

# Human articular chondrocytes, synoviocytes and synovial microvessels express aquaporin water channels; upregulation of AQP1 in rheumatoid arthritis

E. Trujillo<sup>1,2</sup>, T. González<sup>1</sup>, R. Marín<sup>3</sup>, P. Martín-Vasallo<sup>2</sup>, D. Marples<sup>4</sup> and A. Mobasher<sup>5</sup>

<sup>1</sup>Rheumatology Service, University Hospital of the Canaries, La Cuesta, Tenerife, Spain, <sup>2</sup>Laboratory of Developmental Biology, Department of Biochemistry and Molecular Biology, University of La Laguna, La Laguna, Tenerife, Spain <sup>3</sup>Laboratory of Cellular Neurobiology, Department of Physiology, University of La Laguna School of Medicine, Santa Cruz, Tenerife, Spain, <sup>4</sup>School of Biomedical Sciences, University of Leeds, Leeds, United Kingdom and <sup>5</sup>Connective Tissue and Molecular Pathogenesis Research Groups, Faculty of Veterinary Science, University of Liverpool, Liverpool, United Kingdom

**Summary.** Recent studies have shown that aquaporin water channels are expressed in human Meckel's cartilage. The aim of the present investigation was to determine if human articular chondrocytes and synoviocytes express aquaporin 1 (AQP1) water channels and to establish if there are any alterations in AQP1 expression in osteoarticular disorders such as osteoarthritis (OA) and rheumatoid arthritis (RA). Immunohistochemistry was employed semi-quantitatively to compare the expression of AQP1 in human chondrocytes derived from normal, OA and RA joints. PCR, cloning and sequencing confirmed the presence of AQP1 transcripts in chondrocytes. Normal human tissue microarrays including samples of kidney, choroid plexus and pancreas were used as positive controls for AQP1 expression. In most tissues AQP1 was expressed along endothelial barriers. In the kidney AQP1 was present in the glomerular capillary endothelium, proximal tubule and descending thin limbs. AQP1 was also localized to pancreatic ducts and acini and the apical membrane domain of the choroid plexus. Immunohistochemistry showed that AQP1 is expressed in synovial micro-vessels, synoviocytes and predominantly in chondrocytes located in the deep zone of articular cartilage. Image analysis of normal, OA and RA cartilage suggested that AQP1 may be upregulated in RA. This is the first report of AQP1 mRNA and protein expression in articular chondrocytes and synoviocytes. These findings suggest a potential role for AQP1 and possibly other members of the AQP gene family in the movement of extracellular matrix and metabolic water across the membranes of chondrocytes

and synoviocytes for the purposes of chondrocyte volume regulation and synovial homeostasis.

**Key words:** Chondrocyte, Synoviocyte, Synovial microvessel, AQP1, Osteoarthritis, Rheumatoid arthritis, Inflammation, Volume regulation, Tissue microarray, Immunohistochemistry

---

## Introduction

Articular chondrocytes are responsible for synthesizing and maintaining the extracellular matrix of articular cartilage (Cohen et al., 1998). The cartilage matrix consists principally of water (up to 70% of total tissue weight), type II collagen, large aggregating proteoglycans and smaller non-collagenous matrix proteins. The cross-linked collagen network traps aggregating proteoglycans and attracts cations (mainly Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup>) into the matrix. This is followed by movement of water which results in swelling of the proteoglycans, increasing the tension within the collagen network. It is this swelling mechanism that provides the extracellular matrix with the ability to resist tension and shear forces, thereby giving cartilage its ability to resist compression under static and dynamic mechanical load.

The metabolic activity of articular chondrocytes is influenced by physicochemical factors such as the ionic and osmotic environment of cartilage, which in turn alter the shape and volume of the resident cells (Guilak, 2000). Chondrocytes respond in a well-coordinated and adaptive manner to changes in their environment; ionic and osmotic changes in cartilage have been shown to alter cell morphology and affect the viscoelastic mechanical properties of these cells (Guilak, 2002). The mechanisms by which ionic and osmotic changes modulate chondrocyte behaviour are poorly understood.

However, the available information suggests that adaptation to such stimuli involves reorganization of the actin cytoskeleton, changes in the physical properties of the chondrocyte plasma membrane and the activity of channels and transporters therein (Guilak et al., 2002; Mobasheri et al., 1998, 2002a). Recent studies suggest that chondrocytes are able to regulate their volume in response to hyper and hypo-osmotic stress whether isolated or *in situ* (Guilak et al., 1995). Mobilization of osmotically active solutes (osmolytes) and osmotically obliged water and their transport across the chondrocyte membrane accompanies volume regulatory activities.

It is also known that the fenestrated synovial endothelium lining the joint capsule is responsible for production of a nutrient- and proteoglycan-rich synovial fluid (Wallis et al., 1987; O'Hara et al., 1990). Therefore, in this respect the synovium may be regarded as a specialized bi-directional water transporting epithelium. Aquaporin (AQP) water channels may also be involved in the transport of water and small osmolytes across the synoviocyte. Despite this realization, the molecular identity of plasma membrane systems responsible for osmolyte and water transport in chondrocytes and synoviocytes has remained unknown. We have hypothesized that AQP water channels may be involved in mechanotransduction and the regulation of cell volume in chondrocytes (Mobasheri et al., 2002a). Thus, the goals of this study were to determine if AQP1, the archetypal member of water channel family is expressed in human articular chondrocytes and synoviocytes and to quantify the relative expression of AQP1 in pathologies of human cartilage including osteoarthritis (OA) and rheumatoid arthritis (RA).

