.5, the observed gene expression level for Car2 in choroidal tissues of lateral and third ventricles was 'very good' and in choroidal tissues of fourth ventricle was 'good' (Fig 5.45). At E 13.5, the gene expression level observed to be decreased. The observed gene expression level in choroidal tissues of lateral ventricle was 'good', in choroidal tissues of third ventricle was 'fairly good' but in the choroidal tissues of fourth ventricle the gene expression level was 'very good'. A good gene expression was also observed in all of the choroidal tissues at E 14.5. At E 15.5 and E 18.5, the observed gene expression level was 'fairly good' in all of the CPs. During E 18.5 choroidal epithelial specificity was observed.

Thus among all of the four embryonic time points, the observed gene expression for Car2 is high in the choroidal tissues of lateral and third ventricle at E 11.5 and in the choroidal tissues of fourth ventricle at E 13.5.

5.4.6.2 P 4, P 14 and P 28:

Gene expression pattern of Car2 in CP was observed to be changed from 'scattered' in the embryonic CP to 'dense' in the postnatal CP. Thus it is assumed that the protein was produced in higher amount now as compared to the embryonic CP. During P 4, the observed gene expression level was 'fairly good' in the CP of lateral and fourth ventricles and 'good' in the CP of third ventricle. The observed

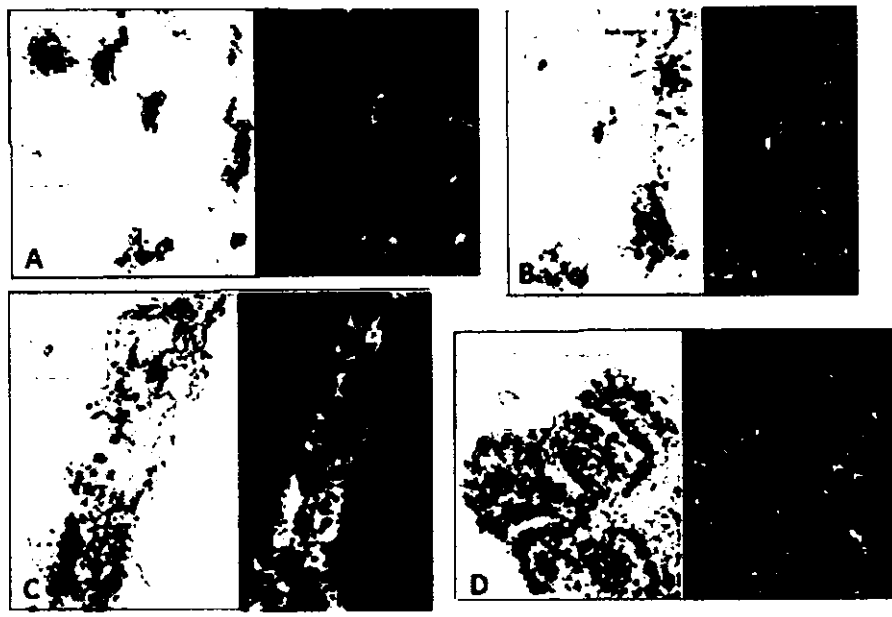


Fig 5.45: ISH image (white) and its corresponding expression images (black) of CAR2. A: Choroidal tissues of lateral ventricle at E 11.5. B: Choroidal tissues of third ventricle at E 11.5. C: Choroidal tissues of fourth ventricle at E 11.5. D: Choroidal tissues of fourth ventricle at E 13.5.

gene expression level was 'fair' in the CP of lateral and third ventricles at P 14 and P 28 whereas it was 'good' in the CP of fourth ventricle at P 14 and 'fairly good' in the CP of fourth ventricle at P 28 (Fig 5.46).

Thus a decline in the gene expression level of Car2 in CP was observed while moving from early embryonic age to the late postnatal age but the expression pattern changed from scattered to dense in postnatal age. The analyses suggest that among the three postnatal ages, the largest production of this protein was by the CP of third ventricle at P 4.

5.4.6.3 Expression of Car2 in Brain other than Choroid Plexus:

Other than choroid plexus, the gene expression for Car2 has been observed in almost every part of the brain with similar expression except at E 15.5 and E 18.5 during which the expression of Car2 appeared only in a small area of pallium and in rhombomeres other than CP. The observed gene expression was higher in CP and lower in the other places during E 15.5 and E 18.5. But during postnatal time points, good gene expression was present in almost every part of the brain (Fig 5.47).

5.4.7 Fgfr-2 (Fibroblast growth factor receptor-2):

The observed overall gene expression for this growth factor was not of excellent level. The gene expression pattern was 'dense' during early embryonic stages but it became 'scattered' in the late embryonic stage and in the postnatal CP. Gene expression for Fgfr-2 did not show any spatial or temporal specificity except in few cases in which it showed some choroidal epithelial specificity.

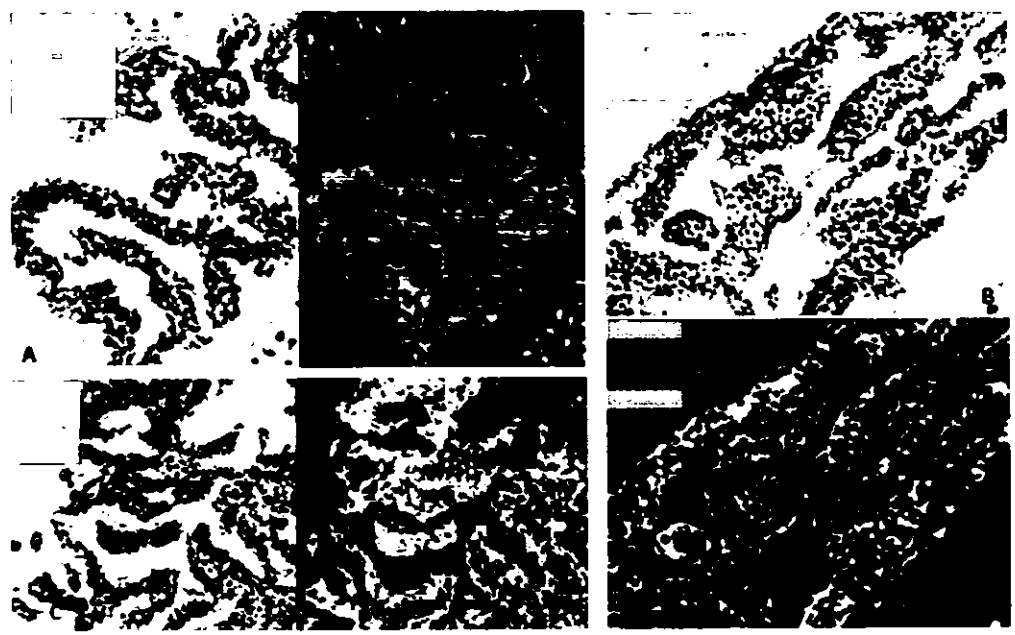


Fig 5.46: ISH image (white) and its corresponding expression images (black) of Car2 in CP of lateral, third and fourth ventricles at P 28. A: CP of lateral ventricle. B: CP of third ventricle. C: CP of fourth ventricle.

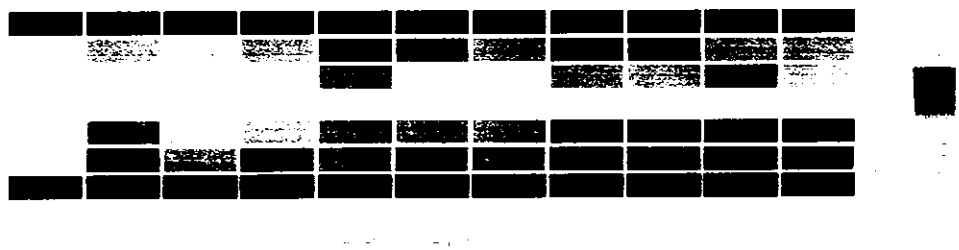


Fig 5.47 : Expression summary of Car2 in Mus musculus brain.

5.4.7.1 E 11.5, E 13.5, E 15.5 and E 18.5:

The observed gene expression level during embryonic growth was mostly 'fairly good' with a few exceptions. During E 11.5, the gene expression showed spatial specificity and it was specific only to the choroidal tissues of fourth ventricle. Thus it is assumed that during E 11.5, only the choroidal tissues of fourth ventricle produced the protein. The observed gene expression level was 'fairly good' and the gene expression pattern was 'dense' (Fig 5.48).

At E 13.5, there observed no such spatial specificity for gene expression like the previous time point and the gene expression was detected in all of the three choroidal tissues. The observed gene expression level was 'good' in the telencephallic choroidal tissues and 'fairly good' in the other two choroidal tissues. The observed gene expression pattern was 'scattered' in the choroidal tissues of third ventricle and 'dense' in rest of the choroidal tissues. It was specific to the choroidal epithelium. This suggests that during E 13.5, only CP epithelium produced the protein and lesser protein is produced by the choroidal tissues of third ventricle as compared to the other two choroidal tissues. At E 15.5, the observed gene expression pattern was 'dense' in the choroidal tissues of all of the ventricle but the expression was specific to CP epithelium for the CP of third ventricle only, while no such specificity was observed in the rest of the two CPs. The observed gene expression level was 'good' in the choroidal tissues of lateral and third ventricles and 'fairly good' in the choroidal tissues of fourth ventricle.

The observed gene expression level at E 18.5 was 'fairly good' in all of the three CPs and the expression pattern was 'scattered' with no epithelial specificity.

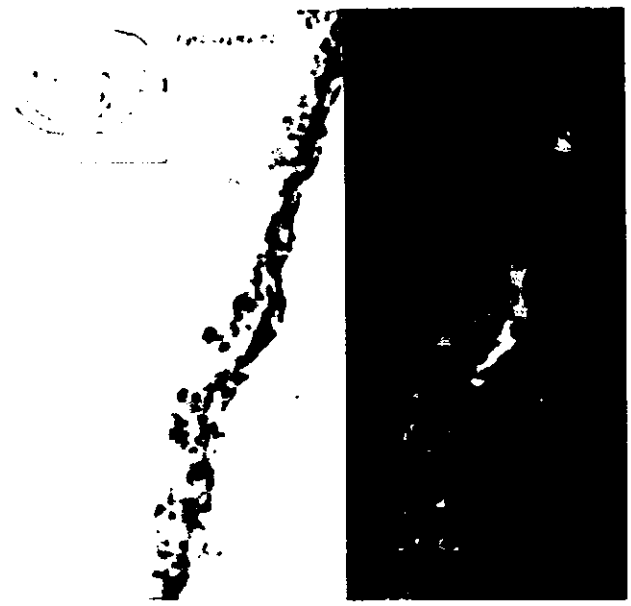


Fig 5.48: ISH image (white) and its corresponding expression images (black) of Fgfr-2 in the CP of fourth ventricle at E 11.5.

This indicates that during this time point, there was lesser production of the growth factor receptor as compared to the previous embryonic time points.

5.4.7.2 P 4, P 14 and P 28:

The observed gene expression pattern in all of the three time points was 'scattered' throughout the structure of CP and the gene expression level was either 'fairly good' or 'fair'. No epithelial specificity was observed for this gene in postnatal CP.

The observed gene expression for Fgfr-2 at P 4 was same as that of the previous time point. The gene expression level was 'fairly good' in all of the three CPs and the gene expression pattern was 'scattered'.

The observed gene expression level at P 14 and P 28 was 'fair' in all of the CPs except the CP of third ventricle at P 28 where the expression level was 'fairly good'. This indicates that during the two later postnatal time points, the CP of third ventricle contributed maximum for the production of the growth factor receptor (Fig 5.49).

5.4.7.3 Expression of Fgfr-2 in Brain other than Choroid Plexus:

During E 11.5, E 13.5 and E 15.5 the gene expression was observed in some parts of mesomere, pallium, subpallium, rhombomeres and prosomeres including the ventricular part (Fig 5.50). But during E 18.5, the gene expression was observed only in ventricular zones. It appeared both in the CP and in the ependymal cells that lines the ventricles. Similar was the case with P 4 but now the expression also appeared in

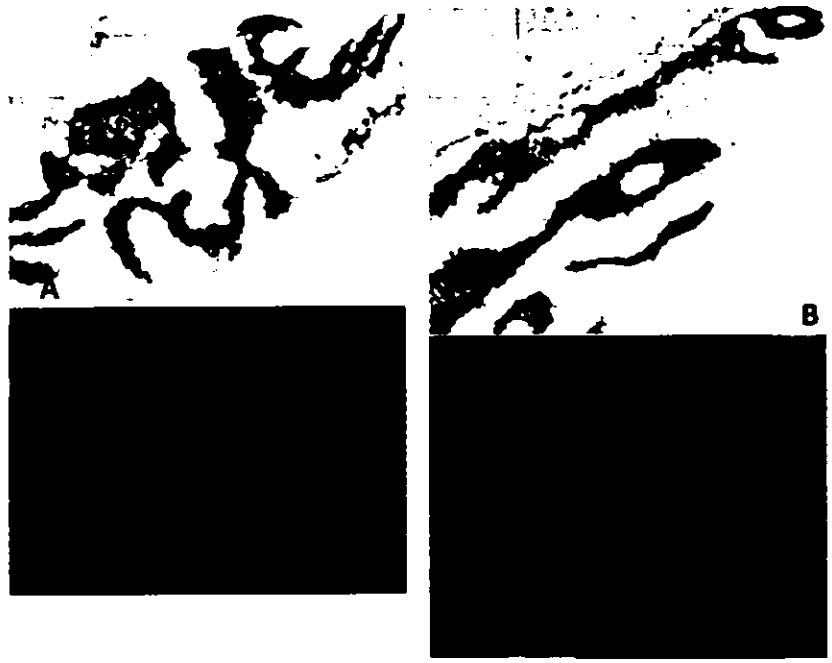


Fig 5.49: ISH image (white) and its corresponding expression images (black) of Fgfr-2 A: CP of third ventricle at P 14. B: CP of third ventricle at P 28.

