

## Altered expression of claudin family proteins in Alzheimer's disease and vascular dementia brains

Mihaela O. Romanitan<sup>a, b</sup>, Bogdan O. Popescu<sup>a, b, \*</sup>, Ștefan Spulber<sup>c</sup>, Ovidiu Băjenaru<sup>b</sup>,  
Laurențiu M. Popescu<sup>a, d</sup>, Bengt Winblad<sup>c</sup>, Nenad Bogdanovic<sup>c</sup>

<sup>a</sup> Laboratory of Molecular Medicine, 'Victor Babeș' National Institute of Pathology, Spl. Independenței, Bucharest, Romania

<sup>b</sup> Department of Neurology, University Hospital Bucharest, 'Carol Davila' University of Medicine and Pharmacy, School of Medicine, Spl. Independenței, Bucharest, Romania

<sup>c</sup> Division of Experimental Geriatrics, Alzheimer's Disease Research Center, Department of NVS, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden

<sup>d</sup> Department of Cellular and Molecular Medicine, 'Carol Davila' University of Medicine and Pharmacy, School of Medicine, Eroilor Sanitari, Bucharest, Romania

Received: August 26, 2009; Accepted: December 4, 2009

### Abstract

Claudins (CIs) are a multigene family of transmembrane proteins with different tissue distribution, which have an essential role in the formation and sealing capacity of tight junctions (TJs). At the level of the blood–brain barrier (BBB), TJs are the main molecular structures which separate the neuronal milieu from the circulatory space, by a restriction of the paracellular flow of water, ions and larger molecules into the brain. Different studies suggested recently significant BBB alterations in both vascular and degenerative dementia types. In a previous study we found in Alzheimer's disease (AD) and vascular dementia (VaD) brains an altered expression of occludin, a molecular partner of CIs in the TJs structure. Therefore in this study, using an immunohistochemical approach, we investigated the expression of CI family proteins (CI-2, CI-5 and CI-11) in frontal cortex of aged control, AD and VaD brains. To estimate the number of CI-expressing cells, we applied a random systematic sampling and the unbiased optical fractionator method. We found selected neurons, astrocytes, oligodendrocytes and endothelial cells expressing CI-2, CI-5 and CI-11 at detectable levels in all cases studied. We report a significant increase in ratio of neurons expressing CI-2, CI-5 and CI-11 in both AD and VaD as compared to aged controls. The ratio of astrocytes expressing CI-2 and CI-11 was significantly higher in AD and VaD as compared to aged controls. The ratio of oligodendrocytes expressing CI-11 was significantly higher in AD and the ratio of oligodendrocytes expressing CI-2 was significantly higher in VaD as compared to aged controls. Within the cerebral cortex, CIs were selectively expressed by pyramidal neurons, which are the ones responsible for cognitive processes and affected by AD pathology. Our findings suggest a new function of CI family proteins which might be linked to response to cellular stress.

**Keywords:** tight junctions • claudins • Alzheimer' disease • vascular dementia

### Introduction

The blood–brain barrier (BBB) is a tightly controlled semi-permeable barrier which prevents the paracellular passage for macro-

molecules and cells from the blood stream into the brain [1]. It consists primarily of capillary endothelial cells packed together by tight junctions (TJs), together with the adjoining astrocyte end-feet and pericytes. TJs are specialized membrane domains at the most apical region of epithelial and endothelial cells, involved in both prevention of solute and water leakage (barrier function) and maintenance of cellular polarity (fence function) [2]. In the brain of vertebrates there are two types of cells which are known to bear TJs or TJs-like structures: vascular endothelial cells and oligodendrocytes [3]. TJs networks are formed by integral membrane proteins, such as junctional associated membrane protein 1 [4],

\*Correspondence to: Bogdan O. POPESCU,  
Laboratory of Molecular Medicine,  
'Victor Babeș' National Institute of Pathology,  
99–101, Spl. Independenței, sector 5,  
Bucharest 050096, Romania.  
Tel.: +40 744 35 34 33  
Fax: +40 21 312 81 02  
E-mail: bogdan.popescu@jcm.org

occludin [5] and the claudin (Cl) family [6]. Occludin and Cls bind to cytoplasmic peripheral membrane proteins, such as ZO-1, ZO-2 or ZO-3, which in turn are connected to the perijunctional actin filament network of the cytoskeleton [1].

Cls are a 20–28 kD multigene family of transmembrane proteins with different tissue distributions, which have an essential role in the formation of TJs strands, and appear to function as the primary seal of the TJs [7]. Among the Cls expressed by brain endothelial cells, Cl-5 is a critical determinant of BBB permeability [8]. Cl-5 is expressed in all tissues, being specifically localized to the endothelia of blood vessels and therefore TJs containing Cl-5 could regulate the permeability of distal vascular territories [9]. However, Cl-1 [10] and Cl-3 [11] were also shown to localize at the BBB level and their expression seems to be altered by various pathological processes. In addition, Cl-1, Cl-2 and Cl-5 are present in the choroid plexus where their expression is regulated by protein kinase C (PKC) [12]. It was suggested that Cls could function by homo- and hetero- oligomerization and a study which employed RNA interference showed that Cl-2, Cl-4 and Cl-7 are permeation operators for the Na<sup>+</sup> and Cl<sup>-</sup> ions. In contrast to other Cls, Cl-2 decreases the tightness of the TJ strands [13] by formation of cation-selective channels [14]. Cl-11 is expressed in TJs of myelin sheaths in brain and of Sertoli cells in testis [15] and it is known as well as oligodendrocyte-specific protein, because it represents a major component of central nervous system (CNS) myelin [16]. Along with other Cl family members, Cl-11 was also found in the epithelial cells of choroid plexus [17]. It was proposed that Cl-11 might be important for the formation and maintenance of myelin and for regulation of oligodendrocytes and Schwann cells proliferation [16].

Considering that the 'sealing' role of TJs is determined by the presence of Cls, the latter is presumably important in pathological situations that involve increased vessel permeability [18]. The integrity of the BBB is compromised in different disorders of the human CNS, most likely by injury or dysregulation of TJs [19]. One example where disruption of BBB is thought to play an important role in the pathophysiology is cerebral microangiopathy [20]. Patients with microangiopathy can present with either isolated or diffuse white matter changes that can ultimately lead to impairment of neuronal flow and cognitive decline, characteristic aspects for most of the dementia disorders [21]. There is mounting evidence that Alzheimer's disease (AD), the most common cause of dementia in the elderly, share vascular risk factors with vascular dementia (VaD), and at least a third of AD cases associates cerebrovascular small vessel disease [22]. In addition to cerebral amyloid angiopathy, AD subjects show profound changes in cerebral microvessels, often independent of amyloid deposition [23]. Moreover,  $\beta$ -amyloid peptide (1–42) might alter BBB integrity by effects on TJs protein complexes [24]. However, based on the current data, the role of TJs proteins is poorly understood in dementia disorders.

Beyond serving the BBB as gatekeepers, TJs proteins could have other physiological functions. Cls have a role in regulation of cell phenotype and growth control and Cl mutations are involved in several human diseases [25]. Furthermore, occludin is

expressed at detectable levels in mouse cultures of neurons and astrocytes [26], which do not form TJs. Moreover, we found in a recent study that occludin is overexpressed in neurons and astrocytes of AD and VaD brains as compared to aged controls [27]. Based on the fact that occludin is a molecular partner of Cls in the TJs structure, in this study we aimed to comparatively investigate the expression of Cl-2, Cl-5 and Cl-11 in human brains of aged control cases, AD and VaD.

## Material and methods

### Brain material

The brain material was obtained from the Huddinge Brain Bank, Stockholm, Sweden in accordance with Swedish law and the permission of the Karolinska University Hospital Ethical Committee. We chose to analyse the cerebral cortex (frontal, Brodmann area 46/9), where specific pathological changes are noted in both AD and VaD [28, 29].

The study was based on evaluation of five aging control, four AD and six VaD post-mortem human brains. The control group included the brains of traffic-accident patients or of patients who had no history of long-term illness or neuropsychiatric disorders. The AD group included brains from patients with clinically and pathologically confirmed AD. Clinical diagnosis was based on combined Diagnostic and Statistical Manual of Mental Disorders (DSM)-III-R [30] and National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [31]. The definite neuropathological diagnosis of AD was determined by using Consortium to Establish a Registry for Alzheimer's disease (CERAD) and National Institute on Aging (NIA) – Reagan Institute Criteria [32, 33]. The VaD group included brains from patients with clinical and pathological diagnosis of VaD. The VaD group consisted of two cases of single strategic stroke, three cases with dementia multiinfarct and one case with diffuse white matter changes. Age and sex of the cases are presented in Table 1.