## Materials and methods

### *Chemicals, tissue culture reagents and antibodies*

Unless otherwise stated, all chemicals were purchased from Sigma/Aldrich (Poole, UK). Chemicals used in molecular biology experiments were molecular biology grade and those used in cell culture procedures were tissue culture grade. Foetal bovine serum was obtained from Sigma/Aldrich. Polyclonal antibodies to rat AQP1 were developed in the laboratory of Dr. D. Marples (Leeds University). Polyclonal AQP1 antibodies were also purchased from Chemicon International (Temecula, CA, USA).

### *Normal, OA and RA cartilage tissues*

Samples of normal human articular cartilage were obtained post mortem from femoral heads of necropsies (normal samples, n=3 broad age range). Samples of diseased human articular cartilage were obtained with informed consent from patients with osteoarthritis OA and RA (n=3 in each case). All experiments were conducted in accordance with current institutional guidelines and with Local Medical Ethics Committee

approval. Cartilage tissue was histologically and morphologically analyzed using established histopathological criteria (Mankin scores; Mankin et al. 1971) by two independent osteoarticular pathologists.

### *Human tissue microarrays*

Tissue microarrays (TMAs) of formalin fixed paraffin embedded samples that included most of the tissues in the human body (including cartilage, synovium and several water transporting epithelia) were obtained from the Cooperative Human Tissue Network of The National Cancer Institute, the National Institutes of Health, Bethesda, MD, USA (<http://faculty.virginia.edu/chtn-tma/home.html>). The TMAs contained 66 anonymized samples of non-neoplastic adult tissues obtained from surgical resection specimens, fixed within one hour of surgical removal from the donors. All the tissues represented on the TMAs were normal. Further details about these arrays may be found on the CHTN website (<http://faculty.virginia.edu/chtn-tma/home.html>).

### *Immunohistochemistry*

The immunohistochemical protocol for AQP1 described below was initially optimized using "test" human TMAs that contained a limited number of water transporting tissues (including human kidney; test microarray code: CHTN2002X1). These arrays were used to titrate immunohistochemical assay parameters and antibody dilutions prior to use of the more comprehensive TMAs. The TMAs were heated at 60 °C for 15 minutes to improve tissue adhesion to the charged glass slides. Prior to immunostaining, TMA slides were deparaffinized in xylene for 20 minutes to remove embedding media and washed in absolute ethanol for 3 minutes. The TMAs were gradually rehydrated in a series of alcohol baths (96%, 85% and 50%) and placed in distilled water for 5 mins. Endogenous peroxidase was blocked for 1 hour in a solution containing 3% hydrogen peroxide and 0.01% sodium azide and the slides washed three times in phosphate buffered saline (PBS). Non-specific antibody binding was blocked by incubating the TMAs for one hour at room temperature (RT) with 20% normal goat serum (NGS) in PBS containing 1% bovine serum albumin and 0.01% sodium azide. Slides were incubated overnight at 4 °C with the polyclonal antibodies to AQP1 diluted 1:100 in PBS containing 1% NGS. After 24 hrs at 4 °C the slides were washed 3 times for 5 min in PBS before incubating with horseradish peroxidase labelled polymer conjugated to affinity purified goat anti-rabbit immunoglobulins for 30 minutes (RT). The sections were washed 3 times for 5 min in PBS before applying liquid DAB+ chromogen (DAKO; 3,3'-diaminobenzidine chromogen solution) for up to 30 seconds. The development of the brown coloured reaction was stopped by rinsing in distilled water. The stained slides were immersed for 5 min in a bath of aqueous hematoxylin (DakoCytomation, Code

## AQP1 in chondrocytes and synoviocytes

No. S3309) to counterstain cell nuclei. The slides were washed for 5 min in running water and dehydrated in a series of graded ethanol baths before rinsing in 3 xylene baths and mounting in DPX (BDH laboratories, UK). The stained TMA slides were scored by two independent investigators and digital images were captured using a Nikon Microphot-FX microscope fitted with a Nikon DXM1200 digital camera.

### Primary chondrocyte isolation

Chondrocytes were isolated using established procedures described previously in detail (Shakibaei et al., 1999). Briefly, human articular cartilage slices (from femoral heads obtained during joint-replacement surgery for femoral neck fractures) were collected in Ham's F-12 medium. Cartilage slices were rinsed with Ham's F-12 medium and digested with 1% pronase (from *Streptomyces griseus*, 7000 units/g) in Ham's F-12 medium containing 2.5% (v/v) foetal calf serum for 2 h at 37 °C and then with 0.2% (v/v) collagenase (from *Clostridium histolyticum*, 0.15 units/mg) in the same solution for 4 h at 37 °C. After rinsing in growth medium, a single-cell suspension was obtained by repeated pipetting and separation from undissolved tissue fragments using a nylon mesh with a pore width of 70 µm. Cells were sedimented by centrifugation at 6000g, rinsed twice in growth medium, and resuspended in growth medium at 1x10<sup>6</sup>/ml. Cell viability was 95% or higher in all experiments described.