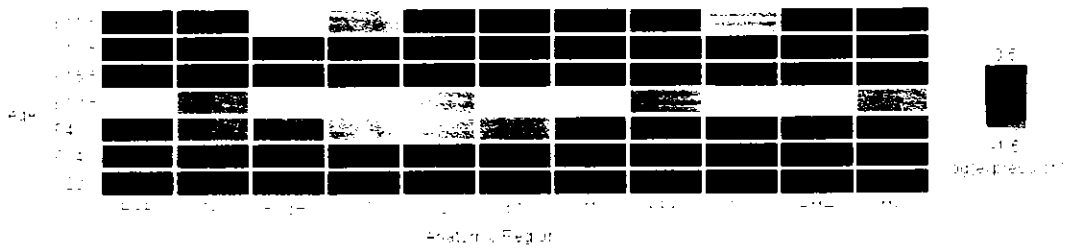


Fig 5.50: Expression summary of Fgfr-2 in Mus musculus brain.

the outer most layer that lines the brain. At P 14 some expression was observed in cerebellum and in some parts of mesomere and rhombomeres other than CP. During P 28 a considerable level of gene expression was observed in almost whole brain.

5.4.8 Htr2C (5-hydroxytryptamine (serotonin) receptor 2C):

For Htr2C, the observed gene expression pattern was 'scattered' in embryonic CP but 'dense' in postnatal CP. Gene expression level varied from 'fairly good' to 'very good'. The detailed results are listed as follows:

5.4.8.1 E 11.5, E 13.5, E 15.5 and E 18.5:

The observed gene expression level and pattern varied among the three CPs throughout the four embryonic time points. During E 11.5 there observed no gene expression in the choroidal tissues of lateral and third ventricles. In choroidal tissues of fourth ventricle the observed gene expression level was 'fairly good' and the expression pattern was 'scattered'. At E 13.5 the observed gene expression level was 'very good' in the choroidal tissues of lateral and third ventricles and 'good' in the choroidal tissues of fourth ventricle. The gene expression pattern was 'scattered' in choroidal tissues of lateral and third ventricles and 'dense' in the choroidal tissues of fourth ventricle. At this time point the observed gene expression was specific to choroidal epithelium. As the development proceeded, the gene expression was observed be decreased and no expression was observed in the choroidal tissues of third ventricle at E 15.5. The observed gene expression level in the choroidal tissues of lateral ventricle was 'fairly good' and 'low' in the choroidal tissues of fourth ventricle. During E 18.5, the observed gene expression level for Htr2c was 'low' in

the CP of lateral ventricle while it was 'fairly good' in the other two CPs. The expression pattern was 'scattered' in all of the CPs and it was specific to the choroid plexus epithelium at this age too.

Thus it is assumed that the largest production of this protein during these four time points was by the CP of lateral and third ventricles at E 13.5 and the smallest production was by the CP of fourth ventricle at E 15.5 and by the CP of lateral ventricle at E 18.5.

5.4.8.2 P 4, P 14 and P 28:

As the development proceeded to postnatal age, there was observed a change in gene expression pattern from 'scattered' to 'dense'. The observed gene expression pattern in all of the CPs was 'dense' throughout the three postnatal time points.

At P 4 and P 14, the observed gene expression level was 'good' in all of the CPs except the CP of third ventricle at P 14 where it was 'fairly good'. During P 4, likewise in the embryonic CP, the observed gene expression was specific to choroidal epithelium. But this was not the case during P 14 and P 28 during which the gene expression was observed in complete CP. During P 28, the observed gene expression level was 'fairly good' in all of the three CPs (Fig 5.51).

5.4.8.3 Expression of Htr2C in Brain other than Choroid Plexus:

Other than choroidal tissues, no good gene expression was observed in any other place in the brain during embryonic development (Fig 5.52). During all of the four embryonic time points, a significant level of gene expression was observed only

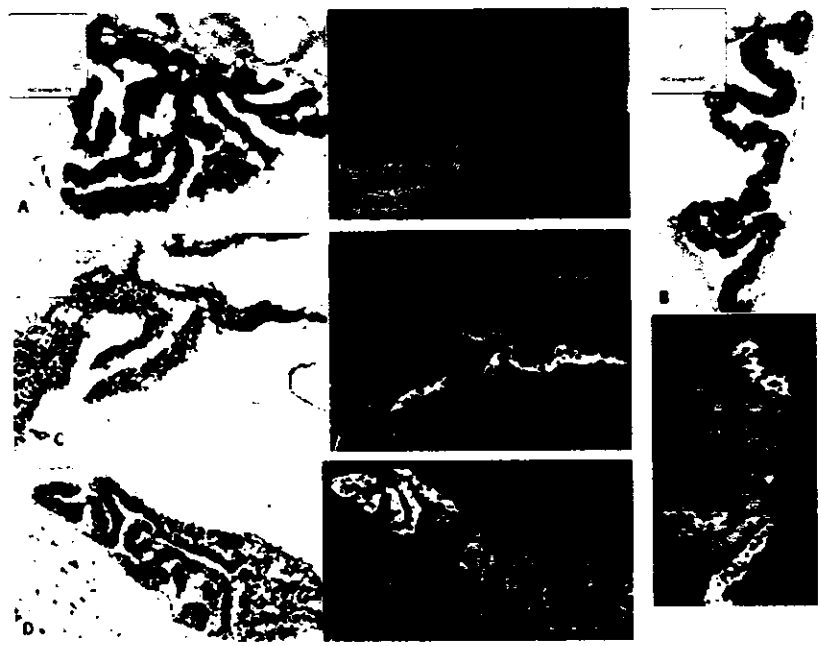


Fig 5.51: ISH image (white) and its corresponding expression images (black) of Htr2C. A: CP of fourth ventricle at P 14. B: CP of lateral ventricle at P 28. C: CP of third ventricle at P 28. D: CP of fourth ventricle at P 28.

Fig 5.52: Expression summary of Htr2C in Mus musculus brain.

in the choroidal tissues. Thus for the embryonic CP, the gene expression is assumed to be CP specific. On the contrary, gene expression of Htr2C was observed in almostevery part of the brain at postnatal age. During P 4 and P 14 the gene expression was observed throughout the brain but it was better in the CP than the other parts of the brain. During P 28, the observed gene expression in the places other than CP decreased again and thus it is assumed the production of the protein by other places decreased as compared to the previous two time points. Thus throughout the seven time point, the observed gene expression in the brain at the places other than CP was higher during P 4 and P 14 but still it was not much good as CP.

5.4.9 Apoe (Apolipoprotein E):

The gene expression for Apolipoprotein E has been detected in all of the CPs. The observed gene expression pattern was 'scattered' in all of the CPs except for few time points. The gene expression did not show any kind of special specificity for CP.

5.4.9.1 E 11.5, E 13.5, E 15.5 and E 18.5:

During the embryonic development, the observed gene expression was scattered in all of the CPs but the overall gene expression was good. During E 11.5, the observed gene expression level was 'very good' in the choroidal tissues of lateral and fourth ventricles and 'fairly good' in the choroidal tissues of third ventricle. At E 13.5, the observed gene expression level was 'excellent' in the choroidal tissues of lateral ventricle and 'very good' in the rest of the two choroidal tissues. At E 15.5, the observed gene expression level was 'very good' in the choroidal tissues of lateral ventricle and 'good' in the rest of the two choroidal tissues. But still the observed

expression level in the choroidal tissues of third ventricle was better than the choroidal tissues of fourth ventricle. At the last embryonic time point of E 18.5, the observed gene expression level was more towards 'good' in all of the choroidal tissues. Thus the observed overall gene expression was good in the choroidal tissues during embryonic development and CP is assumed to produce the protein in a good amount. The observed gene expression level decreased little in the later embryonic time of E 18.5.

5.4.9.2 P 4, P 14, P 28:

During postnatal time points, the observed gene expression level was not as good as that of embryonic CP but the expression pattern was 'dense' in some CPs.

At P 4 the observed gene expression pattern and level was 'scattered' and 'fairly good' in all of the three CPs (Fig 5.53). During P 14 and P 28, the observed gene expression pattern was 'dense' for all of the CPs except the CP of third ventricle at P 28 in which the expression pattern was not much dense as that of the previous time point. Thus during postnatal time points, the protein is assumed to be produced by the CP in a good amount. The gene expression level is lower than that of the early embryonic choroidal tissues but the pattern is 'dense'.

5.4.9.3 Expression of Apoe in Brain other than Choroid Plexus:

Other that choroid plexus, a good gene expression was observed in almost all of the places in the developing mouse brain throughout the seven stages of development (Fig 5.54). Thus any specific location or place in the brain was not identified for gene expression. While excluding the CP, the observed gene expression

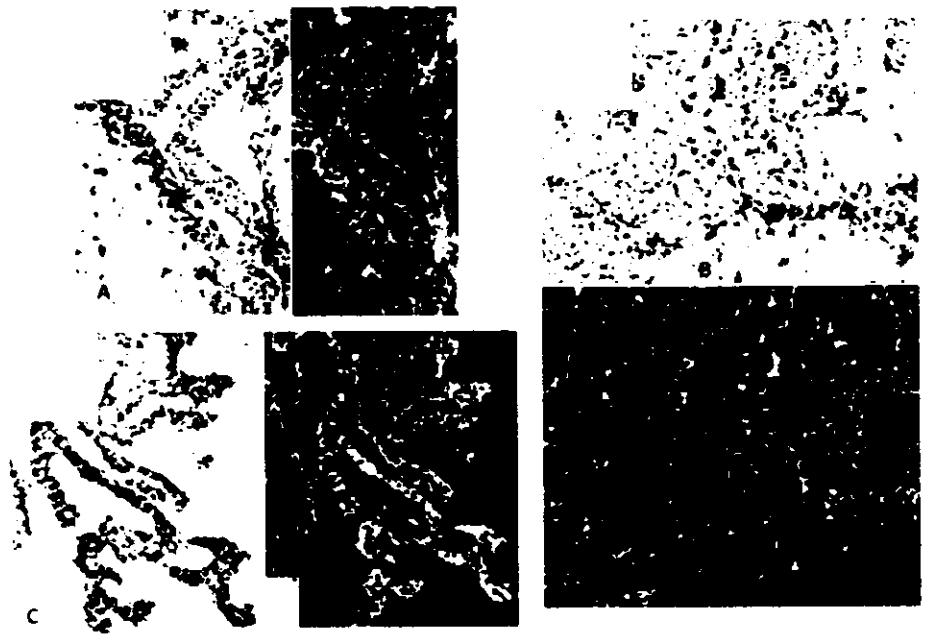


Fig 5.53: ISH image (white) and its corresponding expression images (black) of Apoe at P 4. A: CP of lateral ventricle. B: CP of third ventricle. C: CP of fourth ventricle.

Fig 5.54: Expression summary of Apoe in Mus musculus brain.

in the whole brain was lower during the first three embryonic time points. In all of the three postnatal time points, the observed gene expression for Apoe was higher in whole brain as compared to the early embryonic time points.

5.4.10 Epas1 (Endothelial PAS domain protein 1):

The observed gene expression for Epas1 in CP did not show any spatial specificity with regards to the three CPs but it showed epithelial specificity for some of the CPs.

5.4.10.1 E 11.5, E 13.5, E 15.5 and E 18.5:

During the embryonic development of mouse brain, the observed gene expression for Epas1 in CP was weak in the early and the later embryonic time points which are E 11.5 and E 18.5 whereas it was stronger in the two middle embryonic time points.

During E 11.5, the gene expression was only observed in the choroidal tissues of third and fourth ventricles. The observed gene expression level in the choroidal tissues of third ventricle was 'low' whereas it was 'fair' in the choroidal tissues of fourth ventricle. Thus during this early embryonic time point it is assumed that there was no production of Epas1 by the choroidal tissues of lateral ventricle and the production was very low by the other two choroidal tissues. The observed gene expression pattern in both of the choroidal tissue was 'scattered'.

The observed gene expression level increased at E 13.5 and E 15.5. It was 'fairly good' in the choroidal tissues of lateral ventricle at E 13.5 and in all of the

choroidal tissues at E 15.5, whereas, the observed gene expression level was 'good' in the choroidal tissues of third and fourth ventricles. The observed gene expression pattern was 'scattered' in all of the choroidal tissues during both embryonic ages and it showed choroidal epithelial specificity for all CPs. Thus it is assumed that the production of the protein was higher by the choroidal tissues of third and fourth ventricles at E 13.5. At E 18.5, the gene expression was observed to decrease again. No gene expression was observed in the CP of fourth ventricle and it is of 'negligible' level in the CP of lateral ventricle. In the CP of third ventricle the observed gene expression level and pattern was 'low' and 'scattered' and it showed epithelial specificity. Thus during this time point, only the CP of third ventricle is assumed to produce the protein with a low level of production.