### Sectioning and histology

Immunostaining with antibodies against Cl-2, Cl-5 and Cl-11 was performed on buffered formaldehyde-fixed embedded sections (long-fixation time sections were used). A series of thin sections (7  $\mu$ m) were cut exhaustively in a randomly chosen sagittal plane on the microtome. After the deparaffinization and rehydration procedures the sections were rinsed in distilled water for 5 min. and then heated at 80°C for 20 min in 0.1 M citrate buffer of pH 9. After cooling, the sections were incubated with the primary antibody solution overnight (approximately 16 hrs) at 4°C. The primary antibodies were rabbit polyclonals against Cl-2 (dilution 1:50), Cl-5 (dilution 1:100) or Cl-11 (dilution 1:200) and were purchased from Zymed Laboratories, Inc. (South San Francisco, CA, USA). These primary antibodies were tested in Western blots analysis of rat brain homogenates and no unspecific binding was noted. Thereafter, the sections were treated with a biotinylated secondary antibody (1:300, Vector Laboratories, Burlingame, CA, USA) and with the avidin–biotin–peroxidase complex kit (Vector) with 3,3'-diaminobenzidine-4HCl/H2O2

**Table 1** Age and sex of cases analysed in each study group

Group	Age	Sex
Control	90	F
	88	F
	86	F
	56	M
	70	M
AD	87	F
	86	M
	91	F
	72	F
VaD	87	F
	86	F
	65	M
	72	M
	89	F
	77	F

(DAB, Sigma, St. Louis, MO, USA) as a substrate. Next, the sections were counterstained with haematoxylin–eosin stain for background. Two types of negative controls slides were run for all the sections. The negative controls were run in an identical manner except the incubation with the primary antibody. For the first set of negative controls we omitted the primary antibody, whereas for the second set we added instead of the primary antibody an unspecific immunoglobulin serum at the same concentration. The sections were then mounted and examined under a Nikon microscope.

## Quantification of Cl-expressing brain cells

To estimate the number of Cl-expressing neurons, astrocytes and oligodendrocytes we applied two-dimension quantification with random systematic sampling and the unbiased optical fractionator method [34], similar to a previous study [35]. In brief, the area of interest was delineated in low magnification ( $\times 2.5$ ) using the cursor. Due to the clear anatomical borders, we were able to distinguish the grey matter from the white matter. A meander sampling function of the GRID v2.0 program (Olympus, A/S, Bellarup, Denmark) was used for stepping through the delineated region with a chosen counting frame. Then, a  $100\times$  oil-immersion objective with a numerical aperture of 1.40 was moved into place and the appropriate counting frame superimposed on the screen. The desired horizontal and vertical step lengths, assisted by a highly precise servo-controlled motorized microscopy stage, were dimensioned for the appropriate distance ( $231.22 \mu\text{m}$  [x-step]  $\times$   $231.22 \mu\text{m}$  [y-step]) in between the counting frames ( $1603.9 \mu\text{m}^2$ ). The cells in the space were counted by an optical dissector probe (z-axis). The optical dissector uses the simple rule that a cell is counted if its body cell is in the counter-frame. This procedure ensured the selection of a systematic random sample of sections. The number of cells was expressed as a number per squared millimetre. The neurons could

be easily distinguished from the other types of cells due to the presence of the round nucleus with visible cytoplasm and a single large nucleolus, no heterochromatin. Oligodendrocytes were identified by smaller round or oval dark nuclei with dense chromatin, and they are usually close to the neurons. The astrocytes were recognized as multipolar cells with bigger, paler and less densely packed heterochromatin, without a clear nucleolus and showing a patchy pattern of granules in a rim below the nuclear membrane.

## Statistical analysis

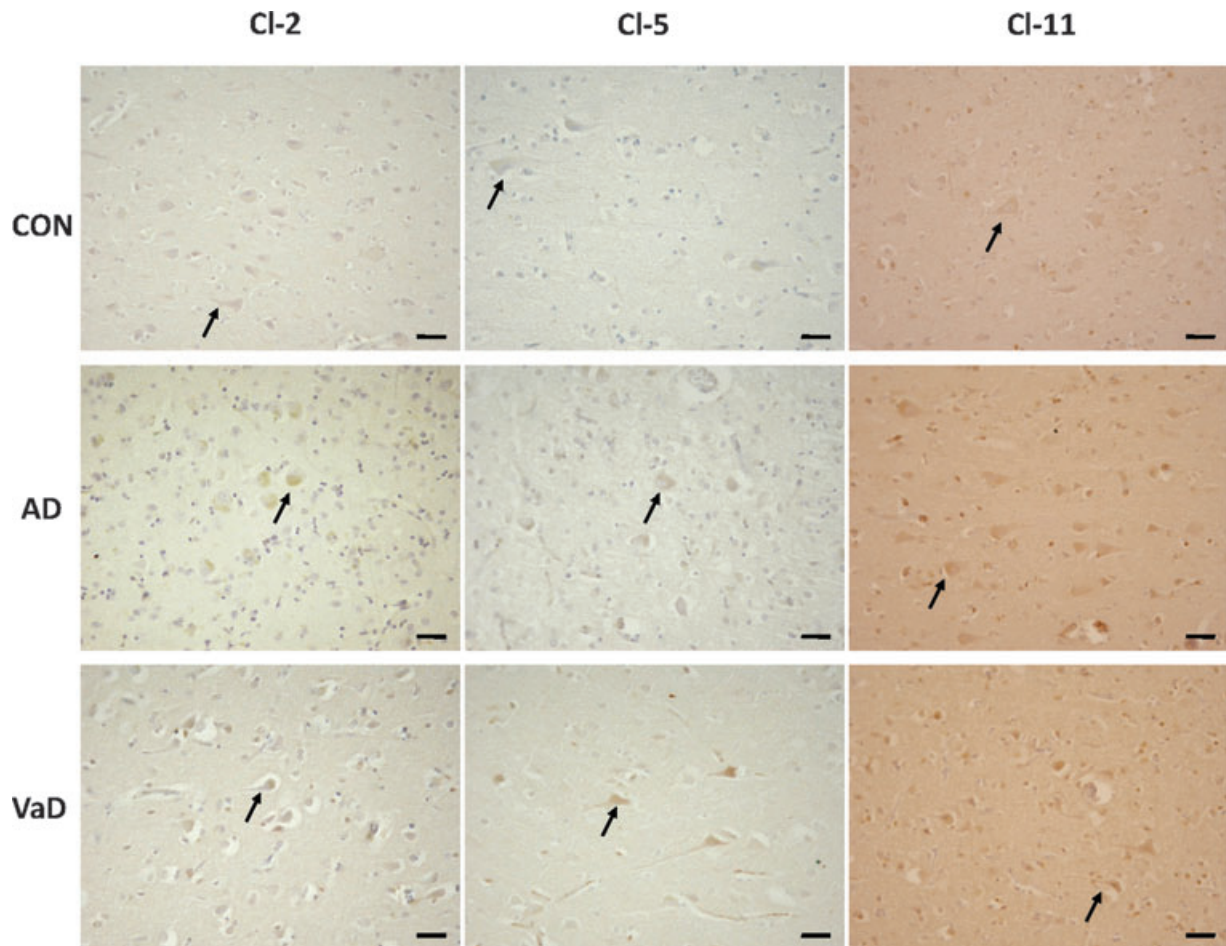
All statistical analyses of the results were performed using Statistica 8.0 software (Statsoft, Tulsa, OK, USA). The neuronal, astrocyte or oligodendrocyte ratio was defined as (number of Cl-expressing cells / total number of cells of respective type)  $\times$  100. Differences within individual cell type were analysed by two-way factorial ANOVA, followed by Fisher's LSD *post hoc* test. For all tests, the significance threshold was set to 0.05. All results are given as mean  $\pm$  standard deviation (S.D.).

## Results

### Expression of claudins in neurons

Our first observation was that selected neurons, in the frontal cortex from aged controls expressed Cl-2, Cl-5 and Cl-11 in detectable amounts (Fig. 1). Expression of Cl-2, Cl-5 and Cl-11 was increased in neurons from frontal cortex of AD and VaD brains as compared to control brains (Table 2 and Fig. 1). The neurons which expressed Cls were in all cases predominantly pyramidal. The neuronal fibres were also stained more intensely in AD and VaD as compared to control (Fig. 1).