### AQP1 immunofluorescence in isolated chondrocytes

Isolated human chondrocytes were fixed in ice-cold methanol for 10 minutes at -20 °C. After removal from the fixative the cells were washed three times with PBS. Non-specific binding was blocked by incubating the cells with PBS containing 10% NGS for 1 hour at RT. Cells were incubated with primary polyclonal antibody to AQP1 (diluted 1:200 in PBS containing 1% NGS) for 2 hours at RT. The cells were then washed three times with PBS, and incubated with FITC-conjugated anti-rabbit IgG (pre-adsorbed with human serum proteins, Sigma) in PBS containing 1% NGS (2 hours at RT). The cells were finally washed three times with PBS and mounted in mounting medium (Vectashield, Vector Laboratories Burlingame CA). Chondrocytes were examined with a Zeiss fluorescence microscope.

### AQP1 immunofluorescence confocal microscopy

Cartilage specimens were frozen in OCT compound (Reichert, Germany) and blocks were stored at -20 °C. Human cartilage sections were cut at 8 µm onto poly-L-lysine coated slides in a temperature-controlled cryostat. Before application of antibodies, sections were allowed to reach room temperature and non-specific binding was blocked by incubation with 10% NGS in PBS (1 hour RT). The sections were incubated with the anti-AQP1 antibody (diluted 1:200) for 24 hrs at 4 °C. After several washes in PBS, sections were incubated for 2 hrs with FITC-conjugated goat anti-rabbit IgG (Sigma, UK; diluted 1:50 in PBS). Finally sections were washed in PBS and mounted using Vectashield (Vector Laboratories) before being examined with a Leica confocal laser scanning microscope. Confocal images were saved as 256x256 TIFF files. Signal intensity was normalized using normal cartilage sections before comparing OA and RA specimens.

### Confocal image analysis

Image analysis was carried out to quantify the intensity of immunofluorescent staining for AQP1 in normal, OA and RA cartilage sections. For this purpose Scion Image software for Windows (version 4.0.2 based on NIH Image for Macintosh) was used.

### PCR, cloning and sequencing

Human articular cartilage and prostate cDNA libraries were obtained from Stratagene (La Jolla, CA, USA) and BD Biosciences Clontech (Palo Alto, CA, USA) respectively. Oligonucleotide primers used to amplify PCR products corresponding to AQP1 and β-actin are shown in Table 1. PCR reactions were carried out in a Perkin Elmer 480 thermal cycler using a PCR master mix (ABgene, UK). The PCR protocol consisted of 1 cycle at 95 °C for 1 min followed by 40 cycles at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min and finally 1 cycle at 72 °C. PCR products were analyzed by electrophoresis using 1% agarose gels. DNA fragments were cloned using a pGEM<sup>®</sup>-T Easy cloning kit (Promega, Madison, WI, USA) and sequenced commercially (MWG-Biotech AG, Ebersberg, Germany). The partial sequences obtained were subsequently deposited in GenBank using the web-based

**Table 1.** Oligonucleotide primer pairs used to detect the presence of AQP1 and β-actin transcripts by PCR.

GENE	OLIGONUCLEOTIDE PRIMERS (5'-3' direction)	PCR PRODUCT SIZE (base pairs)	GenBank ACCESSION No.
AQP1	(F) TTCAAGAAGAAGCTCTTCTGG (R) CAGACCCCTTCTATTTGGGC	810	NM_000385
β-actin	(F) TTCAACTCCATCATGAAGTGTGACGTG (R) CTAAGTCATAGTCCGCCTAGAAGCATT	310	NM_001101

program BankIt (<http://www.ncbi.nlm.nih.gov/BankIt/>).

## Results

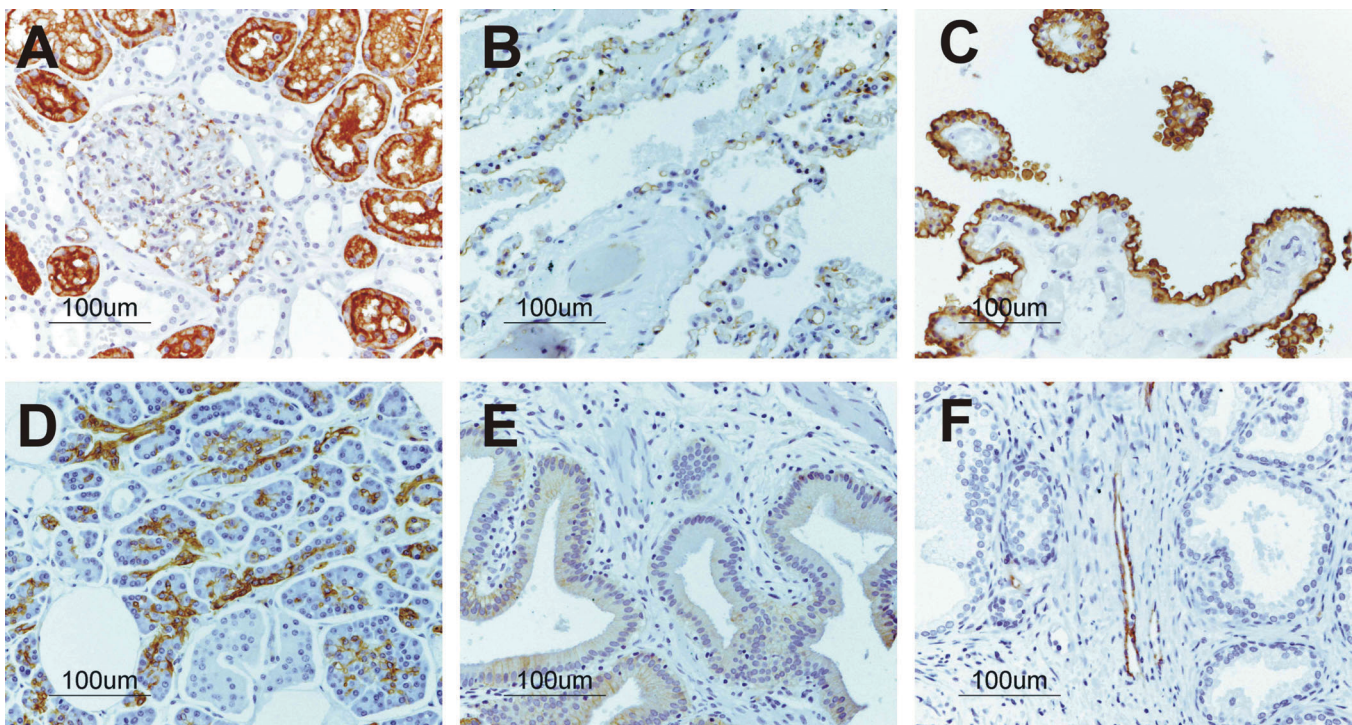
### *Immunohistochemical localization of AQP1 in water transporting tissues*