5.4.10.2 P 4, P 14 and P 28:

The observed gene expression pattern was 'scattered' during the first two postnatal time points P 4 and P 14, but the expression pattern was observed to be 'dense' at the later Postnatal time point of P 28. At P 4, the observed gene expression was 'negligible'. The observed gene expression level in the CP of third ventricle was 'fairly good' but the pattern was 'scattered'. The observed gene expression level in CP of fourth ventricle was 'low' with 'scattered' pattern. Thus during this time point the production of this protein by CP is assumed to be very less because the level of expression was not high and the pattern was very scattered.

At P 14 the observed gene expression level was 'good' in the CP of lateral ventricle and 'fairly good' in the rest of the two CPs. The observed gene expression pattern was still 'scattered'. At P 28, the observed gene expression level was 'fairly

good' in all of the CPs but at this time point the expression pattern was 'dense' in all of the CPs. Thus during this time point, the CP is assumed to produce the protein in large amount as compared to all of the previous time points. The observed gene expression pattern was dense only at P 28 (Fig 5.55) and not at any other of the six time points. This finding suggests that during postnatal development of Mus musculus brain, the production of Epas1 by the CP was low at the early postnatal time point of P 4. As the development proceeded, the production of Epas1 increased.

5.4.10.3 Expression of Epas1 in Brain other than Choroid Plexus:

During development, the observed gene expression for Epas1 has been observed in almost every part of the brain. In all of the embryonic time points, the main areas where gene expression was observed were pallium, subpallium, mesomere, pre optic area and rhombomeres. The observed gene expression of Epas1 was relatively low at E 18.5 in whole brain but was dense and high at P 14 and P 28 in whole brain. At P 14 and P 28, the gene expression was observed to be increased and now it appeared in almost every part of the brain. Thus during the two later postnatal time points, it is assumed that the protein was produced in larger amount in whole brain (Fig 5.56).

5.4.11 Igfbp-7 (Insulin-like growth factor- binding protein 7):

The observed gene expression for Igfbp-7 did not show any specificity with regards to the three CPs. The observed gene expression pattern was 'scattered' in embryonic choroidal tissues and in the CP at early postnatal time point of P 4 whereas

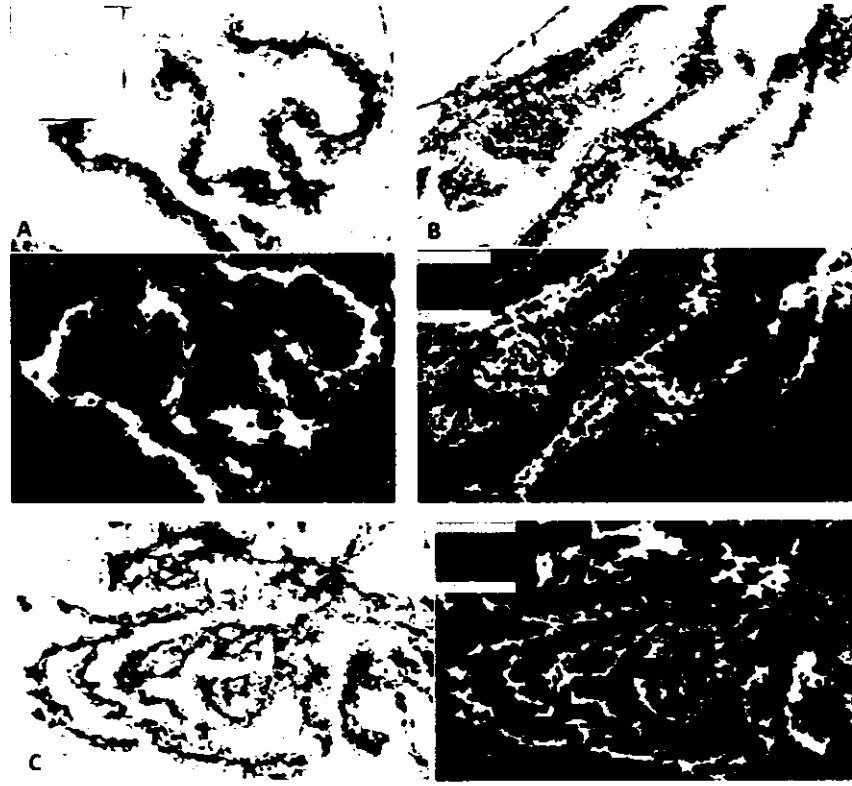


Fig 5.55: ISH image (white) and its corresponding expression images (black) of Epas1. A: CP of lateral ventricle at P 28. B: CP of third ventricle at P 28. C: CP of fourth ventricle at P 28.

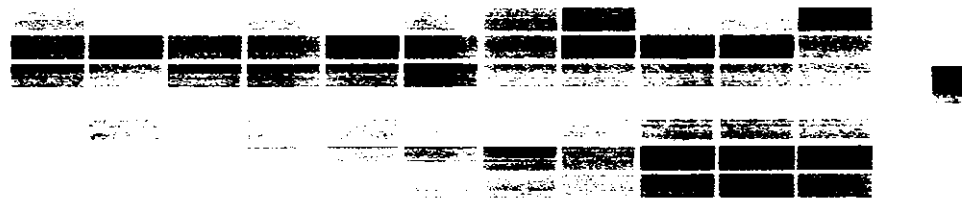


Fig 5.56: Expression summary of Epas1 in Mus musculus brain.

it was 'dense' in the later postnatal time points. The observed gene expression level varied temporally and spatially. For embryonic CP and for the CP of early postnatal time point (P 4), the observed gene expression showed 'inner specificity' for CP. The detailed results for each time point are as follow:

5.4.11.1 E 11.5, E 13.5, E 15.5 and E 18.5:

Gene expression for Igfbp-7 in CP was observed to be increased with the increase in embryonic development. During the early embryonic time point of E 11.5, the observed gene expression was very low. Spatial specificity was also observed during this time point and the gene expression appeared only in the choroidal tissues of fourth ventricle where the observed expression level and expression pattern was 'low' and 'dense'. Thus it is assumed that there was very less production of this binding protein by CP at E 11.5. At E 13.5, the gene expression was observed in all of the choroidal tissues but the gene expression level was 'negligible' in the choroidal tissues of third ventricle. Thus it is suggested that the protein was produced by the choroidal tissues of lateral and fourth ventricles only during E 13.5. The observed gene expression level was 'fairly good' in the choroidal tissue of lateral ventricle whereas it was 'good' in the choroidal tissues of fourth ventricle.

At E 15.5, the observed gene expression level was 'fair' in the choroidal tissues of lateral ventricle, 'negligible' in the choroidal tissues of third ventricle and 'very good' in the choroidal tissues of fourth ventricle. Thus just like the previous time point, the choroidal tissues of fourth ventricle is assumed to produce the protein in higher amount as compared to the other two choroidal tissues at E 15.5 and the choroidal tissues of third ventricle did not produce the protein. The observed level of

gene expression was higher in the CP of fourth ventricle at E 15.5 as compared to that of E 13.5. At E 18.5, the observed gene expression level in CP was highest among the four embryonic time point. The observed gene expression level was 'good' in the CP of lateral ventricle and 'very good' in the CP of third and fourth ventricles. Thus out of the four time points of embryonic development, the CP is assumed to produce the protein in higher amount at E 18.5.

During embryonic development spatial analysis regardless of the time points suggested that the CP of fourth ventricle produced the protein in higher amount and CP of third ventricle produced the protein in lower amount.

5.4.11.2 P 4, P 14 and P 28:

With proceed in the development to postnatal stage there observed a decrease in gene expression of Igfbp-7. The observed gene expression pattern also became 'dense' for the last two postnatal time points P 14 and P 28 and during these two time points the gene expression does not showed choroidal epithelial specificity. At P 4, the observed gene expression level was 'low' in the CP of lateral ventricles, 'fairly good' in the CP of third ventricle and 'good' in the CP of fourth ventricle. Thus during this time point, the CP of fourth ventricle is assumed to produce the protein in higher amount. At P 14 and P 28, the observed gene expression level was 'good' in all of the three CPs and the observed gene expression pattern was 'dense' (Fig 5.57).

5.4.11.3 Expression of Igfbp-7 in Brain other than Choroid Plexus:

The observed overall gene expression of Igfbp-7 in developing Mus musculus brain was not of excellent level except at the time point P 14 (Fig 5.58).

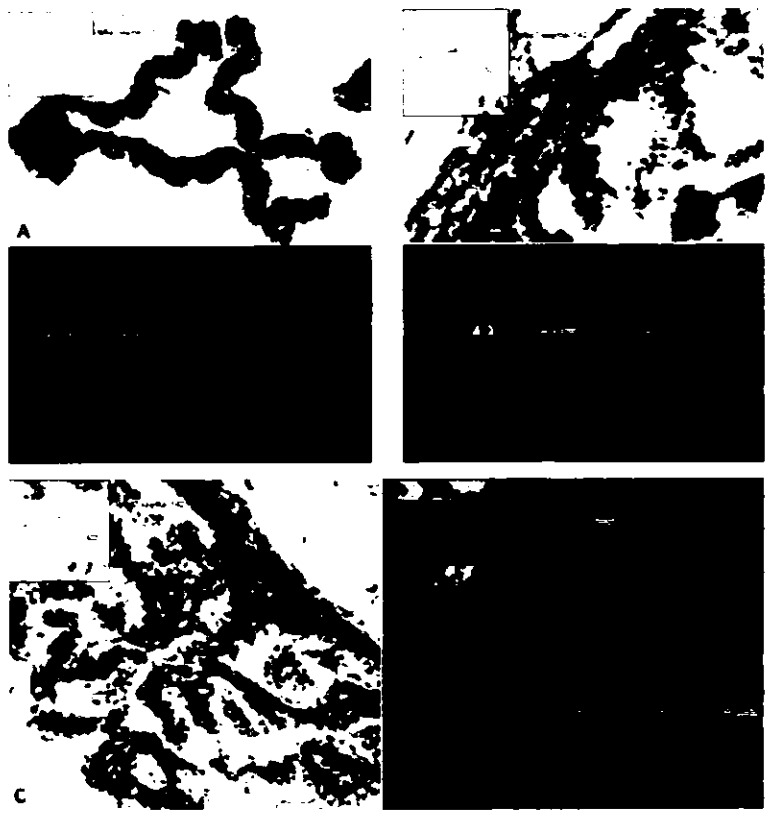


Fig 5.57: ISH image (white) and its corresponding expression images (black) of Igfbp-7 at P 28. A: CP of lateral ventricle. B: CP of third ventricle. C: CP of fourth ventricle.

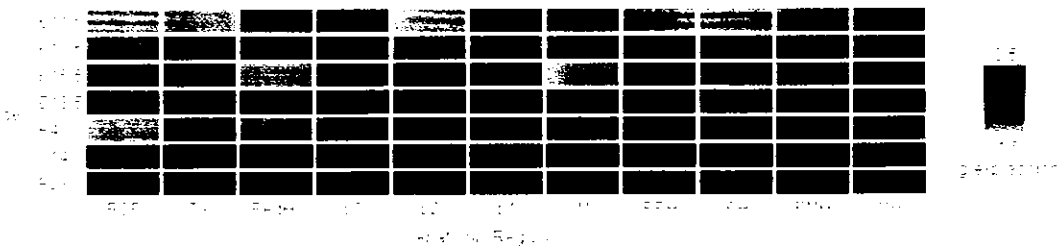


Fig 5.58: Expression summary of Igfbp-7 in Mus Musculus brain.

At P 14 the observed expression was of maximum level in all the major parts of the brain. During embryonic ages, the observed expression level was higher for E 18.5.

5.5 GEDDMCP (gene expression data of developing mouse choroid plexus):

GEDDMCP is the database which was created and fed with the resultant data of this research for Choroid plexus. To make a user friendly access to this database, a website has been constructed and the database was linked to this web site. A user can retrieve gene expression data for all of the 20 genes either in the form of gene expression images (a total of 21 images for each gene except for Ttr which has 42 images) or in the form of gene report one for each gene. Each report contains three things:

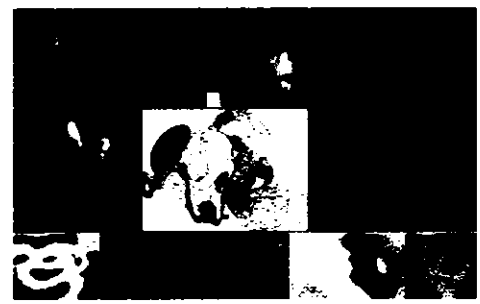
1. Functional description of protein.
2. Whole brain gene expression summary for all of the seven time points.
3. Presence and role of protein in CSF.

From the home page of this website (Fig 5.59) a user can navigate one of the three locations by selecting anyone at a time. These three locations contains three datasets which are 'All genes' (Fig 5.60), 'temporally expressed genes' (Fig 5.61) and 'spatially expressed genes' (Fig 5.62). From 'All genes' a user can retrieve data about 'CP-Specific genes' (Fig 5.63) and 'non CP-Specific genes'. From the other two locations a user can get temporal and spatial gene expression data. Flow of forms is described in flow chart given in Fig 5.64.