Subsequently, we used a quantitative approach in order to estimate the number of Cl-expressing neurons in control, AD and VaD groups. Table 2 presents comparatively the quantitative data of neurons/ $\text{mm}^2$  and Cl-2, Cl-5 and Cl-11<sup>+</sup> neurons/ $\text{mm}^2$  in control, AD and VaD groups, respectively. The number of neurons did not significantly differ between the groups analysed. The area chosen for counting was similar in all cases (with around 100 counting frames/area) and there was no significant difference in the density (number of neurons/area) between the cases analysed. Qualitatively, we found no areas with marked heterogeneity in distribution of Cls. The ratios of Cl-2, Cl-5 and Cl-11-expressing neurons were significantly higher in the AD group ( $54.02 \pm 1.77$ ,  $P < 0.05$ ,  $39.82 \pm 3.75$ ,  $P < 0.05$  and  $71.62 \pm 9.19$ ,  $P < 0.05$ , respectively) and in the VaD group ( $38.07 \pm 6.97$ ,  $P < 0.05$ ,  $26.56 \pm 4.44$ ,  $P < 0.05$  and  $68.34 \pm 7.19$ ,  $P < 0.05$ , respectively) as compared to the control group ( $7.30 \pm 1.36$ ,  $9.62 \pm 13.08$  and  $23.77 \pm 5.00$ , respectively), as shown in Fig. 2. The ratio of Cl-2-expressing neurons was significantly higher in the AD group as compared to the VaD group ( $P < 0.05$ ). The ratios of Cl-5 and Cl-11-expressing neurons did not differ significantly between AD and VaD groups.



**Fig. 1** Expression of CI-2, CI-5 and CI-11 in control (CON), AD and VaD brains, in the frontal cortex, Brodmann area 46/9. CI-expressing cells are brownish, as a result of the immunohistochemical staining method with anti-CI sera detected with avidin–biotin–peroxidase complex kit and DAB substrate. Expression of CI-2, CI-5 and CI-11 was increased in neurons and neuronal fibres in AD and VaD brains, as compared to control brains. Expression of CIs was noticed mainly in the pyramidal neurons in CON, AD and VaD. Arrows are indicating CI-expressing neurons. Bars: 30  $\mu$ m.

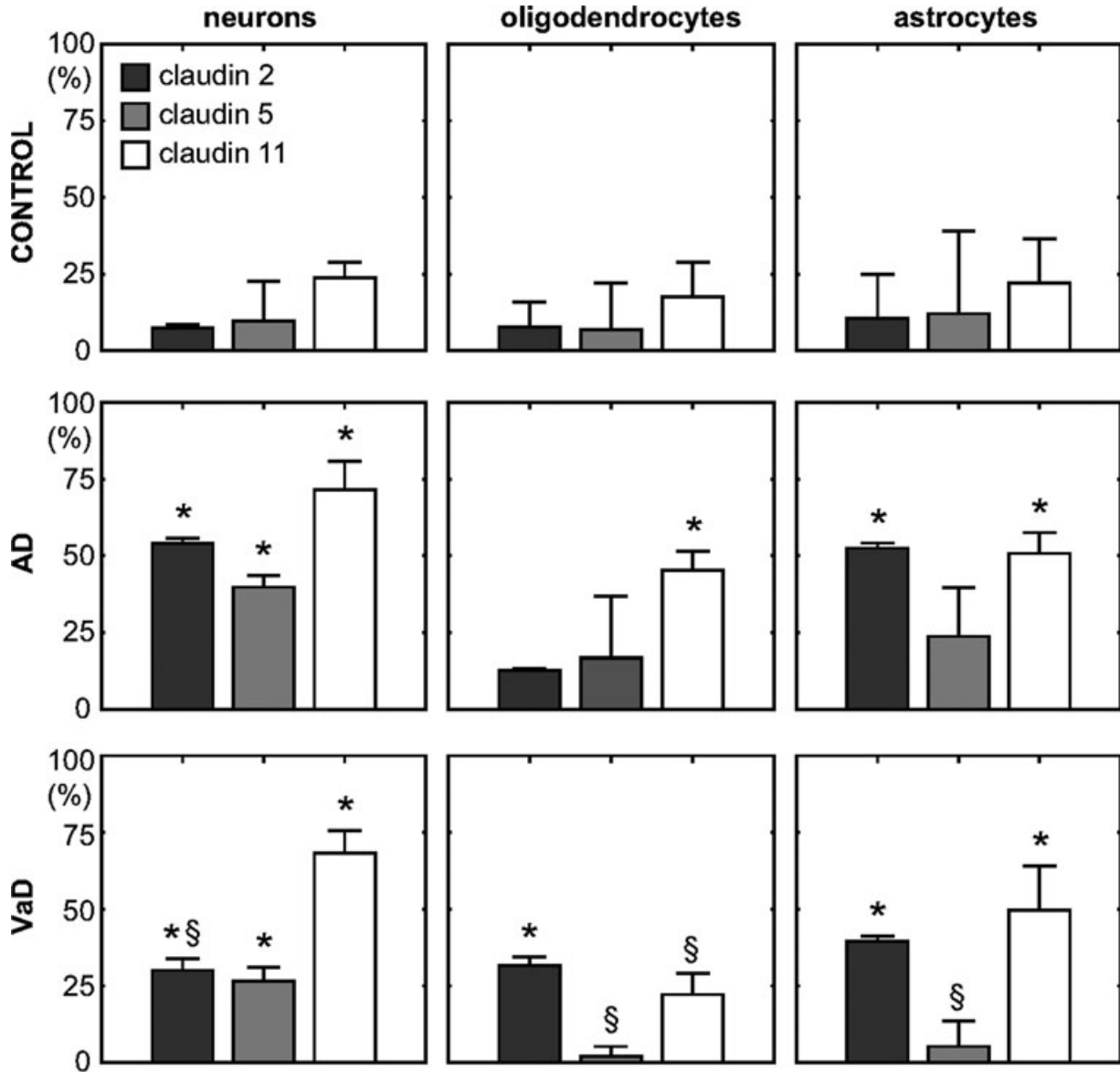
**Table 2** Quantification of total number of neurons and CI-expressing neurons (positive neurons) in the frontal cortex region in control (CON, five cases), AD (four cases) and VaD (six cases) groups. All values were truncated to two decimals. S.D. = standard deviation

Group	Total number of neurons						Number of positive neurons					
	CI-2		CI-5		CI-11		CI-2		CI-5		CI-11	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
CON	33.47	4.79	31.33	4.82	32.53	5.78	2.47	0.73	3.20	4.56	7.80	2.64
AD	32.17	3.87	33.58	2.20	32.58	0.32	17.42	2.63	13.33	0.98	23.33	3.02
VaD	27.00	4.49	27.83	3.13	28.44	2.93	10.44	3.12	7.39	1.48	19.44	2.93

### Expression of claudins in oligodendrocytes

Further on, we evaluated the expression of CI-2, CI-5 and CI-11 in oligodendrocytes in the frontal region of aged control, AD and VaD brains. In the grey matter of the frontal cortex, selected oligoden-

droglial cells expressed CI-2, CI-5 and CI-11 in all AD, VaD and control brains (Fig. 3). In order to estimate the number of CI-expressing oligodendrocytes in control, AD and VaD groups we used the same quantitative approach as described for the neurons. Table 3 presents comparatively the quantitative data of



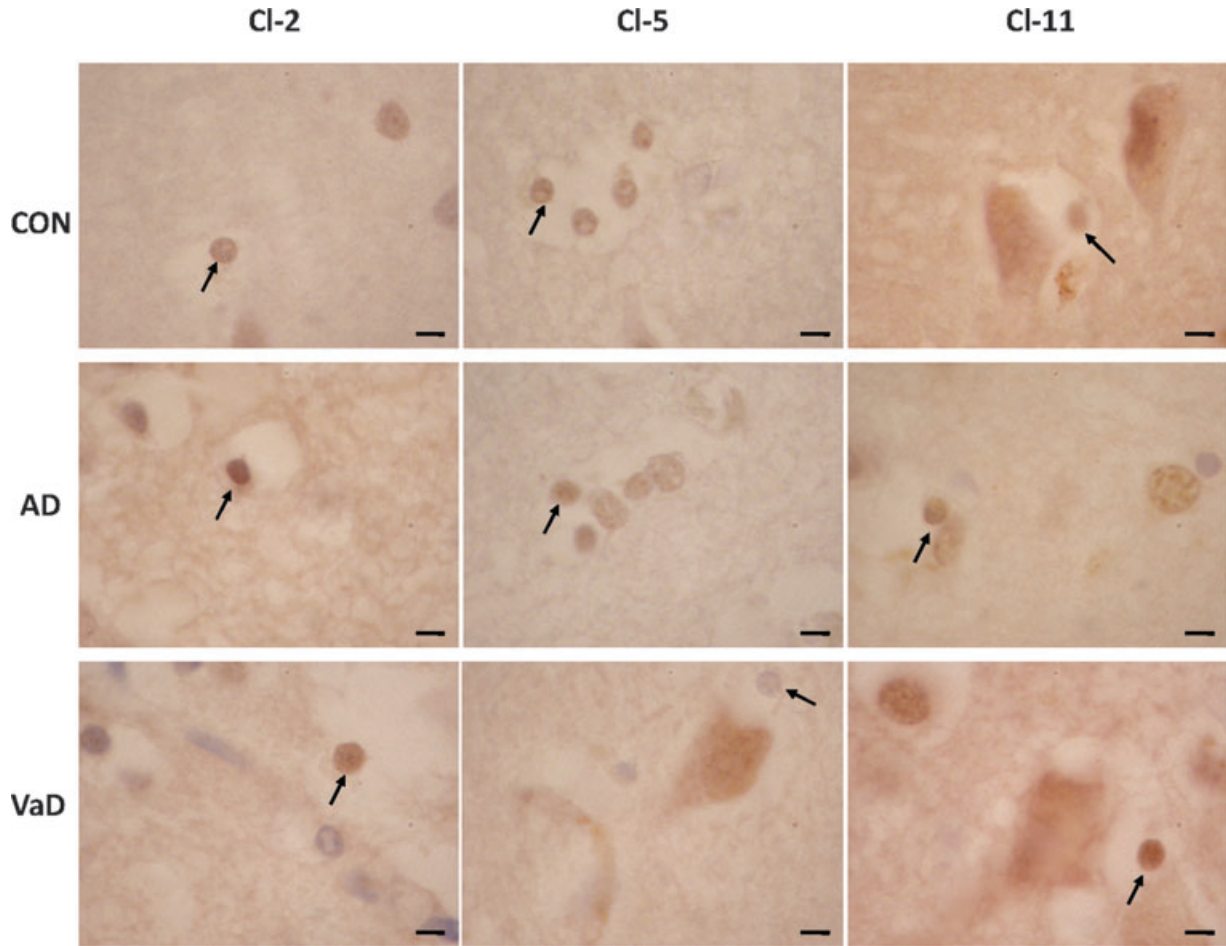
\* -  $p < 0.05$  compared to CONTROL  
 § -  $p < 0.05$  compared to AD