Normal human tissue microarrays (TMAs) from the Cooperative Human Tissue Network (CHTN) of The National Cancer Institute were used as the most suitable positive controls for AQP1 protein expression since they contain numerous types of water transporting epithelial tissues. The most abundant AQP1 expression was noted in the renal cortex, the lungs, choroid plexus and pancreas (Fig. 1). In the kidney AQP1 was present in the glomerular capillary endothelium and in the apical (brush border) and basolateral membrane domains of proximal tubule segments (Fig. 1A). AQP1 immunolabeling was also observed in the squamous epithelium lining the descending thin limbs of the loop of Henle which accounts for the high water permeability of this nephron segment in the medulla. AQP1 expression was not detected in the thick ascending limb and medullary collecting ducts (data not shown). These results are in agreement with previous studies in normal

human kidney (Agre et al., 1993; Devuyst et al., 1996; Maunsbach et al., 1997). AQP1 expression was also evident in the lung, choroid plexus and pancreas (Fig. 1, panels B, C and D). Heavy AQP1 immunolabeling was observed in the apical membrane of choroid plexus epithelial cells (Fig. 1, panel C) and basolateral membranes of the gallbladder epithelium (Fig. 1, panel E). These results are consistent with recent observations in the rat choroid plexus (Speake et al., 2003) and in the ductal system of the rat exocrine pancreas (Hurley et al., 2001). In sections of human prostate much weaker AQP1 immunostaining was observed in prostatic epithelial cells (Fig. 1, panel F) which is consistent with previous observations in the rat prostate (Brown et al., 1995). AQP1 expression was also seen along all endothelial barriers a finding that is consistent with many other reports in the literature.

### *Immunohistochemical localization of AQP1 in chondrocytes and synoviocytes*

Expression of AQP1 in human cartilage and synovium is shown in Fig. 2. These tissues were also represented on the CHTN normal human TMAs along with the water transporting epithelial tissues shown in

