

## SHORT REPORT

Water transport becomes uncoupled from K<sup>+</sup> siphoning in brain contusion, bacterial meningitis, and brain tumours: immunohistochemical case review

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*J Clin Pathol* 2003;56:972-975

Specimens of normal human brain, contused brain, brain with bacterial meningitis, and brain tumours were immunolabelled for aquaporin 4 (AQP4) and Kir4.1. In normal brain tissue, AQP4 and Kir4.1 were detected around the microvessels. In pathological brain tissue, AQP4 was upregulated in astrocytes in oedematous regions and Kir4.1 was upregulated in astrocytes in damaged brain. Changes in  $\alpha$  syntrophin expression paralleled those of AQP4 and Kir4.1. The following hypothesis is proposed: in astrocytes, under normal conditions, AQP4 couples water transport with Kir4.1 mediated K<sup>+</sup> siphoning, but in pathological states, AQP4 facilitates the flow of brain oedema fluid, and Kir4.1 buffers increased extracellular K<sup>+</sup>.

Aquaporin 4 (AQP4), the major water channel of rodent<sup>1</sup> and human<sup>2</sup> brains, is expressed in astrocyte foot processes, glia limitans, and ependyma, but its function remains unknown. Kir4.1, the inward rectifying K<sup>+</sup> channel of rodent astrocytes, buffers neurotoxic rises in extracellular K<sup>+</sup> and localises in perimicrovessel astrocyte processes,<sup>3,4</sup> but data from humans are limited. In rodents,  $\alpha$  syntrophin recruits AQP4 to astrocyte foot processes<sup>1</sup> and Kir4.1 to retinal Müller cell foot processes,<sup>5</sup> but the situation in humans is unknown.

“Kir4.1, the inward rectifying K<sup>+</sup> channel of rodent astrocytes, buffers neurotoxic rises in extracellular K<sup>+</sup> and localises in perimicrovessel astrocyte processes, but data from humans are limited”

AQP4 may work with Kir4.1, allowing for K<sup>+</sup> siphoning into astrocytes during high neuronal activity,<sup>5,6</sup> or participate in the formation of brain oedema.<sup>7-9</sup> We explored these ideas by immunodetecting AQP4, Kir4.1, and  $\alpha$  syntrophin in normal and pathological human brain tissue obtained from 10 patients.

## METHODS

## Patients

Our study was approved by the local ethics committee. We analysed resected brain tumours, contused brain, and biopsied tissue from the ventricular drain site of a patient with bacterial meningitis. Normal brain tissue was obtained from temporal lobectomies for epilepsy. All patients had magnetic resonance imaging to detect brain oedema.

## Immunolabelling

Formalin fixed, paraffin wax embedded tissue was processed as previously described.<sup>2,10</sup> Antibodies were rabbit anti-AQP4 (Chemicon, Chandler's Ford, Hampshire, UK), rabbit anti-Kir4.1 (Alomone, Silverstone, Towcester, UK), rabbit anti- $\alpha$  syntrophin (Sigma, Poole, Dorset, UK), and biotinylated goat antirabbit immunoglobulin (Sigma). Immunoreactivity was visualised using avidin-biotin complex and diaminobenzidine. Omission of the primary antibody or use of a blocking peptide abolished immunoreactivity. Tissue sections were counterstained with cresyl violet or haematoxylin. Immunolabelling was graded as 0 (no labelling), + (perimicrovessel labelling only), or ++ (labelling of many cells).