GEDDMCP

Gene Expression Data of Developing Mouse Choroid Plexus

Background
Introduction
Sources
Contact Us
FAQ



— select —
— select —
All genes
Spatial gene expression
Temporal gene expression

Developing mouse choroid plexus gene expression analysis is an interesting project. This project provides information to the researchers about spatial and temporal gene expression data specifically for choroid plexus of developing mouse brain

Fig 5.59: Home page of GEDDMCP.

List of the genes which are expressed in Choroid Plexus of Developing mouse brain

— select —
— select —
Cp specific genes
non Cp specific genes

Gene ID	Gene Symbol	Gene Name
11421	Ace	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1
11669	Aldh2	Aldehyde dehydrogenase 2, mitochondrial
11744	Anxa11	Annexin A11
11816	Apoe	Apolipoprotein E
12349	Car2	Carbonic anhydrase 2
12759	Clu	Clusterin
13819	Epas1	Endothelial PAS domain protein 1
14164	Fgf2	Fibroblast growth factor 2
15560	Htr2c	5-hydroxytryptamine (serotonin) receptor 2C

Fig 5.60: Web page of GEDDMCP displaying list of all CP genes.

Temporal Gene expression

1) List of genes whose gene expression is temporally specific to some of the time points.

Gene / Time point	Tgfb-2 (Transforming growth factor beta-2)	Igfbp-3 (Insulin-like growth factor-binding protein 3)	Anxa 11 (Annexin A11)	A2m (alpha-2-macroglobulin)	Aldh-2 (Aldehyde dehydrogenase 2)	CD 164 (antigen)	Hspb8 (Heat shock protein 8)
E 11.5 TCh	----	----	----	----	----	----	----
E 11.5 P2Ch/P3Ch	----	----	----	----	----	----	----
E 11.5 V4	----	----	----	----	----	----	S
E 13.5 TCh	----	I,S	----	----	S	S	----
E 13.5 P2Ch/P3Ch	----	I,S	----	----	S	S	----
E 13.5 V4	----	I,S	----	----	S	S	----
E 15.5 TCh	----	I,S	----	----	S	----	----

Fig 5.61: Temporal gene expression web page of GEDDMCP.

Spatially Expressed Genes

List of Genes expressed in CP of 'Lateral ventricles':

Gene ID	Gene Symbol	Gene Name	E11.5 lateral controls	E12.5 lateral controls	E13.5 lateral controls	E14.5 lateral controls	E15.5 VL	E16.5 lateral controls	E17.5 VL	E18.5 VL	P1 lateral controls	P2 lateral controls	P4 lateral controls	P
11421	Ace	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	E11.5 VL	E12.5 VL	E13.5 VL	E14.5 VL	E15.5 VL	E16.5 VL	E17.5 VL	E18.5 VL	P1 VL	P2 VL	P4 VL	P
11669	Aldh2	Aldehyde dehydrogenase 2 mitochondrial	E11.5 VL	E12.5 VL	E13.5 VL	E14.5 VL	E15.5 VL	E16.5 VL	E17.5 VL	E18.5 VL	P1 VL	P2 VL	P4 VL	P
11744	Anxa11	Annexin A11	E11.5 VL	E12.5 VL	E13.5 VL	E14.5 VL	E15.5 VL	E16.5 VL	E17.5 VL	E18.5 VL	P1 VL	P2 VL	P4 VL	P
11816	Apoe	Apolipoprotein E	E11.5 VL	E12.5 VL	E13.5 VL	E14.5 VL	E15.5 VL	E16.5 VL	E17.5 VL	E18.5 VL	P1 VL	P2 VL	P4 VL	P
12349	Car2	Carbonic anhydrase 2	E11.5 VL	E12.5 VL	E13.5 VL	E14.5 VL	E15.5 VL	E16.5 VL	E17.5 VL	E18.5 VL	P1 VL	P2 VL	P4 VL	P
12759	Clu	Clusterin	E11.5 VL	E12.5 VL	E13.5 VL	E14.5 VL	E15.5 VL	E16.5 VL	E17.5 VL	E18.5 VL	P1 VL	P2 VL	P4 VL	P

Fig 5.62: Spatially expressed gene web page of GEDDMCP.

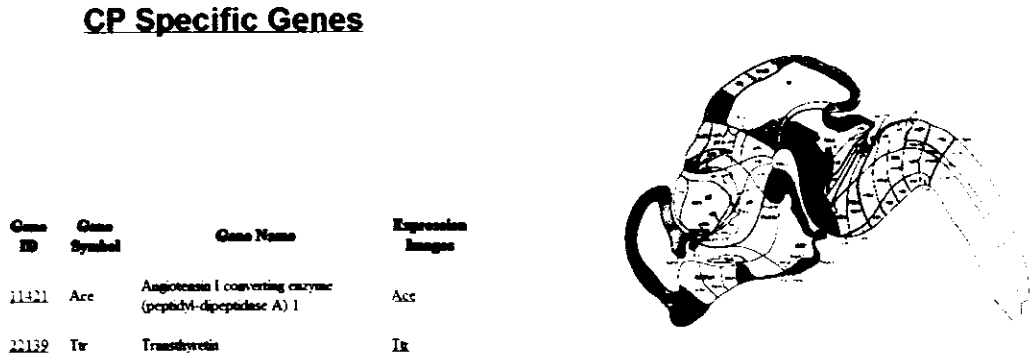


Fig 5.63: Web page of GEDDMCP displaying list of CP-Specific genes.

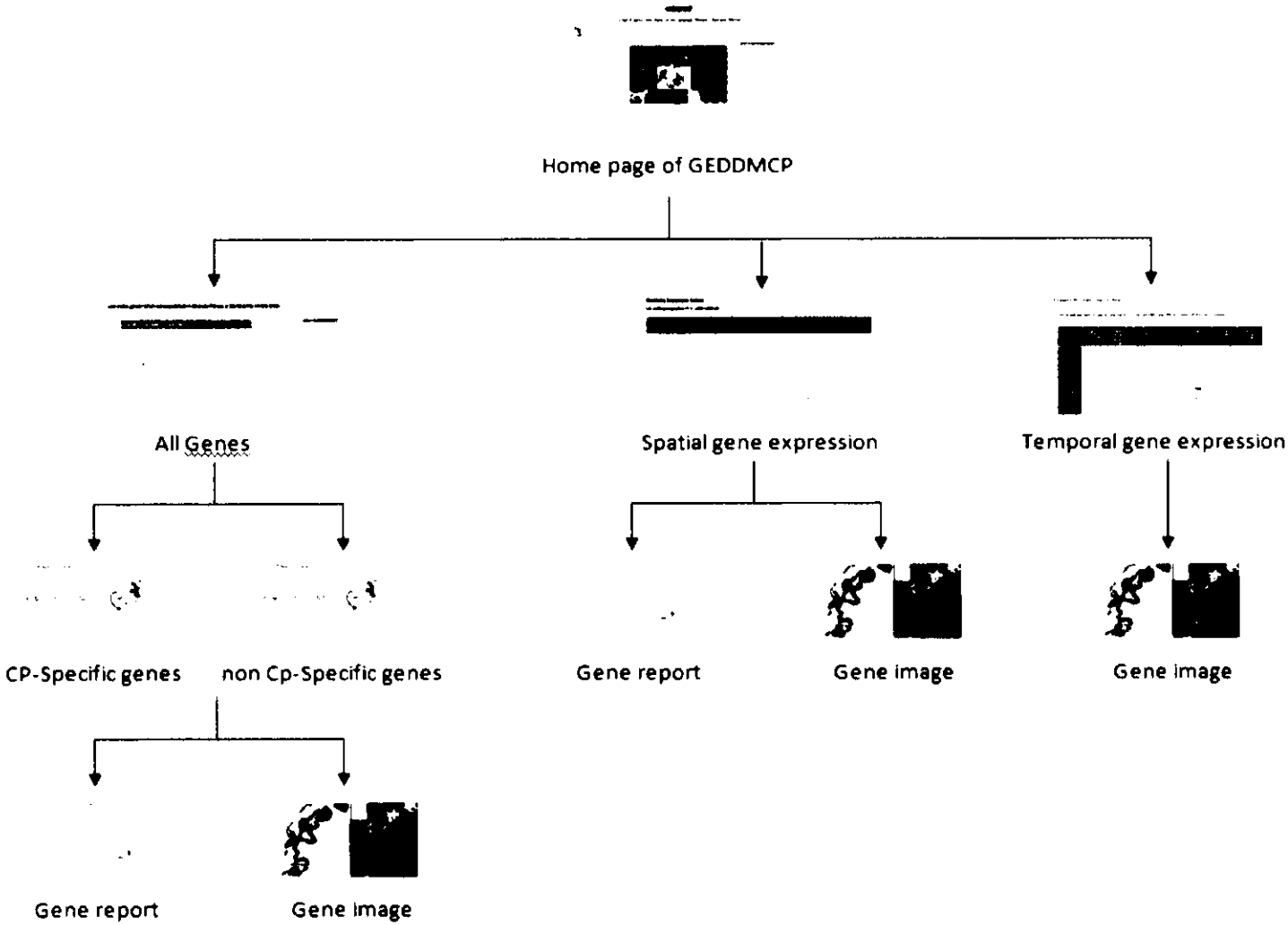


Fig 4.64: Flow chart of GEDDMCP.

CHAPTER 6

DISCUSSION

Choroid plexus is known to secrete various factors into CSF along with CSF itself (Siegel *et al.*, 1999, Speake *et al.*, 2001 and Brown *et al.*, 2004) into the ventricles of the brain which then circulates throughout the brain and delivers these factors to other parts of the brain (Redzic *et al.*, 2005). During embryonic development, the embryonic CSF plays regulatory roles in cortical cell proliferation and maintenance (Zappaterra *et al.*, 2007) and thus CSF plays vital role for the development of brain from very early embryonic time points (Miyan *et al.*, 2003). To determine the factors which are specifically produced by choroid plexus and secreted into CSF in very early stage of development of mouse brain a spatial and temporal gene expression analysis has been carried out for the three CPs across four embryonic and three postnatal time points of development of Mus musculus brain.

After carrying out the detailed spatial and temporal gene expression data analysis for developing mouse choroid plexus, it has been found that the gene expression level and pattern is different for every gene. Four types of expression patterns have been found for these 20 genes regardless of the expression level. In some cases, almost all of the cells of choroid plexus were expressing the gene and thus the pattern was simply named as dense. Dense expression have been observed for the CP-specific gene Transthyretin and

for some non CP-specific genes too which includes Insulin-like growth factor 2 insulin-like growth factor binding protein-2, Clusterin and some time points for Aldehyde dehydrogenase 2, Carbonic anhyderase 2, CD-164 (antigen), 5-hydroxytryptamine (serotonin) receptor 2C, Apolipoprotein E and Insulin-like growth factor binding protein-7. On the contrary, for some genes, only few cells of choroid plexus showed expression and thus it was said that the pattern was scattered or rare. Scattered expression pattern have been seen for a CP-specific enzyme the Angiotensin I converting enzyme and for some of non CP-specific genes which were transforming growth factor beta-2, inositol 1,4,5-triphosphate receptor, type 1, Annexin A11, alpha-2-macroglobulin, angiopoietin-like 2, Carbonic anhydrase 2, Cd164, Fibroblastic growth factor receptor-2, Apolipoprotein-2, Endothelial PAS domain protein 1 and Heat shock protein-8 . In the third case, only the outer layer which is the choroid plexus epithelium (composed of the modified ependymal cells) expressed the particular gene and not the inner layers of CP which indicated that the gene expression was confined only to the CP epithelium. Both dense and scattered patterns have been found in this type of gene expression specificity. Dense epithelial specific gene expression pattern have been observed for Clusterin, Insulin-like growth factor- binding protein 2 and for certain time points of Angiotensin I converting enzyme and 5-hydroxytryptamine (serotonin) receptor 2C. As the choroidal epithelium is known to secrete factors into CSF (Brown *et al.*, 2004 and Thouvenot *et al.*, 2006) and the presence of the above mentioned proteins in CSF have already been reported thus it can be said that these proteins have been secreted into CSF by CP. Similarly, in the fourth type of expression pattern, the expression was confined to the innermost layer of the structure of CP and was not observed in the outer ependymal layer

and for this type of expression also, both cases of scattered and dense pattern have been seen. Scattered inner expression pattern appeared in some time points for Insulin-like growth factor- binding protein 7, Endothelial PAS domain protein 1, and Insulin-like growth factor binding protein 4 and for Insulin-like growth factor- binding protein 3 in all of the time points. Inner dense expression pattern have been seen in the case of Insulin-like growth factor- binding protein-4 and only in CP of lateral ventricle at P 4 for Insulin-like growth factor- binding protein-7. In this fourth type of gene expression pattern the staining is in choroidal capillaries only and thus it is assumed that the factors were synthesized by choroidal capillaries and may be transported across from blood to CSF through CP because their presence in the CSF have already been reported and it is already been known that some proteins are transferred across the blood plasma to CSF through CP (Johansson *et al.*, 2006 and Liddelow *et al.*, 2009).