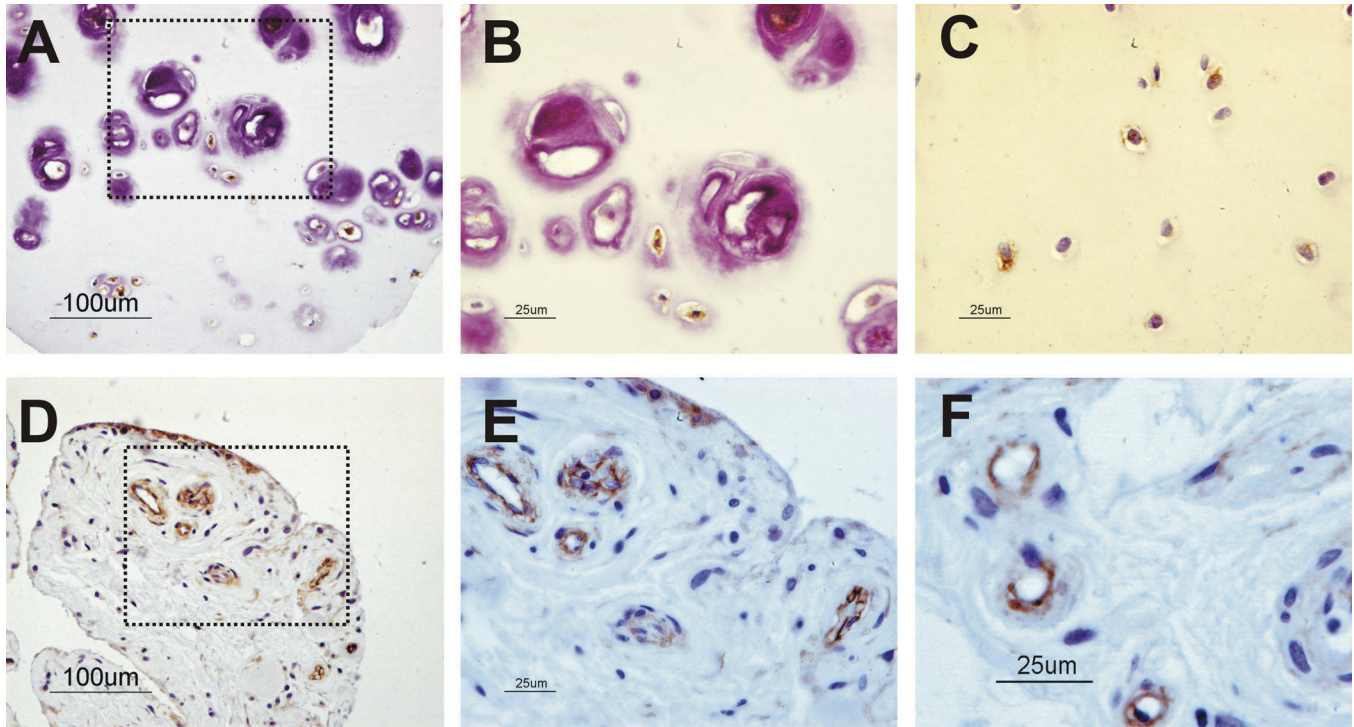


**Fig. 1.** Expression of AQP1 in water transporting tissues including kidney cortex (A), lung (B), choroid plexus (C), pancreas (D), gallbladder (E) and prostate (F). In the kidney (A) AQP1 was present in the glomerular capillary endothelium and in the apical (brush border) membrane domain of proximal tubule segments. In the lung (B) AQP1 was expressed in microvascular endothelia. In the choroid plexus (C) AQP1 was expressed in the apical membrane of epithelial cells. In the pancreas AQP was seen in the duct epithelia and acini (D). AQP1 expression was observed in the duct epithelia of the gallbladder (E). AQP1 expression was also evident in vascular endothelial cells lining small capillaries in the prostate. Bar: 100  $\mu$ m.

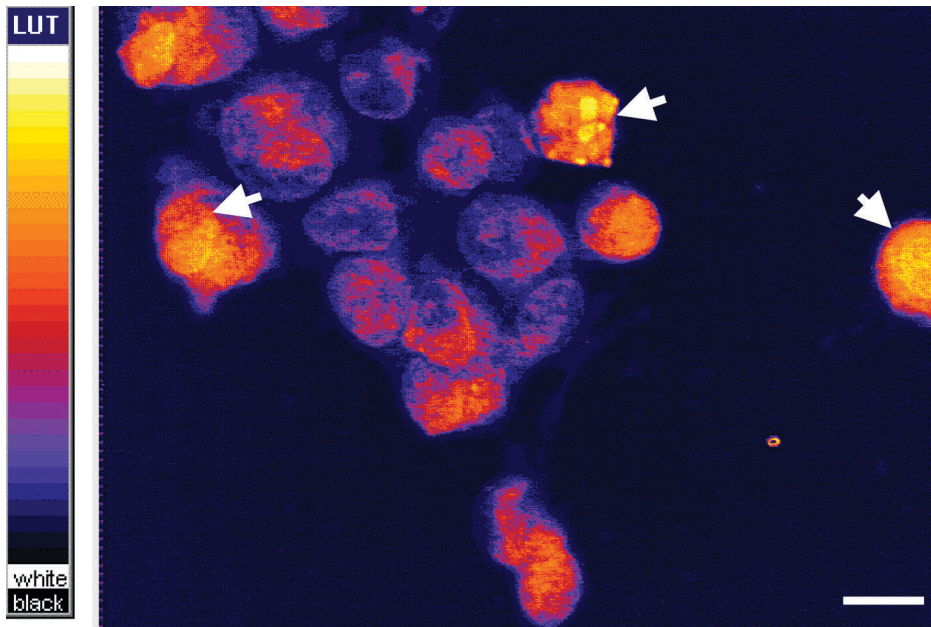
*AQP1 in chondrocytes and synoviocytes*

Fig. 1. Immunohistochemical staining demonstrated AQP1 localization around articular chondrocytes, synoviocytes and in synovial capillary vascular

endothelial cells. It is important to note that all the tissues on the microarrays were subjected to the same experimental conditions (i.e. the same antibody