Factorial ANOVA, followed by Fisher's LSD post-hoc test.

**Fig. 2** Ratios of CI-2, CI-5 and CI-11-expressing cells in control, AD and VaD brains, in the frontal cortex, Brodmann area 46/9. The neuronal, astrocyte or oligodendrocyte ratio was defined as (number of CI-expressing cells / total number of cells)  $\times$  100. To compare the cell ratio between controls, AD and VaD (two by two) factorial ANOVA has been used, followed by the Fisher's LSD *post hoc* test. Error bars mark the S.D.

oligodendrocytes/mm<sup>2</sup> and CI-2, CI-5 and CI-11<sup>+</sup> oligodendrocytes/mm<sup>2</sup> in control, AD and VaD groups, respectively. As shown in Fig. 2, the ratio of CI-2-expressing oligodendrocytes was signif-

icantly higher in the VaD group ( $17.61 \pm 11.01$ ,  $P < 0.05$ ), but not in the AD group ( $12.77 \pm 0.71$ ), as compared to the control group ( $7.74 \pm 8.16$ ). The ratio of CI-5-expressing oligodendrocytes did



**Fig. 3** Expression of CI-2, CI-5 and CI-11 in oligodendrocytes in control (CON), AD and VaD brains, in the frontal cortex, Brodmann area 46/9. CI-expressing cells are brownish, as a result of the immunohistochemical staining method with anti-CI sera detected with avidin–biotin–peroxidase complex kit and DAB substrate. Oligodendrocytes are indicated by arrows. Bars: 5  $\mu$ m.

not significantly differ in the AD ( $16.75 \pm 20.08$ ) and VaD ( $2.03 \pm 3.17$ ) groups as compared to the control group ( $6.80 \pm 15.21$ ). However, the ratio of CI-5-expressing oligodendrocytes was significantly lower in VaD group as compared to the AD group ( $P < 0.05$ ). The ratio of CI-11-expressing oligodendrocytes was significantly higher in the AD group ( $45.32 \pm 6.21$ ,  $P < 0.05$ ), but not in the VaD group ( $22.18 \pm 6.91$ ), as compared to the control group ( $17.62 \pm 11.18$ ). Similar to CI-5 ratios, the ratio of CI-11-expressing oligodendrocytes was significantly lower in VaD group as compared to the AD group ( $P < 0.05$ ).

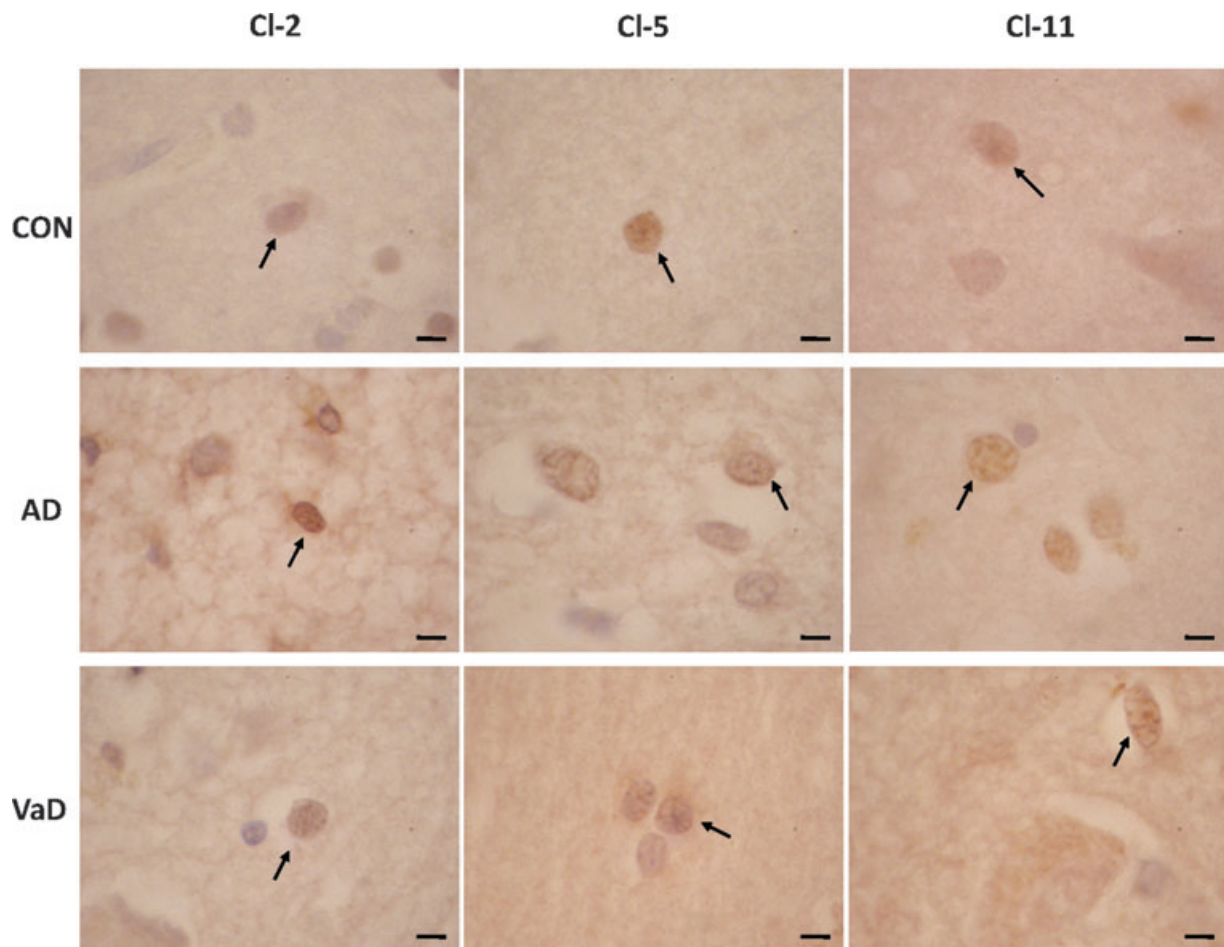
### Expression of claudins in astrocytes

For evaluation of CI expression in astrocytes we followed the same procedure as for neurons and oligodendrocytes. In the

frontal cortex examined, we found astrocytes expressing CI-2, CI-5 and CI-11 in all AD, VaD and control brains (Fig. 4). Table 4 presents comparatively the quantitative data of astrocytes/mm<sup>2</sup> and CI-2, CI-5 and CI-11<sup>+</sup> astrocytes/mm<sup>2</sup> in control, AD and VaD groups, respectively. As Fig. 2 shows, the ratios of CI-2 and CI-11-expressing astrocytes were significantly higher in the AD group ( $52.47 \pm 1.67$ ,  $P < 0.05$  and  $50.79 \pm 6.76$ ,  $P < 0.05$ ) and in the VaD group ( $37.75 \pm 6.86$ ,  $P < 0.05$  and  $49.73 \pm 14.36$ ,  $P < 0.05$ ) as compared to the control group ( $10.51 \pm 14.45$  and  $22.00 \pm 14.43$ ). Similar to the result obtained for oligodendrocytes, the ratio of CI-5-expressing astrocytes did not significantly differ in the AD ( $23.74 \pm 16.01$ ) and VaD ( $5.28 \pm 8.22$ ) groups as compared to the control group ( $12.08 \pm 27.01$ ). Again as for the oligodendrocytes, the ratio of CI-5-expressing astrocytes was significantly lower in VaD group as compared to the AD group ( $P < 0.05$ ).