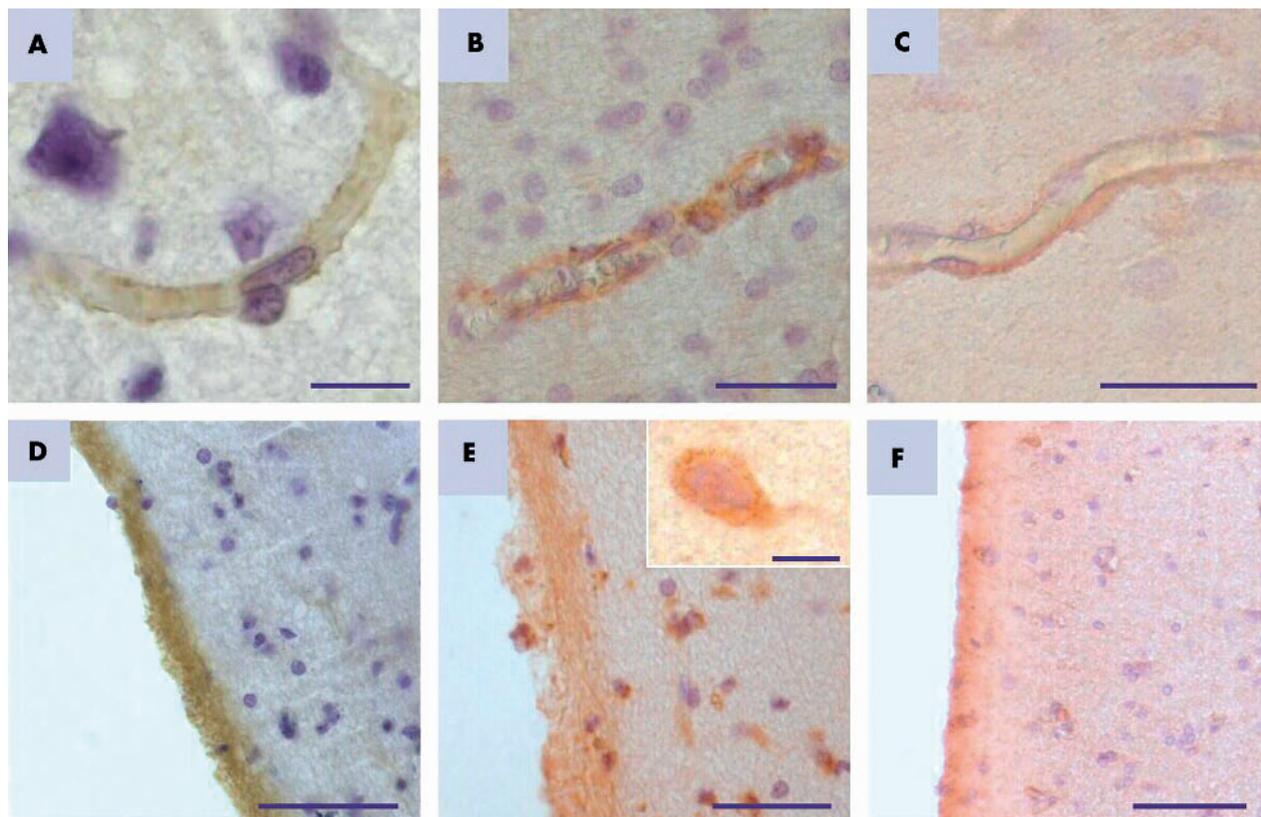
## RESULTS

Table 1 summarises the details of the patients investigated in our study.

**Table 1** Patient details and expression of AQP4, Kir4.1, and  $\alpha$  syntrophin in the different specimens

Histology	Age	Sex	Side	Site	Cell type	AQP4	Kir4.1	Syn	Oedema
Normal	39	F	R	Temporal	Astrocyte	+	+	+	No
					Neurone	0	++	0	No
Normal	65	M	R	Temporal	Astrocyte	+	+	+	No
					Neurone	0	++	0	No
Glioblastoma	54	M	R	Frontal	Astrocyte/astrocytoma	++	++	++	Yes
Glioblastoma	57	M	R	Temporal	Astrocyte/astrocytoma	++	++	++	Yes
Oligodendroglioma	34	F	L	Frontal	Oligodendroglioma	0	++	++	Yes
					Peritumour astrocyte	++	+	++	Yes
Carcinoma	76	F	L	Cerebellum	Cancer cell	0	++	++	Yes
					Peritumour astrocyte	++	+	++	Yes
Carcinoma	68	M	R	Cerebellum	Cancer cell	0	++	++	Yes
					Peritumour astrocyte	++	+	++	Yes
Contusion	69	M	L	Frontal	Astrocyte	++	++	++	Yes
Contusion	23	M	R	Frontal	Astrocyte	++	++	++	Yes
Bacterial meningitis	67	M	R	Frontal	Astrocyte	++	++	++	Yes

AQP4, aquaporin 4; F, female; L, left; M, male; R, right; Syn,  $\alpha$  syntrophin.



**Figure 1** Normal brain immunolabelled for (A, D) AQP4, (B, E) Kir4.1, and (C, F)  $\alpha$  syntrophin. Expression is seen around (A–C) microvessels and (D–F) in the glia limitans and pia. No other regions labelled with these antibodies except for some neurones, which were immunopositive for Kir4.1 (E, insert). Bars, (A) 20  $\mu$ m; (B) 100  $\mu$ m; (C) 50  $\mu$ m; (D) 100  $\mu$ m; (E) 100  $\mu$ m and 10  $\mu$ m (insert); and (F) 150  $\mu$ m.