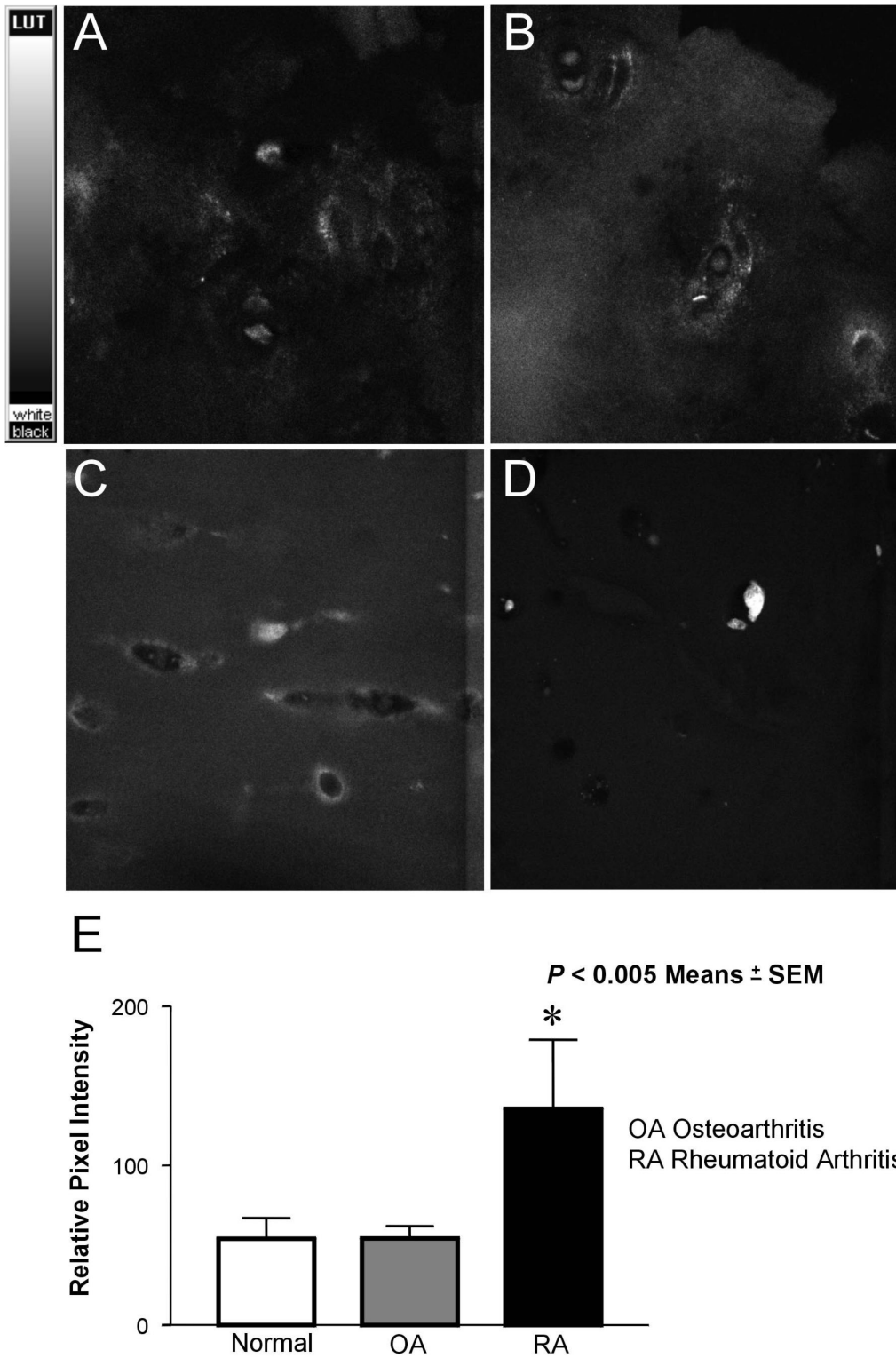
**Fig. 2.** Expression of AQP1 in human cartilage and synovium represented on the CHTN normal human tissue microarrays (TMAs). Immunohistochemical staining with anti-AQP1 polyclonal antibody showed localization of the DAB reaction around articular chondrocytes (**panel A** and enlarged boxed area in **panel B**). AQP1 was also present in synoviocytes and in small capillary vascular endothelial cells (**panel D** and enlarged boxed area in **panel E**). **Panel C** shows AQP1 expression in chondrocytes from the middle zone of articular cartilage. **Panel F** is a higher magnification view of AQP1 expression in endothelial barriers of capillaries that constitute the synovial microcirculation.



**Fig. 3.** Evidence for expression of AQP1 in freshly isolated human articular chondrocytes. Immunofluorescence micrograph of methanol fixed chondrocytes probed with polyclonal antibodies to AQP1. The look-up table shown on the left gives an indication of staining intensity. Bar: 10  $\mu$ m.

dilutions, incubation times, temperature and DAB substrate concentration). Therefore, the AQP1 immunohistochemical results obtained with the samples

of cartilage and synovium shown in Fig. 2 may be directly and quantitatively compared with the AQP1 immunostaining data derived from the water transporting



**Fig. 4.** Immunofluorescence confocal localization of AQP1 expression in normal (A), osteoarthritic (B and C) and rheumatoid (D) human articular chondrocytes. The cartilage sections were stained using a polyclonal antibody to AQP1 and secondary FITC-conjugated anti-rabbit IgG before analysis by immunofluorescence confocal microscopy. Data analysis was performed by Scion Image on five representative confocal slices taken through each specimen. The look-up table (LUT) on the left of panel A gives an indication of pixel intensity.

## AQP1 in chondrocytes and synoviocytes

epithelial tissues shown in Fig. 1. The strongest AQP1 expression was observed in chondrocytes that resided in the deep zone of articular cartilage (Fig. 2, Panel B). In the synovium weak AQP1 expression was detected in synoviocytes and moderate AQP1 expression was noted in the synovial micro-vessels (Fig. 2, panels D, E and F).

### Immunofluorescence localization of AQP1 in isolated chondrocytes

The results of immunofluorescence studies on freshly isolated human chondrocytes confirmed the presence of AQP1 (Fig. 3). The fluorescence signal was not uniform in all cells which suggests that the expression of AQP1 in chondrocytes varies depending on the cartilage zone from which they originate and confirms the immunohistochemical data shown in Fig. 2.

### Immunofluorescence confocal studies of AQP1 expression in normal, OA and RA cartilage

Immunofluorescence confocal studies on human cartilage sections from normal subjects and from patients with OA and RA also demonstrated that AQP1 is expressed in normal human articular cartilage (Fig. 4). Image analysis of immunofluorescence confocal micrographs showed no significant differences in the expression of AQP1 in normal and OA cartilage. However, in all three RA samples examined, the

intensity of AQP1 fluorescence was elevated in chondrocytes ( $P < 0.005$ , Student's t-test) suggesting that AQP1 is upregulated in chondrocytes in RA (see the data summarized in Table 2 for a comparison of fluorescence pixel intensities).