All of these proteins have been found to perform vital functions for the development of the brain and for normal functioning of the brain and this have already been reported in the published literature. The two growth factors IGF-2 and Tgfb-2 regulates cell growth and proliferation and elevated levels or deficiency of these proteins in CSF can lead to serious diseases and disorders (Bai *et al.*, 2006 and Swardfager *et al.*, 2010). Four were insulin like growth factor binding proteins Igfbp-2, Igfbp-3, Igfbp-4 and Igfbp-7. During cell growth and development they modulate the activity and availability of IGFs (Yamanaka *et al.*, 1997, Baxter *et al.*, 2000, Firth *et al.*, 2002 and Chesik *et al.*, 2007). Two genes Ttr and Apoe encode carrier proteins. Ttr is CP-specific transport protein in brain which carries retinol (vitamin) and thyroxin (thyroid hormone)

and thus plays regulatory role during growth and development (Herbert *et al.*, 1986). Apoe is the main lipid carrier protein in CNS and in embryonic CSF it is thought to be required for neural differentiation (Parada *et al.*, 2008). Two genes encode enzymes and one encodes isozyme and these were Ace, Aldh-2 and Car-2. Ace is CP-specific gene and it regulates Angiotensin. Aldh-2 catalysis conversions of aldehydes to their corresponding carboxylic acids and Car-2 catalyses the reversible reaction of carbon dioxide hydration and HCO_3^- dehydration. When in CSF, the two enzymes have been found to be associated with various serious conditions like schizophrenia, Alzheimer disease and Parkinson disease (Zubenko *et al.*, 1985, Wahlbeck *et al.*, 2000 and Bai *et al.*, 2011). Inhibition of Car-2 reduces the formation of CSF by 30-50% (Swenson, 2003). The binding protein Anxa11 is calcium and phospholipid binding protein and is expressed in CP and plays vital role in CSF secretion as the secretion occurred as a result of coordinated transport of calcium and other ions and water across the epithelial layer (Speake *et al.*, 2001 and Pollay, 2010). Hspb8 is a small heat shock protein and it acts as molecular chaperons (Li *et al.*, 2004). The gene Alpha 2 macroglobulin (A2m) inhibits proteinases and acts as cytokine transporter and also serves as a parameter for the condition of blood-CSF barrier (Schliep and Felgenhauer, 1974 and Borth, 1992). Clusterin has a variety of functions and it acts as molecular chaperon, transporting protein for lipoproteins, modulator of cell-cell interactions, tumour suppressor gene, preapototic and antiapoptotic factor (Poon *et al.*, 2002, Leskov *et al.*, 2003 and Wyatt, 2009). Many studies show that Clusterin is contained in cerebrospinal fluid and the protein is thought to be associated with Alzheimer's disease (Sihlbom *et al.*, 2008 and Yerbury *et al.*, 2010). Epas1 is the transcription factor that induces the genes regulated by oxygen. The

gene is expressed selectively in vascular endothelial cells (Tian *et al.*, 1997, and Skuli *et al.*, 2009) and the study clearly show the expression of this gene in vascular endothelial cells of choroidal capillary.

Overall, no fixed or specific timing and sequence of synthesis for these 20 molecules has been observed in the three CP locations. But as far as individual gene expression was concerned, there were genes which expressed themselves in embryonic stages but not in postnatal time points and vice versa. Like in the case of Transforming growth factor beta 2, the embryonic CP did not produce the growth factor except the CP of fourth ventricle at E 18.5, but the postnatal CP produced it. Similar is the case with Annexin A 11 where there was no expression of this gene in CP in embryonic time points but there was expression in CP of postnatal ages. This observation supports the study of Weise *et al.*, 1993 and Parada *et al.*, 2008 in which they suggested that proteins may have different effects in the immature and mature nervous system. The protein was not produced by the CP and thus it was not secreted into CSF by the embryonic brain but there are chances of the protein being secreted into CSF in the postnatal brain. This can be due to the reason that the brain function and structure experience dramatic changes from embryonic to postnatal development (Andersen, 2003 and Han *et al.*, 2009). Further wet lab experimentation is needed to prove the hypothesis. Until now certain researchers have studied the gene expression of CP- Specific gene Ttr during development of mouse brain, in which they have concluded that Ttr first appears in CP of fourth ventricle (Tiziana *et al.*, 1993 and Thomas *et al.*, 1988). However this study indicates that it is not the case, as it appeared in all of the CPs throughout development

Some other cases have been observed where there was no production of the molecule during early time points of development which are E 11.5, E 13.5 and even E 15.5. but as the development proceeds CPs started to produce the molecule in late embryonic stages and continued to produce it in postnatal ages. This has been observed in the case of Insulin-like growth factor binding protein-3 in which choroidal tissues of E 11.5 did not produce the protein, Insulin-like growth factor binding protein-4 which was not being produced by telencephallic choroidal tissues at E11.5, Alpha-2 microglubulin which started to show its expression at E 15.5 only in fourth ventricle and then continued to express in the later ages in all of the CPs, Fibroblastic growth factor-2 which was not produced by telencephallic choroidal tissues and choroidal tissues of prosomere only at E 11.5 and similar were the cases for 5-hydroxytryptamine (serotonin) receptor 2C, Endothelial PAS domain protein 1, Heat shock protein 8 and Insulin-like growth factor-binding protein-7 respectively. From this scenario, it is hypothesised that in very early stages of embryonic development, as only the basic structure of the brain is there and the number of cells which needs the molecule are less, and lesser functions have to be performed by the brain as compared to the later time points. That is why these proteins are not being produced by choroid plexus and ultimately are not secreted into CSF in very early stages of development.

No specific sequence for synthesis of these 20 molecules by the three CPs has been found. But it has been observed that in certain cases the gene expression first appeared in the CP of fourth ventricle at a certain age and then in the other two CPs in the later age. Such cases have been reported for six genes which are Fibroblast growth factor

receptor-2, 5-hydroxytryptamine (serotonin) receptor 2C, Heat shock protein 8, Insulin-like growth factor- binding protein 7, Alpha-2-macroglobulin and Transforming growth factor beta-2. It has also been found for two genes, Insulin-like growth factor- binding protein-4 and Endothelial PAS domain protein-1, that during development the gene expression first appeared in choroidal tissues of both the third and fourth ventricle at an earlier time point and later it appeared in the lateral ventricle. But there was no case where choroidal tissues of lateral ventricles produced a protein before the other two CPs. Thus, this study showed that the CP of lateral ventricle did not start to produce CSF proteins before the other two CPs.

For the one CP-specific gene Transthyretin, no timing and sequence of synthesis of the protein by the three CPs at all the seven time points have been observed. All of the CPs produced the protein in all of the seven time points. This can be due to the reason that the need of these proteins by the brain cells is only met by CP throughout the development and thus the protein is produced efficiently without any pause throughout the development. The excellent expression level of this gene in CP indicated that the protein was being produced by CP in a very good amount and to meet the requirement of this protein by whole brain. For the other CP-specific gene Angeotensin I converting enzyme, the gene was switched on at late embryonic time point E 15.5 but there was no sequence of switching on or off of the gene among the three CPs and all were switched like once for all.

While observing all the three CPs individually, for their contribution in secreting the molecules into CSF, no specific rule or pattern has been observed about less or more

contribution from any one of the three CPs. The gene expression level between the three CPs varied among the different genes and all of the 20 genes had their own variations in gene expression level. Hence it can be said that there was no observed hard and fast rule that CP of lateral ventricle or third ventricle or fourth ventricle contributes more in the production of molecules and their secretion into CSF than the rest of the two CPs.

Out of these 20 genes four genes were found for which literature does not report their protein products in CSF but this study has observed their gene expression in developing mouse CP which suggests their production in developing mouse CP and secretion into CSF. These four genes are Annexin A11, Aldehyde dehydrogenase-2, heat shock protein 8 and Inositiol 1,4,5-triphosphate receptor type 1. The suggested reason is that the functions of all of these four gene products are somehow associated with CSF. For instance, Anxa11 is a Ca^{2+} binding protein (Gerke and Moss, 2001) and Itpr-1 is a Ca^{2+} release channel which is involved in early development and controls Ca^{2+} dependant cell functions (Monkawa *et al.*, 1998 and Mikoshiba, 2006) and the mechanism of CSF secretion involves movement of Ca^{2+} (Brown *et al.*, 2004) therefore these proteins might be involved with CSF secretion from CP. Similarly Heat shock binding proteins are particularly abundant in nerve cells (Kampinga *et al.*, 2009) and Marques *et al.*, in their 2011 study showed that the genes encoding for molecules that modulate adult neural stem cell proliferation and fate are transcribed in the CP and distributed via CSF therefore this protein might be secreted into CSF from CP. The protein Aldh-2 degrades toxic aldehydes and works for the prevention of neurodegenerative diseases (Ohsawa *et al.*, 2008) and both CP and CSF are thought to have close relations with neurodegenerative

diseases specially Alzheimer's disease (Serot *et al.*, 2003 and Carrette *et al.*, 2003) therefore this protein is might be secreted into CSF from CP.

In case of enzymes, CP was not found to be producing the protein in good amount during embryonic development but the production increased during the postnatal development and the highest production of enzyme by the CPs was during the last postnatal stage which was analyzed for gene expression and it was P 28. For both of the enzymes whose gene expression was analyzed in this study, the CPs did not produce any amount of them during the very early embryonic time point of E 11.5.

Majority of these 20 CP genes have been found to be associated with the Alzheimer's disease and for some genes varied levels of their proteins into CSF during Alzheimer's disease have been reported in the published literature. These CP genes include Inositol 1,4,5-triphosphate receptor, type 1, Apolipoprotein E, Transthyretin, Alpha-2-macroglobulin, angiotensin I converting enzyme, Clusterin, transforming growth factor beta 2, insulin like growth factor binding protein-3, insulin like growth factor binding protein-2, Aldehyde dehydrogenase 2 (mitochondrial)., Carbonic anhydrase II and 5-hydroxytryptamine (serotonin) receptor 2C. The gene expression of all these genes have been analyzed in this study and it is suggested that as these proteins are secreted into CSF by the CP thus CP may have a role in Alzheimer's disease. Close relation between CP, CSF proteins and Alzheimer's disease have also been reported in various literatures (Serot *et al.*, 2003).

Other than CP, the gene expression of almost all of these 20 molecules has been found in the ependymal cell layer that lines the ventricles. Some past studies also hypothesized that the extra choroidal CSF is secreted into the ventricles by the ependymal cells that lines them (Perez-Figares *et al.*, 2001, Edsbagge *et al.*, 2004 and Veening, 2010). Through this study it is concluded that this hypothesis may be a fact because all of the genes analyzed during this study showed their gene expression in these ependymal cells along with the CP. Even in the very early stage of embryonic development of E 11.5 when there does not exist any structure of choroid plexus, these ependymal cells do show the gene expression of these molecules. These ependymal cells are known to undergo modifications during brain development and thus differentiate into choroid plexus epithelium. In the later ages and in the adult brain this choroid plexus epithelium is continuous with the ependymal cell layer that lines the ventricles and this lining connects the three CPs in the ventricular system. Gene expression analysis of this study suggest that in majority of the cases, this ependymal layer continues to show the gene expression from the early embryonic time points to the later postnatal ages. Expression levels of these genes in ependymal cell lining are almost the same as in CP or lower than that.

Uptil now there was no online database available to the researchers for gene expression data of developing mouse Choroid plexus. As a contribution to the field of Bioinformatics a SQL database have been designed based on the research data and a web site named as GEDDMCP (Gene Expression Analysis of Developing Mus musculus

Choroid Plexus) have been created. Gene expression data only for the choroid plexus of Mus musculus can be accessed by the researchers on this web site.

CONCLUSION AND FUTURE WORK

Temporal and spatial gene expression analysis of choroid plexus reveals that the gene expression level and pattern for every CP gene is unique and there is some sort of switching 'on and off' of different CP genes temporally and spatially but there is not any group of proteins that share the similar behavior for switching on and off at the three locations. There is also some sequence of switching on and off of these CP genes but it varies with individual genes. It has been found that CP starts to produce some proteins from very early embryonic stage even before E 15.5 when the CP is not fully developed and it is in the form of choroidal tissues. As all of these CP gene products are present in CSF or have close relation with CSF thus it is concluded that these factors are secreted into CSF by the CP. All of these proteins are known to perform vital functions for the development and normal functioning of the brain thus the CP is said to be playing vital roles for the development of the brain by producing these factors and secreting into CSF which transport them globally to the brain. Spatially no CP has been found to be contributing more or less for the production of these proteins as compared to the other two CPs and thus it cannot be said that anyone of the three CPs contribute more or less in the production of CSF factors.

Literature does not report Anxa11, Aldh-2 and Itpr-1in CSF but this study has observed its gene expression in developing mouse CP and their functions suggest that they might have some relation with CSF. The findings of this study suggest that other

than CP, these CP genes are also being produced by the ependymal cells that line the ventricles and thus they can be thought of contributing in the secretion of extra choroidal CSF. Further, almost all of these CP genes have been reported to have some relation with Alzheimer's disease too.

Ultimate benefit of this laborious study is the development of a user friendly tool GEDDMCP which contains resultant data of this study. So that in future no one will have to go through the same laborious, time consuming steps which were necessary for this study.