### AQP4

As expected, normal brain expressed AQP4 around microvessels, in the glia limitans, and the pia (fig 1A, D). AQP4 was upregulated in astrocytes in contused brain (fig 2A), bacterial meningitis (fig 2D), brain around carcinoma (fig 2G) and oligodendroglioma (fig 2J), and in glioblastoma multiforme (fig 2M).

### Kir4.1

In normal brain, Kir4.1 was seen around microvessels (fig 1B), in the glia limitans/pia (fig 1E), and in occasional neurones in cortical layers I and II (fig 1E insert). In brain contusion (fig 2B) and brain surrounding carcinoma and oligodendroglioma, Kir4.1 expression was also seen around microvessels. Kir4.1 was upregulated in astrocytes in bacterial meningitis (fig 2E), contusion (fig 2B), and in carcinoma (fig 2H), oligodendroglioma (fig 2K), and glioblastoma cells (fig 2N). Gemistocytes in glioblastoma showed pronounced Kir4.1 immunoreactivity (fig 2N insert).

### $\alpha$ Syntrophin

In normal brain,  $\alpha$  syntrophin was only seen around microvessels (fig 1C), in the glia limitans, and in the pia (fig 1F).  $\alpha$  Syntrophin was upregulated in astrocytes in cerebral contusion (fig 2C), bacterial meningitis (fig 2F), and peritumour brain. Carcinoma (fig 2I), oligodendroglioma (fig 2L), and glioblastoma (fig 2O) cells also expressed Kir4.1. Gemistocytes within glioblastoma strongly expressed  $\alpha$  syntrophin (fig 2O, insert).

### DISCUSSION

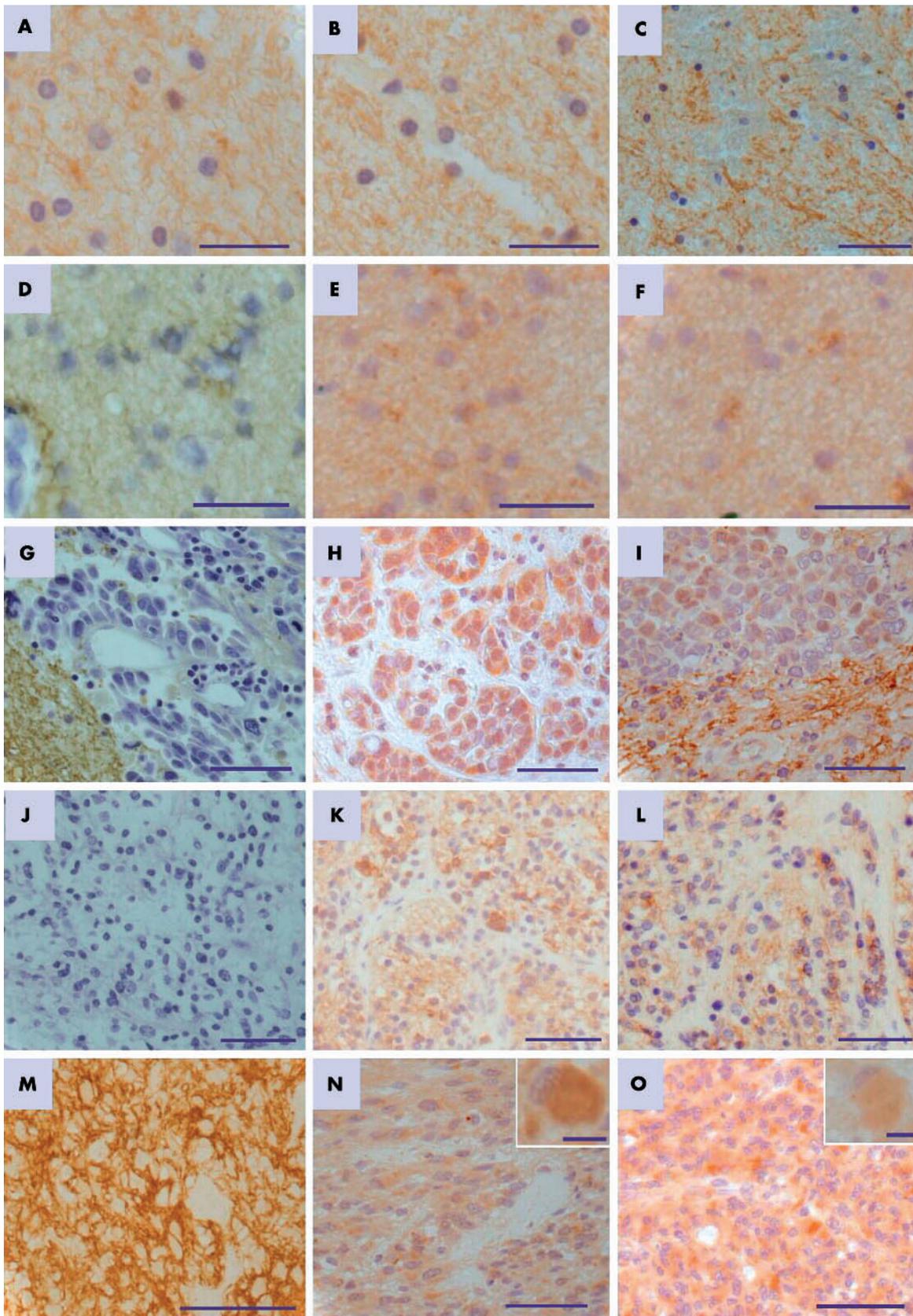
In normal human brain, the pattern of AQP4 expression in astrocytes correlates with that of Kir4.1, supporting the