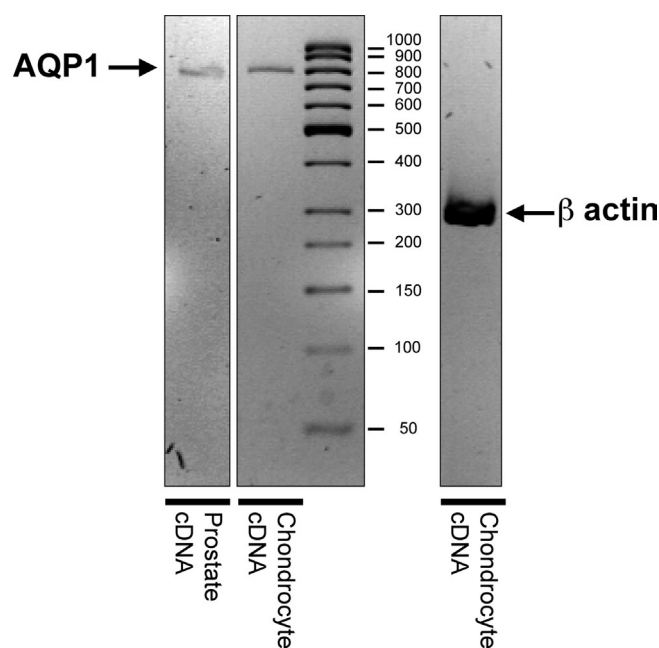
### PCR, cloning and sequencing of AQP1 from a human cartilage cDNA library

PCR confirmed that normal human chondrocytes express mRNA for AQP1. An 810 base pair product corresponding to AQP1 was detected in cDNA derived from normal human cartilage. An identical PCR product was detected in a human prostate cDNA library which was used as a positive control since the prostate gland consists of a fibromuscular stroma and a water and salt transporting epithelial tissue (Fig. 5). The PCR product obtained from the human cartilage cDNA library was commercially sequenced and the sequence obtained was 100% identical to nucleotides 51 to 860 of an existing *Homo sapiens* AQP1 sequence in GenBank (accession number NM\_000385). The partial AQP1 sequence obtained from the chondrocyte cDNA was deposited in GenBank under accession number AF480415.

## Discussion

The major results of this investigation are as follows: (1) AQP1 is expressed in human articular chondrocytes, synovial micro-vessels and synoviocytes; (2) AQP1 expression is not uniform across different cartilage zones, and the chondrocytes exhibiting the highest expression levels are in deep zone; (3) AQP1 expression appears to be maintained in isolated chondrocytes but the AQP1 protein level is not uniform in all isolated cells confirming the heterogeneous and zone-dependent expression; (4) AQP1 is expressed in normal, OA and RA cartilage but immunofluorescence confocal studies suggest that it may be upregulated in RA cartilage.

Articular chondrocytes are particularly sensitive to the ionic and osmotic composition of their physicochemical environment. In response to osmotic stress chondrocytes re-organize their actin cytoskeleton and activate various plasma membrane transport mechanisms to regulate cell volume. This regulation is essential for the maintenance of the extracellular matrix since the metabolic activity of chondrocytes is



**Fig. 5.** Evidence for expression of AQP1 in human articular cartilage. Transcripts for AQP1 and  $\beta$ -actin (used as an internal PCR control) were detected in a cDNA library of human articular cartilage. AQP1 expression was also detected in a human prostate cDNA library.

**Table 2.** Pixel intensities of the immunofluorescence signal for AQP1 in confocal images of normal, OA and RA cartilage.

	NORMAL CARTILAGE	OA CARTILAGE	RA CARTILAGE
Pixel Intensity	54.09	54.32	135.61
SEM	12.92	7.69	43
N	3	3	3

influenced by alterations in the osmotic environment of cartilage tissue (Guilak et al., 2002). Accordingly, chondrocytes possess the capacity to regulate their volume in response to swelling or shrinkage; they do this by using ion and osmolyte transporters which may be used to accumulate or dispose of ions and osmotically active substances during regulatory volume increase and regulatory volume decrease respectively (reviewed in Mobasheri et al., 1998). The transport of osmotically obliged water across the chondrocyte plasma membrane constitutes an important component of this regulatory mechanism (Trujillo et al., 2000).

The synovium is a thin, mesenchyme-derived tissue responsible for the generation, encapsulation, and drainage of synovial fluid in load-bearing joints. Synovial fluid passes between a discontinuous layer of highly specialized synoviocytes (specialized fibroblasts; Edwards, 1999) that line the surface and drains into a sub-synovial lymphatic system (Poli et al., 2001). Therefore the synovium is, in some respects, very similar to an epithelial barrier. Synoviocytes like chondrocytes are mesenchyme-derived cells and are responsible for the production of the synovial fluid which contains water, nutrients, growth factors, dissolved gases and proteoglycans (O'Hara et al., 1990). In synovitis, an inflammatory disorder of the synovium, a breakdown of the endothelial barrier leads to the development of a protein-rich effusion in the joint cavity (Wallis et al., 1987). It has been established that pro-inflammatory cytokines such as interleukin 1 beta (IL-1 $\beta$ ) and tumour necrosis factor alpha (TNF- $\alpha$ ) are involved in this process (Arend, 2001). It is also highly likely that water transport and AQP proteins may be involved in this process as well.