**Table 3** Quantification of total number of oligodendrocytes and CI-expressing oligodendrocytes (positive oligodendrocytes) in the frontal cortex region in control (CON, five cases), AD (four cases) and VaD (six cases) groups. All values were truncated to two decimals. S.D. = standard deviation

Group	Total number of oligodendrocytes						Number of positive oligodendrocytes					
	CI-2		CI-5		CI-11		CI-2		CI-5		CI-11	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
CON	53.47	4.27	54.47	5.29	57.60	5.58	4.27	4.65	3.40	7.60	10.13	6.38
AD	57.25	4.72	46.50	1.91	53.50	7.10	7.33	0.98	7.75	9.29	23.92	1.97
VaD	43.78	5.29	39.44	1.99	47.44	5.62	7.78	4.96	0.78	1.22	10.67	3.92



**Fig. 4** Expression of CI-2, CI-5 and CI-11 in astrocytes in control (CON), AD and VaD brains, in the frontal cortex, Brodmann area 46/9. CI-expressing cells are brownish, as a result of the immunohistochemical staining method with anti-CI sera detected with avidin–biotin–peroxidase complex kit and DAB substrate. Astrocytes are indicated by arrows. Bars: 5  $\mu$ m.

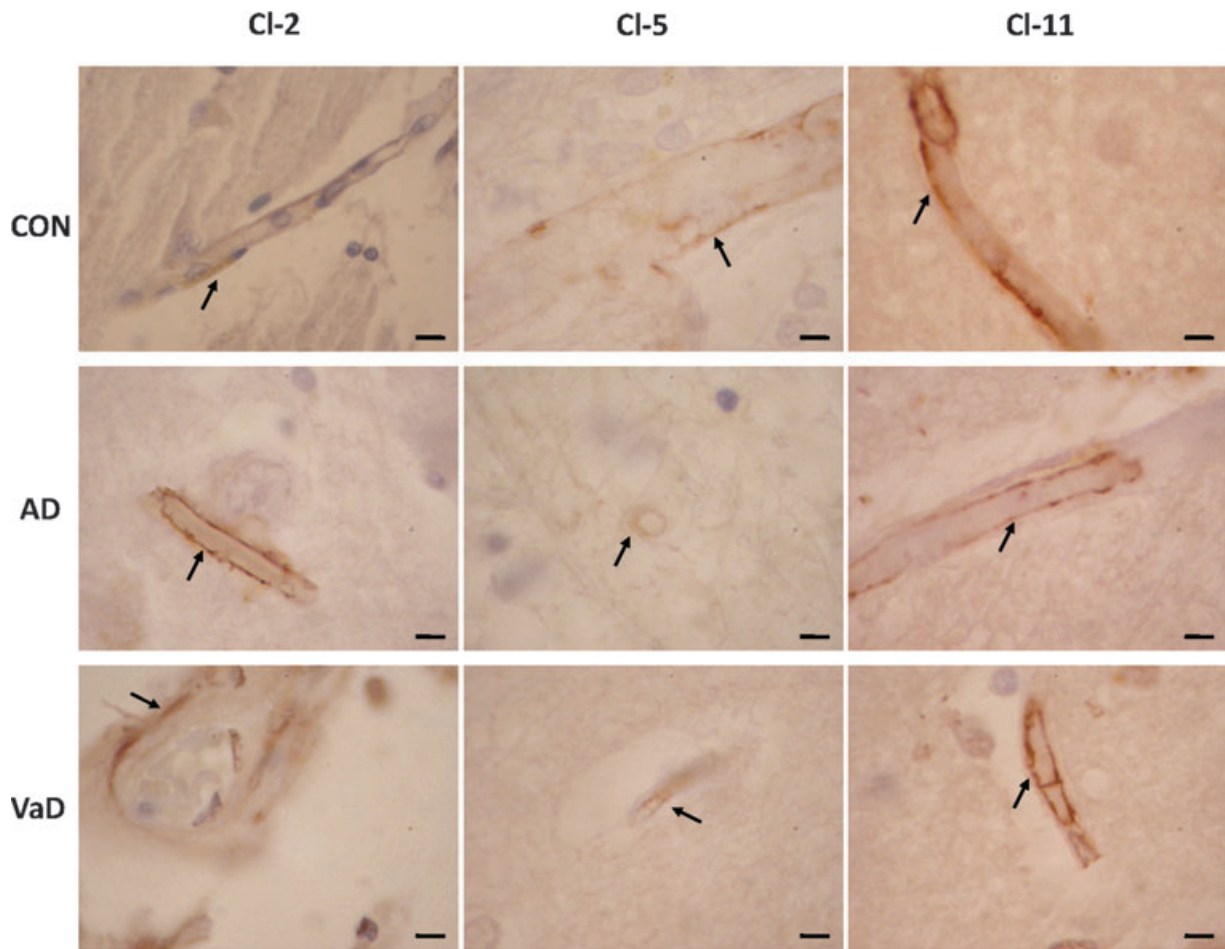
### Expression of claudins in cerebral vessels

At the level of brain microvasculature in AD, VaD and control brains we observed that expression of CIs localized selectively in

some of the endothelial cells or in the media layer (Fig. 5). The level of expression of CI-2, CI-5 and CI-11 was variable from one vessel to another in all cases studied and no evident qualitative difference between AD, VaD and aged control groups was observed.

**Table 4** Quantification of total number of astrocytes and CI-expressing astrocytes (positive astrocytes) in the frontal cortex region in control (CON, five cases), AD (four cases) and VaD (six cases) groups. All values were truncated to two decimals. S.D. = standard deviation

Group	Total number of astrocytes						Number of positive astrocytes					
	CI-2		CI-5		CI-11		CI-2		CI-5		CI-11	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
CON	35.40	2.77	37.53	8.17	38.47	2.29	3.67	5.16	4.07	9.09	8.40	5.47
AD	33.92	3.06	40.17	4.85	43.33	6.02	17.83	2.13	9.75	7.28	21.83	2.74
VaD	31.00	4.27	34.83	1.44	35.61	4.06	11.50	1.07	1.89	2.93	17.78	5.63



**Fig. 5** Expression of CI-2, CI-5 and CI-11 in brain microvessels in control (CON), AD and VaD brains, in the frontal cortex, Brodmann area 46/9. CI-expressing cells are brownish, as a result of the immunohistochemical staining method with anti-CI sera detected with avidin–biotin–peroxidase complex kit and DAB substrate. Endothelial cells expressing CI-2, CI-5 or CI-11 are indicated by arrows. Bars: 5  $\mu$ m.

## Discussion

To our knowledge, this is the first report on different CI isoforms being expressed in the human brain, not only by endothelial cells and oligodendrocytes, but also by astrocytes and neurons. From

the CI family of proteins, CI-1 [10], CI-3 [11] and CI-5 [8] are known to localize to the brain endothelial cells. In addition to CI-5, we found CI-2 and CI-11 to be expressed by endothelial cells in aged controls, as well as in AD and VaD brains. We were able to identify microvessels in the frontal cortex expressing CI-2, CI-5 or



Cl-11 in various amounts in all brains investigated. We noted no qualitative difference in the distribution or amount of expression of these proteins in vessels between AD, VaD and aged control brains. However, we found significant changes in the expression pattern of CIs which differentiate the AD and VaD from control brains. Thus, Cl-2 or Cl-5 is expressed by approximately one tenth and Cl-11 by one fourth of cortical frontal neurons in aged control brains, cells which have not been previously known to form TJs. In AD and VaD brains two to eight times more neurons in the frontal cortex expressed these CI isoforms in detectable amounts. These CI-expressing neurons were mainly of pyramidal type, which are involved in cognition and are typically affected by AD pathology. Importantly, while Cl-5 and Cl-11 are known to strengthen the TJs and to increase the transendothelial resistance [41], Cl-2 induces a leaky strand type [13]. Therefore, the permeability of the BBB is presumably decided by a complex interplay of differential CI isoform regulation, suggesting that increased CI expression might be a part of the cellular response to stress.

CIs are expressed in a tissue-dependent manner and more than two different CI isoforms are typically found in the most cell types, conceivably influencing the TJs barrier function properties [36]. Expression of CIs in endothelial cells seems to be regulated by both blood stream generated factors, and signals derived from astrocytes and pericytes [37–39]. However, regulation of CIs expression might be operated by different signalling systems in different cell types. Ageing itself decreases expression of CIs and occludin at the level of brain microvessels, altering the BBB permeability [40]. Disruption of BBB is now increasingly documented in ageing, vascular and degenerative diseases and this disruption might contribute to the progression of the pathology [1].