hypothesis that AQP4 provides the water flux necessary for Kir4.1 mediated  $K^+$  siphoning. In pathological tissue, astrocyte AQP4 expression becomes dissociated from Kir4.1 expression (table 1), suggesting that AQP4 function is unrelated to  $K^+$  siphoning. The correlation between the upregulation of astrocyte AQP4 expression and the presence of brain oedema (table 1) suggests that AQP4 may participate in the formation or absorption of brain oedema, regardless of the pathology.

Increased AQP4 expression in astrocytes was found in cytotoxic (bacterial meningitis) and vasogenic (brain tumours) oedema. AQP4 null mice are protected from cytotoxic oedema,<sup>7</sup> but it is unknown whether they are also protected from vasogenic oedema. The effects of dexamethasone and mannitol on the expression of AQP4, Kir4.1, and  $\alpha$  syntrophin also merit investigation.<sup>9</sup>

“The correlation between the upregulation of astrocyte AQP4 expression and the presence of brain oedema suggests that AQP4 may participate in the formation or absorption of brain oedema, regardless of the pathology”

Normal human brain expresses Kir4.1 and  $\alpha$  syntrophin in a similar manner to rodent brain.<sup>1,3,4</sup> However, unlike rodents, humans also express Kir4.1 in some neurones. The increased Kir4.1 expression in bacterial meningitis and contusion probably boosts astrocyte  $K^+$  buffering capacity when  $[K^+]_o$  rises as a result of cell death. Kir4.1 in adenocarcinoma, oligodendroglioma, and glioblastoma cells may represent aberrant expression. Because the upregulation of AQP4 or Kir4.1 expression is accompanied by a similar increase in  $\alpha$  syntrophin in astrocytes,  $\alpha$  syntrophin may



**Figure 2** Pathological brain immunolabelled for (A, D, G, J, M) AQP4, (B, E, H, K, N) Kir4.1, and (C, F, I, L, O)  $\alpha$  syntrophin. (A–C) Contused brain cortex, (D–F) bacterial meningitis, (G–I) metastatic carcinoma, (J–L) oligodendroglioma, and (M–O) glioblastoma multiforme. Note peritumour brain in (G) (bottom left) and (I) (bottom). Inserts in (N) and (O) show gemistocytoma from the respective pictures. Bars, 10  $\mu$ m (N, O inserts); 50  $\mu$ m (A, B, D–F); and 100  $\mu$ m (C, G–O).

### Take home messages

- We propose that, in astrocytes, normally AQP4 couples water transport with Kir4.1 mediated K<sup>+</sup> siphoning, but in pathological states, AQP4 facilitates the flow of brain oedema fluid, and Kir4.1 buffers increased extracellular K<sup>+</sup>
- A larger study to validate our ideas is warranted
- Uncovering the molecular mechanisms responsible for changes in AQP4, Kir4.1, and  $\alpha$  syntrophin expression in brain pathologies might improve our understanding of the pathophysiology of brain oedema

provide the necessary scaffolding to guide AQP4 and Kir4.1 expression in disease states as in healthy tissue.<sup>1–5</sup>

This heterogeneous case series provides the basis for a larger study to validate our ideas. The molecular mechanisms responsible for changes in AQP4, Kir4.1, and  $\alpha$  syntrophin expression in brain pathologies remain unclear. Given the mounting evidence implicating AQP4 in the formation of brain oedema,<sup>2–9</sup> uncovering these mechanisms may improve our understanding of the pathophysiology of this important clinical problem.

### ACKNOWLEDGEMENTS

Supported by the Neuroscience Research Foundation and a Wellcome Trust Clinician Scientist Fellowship to MCP.

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Accepted for publication 6 June 2003

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