Further possible implications of the CP project are as follows:

1. Wet lab experiments for individual gene to examine the level of these factors secreted into the CSF by CP and to determine the complete pathway for each protein starting from expression in CP, secreted into CSF and utilization by cells of the developing brain.
2. Further dry lab or wet lab experiments can be conducted to determine that:
    - Why the expression in choroid plexus for some genes differs in embryonic and post natal time points? And
    - Why some of the factors are not present in Blood-brain barrier in the very early time points of E11.5 and E 13.5?
    - For Fibroblast growth factor receptor-2, 5-hydroxytryptamine (serotonin) receptor 2C, Heat shock protein 8, Insulin-like growth factor- binding protein 7 and Transforming growth factor beta-2, why the gene expression first

appears in fourth ventricle during development of the brain and then in the other two CPs later.

3. Experiments can be conducted to report the relation of Anxa11, Aldh-2, Itpr-I and Hspb-8 with CSF and to determine that either they are secreted from CP to CSF or they are the house keeping genes of CP.

REFERENCES

Aldred A.R., C. M. Brack and G. Schreiber. 1995. The cerebral expression of plasma protein genes in different species. Comparative Biochemistry and Physiology; 111B: 1–15.

Andersen S. L. 2003. Trajectories of brain development: point of vulnerability or window of opportunity?. Neuroscience and Biobehavioral Reviews; 27(1-2): 3–18.

Annerbrink K., E. G. Jönsson, M. Olsson and S. Nilsson. 2010. Associations between the angiotensin-converting enzyme insertion/deletion polymorphism and monoamine metabolite concentrations in cerebrospinal fluid. Psychiatry Research; 179: 231–234.

Aronow B. J., S. D. Lund, T. L. Brown, J. A. Harmony and D. P. Witte. 1993. Apolipoprotein J expression at fluid-tissue interfaces: potential role in barrier cytoprotection. Proceedings of National Academy of Science U.S.A.; 90: 725–729.

Bae G. U., U. Gaio, Y. J. Yang, H. J. Lee, J. S. Kang and R. S. Krauss. 2008. Regulation of Myoblast Motility and Fusion by the CXCR4-associated Sialomucin, CD164. The Journal of Biological Chemistry; 283: 8301–8309.

Bai B., B. Chen, P. Jiang, Z. J. Liu, N. C. Huang, Z. C. Gong, X. Z. Du. 2006. Changes of serum and cerebrospinal fluid insulin-like growth factor-II levels in neonates with hypoxic-ischemic encephalopathy. Zhongguo Dang Dai Er Ke Za Zhi; 8(3):187–90.

Bai J. and Y. Mei. 2011. Overexpression of Aldehyde Dehydrogenase-2 Attenuates Neurotoxicity Induced by 4-Hydroxynonenal in Cultured Primary Hippocampal Neurons. Neurotoxicity Research; 19(3): 412-422.

Baker J., J. P. Liu, E. J. Robertson and A. Efstratiadis. 1993. Role of insulin-like growth factors in embryonic and postnatal growth. Cell; 75(1): 73–82.

Brar A. K. and S. D. Chernausek. 1993. Localization of insulin-like growth factor binding protein-4 expression in the developing and adult rat brain: analysis by in situ hybridization. Journal of Neuroscience Research; 35(1): 103–14.

Batemen A. 2007. EDITORIAL. Nucleic Acids Research, Database issue; 35: D1–D2.

Baxter R. C. 2000. Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities. American Journal of Physiology; 278: E967–E976.

Birney E. and M. Clamp. 2004. Biological database design and implementation. Briefings in Bioinformatics; 5(1): 31–38.

Borth W. 1992. α_2-Macroglobulin, a multifunctional binding protein with targeting characteristics. FASEB; 6: 3345–3353.

Brown P. D., S. L. Davies, T. Speake and I. D. Millar. 2004. Molecular Mechanisms of Cerebrospinal Fluid Production. Neuroscience; 129(4): 957–970.

Buxbaum J. N. and N. Reixach. 2009. Transthyretin: the servant of many masters. Cellular and Molecular Life Sciences; 66(19): 3095–101.

Carrette O., I. Demalte, A. Scherl, O. Yalkinoglu, G. Corthals, P. Burkhard, D. F. Hochstrasser and J. C. Sanchez. 2003. A panel of cerebrospinal fluid potential biomarkers for the diagnosis of Alzheimer's disease. Proteomics; 3:1486–1494.

Chanoine J. P., S. Alex, S. L.Fang, S. Stone, J. L. Leonard, J. Korhle and L. E. Braverman. 1992. Role of transthyretin in the transport of thyroxine from the blood to the choroid plexus, the cerebrospinal fluid, and the brain. Endocrinology; 130: 933–938.

Chesik D., J. D. Keyser and N. Wilczak. 2007. Insulin-like growth factor binding protein-2 as a regulator of IGF actions in CNS: Implications in multiple sclerosis. Cytokine and Growth Factor Reviews; 18(3): 267–278.

Chodobska J. S., C. L. Pascale, A. N. Pfeffer, C. Coulter and A. Chodobski. 2007. Expression of junctional proteins in choroid plexus epithelial cell lines: a comparative study. Cerebrospinal Fluid Research; 4:11.

Clemmons D. R. 1997. Insulin-like growth factor binding proteins and their role in controlling IGF actions. Cytokine and Growth Factor Rev; 8(1): 45–62.

Coates D. 2003. Molecules in focus The angiotensin converting enzyme (ACE). The International Journal of Biochemistry & Cell Biology; 35: 769–773.

Conrad J., M. Paul and T. Rosemarie. 2004. Homeostatic capabilities of the choroid plexus epithelium in Alzheimer's disease. Cerebrospinal Fluid Research; 1: 3.

Corbo M., C. Lunetta, P. Magni, E. Dozio, M. Ruscica, L. Adobbati and V. Silani. 2010. Free insulin-like growth factor (IGF)-1 and IGF-binding proteins-2 and -3 in serum and cerebrospinal fluid of amyotrophic lateral sclerosis patients. European Journal of Neurology; 17(3): 398–404.

Crippa V., D. Sau, P. Rusmini, A. Boncoraglio and E. Onesto. 2010. The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). Human Molecular Genetics; 19(17): 3440–56.

Cuff A. J., G. M. P. Coates, T. J. R. Cutts and M. Rae. 2011. The Ensembl Computing Architecture. Genome Research; 14: 971–975.

De Maio A. 1999. Heat shock proteins: facts, thoughts, and dreams. Shock; 11(1): 1–12.

Doyonnas R., J. Y. H. Chan and L. H. Butler. 2000. CD164 Monoclonal Antibodies That Block Hemopoietic Progenitor Cell Adhesion and Proliferation Interact with the First Mucin Domain of the CD164 Receptor. The Journal of Immunology; 165: 840–851.

Edsbagge M., M. Tissel, L. Jacobsson and C. Wikkelso. 2004. Spinal cerebrospinal fluid absorption in healthy individuals. American Journal Physiology-Regulatory, Integrative and Comparative Physiology; 287: 1450–1455.

Emerich D. F., A. V. Vasconcellos, R. B. Elliott, S. J. M. Skinner and C. V. Borlongan. 2004. The choroid plexus: function, pathology and therapeutic potential of its transplantation. Expert Opinion on Biological Therapy; 4(8): 1191–1201.

Erickson J. A., R. Cousin, J. T. Wu and E.R. Ashwood. 2003. Quantitative Spectrophotometric Microplate Assay for Angiotensin-converting Enzyme in Cerebrospinal Fluid. Clinical Chemistry; 49: 970–972.

Eswarakumar V. P., I. Lax and J. Schlessinger. 2005. Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev.;16:139–49.

Ezzat S. and S. L. Asa. 2005. FGF receptor signaling at the crossroads of endocrine homeostasis and tumorigenesis. Horm Metab Res; 37: 355–60.

Fang Q., A. Strand, W. Law, V. M. Faca, M. P. Fitggibbon, N. Hamel, B. Howle, X. Liu, D. H. May, G. Poschmann, L. Roy, K. Stuhler, W. Ying, J. Zhang, Z. Zhang, J. J. M. Bergeron, S. Hamash, F. He, B. R. Leavitt, H. E. Meyer, X. Qian and M. W. Mcintosh. 2009. Brain-specific protein decline in the cerebrospinal fluid of humans with Huntington disease, Molecular and cellular proteomics; 8(3): 451-466.

Feldman S. R., S. L. Gonias and S. V. Pizzo. 1985. Model of a2-macroglobulin structure and function. Proceedings of National Academy of Science of the USA; 82: 5700–5704.

Firth S. M. and R. C. Baxter. 2002. Cellular Actions of the Insulin-Like Growth Factor Binding Proteins. Endocrine Reviews; 23(6): 824–854.

Gerke V. and S. E. Moss. 2001. Annexins: From Structure to Function. Physiol Rev; 82: 331–371.

Han M. E., H. J. Kim, Y. S. Lee, D. H. Kim, J. T. Choi, C. S. Pan, S. Yoon, S. Y. Baek, B. S. Kim, J. B. Kim and S. O. Oh. 2009. Regulation of cerebrospinal fluid production by caffeine consumption. BMC Neurosci; 10: 110.

Hana X., X. Wua, W. Chungb, T. Lia, A. Nekrutenkob, N. Altmanb, G. Chena and H. Maa. 2009. Transcriptome of embryonic and neonatal mouse cortex by high-throughput RNA sequencing. PNAS; 106:31: 12741–12746.

Hatters D. M., C. A. Peters-Libeu and K. H. Weisgraber. 2006. Apolipoprotein E structure: insights into function. Trends in Biochemical Sciences; 31(8): 445–454.

Herbert J., J. N. Wilcox, K. T. Pham, R. T. Jr. Fremeau, M. Zeviani, A. Dwork, D. R. Soprano, A. Makover, D. S. Goodman and E. A. Zimmerman. (1986). Transthyretin: A choroid plexus-specific transport protein in human brain: The 1986 S. Weir Mitchell Award. Neurology; 36(7): 900–11.

Heine U. I., E. E. Munoz and K. C. Flanders. 1991. Role of transforming growth factor-B in the development of the mouse embryo. Journal of Cell Biology; 115(4): 1091–1105.

Hill M. J. and G. P. Reynolds. 2007. 5-HT2C receptor gene polymorphisms associated with antipsychotic drug action alter promoter activity. Brain Res; 1149: 14–7.

Hoeflich A. and R. Reisinger. 2001. Insulin-like Growth Factor-binding Protein 2 in Tumorigenesis: Protector or Promoter?, Cancer Research; 61: 8601–8610.

Holmes C., M. J. Arranz, J. F. Powell, D. A. Collier and S. Lovestone. 1998. 5-HT2A and 5-HT2C receptor polymorphisms and psychopathology in late onset Alzheimer's disease. Hum Genet; 7: 1507–1509.

Hoyer D., J. P. Hannon and G. R. Martin. 2002. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav; 71: 533–54.

Iseki S., A. O. M. Wilkie and G. M. Morriss-Kay. 1999. Fgfr1 and Fgfr2 have distinct differentiation- and proliferation-related roles in the developing mouse skull vault. Development; 126: 5611–5620.

Jacob R. and H. Yadong. 2004. Yadong. ApoE genotype accounts for the vast majority of AD risk and AD pathology. Elsevier; 25(5): 641– 659.

Jimenez A. J., L. P Fernandez and P. J. M. Figares. 2001. Neural input and neural control of the subcommissural organ. Microscopy Research and Technique; 52: p. 520–533.

Johansson P. A., K. M. Dziegielewska, C. J. Ek, M. D. Habgood, S. A. Liddelow, A. M. Potter, H. B. Stolp and N. R. Saunders. 2006. Blood-CSF barrier function in the rat embryo. European Journal of Neuroscience; 24: P. 65–76.

Jones S. E. and C. Jomary. 2002. Clusterin. International Journal of Biochemistry and Cell Biology; 34(5): 427–31.

Kalat J. W. 2008. Biological Physiology, 10th edition. Cengage Learning. Belmont, USA. pp: 96.

Kampinga H. H., J. Hageman, M. J. Vos, H. Kubota, R. M. Tanguay and E. A. Bruford. 2009. Guidelines for the nomenclature of the human heat shock proteins. Cell Stress and Chaperones; 14(1): 105–111.

Kim H. S., S. R. Nagalla, Y. Oh, E. Wilson, C. T. Roberts, Jr. and R. G. Rosenfeld. 1997. Identification of a family of low-affinity insulin-like growth factor binding proteins (IGFBPs): characterization of connective tissue growth factor as a member of the IGFBP superfamily. Proceedings of National Academy of Science of U S A; 94: 12981–6.

Kingsley D. M. 1994. The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. Genes & Development; 8: 133–146.

Korovaitseva G. I. and S. Premkumar. 1999. Alpha-2 macroglobulin gene in early- and late-onset Alzheimer disease. Neurosci Lett; 271(2): 129–31.

Lappalainen J., J. C. Long, M. Virkkunen, N. Ozaki, D. Goldman and M. Linnoila. 1999. 5-HTR2C Cys23Ser polymorphism in relation to CSF monoamine metabolite concentrations and DSM-III-R psychiatric diagnoses. Biol Psychiatry; 46(6): 821–826.