Alteration of the brain microvessels is common to both AD and VaD [42] and chronic brain hypoperfusion as well [43]. A recent report demonstrated that in rats exposed to cerebral hypoperfusion, an up-regulation of Cl-3, which accompanies the BBB breakdown, occurs in endothelial cells [44]. The profile of CI isoforms expression can be changed by a reduced blood flow not only in the brain, but in other organs as well, such as kidney, where levels of Cl-7 have been found increased in an aging kidney rat model [45]. Ischemia and hypoxia are able to trigger a multitude of cellular responses, including changes in gene expression [46]. Our findings of increased level of CIs in neurons and, to some extent, in glial cells as well, might be explained by hypoxia-induced gene regulation. Moreover, as recently suggested for occludin, the increased levels of CIs found in dementia brains might reflect an autophagy of the TJ proteins by neighbouring cells after the BBB breakdown triggered by chronic hypoxia [47].

Several studies indicated a disturbance of function of the hypothalamic–pituitary–adrenocortical (HPA) axis in both AD and VaD, with increased plasma and urinary cortisol levels, as compared to aged controls [48, 49]. Besides increasing the amyloid- $\beta$  and  $\tau$  pathology, at least in an AD mouse model [50] and triggering neuronal cell death [51], glucocorticoids (GC) have been shown to up-regulate expression of Cl-5 in murine brain [52] and of other CI isoforms in cultures from Atlantic Salmon gill [53]. Therefore, our findings of increased expression of different CI isoforms in AD and

VaD brains might be explained by the effect of an improper HPA axis functioning. Moreover, the different profile of CI isoforms expression in different cell types could be explained by a recent hypothesis on the combined effect of GC and brain ageing, which states that ageing selectively increases GC efficacy in neurons while decreasing GC efficacy in glial cells, by an age-related phenotype change [54]. A GC-related susceptibility for AD, based on a genetic variation in the GC system, has been reported as well [55].

We found that expression of Cl-5 is significantly increased in neurons, but not in oligodendrocytes and astrocytes, in AD and VaD brains as compared to aged control brains. Based on experiments in endothelial cell cultures it has been suggested that  $\beta$ -amyloid does not influence directly the expression of Cl-5 [56]. One recent study [39] reported that Cl-5 and occludin are down-regulated by vascular endothelial growth factor (VEGF)-A, released by astrocytes. VEGF-A signalling is known to be involved as well in the ischemic preconditioning of neurons, by activation of cAMP response element-binding protein pathway [57] and in neuroprotection against excitotoxicity [58]. Therefore, a decrease in VEGF-A availability could explain both the increased Cl-5 expression in neurons in AD and VaD found in this study and the increase in occludin expression, reported before [27]. Furthermore, diminished VEGF activity in brain microvessels was found in areas of AD brains with pathological hallmarks of the disease [59] and VEGF is significantly decreased in serum of AD patients [60].

Transforming growth factor (TGF)- $\beta$  is a member of a family of dimeric polypeptide growth factors involved in cellular differentiation, proliferation, anti-inflammatory signalling, reparative and neuroprotective functions. Increased levels of TGF- $\beta$  seem to enhance the AD pathology and an experimental blockade of TGF- $\beta$ -Smad 2/3 signalling in an AD transgenic mouse model resulted in a 90% clearance of  $\beta$ -amyloid brain deposits [61]. TGF- $\beta$  is elevated in plasma [62] and in CSF [63] of AD and VaD patients and its expression is induced following cerebral ischemia with neuroprotective consequences [64]. In addition, TGF- $\beta$  had been found to induce expression of Cl-2 and occludin through activation of ERK/MAPK signalling [65]. Therefore, our finding of increased Cl-2 expression in AD and VaD brains, in both neurons and astrocytes, is in the line with the above mentioned studies and might be an effect of TGF- $\beta$  increased signalling. Interestingly enough, the decrease of neuronal TGF- $\beta$  signalling in a mouse AD model resulted in neurodegeneration,  $\beta$ -amyloid accumulation and dendritic loss, which suggests that the increased TGF- $\beta$  level found in dementia patients is part of an endogenous protective response. Consequently, it is possible that overexpression of CIs in brain cells in AD and VaD to be a downstream result, at the phenotype level, of this protective response. However, a limitation of our study remains the low number of brains analysed and therefore confirmation of these mechanisms in cell culture systems or animal models would be important.

Similar to TGF- $\beta$ , the level of basic fibroblast growth factor-2 (FGF-2) is markedly elevated in AD brains [66] and, on the other hand, in cortical organotypic slice cultures of mice, FGF-2 had been shown to stimulate expression of Cl-5 and Cl-3 and to conserve the integrity of TJs [67]. Therefore, the high FGF levels

might trigger as well an increase in CIs expression, with a possible protective role.

PKC is a potent modulator of the non-amyloidogenic pathway of the amyloid precursor protein (APP) processing, being involved as well in synaptic remodelling and induction of protein synthesis. PKC levels are significantly decreased in AD and VaD brains [68]. PKC activation had been reported to down-regulate CI-2 [12, 69] and to phosphorylate and down-regulate CI-5, with subsequent alteration of TJs properties [12, 70]. Therefore, the general reduction in PKC activity found in dementia brains could account for the increased levels of CIs found in our study. Moreover, phospholipase D (PLD) expression and activity are up-regulated in AD brains [71]. Phosphatidic acid (PA), a product of PLD, and lysophosphatidic acid (LPA) have been found significantly increased in AD brains as well [72, 73]. PLD and its end-products seem to have multiple links to AD pathogenesis. Although PLD1 colocalizes and interacts with APP [71] and promotes the trafficking and cell surface accumulation of presenilin 1/ $\gamma$  secretase complex [74], PLD 2 has been reported to regulate endothelial barrier permeability through cytoskeleton reorganization and occludin down-regulation, in cell culture experiments [75]. LPA, which in contrast to PA [76] has a pro-apoptotic effect on neurons [77], is able to increase the permeability of TJs without triggering a relocalization of TJs-associated proteins. All these PLD pathway effects might participate to alteration of BBB properties in AD patients. However, no studies explored the effect of PLD on expression of CIs in neurons or glial cells.

CI-11 was classically known to be localized to TJs of myelin sheaths in brain and of Sertoli cells in testis [9]. The expression of CI-11 in endothelial cells was recently reported in human corpus cavernosum [78]. In line with this study, we report here, presumably for the first time, expression of CI-11 in human brain endothelial cells. Moreover, we found that CI-11 is expressed by a large population of neurons, oligodendrocytes and astrocytes in ageing controls and significantly by more of these cells in AD and VaD brains. Both estrogens and testosterone have neuroprotective roles against AD-related insults, being also able to reduce  $\beta$ -amyloid accumulation [79]. Meanwhile, testosterone has been also reported to be an important operator of CI-11 gene expression, physiological concentrations increasing the amount of CI-11 mRNA two- to threefold within 3 days in rat Sertoli cells *in vitro* [80]. However the same study demonstrated that estradiol had no effect on CI-11 expression. Serum androgen levels are not different in male aged controls and AD patients [81] and we found no difference in CI-11 expression between male and female brains. In

addition, Kaitu'u-Lino *et al.* demonstrated that gonadotropins stimulate CI-11 expression as well and follicle stimulating hormone (FSH) and luteinizing hormone (LH) serum levels have been found significantly increased in patients with dementia [82]. LH is also involved in regulation of presenilin 1 and 2 genes [83], which are the responsible for amyloid processing [84]. LH and FSH could be important players in the pathogenesis of dementia, since their receptors follow a regional pattern of the neuronal populations affected in AD [85] and could operate as well the increased expression of CI-11 in dementia brains reported in our study.

In conclusion we describe, to our knowledge for the first time, the pattern of expression of CI-2, CI-5 and CI-11 in neurons, astrocytes, oligodendrocytes and microvessels in human brains. In both AD and VaD, significantly more neurons in the frontal cortex express all CI isoforms examined as compared to controls. More astrocytes in both AD and VaD brains express CI-2 and CI-11 as compared to aged control brains. More oligodendrocytes express CI-11 in AD and CI-2 in VaD as compared to controls. This complex up-regulation of expression of different CI isoforms in demented brains might be a result of an endogenous protective response. Our findings could be important to elucidate new pathogenic pathways in dementia disorders and to add new functions to the heterogeneous CI family of proteins.

## Acknowledgements

This study has been supported by Marie Curie–Eurogendis fellowship program, Swedish Brain Power Program and Alzheimer Foundation, Sweden and by the Romanian Ministry of Education and Research, the National Innovation, Research and Development Plan – grants no. 41–013/2007, 61–019-P2/2007 and PN 06.26–01.01/2007. We thank Inga Volkmann for excellent technical support, Anca Catrina for providing us the unspecific immunoglobulin serum for the negative controls and Cătălin Manole for critically reading the manuscript. Our gratefulness is towards the families that donated the brain material for scientific research.

## Disclosure

The scientific work of Dr. Nenad Bogdanovic and results in this paper are not subject of commercial interest but pure academic as a part of his academic position at Karolinska Institutet in Sweden.

## References

1. Popescu BO, Toescu EC, Popescu LM, *et al.* Blood-brain barrier alterations in ageing and dementia. *J Neurol Sci.* 2009; 283: 99–106.
2. Schneeberger EE, Lynch RD. The tight junction: a multifunctional complex. *Am J Physiol Cell Physiol.* 2004; 286: C1213–28.
3. Rubin LL. The blood-brain barrier in and out of cell culture. *Curr Opin Neurobiol.* 1991; 1: 360–3.
4. Martin-Padura I, Lostaglio S, Scheneemann M, *et al.* Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distrib-
5. Furuse M, Hirase T, Itoh M, *et al.* Occludin: a novel integral membrane protein localizing at the tight junctions. *J Cell Biol.* 1993; 123: 1777–88.
6. Furuse M, Hirase T, Itoh M, *et al.* Occludin: a novel integral membrane protein localizing at the tight junctions and regulates monocytes transmigration. *J Cell Biol.* 1998; 142: 117–27.

6. **Sonoda N, Furuse M, Sasaki H, et al.** Clostridium perfringens enterotoxin fragment removes specific claudins from tight junction strands: evidence for direct involvement of claudins in tight junction barrier. *J Cell Biol.* 1999; 147: 195–204.
7. **Tsukita S, Furuse M, Itoh M.** Multifunctional strands in tight junctions. *Nat Rev Mol Cell Biol.* 2001; 2: 285–93.
8. **Nitta T, Hata M, Gotoh S, et al.** Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. *J Cell Biol.* 2003; 161: 653–60.
9. **Morita K, Furuse M, Fujimoto K, et al.** Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands *Proc Natl Acad Sci USA.* 1999; 96: 511–6.
10. **Huber JD, Witt KA, Hom S, et al.** Inflammatory pain alters blood-brain barrier permeability and tight junctional protein expression. *Am J Physiol Heart Circ Physiol.* 2001; 280: H1241–8.
11. **Wolburg H, Wolburg-Buchholz K, Kraus J, et al.** Localization of claudin-3 in tight junctions of the blood-brain barrier is selectively lost during experimental autoimmune encephalomyelitis and human glioblastoma multiforme. *Acta Neuropathol.* 2003; 105: 586–92.
12. **Lippoldt A, Liebner S, Andbjør B, et al.** Organization of choroid plexus epithelial and endothelial cell tight junctions and regulation of claudin-1, -2 and -5 expression by protein kinase C. *Neuro Report.* 2000; 11: 1427–31.
13. **Furuse M, Furuse K, Sasaki H, et al.** Conversion of Zonulae occludentes from tight to leaky strand type by introducing claudin-2 into Madin-Darby canine kidney cells. *J Cell Biol.* 2001; 153: 263–72.
14. **Amasheh S, Meiri N, Gitter AH, et al.** Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. *J Cell Sci.* 2002; 115: 4969–76.
15. **Morita K, Sasaki H, Fujimoto K, et al.** Claudin-11/OSP- based tight junctions of myelin sheaths in brain and Sertoli cells in testis. *J Cell Biol.* 1999; 145: 579–88.
16. **Bronstein JM, Tiwari-Woodruff S, Buznikov AG, et al.** Involvement of OSP/claudin-11 in oligodendrocyte membrane interactions: role in biology and disease. *J Neurosci Res.* 2000; 59: 706–11.
17. **Wolburg H, Wolburg-Buchholz K, Liebner S, et al.** Claudin-1, claudin-2 and claudin-11 are present in tight junctions of choroid plexus epithelium of the mouse. *Neurosci Lett.* 2001; 307: 77–80.
18. **Miyamori H, Takino T, Kobayashi Y, et al.** Claudin promotes activation of Pro-MMP-2 mediated by membrane-type matrix metalloproteinases. *J Biol Chem.* 2001; 276: 28204–11.
19. **Rubin LL, Staddon JM.** The cell biology of the blood-brain barrier. *Annu Rev Neurosci.* 1999; 22: 11–28.
20. **Nag S.** Pathophysiology of blood-brain barrier breakdown. *Methods Mol Med.* 2003; 89: 97–119.
21. **Kreuter J.** Neuroparticulate systems for brain delivery of drugs. *Adv Drug Deliv Rev.* 2001; 47: 65–81.
22. **Kalaria RN.** Vascular factors in Alzheimer's disease. *Int Psychogeriatr.* 2003; 15: 47–52.
23. **Thal DR, Griffin WS, Braak H.** Parenchymal and vascular Abeta-deposition and its effects on the degeneration of neurons and cognition in Alzheimer's disease. *J Cell Mol Med.* 2008; 12: 1848–62.
24. **Marco S, Skaper S.** Amyloid beta-peptide (1–42) alters tight junction protein distribution and expression in brain microvessel endothelial cells. *Neurosci Lett.* 2006; 401: 219–24.
25. **Findley MK, Koval M.** Regulation and roles for claudin-family tight junction proteins. *IUBMB Life.* 2009; 61: 431–7.
26. **Bauer H, Stelzhammer W, Fuchs R, et al.** Astrocytes and Neurons Express the Tight Junction-Specific Protein Occludin *in vitro*. *Exp Cell Res.* 1999; 250: 434–8.
27. **Romanitan MO, Popescu BO, Winblad B, et al.** Occludin is overexpressed in Alzheimer's disease and vascular dementia. *J Cell Mol Med.* 2007; 11: 569–79.
28. **Bailey TL, Rivara CB, Rocher AB, et al.** The nature and effects of cortical microvascular pathology in aging and Alzheimer's disease. *Neuro Res.* 2004; 26: 573–8.
29. **Viswanathan A, Gray F, Bousser MG, et al.** Cortical neuronal apoptosis in CADASIL. *Stroke.* 2008; 37: 2690–5.
30. **American Psychiatric Association.** Committee on Nomenclature and Statistics. *Diagnostic and statistical manual of mental disorders.* 4th ed. Washington, DC: American Psychiatric Association; 1994. pp. 133–45.
31. **McKhann G, Drachmann D, Folstein M, et al.** Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work group under the auspices of the Department of Health and Human Services. *Neurology.* 1984; 34: 939–44.
32. **Bogdanovic N, Morris JH.** Diagnostic criteria for Alzheimer's disease in multicentre brain banking In: Cruz-Sanches FF, Ravid R, Cuzner ML, editors. *Neuropathological diagnostic criteria for brain banking.* Amsterdam: IOS Press; 1995. pp. 20–9.
33. **National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer Disease.** Consensus recommendations for the post-mortem diagnosis of Alzheimer's disease. *Neurobiol Aging.* 1997; 18: S1–2.
34. **West MJ, Slomianka L, Gundersen HJ.** Unbiased stereological estimation of the total number of neurons in the subdivisions of rat hippocampus using the optical fractionator. *Anat Rec.* 1991; 231: 482–97.
35. **Lasn H, Winblad B, Bogdanovic N.** Neuroglia in the inferior olivary nucleus during normal aging and Alzheimer's disease. *J Cell Mol Med.* 2006; 10: 145–56.
36. **Förster C.** Tight junctions and the modulation of barrier function in disease. *Histochem. Cell Biol.* 2008; 130: 55–70.
37. **Kröll S, El-Gindi J, Thanabalasundaram G, et al.** Control of the blood-brain barrier by glucocorticoids and the cells of the neurovascular unit. *Ann NY Acad Sci.* 2009; 1165: 228–39.
38. **Ronaldson PT, Demarco KM, Sanchez-Covarrubias L, et al.** Transforming growth factor-beta signaling alters substrate permeability and tight junction protein expression at the blood-brain barrier during inflammatory pain. *J Cereb Blood Flow Metab.* 2009; 29: 1084–98.
39. **Argaw AT, Gurfein BT, Zhang Y, et al.** VEGF-mediated disruption of endothelial CLN-5 promotes blood-brain barrier breakdown. *Proc Natl Acad Sci USA.* 2009; 106: 1977–82.
40. **Bake S, Friedman JA, Sohrabji F.** Reproductive age-related changes in the blood brain barrier: expression of IgG and tight junction proteins. *Microvasc Res.* 2009; 78: 413–24.
41. **Furuse M, Tsukita S.** Claudins in occluding junctions of humans and flies. *Trends Cell Biol.* 2006; 16: 181–8.
42. **Farkas E, Luiten PG.** Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog Neurobiol.* 2001; 64: 575–611.
43. **de Jong GI, de Vos RAI, Jansen Steur ENH, et al.** Cerebrovascular hypoperfusion: a risk factor for Alzheimer's disease? *Ann NY Acad Sci.* 1997; 826: 56–74.
44. **Shin JS, Hyun SY, Kim DH, et al.** Chronic hypoperfusion increases claudin-3 immunoreactivity in rat brain. *Neurosci Lett.* 2008; 445: 144–8.