Lee C.Y. and M. M. Rechler. 1996. Proteolysis of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in 150-kilodalton IGFBP complexes by a cation-dependent protease activity in adult rat serum promotes the release of bound IGF- I, Endocrinology. 13; 2051–2058.

Lehtinen M. K., M. W. Zappaterra, X. Chen, Y. J. Yang , A. D. Hill, M. Lun, T. Maynard, D. Gonzalez, S. Kim, P. Ye, A. J. D'Ercole, E. T. Wong, A. S. LaMantia and C. A. Walsh. 2011. The Cerebrospinal Fluid Provides a Proliferative Niche for Neural Progenitor Cells. Neuron; 69(5): 893–905.

Liddelow S. A., K. M. Dziegielewska, C. J. Ek, P. A. Johansson, A. M. Potter and N. R. Saunders. 2009. Cellular transfer of macromolecules across the developing choroid plexus of *Monodelphis domestica*. European Journal of Neuroscience; 29: 253–266.

Leduc V., D. Domenger, L. D. Beaumont, D. Lalonde, S. B. Jasmin and J. Poirier. 2011. Function and Comorbidities of Apolipoprotein E in Alzheimer's disease. International Journal of Alzheimer's disease; 22.

Leskov K. S., D. Y. Klokov, J. Li, T. J. Kinsella and D. A. Boothman. 2003. Synthesis and functional analyses of nuclear clusterin: a cell death protein. J. Biol. Chem.; 278: 11590-11600.

Li Y., J. Xiang and C. Duan. 2005. Insulin-like Growth Factor-binding Protein-3 Plays an Important Role in Regulating Pharyngeal Skeleton and Inner Ear Formation and Differentiation. J. of Biological Chemistry; 280: 3613–3620.

Li Z. and P. Srivastava. 2004. Heat-shock proteins. Current Protocols in Immunology.

Link H., O. Zettervall and G. Blennow. 1972. Individual cerebrospinal fluid (CSF) proteins in the evaluation of increased CSF total protein. Journal of neurology; 203(2): 119–132.

Maren T. H. and L. E. Broder. 1970. The role of Carbonic Anhydrase in anion secretion into Cerebrospinal fluid. Journal of Pharmaceutical and Experimental Therapeutics; 172(2): 197–202.

Marques F., J. C. Sousa, G. Coppola, F. Gao, R. Puga, H. Brentani, D. H. Geschwind, N. Sousa, M. Correia-Neves and J. A. Palha. 2011. Transcriptome signature of the adult mouse choroid plexus. Fluids and Barriers of the CNS; 8:10.

Marti H. J. H., M. Bernaudin, A. Bellail, H. Schoch, M. Euler, E. Petit and W. Risau. 2000. Hypoxia-Induced Vascular Endothelial Growth Factor Expression Precedes Neovascularization after Cerebral Ischemia. American Journal of Pathology; 156: 965–76.

Miguel C., T. Takahiko and R. Agueda. 1999. Functional and structural properties of lipid-associated apolipoprotein J (clusterin). Biochem. J.; 344: 375–383.

Mikoshiba K. 2006. Inositol 1,4,5-trisphosphate (IP3) receptors and their role in neuronal cell function. Journal of Neurochemistry; 97: 1627–1633.

Miyan J. A., M. Nabiyouni and M. Zendah. 2003. Development of the brain: a vital role for cerebrospinal fluid. Canadian Journal of Physiology Pharmacology; 81: 317–328.

Monkawa T., M. Hayashi and A. Miyawaki. 1998. Localization of inositol 1,4,5-trisphosphate receptors in the rat kidney. Kidney International; 53: 296–30.

Morrione A. and B. Valentinis. 1997. Insulin-like growth factor II stimulates cell proliferation through the insulin receptor. PNAS; 94(8): 3777–3782.

Netsky M. G. and S. Shuangshoti. 1975. Origin of choroid plexus and ependyma. In: The Choroid Plexus in Health and Disease. Eds. In chief: M. G. Netsky and S. Shuangshoti. University Press of Virginia. Charlottesville; pp. 3–18.

Nicola M., F. Vince and C. Luca. 2003. Serum Transthyretin Monomer as a Possible Marker of Blood-to-CSF Barrier Disruption. The journal of Neuroscience. 23(5): 1949–1955.

Nilsson C., A. M. Lindvall and C. Owman. 1992. Neuroendocrine regulatory mechanisms in the choroid plexus-cerebrospinal fluid system. Brain research reviews; 17: 109–13.

Nillson C., B. M. Hultberg and S. Gammeltoft. 1996. Autocrine role of insulin-like growth factor II secretion by the rat choroid plexus. European Journal of Neuroscience; 8: 629– 635.

Nilselid A.M., P. Davidsson, K. Nägga, N. Andreasen, P. Fredman and K. Blennow. 2006. Clusterin in cerebrospinal fluid: analysis of carbohydrates and quantification of native and glycosylated forms. Neurochem Int.; 48(8): 718–28.

O'Dell S. D. and I. N. M. Day. 1998. Molecules in focus Insulin-like growth factor II (IGF-II). The International Journal of Biochemistry & Cell Biology; 30(7): p.767–771.

Oh Y. and S. R. Nagalla. 1996. Synthesis and Characterization of Insulin-like Growth Factor-binding Protein (IGFBP)-7 RECOMBINANT HUMAN mac25 PROTEIN

SPECIFICALLY BINDS IGF-I AND –II. The journal of biological chemistry; 271(48): 30322–30325.

Ohsawa I., K. Nishimaki and Y. Murakami. 2008. Age-Dependent Neurodegeneration Accompanying Memory Loss in Transgenic Mice Defective in Mitochondrial Aldehyde Dehydrogenase 2 Activity. The Journal of Neuroscience; 28(24): 6239 – 6249.

Palha J. A. 2002. Transthyretin as a thyroid hormone carrier: function revisited. Clinical Chemistry and Laboratory Medicine; 40(12): 1292–300.

Parkkila A. K., S. Parkkila, M. Reunanen and O. Niemela. 1997. Carbonic anhydrase II in the cerebrospinal fluid: its value as a disease marker. European Journal of Clinical Investigation; 27(5): 392–397.

Parvas M., C. Parada and D. Bueno. 2008. A blood–CSF barrier function controls embryonic CSF protein composition and homeostasis during early CNS development. Developmental Biology; 321(1): 51–63.

Parvas M. and D. Bueno. 2010. The embryonic blood-CSF barrier controls E-CSF osmolarity during early CNS development. The Journal of Neuroscience Research; 88(6): 1205–1212.

Parada C., G. J. C. Escolà, and D. Bueno. 2008. Low-density lipoproteins from embryonic cerebrospinal fluid are required for neural differentiation. Journal of Neuroscience Research; 86(12): 2674–2684.

Pazos A., D. Hoyer and J. M. Palacios. 1984. The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site. European Journal of Pharmacology; 106: 530–4.

Pelton R.W. and B. Saxena. 1991. Immunohistochemical Localization of TGF1, TGFB2, and TGFB3 in the mouse embryo: Expression Patterns Suggest Multiple Roles during Embryonic Development. Journal of Cell Biology; 115(4): 1091–1105.

Peng G. S. and S. J. Yin. 2009. Effect of the allelic variants of aldehyde dehydrogenase*ALDH2*2* and alcohol dehydrogenase *ADH1B*2* on blood acetaldehyde concentrations. Human Genomics; 3: 121–127.

Pez-Bermejo A. L., J. Khosravi and C. L. Corless. 2003. Generation of Anti-Insulin-Like Growth Factor-Binding Protein-Related Protein 1 (IGFBP-rP1/MAC25) Monoclonal Antibodies and Immunoassay: Quantification of IGFBP-rP1 in Human Serum and Distribution in Human Fluids and Tissues. The Journal of Clinical Endocrinology & Metabolism; 88(7): 3401–3408.

Pollay M. 2010. The function and structure of the cerebrospinal fluid outflow system. Cerebrospinal Fluid Research; 7:9.

Preston J. E. 2001. Ageing choroid plexus-cerebrospinal fluid system. Microscopy Research and Technique; 52: 31–3.

Perez-Figares J. M., A. J. Jimenez and E. M. Rodriguez. 2001. Subcommissural organ, cerebrospinal fluid circulation, and hydrocephalus. Microscopy Research and Technique; 52: 591–607.

Poon S., T. M. Treweek and M. R. Wilson. 2002. Clusterin is an extracellular chaperone that specifically interacts with slowly aggregating proteins on their off-folding pathway. EEBS letters; 513(2-3): 259–266.

Quraishe S., A. Asuni, W. C. Boelens, V. O'Connor and A. Wyttenbach. 2008. Expression of the small heat shock protein family in the mouse CNS: differential anatomical and biochemical compartmentalization. Neuroscience; 153(2): 483–91.

Rajaram S., D. J. Baylink and S. Mohan. 1997. Insulin-Like Growth Factor-Binding Proteins in Serum and Other Biological Fluids: Regulation and Functions. Endocrine Reviews; 18 (6): 801–831.

Ramaswamy G., Q. Xu, Y. Huang and K. H. Weisgraber. 2005. Effect of Domain Interaction on Apolipoprotein E Levels in Mouse Brain. The Journal of Neuroscience; 25(46): 10658–10663.

Ranganathan T. S. 2000. A text book of human anatomy, S. Chand and Company Limited; India. pp: 622–702.

Rao N. M. and E. G. P. Udupa. 2007. Angeotensin converting enzyme from sheep mammary, lingual and other tissues. Indian Journal of Exp. Biology; 45: 1003–1006.

Redzic Z. B, J. E. Preston, J. A. Duncan, A. Chodobski and J. S. Chodobska. 2005. The choroid plexus-cerebrospinal fluid system: from development to aging. Current Topics in Developmental Biology; 71:1–52.

Reiber H. 2003. Proteins in cerebrospinal fluid and blood: barriers, CSF flow rate and source-related dynamics. Restorative Neurology and Neuroscience; 21: 79–96.

Reid S. and P. Ferretti. 2003. Differential expression of fibroblast growth factor receptors in the developing murine choroid plexus. Brain Res Dev Brain Res; 141: 15–24.

Rensink A. A., H. Gellekink, I. Otte-Holler, H. J. ten Donkelaar, R. M. de Waal, M. M. Verbeek and B. Kremer. 2002. Expression of the cytokine leukemia inhibitory factor and pro-apoptotic insulin-like growth factor binding protein-3 in Alzheimer's disease. Acta Neuropathol (Berl); 104: 525–533.

Reynolds G. P., M. J. Hill and S. L. Kirk. 2006. The 5-HT2C receptor and antipsychoticinduced weight gain - mechanisms and genetics. Journal of Psychopharmacology; 20: 15–8.

Reynolds G. P., Z. J. Zhang and X. B. Zhang. 2002. Association of antipsychotic drug-induced weight gain with a 5-HT2C receptor gene polymorphism. Lancet; 359: 2086–7.

Reynolds D. 1997. Apolipoprotein E. Stanislaus Journal of Biochemical Reviews; 2.

Richardson S. J. 2007. Cell and Molecular Biology of Transthyretin and Thyroid Hormones. International Review of Cytolog; 285: 137–193.

Riddell D. R., H. Zhou, K. Atchison, H. K. Warwick and P. J. Atkinson. 2008. Impact of Apolipoprotein E (ApoE) Polymorphism on Brain ApoE Levels. J Neuroscience; 28(45): 11445–11453.

Riikonen R., I. Makkonen, R. Vanhala, U. Turpeinen, J. Kuikka and H. Kokki. 2006. Cerebrospinal fluid insulin-like growth factors IGF-1 and IGF-2 in infantile autism. Developmental Medicine and Child Neurology; 48(9): 751–5.

Rösman J. B. 2008. Influence of Serotonin-related Genes on Behavior and Body weight. Doctoral Thesis; Goteborgs University Publishers, Sweden.

Roghani M., C. Lassarre, J. Zapf, G. Povoa and M. Binoux. 1991. Two insulin-like growth factor (IGF)-binding proteins are responsible for the selective affinity for IGF-II of cerebrospinal fluid binding proteins. Journal of Clinical Endocrinology and Metabolism; 73(3): 658–66.

Ruan W., S. Zhu, H. Wang and F. Xu. 2010. IGFBP-rP1, a potential molecule associated with colon cancer differentiation. Molecular Cancer; 9: 281.

Rudrasingham V. and F. W. D. Vrièze. 1999. α-2 macroglobulin gene and Alzheimer disease. Nature Genetics; 22: 17–19.

Schliep G. and K. Felgenhauer. 1978. Serum-CSF protein gradients, the blood-CSF barrier and the local immune response. Journal of neurology; 218(2): 77–96.

Schliep G. and K. Felgenhauer. 1974. The α_2-macroglobulin level in cerebrospinal fluid; a parameter for the condition of the blood-CSF barrier. Journal of Neurology; 207(3): 171–181.

Schreiber G., T. M. Pettersson, B. R. Southwell, A. R. Aldred, P. J. Harms, S. J. Richardson, R. E. H. Wettenhall, W. Duan and S. C. Nicol. (1993). Transthyretin gene expression evolved more recently in the liver than in the brain. Comparative Biochemistry Physiology; 105B: 317–32.

Schultz K., K. Nilsson and J. E. Nielsen. 2010. Transthyretin as a potential CSF biomarker for Alzheimer's disease and dementia with Lewy bodies: effects of treatment with cholinesterase inhibitors. European Journal of Neurology; 17(3): 456–60.