45. **Chen G, Bridenbaugh EA, Akintola AD, et al.** Increased susceptibility of aging kidney to ischemic injury: identification of candidate genes changed during aging, but corrected by caloric restriction. *Am J Physiol Renal Physiol.* 2007; 293: F1272–81.
46. **Van Elzen R, Moens L, Dewilde S.** Expression profiling of the cerebral ischemic and hypoxic response. *Expert Rev Proteomics.* 2008; 5: 263–82.
47. **Zlokovic BV.** The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron.* 2008; 57: 178–201.
48. **Gottfries CG, Balldin J, Blennow K, et al.** Hypothalamic dysfunction in dementia. *J Neural Transm Suppl.* 1994; 43: 203–9.
49. **Maeda K, Tanimoto K, Terada T, et al.** Elevated urinary free cortisol in patients with dementia. *Neurobiol Aging.* 1991; 12: 161–3.
50. **Green KN, Billings LM, Roozendaal B, et al.** Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. *J Neurosci.* 2006; 26: 9047–56.
51. **Popescu BO, Popescu LM.** Neuronal apoptosis triggered by anti-Fas (CD95, APO-1) antibody is enhanced by dexamethasone. *J Med Biochem.* 2000; 4: 1–14.
52. **Burek M, Förster CY.** Cloning and characterization of the murine claudin-5 promoter. *Mol Cell Endocrinol.* 2009; 298: 19–24.
53. **Tipmark CK, Jørgensen C, Brande-Lavridsen N, et al.** Effects of cortisol, growth hormone and prolactin on gill claudin expression in Atlantic salmon. *Gen Comp Endocrinol.* 2009; 163: 270–7.
54. **Landfield PW, Blalock EM, Chen KC, et al.** A new glucocorticoid hypothesis of brain aging: implications for Alzheimer's disease. *Curr Alzheimer Res.* 2007; 4: 205–12.
55. **de Quervain DJ, Poirier R, Wollmer MA, et al.** Glucocorticoid-related genetic susceptibility for Alzheimer's disease. *Hum Mol Genet.* 2004; 13: 47–52.
56. **Tai LM, Holloway KA, Male DK, et al.** Amyloid-beta-induced occludin down-regulation and increased permeability in human brain endothelial cells is mediated by MAPK activation. *J Cell Mol Med.* 2009. DOI: 10.1111/j.1582-4934.2009.00717.x.
57. **Lee HT, Chang YC, Tu YF, et al.** VEGF-A/VEGFR-2 signaling leading to cAMP response element-binding protein phosphorylation is a shared pathway underlying the protective effect of preconditioning on neurons and endothelial cells. *J Neurosci.* 2009; 29: 4356–68.
58. **Moser KV, Humpel C.** Vascular endothelial growth factor counteracts NMDA-induced cell death of adult cholinergic neurons in rat basal nucleus of Meynert. *Brain Res Bull.* 2005; 65: 125–31.
59. **Provias J, Jeynes B.** Neurofibrillary tangles and senile plaques in Alzheimer's brains are associated with reduced capillary expression of vascular endothelial growth factor and endothelial nitric oxide synthase. *Curr Neurovasc Res.* 2008; 5: 199–205.
60. **Mateo I, Llorca J, Infante J, et al.** Low serum VEGF levels are associated with Alzheimer's disease. *Acta Neurol Scand.* 2007; 116: 56–8.
61. **Town T, Laouar Y, Pittenger C, et al.** Blocking TGF-beta-Smad2/3 innate immune signaling mitigates Alzheimer-like pathology. *Nat Med.* 2008; 14: 681–687.
62. **Malaguarnera L, Motta M, Di Rosa M, et al.** Interleukin-18 and transforming growth factor-beta 1 plasma levels in Alzheimer's disease and vascular dementia. *Neuropathology.* 2006; 26: 307–12.
63. **Tarkowski E, Issa R, Sjögren M, et al.** Increased intrathecal levels of the angiogenic factors VEGF and TGF-beta in Alzheimer's disease and vascular dementia. *Neurobiol Aging.* 2002; 23: 237–43.
64. **Hu Z, Fan J, Zeng L, et al.** Transient cerebral ischemia leads to TGF-beta2 expression in Golgi apparatus organelles. *Curr Neurovasc Res.* 2008; 5: 178–184.
65. **Howe KL, Reardon C, Wang A, et al.** Transforming growth factor-beta regulation of epithelial tight junction proteins enhances barrier function and blocks enterohemorrhagic Escherichia coli O157:H7-induced increased permeability. *Am J Pathol.* 2005; 167: 1587–97.
66. **Stopa EG, Gonzalez AM, Chorsky R, et al.** Basic fibroblast growth factor in Alzheimer's disease. *Biochem Biophys Res Commun.* 1990; 171: 690–6.
67. **Bendfeldt K, Radojevic V, Kapfhammer J, et al.** Basic fibroblast growth factor modulates density of blood vessels and preserves tight junctions in organotypic cortical cultures of mice: a new *in vitro* model of the blood-brain barrier. *J Neurosci.* 2007; 27: 3260–7.
68. **Cowburn RF, O'Neill C, Bonkale WL, et al.** Receptor-G-protein signalling in Alzheimer's disease. *Biochem Soc Symp.* 2001; 67: 163–75.
69. **Angelow S, Zeni P, Höhn B, et al.** Phorbol ester induced short- and long-term permeabilization of the blood-CSF barrier *in vitro*. *Brain Res.* 2005; 1063: 168–79.
70. **Stamatovic SM, Dimitrijevic OB, Keep RF, et al.** Protein kinase Calpha-RhoA cross-talk in CCL2-induced alterations in brain endothelial permeability. *J Biol Chem.* 2006; 281: 8379–88.
71. **Jin JK, Ahn BH, Na YJ, et al.** Phospholipase D1 is associated with amyloid precursor protein in Alzheimer's disease. *Neurobiol Aging.* 2007; 28: 1015–27.
72. **Jin JK, Kim NH, Lee YJ, et al.** Phospholipase D1 is up-regulated in the mitochondrial fraction from the brains of Alzheimer's disease patients. *Neurosci Lett.* 2006; 407: 263–7.
73. **Umemura K, Yamashita N, Yu X, et al.** Autotaxin expression is enhanced in frontal cortex of Alzheimer-type dementia patients. *Neurosci Lett.* 2006; 400: 97–100.
74. **Liu Y, Zhang YW, Wang X, et al.** Intracellular trafficking of presenilin 1 is regulated by beta-amyloid precursor protein and phospholipase D1. *J Biol Chem.* 2009; 284: 12145–52.
75. **Zeiller C, Mebarek S, Jaafar R, et al.** Phospholipase D2 regulates endothelial permeability through cytoskeleton reorganization and occludin downregulation. *Biochim Biophys Acta.* 2009; 1793: 1236–49.
76. **Popescu AT, Vidulescu C, Stanciu CL, et al.** Selective protection by phosphatidic acid against staurosporine-induced neuronal apoptosis. *J Cell Mol Med.* 2002; 6: 433–438.
77. **Steiner MR, Holtzberg FW, Keller JN, et al.** Lysophosphatidic acid induction of neuronal apoptosis and necrosis. *Ann NY Acad Sci.* 2000; 905: 132–41.
78. **Wessells H, Sullivan CJ, Tsubota Y, et al.** Transcriptional profiling of human cavernosal endothelial cells reveals distinctive cell adhesion phenotype and role for claudin 11 in vascular barrier function. *Physiol Genomics.* 2009; 39: 100–8.
79. **Pike CJ, Carroll JC, Rosario ER, et al.** Protective actions of sex steroid hormones in Alzheimer's disease. *Front Neuroendocrinol.* 2009; 30: 239–58.
80. **Kaitu'u-Lino TJ, Sluka P, Foo CF, et al.** Claudin-11 expression and localisation is regulated by androgens in rat Sertoli cells *in vitro*. *Reproduction.* 2007; 133: 1169–79.
81. **Ponholzer A, Madersbacher S, Rauchenwald M, et al.** Serum androgen levels and their association to depression

- and Alzheimer dementia in a cohort of 75-year-old men over 5 years: results of the VITA study. *Int J Impot Res.* 2009; 21: 187–91.
82. **Bowen RL, Isley JP, Atkinson RL.** An association of elevated serum gonadotropin concentrations and Alzheimer disease? *J Neuroendocrinol.* 2000; 12: 351–4.
83. **Rimon E, Sasson R, Dantes A, et al.** Gonadotropin-induced gene regulation in human granulosa cells obtained from IVF patients: modulation of genes coding for growth factors and their receptors and genes involved in cancer and other diseases. *Int J Oncol.* 2004; 24: 1325–38.
84. **Popescu BO, Ankarcrona M.** Neurons bearing presenilins: weapons for defense or suicide? *J Cell Mol Med.* 2000; 4: 249–261.
85. **Casadesus G, Garrett MR, Webber KM, et al.** The estrogen myth: potential use of gonadotropin-releasing hormone agonists for the treatment of Alzheimer's disease. *Drugs R D.* 2006; 7: 187–93.