Schweisfurth H. and S. Schiöberg-Schiegnitz. 1984. Assay and biochemical characterization of angiotensin-I-converting enzyme in cerebrospinal fluid Enzyme; 32: 12–19.

Segal M. B. 2000. The choroid plexuses and the barriers between the blood and the cerebrospinal fluid. Cellular and Molecular Neurobiology; 20: 183–196.

Serot J. M., M. C. Béné and G. C. Faure. 2003. Choroid Plexus, Ageing of the Brain, and Alzheimer's disease. Frontiers in Bioscience; 8: s515–521.

Siegel G. J., B. W. Agranoff, R. W. Albers, S. K. Fisher and M. D. Uhler. 1999. Basic Neurochemistry: Molecular, Cellular and Medical Aspects, 6th edition. Raven Press, NY; 671–689.

Siegel A. and H. N. Sapru. 2009. Essential Neuroscience, 2nd edition. Lippincott Williams & Wilkins; Philedelphia, USA. pp: 28.

Sihlbom C., P. Davidsson, M. Sjögren, L. O. Wahlund and C. L. Nilsson. 2008. Structural and quantitative comparison of cerebrospinal fluid glycoproteins in Alzheimer's disease patients and healthy individuals. Neurochem Res.; 33(7): 1332–40.

Skidgel R. A. 1993. Basic science aspects of angeotensin converting enzyme and its inhibitors. Fundamental Clin Cardiol (Heart Failure); 15: 399.

Skipor J. and J. C. Thiery. 2008. The choroid plexus–cerebrospinal fluid system: undervaluated pathway of neuroendocrine signaling into the brain. Acta Neurobiol Exp (Wars); 68: 414–428.

Skuli N., L. Liu, A. Runge, A. Wang and L. Yuan. 2009. Endothelial deletion of hypoxia-inducible factor–2α (HIF-2α) alters vascular function and tumor angiogenesis. Blood; 114(2). 469–477.

Sobanska L. G., E. Pirraglia and M. Brys. 2009. The effects of normal aging and ApoE genotype on the levels of CSF biomarkers for Alzheimer's disease. Neurobiology of Aging; 30: 672–681.

Söderqvist F., L. Hardell, M. Carlberg and K. H. Mild. 2010. Radiofrequency fields, transthyretin, and Alzheimer's disease. Journal of Alzheimers Disease; 20(2): 599–606.

Speake T., C. Whitwell, H. Kajita, A. Majid and P. D. Brown. 2001. Mechanisms of CSF secretion by the choroid plexus. Microscopy Research and Technique; 52: 49–59.

Sprenger C. C., M. E. Vail, K. Evans, J. Simurdak and S. R. Plymate. 2002. Over-expression of insulin-like growth factor binding protein-related protein-1(IGFBP-rP1/mac25) in the M12 prostate cancer cell line alters tumor growth by a delay in G1 and cyclin A associated apoptosis. Oncogene; 21: 140–147.

Standring S. 2008. Gray's Anatomy: The Anatomical Basis of Clinical Practice, 40th edition. Churchill-Livingstone, Elsevier; 237–390.

Stenvers K. L., E. M. Zimmermann, M. Gallagher and P. K. Lund. 1994. Expression of insulin-like growth factor binding protein-4 and -5 mRNAs in adult rat forebrain. J Comp Neurol; 339(1): 91–105.

Stylianopoulou F., J. Herbert, M. B. Soares and A. Efstratiadis. 1988. Expression of the insulin-like growth factor II gene in the choroid plexus and the leptomeninges of the adult rat central nervous system. Proceedings of National Academy of Science; 85: 141–145.

Sullivan G. M., J. A. Hatterer and J. Herbert. 1999. Low Levels of Transthyretin in the CSF of Depressed Patients. American Journal of Psychiatry; 156: 710–715.

Sultana R., H. F. Poon, J. Cai, W. M. Pierce, M. Merchant, J. B. Klein, W. R. Markesbery and D. A. Butterfield. 2006. Identification of nitrated proteins in Alzheimer's disease brain using a redox proteomics approach. Neurobiology of Disease; 22(1): 76–87.

Swardfager W., K. Lanctôt, L. Rothenburg, A. Wong, J. Cappell and N. Herrmann. 2010. A meta-analysis of cytokines in Alzheimer's disease. Biological Psychiatry.15; 68(10): 930–41.

Swenson E. R. 2003. A comparative approach to carbonic anhydrase: the work of Thomas H. Maren. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology; 136(2): 229–241.

Szmydynger-Chodoska J., A. Chodobski and C. E. Johanson. 1994. Postnatal developmental changes in blood flow to choroid plexus and cerebral cortex of the rat. American journal of physiology; 266: 1488–1492.

Takashi H., S. Naoto and K. Takayuki. 1993. Immunohistochemical localization of fibroblast growth factor receptors in the rat mandibular condylar cartilage and tibial cartilage. Journal of Bone and Mineral Metabolism; 17(4): 274–282.

Tham A., A. Nordberg, F. E. Grissom, C. Carlsson-Skwirut, M. Viitanen and V. R. Sara. 1993. Insulin-like growth factors and insulin-like growth factor binding proteins in cerebrospinal fluid and serum of patients with dementia of the Alzheimer type. Journal of Neural Transmission. Parkinson's Disease and Dementia Section; 5(3): 165–76.

Thomas T., B. Power, P. Hudson, G. Schreiber and M. Dziadek. 1988. The expression of transthyretin mRNA in the developing rat brain. Developmental Biology; 128(2): 415–427.

Thouvenot E., M. Lafon-Cazal, E. Demettre, P. Jouin, J. Bockaert and P. Marin. 2006. The proteomic analysis of mouse choroid plexus secretome reveals a high protein secretion capacity of choroidal epithelial cells. Proteomics; 6: 5941–5952.

Tian H., S. L. McKnight and D. W. Russell. 1997. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes Dev; 11: 72–82.

Tiziana C., M. L. Rober, S. Fotini and H. Joseph. 1993. Differential Expression of the Insulin-Like Growth Factor II and Transthyretin Genes in the Developing Rat Choroid Plexus. Journal of Neuropathology & Experimental Neurology; 52(2):153–162.

Valdez-Cruz N. A., O. T. Ramírez and M. A. Trujillo-Roldán. 2011. Molecular responses of E. coli caused by heat stress and recombinant protein production during temperature induction. Bioeng Bugs; 2(2): 105–10.

Vance J. E. and H. Hayashi. 2010. Formation and function of apolipoprotein E-containing lipoproteins in the nervous system. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids; 1801(8): 806–818.

Vasiliou V. and D. W. Nebert. 2005. Analysis and update of the human aldehyde dehydrogenase (ALDH) gene family. Human Genomics; 2(2): 138–43.

Veening J. G. and H. P. Barendregt. 2010. The regulation of brain states by neuroactive substances distributed via the cerebrospinal fluid: a review. Cerebrospinal Fluid Research; 7:1.

Vio K., S. Rodriguez, C. R. Yulis, C. Oliver and E. M. Rodriguez. 2008. The subcommissural organ of the rat secretes Reissner's fiber glycoproteins and CSF-soluble

proteins reaching the internal and external CSF compartments. Cerebrospinal Fluid Res; 5:3.

Vogel S., M. V. Gamble and W. S. Blaner. 1999. Biosynthesis, absorption, metabolism and transport of retinoids. Springer Verlag; 31–39.

Wahlbeck K., A. Ahokas, H. Nikkila, K. Miettinen and R. Rimo'n. 2000. Cerebrospinal fluid angiotensin-converting enzyme (ACE) correlates with length of illness in schizophrenia. Schizophrenia Research; 41: 335–340.

Wajapeyee N., R. W. Serra, X. Zhu, M. Mahalingam and M. R. Green. 2008. Oncogenic BRAF induces senescence and apoptosis through pathways mediated by the secreted protein IGFBP7. Cell; 132: 363–374.

Walter S. and J. Buchner. 2002. Molecular chaperones—cellular machines for protein folding. Angewandte Chemie International Eddition Engl.; 41: 1098–1113.

Watt S. M., H. J. Bühring, I. Rappold, J. Y. Chan, J. Lee-Prudhoe, T. Jones, A. C. Zannettino, P. J. Simmons, R. Doyonnas, D. Sheer and L. H. Butler. 1998. CD164, a novel sialomucin on CD34(+) and erythroid subsets, is located on human chromosome 6q21. Blood; 92 (3): 849–66.

Weise B., T. Janet and C. Grothe. 1993. Localization of bFGF and FGF-receptor in the developing nervous system of the embryonic and newborn rat. Journal of Neuroscience Research; 34: 442–453.

Welches W. R., K. B. Brosnihan and C. M. Ferrario. 1993. A comparison of the properties and enzymatic activities of three angiotensin processing enzymes: angiotensin converting enzyme, prolyl endopeptidase and neutral endopeptidase. 24.11. Life Sciences; 52: 1461–1480.

Wilson E. M., Y. OH and R. G. Rosenfeld. 1997. Generation and characterization of an IGFBP-7 Antibody: Identification of 31kD IGFBP-7 in human biological fluids and

Hs578T human breast cancer conditioned media. J Clinical Endocrinology and Metabolism; 82(4): 1301–1303.

Wostyn P., K. Audenaert and P. P. D. Deyn. 2011. Choroidal Proteins Involved in Cerebrospinal Fluid Production may be Potential Drug Targets for Alzheimer's Disease Therapy. Perspectives Medicinal Chemistry; 5: 11–17.

Wyatt A. R. 2009. The role of clusterin (CLU) in extracellular protein folding quality control, PhD thesis. School of Biological Sciences. University of Wollongong.

Yamanaka Y., E. M. Wilson, R. G. Rosenfeld and Y. Oh. 1997. Inhibition of Insulin Receptor Activation by Insulin-like Growth Factor Binding Proteins. The Journal of Biological Chemistry; 272(49): 30729–30734.

Yamauchi K., M. Tozuka, T. Nakabayashi and M. Sugano. 1999. Apolipoprotein E in Cerebrospinal Fluid: Relation to Phenotype and Plasma Apolipoprotein E Concentrations. Clinical Chemistry; 45: 497–504.

Yazaki N., Y. Hosoi, K. Kawabata, A. Miyake, M. Minami, M. Satoh, M. Ohta, T. Kawasaki and N. Itoh. 1994. Differential expression patterns of mRNAs for members of the fibroblast growth factor receptor family, FGFR-1-FGFR-4, in rat brain. J Neurosci Res; 37: 445–452.

Yuan X. and D. M. Desiderio. 2005. Human cerebrospinal fluid peptidomics. J Mass Spectrom; 40: 176–181.

Yerbury J. J. and M. R. Wilson. 2010. Extracellular chaperones modulate the effects of Alzheimer's patient cerebrospinal fluid on Abeta(1-42) toxicity and uptake. Cell Stress Chaperones; 15(1): 115–21.

Zannettino A. C. 2001. CD164. Journal of Biological Regulators and Homeostatic Agents; 15(4): 394–6.

Zannettino A. C. W., H. –J Bühring, S. Niutta, S. M. Watt, M. A. Benton and P. J. Simmons. 1998. The Sialomucin CD164 (MGC-24v) Is an Adhesive Glycoprotein Expressed by Human Hematopoietic Progenitors and Bone Marrow Stromal Cells That Serves as a Potent Negative Regulator of Hematopoiesis. Blood; 92(8): 2613–2628.

Zappaterra and M. Dylan. 2008. Analysis of cerebrospinal fluid in brain development. Harvard University; 324 pages.

Zappaterra M. D, S. N. Lisgo, S. Lindsay, S. P. Gygi, C. A. Walsh and B. A. Ballif. 2007. A Comparative Proteomic Analysis of Human and Rat Embryonic Cerebrospinal Fluid. Journal of proteome research; 6 (9): 3537–3548.

Zheng W., Y. M. Lu, G. Y. Lu. 2001. Transthyretin, Thyroxine and Retinol-binding Protein in Human Cerebrospinal Fluid: Effect of Lead Exposure. Toxicological Sciences; 61: 107–114.

Zimatkin S. M. 1991. Histochemical study of aldehyde dehydrogenase in the rat CNS. Journal of Neurochemistry; 56(1): 1–11.

Zimatkin S. M., O. IuM, 1998. Aldehyde dehydrogenase activity in the barrier brain structures. Biull Eksp Biol Med; 106(9): 283–4.

Zougman A., B. Pilch, A. Podtelejnikov, M. Kiehntopf, C. Schnabel, C. Kumar and M. Mann. 2008. Integrated Analysis of the Cerebrospinal Fluid Peptidome and Proteome. Journal of Proteome Research; 7: 386–399.

Zarros A. C., K. S. Kalopita and S. T. Tsakiris. 2005. Serotoninergic impairment and aggressive behaviour in Alzheimer's disease. Acta Neurobiol; 65: 277–286.

Zubenko G. S., L. Volicer, L. K. Direnfeld, M. Freeman, P. J. Langlais and R. A. Nixon. 1985. Cerebrospinal fluid levels of angiotensin-converting enzyme in Alzheimer's disease, Parkinson's disease and progressive supranuclear palsy. Brain Res; 328: 215–221.