Aquaporins are expressed in a variety of water transporting epithelia and many other tissues where they facilitate water transport across the cell membrane (Borgnia et al., 1999). It is generally accepted that AQP1 (previously also known as AQP-CHIP or CHIP28) is the ubiquitously expressed, archetypal water channel (Agre et al., 1993). Although a great deal is known about the expression, regulation and physiological function of aquaporins in absorptive and secretory epithelia, thus far, there are only two indirect reports of aquaporin expression in skeletal cells. Early developmental studies in the rat have demonstrated abundant AQP1 mRNA in the mesenchyme surrounding developing calcified bone (Bondy et al., 1993). In addition very recent evidence from studies in developing orofacial tissues confirm that AQP1 is expressed in human Meckel's cartilage along with several other members of the AQP gene family (Wang et al., 2003). The results of this study confirm that AQP1 is also expressed in human articular chondrocytes and in synoviocytes. This is the first time AQP1 expression has been reported in chondrocytes and synoviocytes using TMA technology and immunohistochemistry.

Although comparisons of normal, OA and RA cartilage showed no difference between AQP1

expression in normal and OA, a significant upregulation was observed in RA cartilage. The apparent upregulation of AQP1 in RA cartilage is intriguing and may be related to an increased demand for glucose metabolism and water transport in inflammatory conditions where chondrocyte metabolism is drastically altered by catabolic cytokines such as IL-1 $\beta$  and TNF- $\alpha$ . Recent studies suggest that multiple isoforms of the facilitative glucose transporter gene family (GLUT/SLC2A) are expressed in human chondrocytes (Mobasheri et al., 2002b; Richardson et al., 2003). In addition, the transport of glucose is significantly increased by IL-1 $\beta$  and TNF- $\alpha$  through upregulation of "inducible" facilitative glucose transporters (Shikhman et al., 2001; Richardson et al., 2003). This study also highlights the fact that AQP1 is expressed in synovial micro-vessels in addition to endothelial capillaries. It is possible that AQP1 expression may change in inflammatory synovitis contributing to the development of a protein-rich effusion in the joint cavity (Wallis et al., 1987).

Aside from our observations in cartilage and synovium, the immunohistochemical data obtained from the TMAs confirms the data presented in several recent reports of AQP1 expression in the kidney (Devuyst et al., 1996; Mobasheri et al., 1997), lung (Song et al., 2001), pancreas (Hurley et al., 2001; Burghardt et al., 2003) and choroid plexus (Speake et al., 2003). However, this study is the only study that confirms the distribution of AQP1 in the abovementioned human tissues and compares them with cartilage and synovium using TMA technology. Furthermore, this is the second report of AQP1 expression in the human gallbladder confirming that AQP1 may be involved in bile formation and dietary fat processing in humans as well as mice (Ma et al., 2001; Mobasheri and Marples, 2004).

The presence of AQP1 in chondrocytes and synoviocytes supports a role for AQP1 mediated water transport across the synovial micro-vessels and the plasma membrane of chondrocytes and synoviocytes. The water crossing the chondrocyte membrane is likely to be metabolic or extracellular matrix water and hence AQP1 may be important in allowing chondrocytes to respond to changes in their ionic and osmotic environment by volume regulatory behaviour. AQP1 in the synovium is likely to be involved in regulating the bi-directional flow of water across the fenestrated synovial endothelium. Future studies will focus on the molecular identification and cellular localization of other aquaporins in cartilage and synovium, particularly those with the capacity to transport glycerol and other osmolytes (Borgnia et al., 1999).

**Note added in proof:** While this paper was being reviewed, we published a study in which we used tissue microarrays for a semi-quantitative histomorphometric analysis of AQP1 expression in over sixty of normal human tissue (Mobasheri and Marples, 2004). The results of that paper also confirm the presence of AQP1 in chondrocytes and synoviocytes.



## *AQP1 in chondrocytes and synoviocytes*

---

*Acknowledgements.* Supported by grants from the Teaching Company Directorate (Teaching Company Scheme, Department of Trade and Industry, UK), the University of Liverpool Research Development Fund and Programa Sectorial de Promocion General del Conocimiento, Ministerio de Educacion y Cultura, Spain. We are grateful to B. Masrani and R. Gadelrab for technical assistance. We would like to dedicate this paper to Dr. Carolyn Bondy (Developmental Endocrinology Branch, National Institute of Child Health, Bethesda, USA) in recognition of her original observation of AQP1 in developing bone and her outstanding contributions to the field.

---

### References

- Agre P., Preston G.M., Smith B.L., Jung J.S., Raina S., Moon C., Guggino W.B. and Nielsen S. (1993). Aquaporin-CHIP: the archetypal molecular water channel. *Am. J. Physiol.* 265, F463-F476.
- Arend W.P. (2001). Cytokine imbalance in the pathogenesis of rheumatoid arthritis: the role of interleukin-1 receptor antagonist. *Semin. Arthritis Rheum.* 30, 1-6.
- Bondy C., Chin E., Smith B.L., Preston G.M. and Agre P. (1993). Developmental gene expression and tissue distribution of aquaporin-CHIP. *Proc. Natl. Acad. Sci. USA* 90, 4500-4504.
- Borgnia M., Nielsen S., Engel A. and Agre P. (1999). Cellular and molecular biology of the aquaporin water channels. *Annu. Rev. Biochem.* 68, 425-458.
- Brown D., Verbavatz J-M., Valenti G., Lui B. and Sabolić I. (1995). Localization of the CHIP28 water channel in reabsorptive segments of the rat male reproductive tract. *Eur. J. Cell Biol.* 61, 264-273.
- Burghardt B., Elkaer M.L., Kwon T.H., Racz G.Z., Varga G., Steward M.C. and Nielsen S. (2003). Distribution of aquaporin water channels AQP1 and AQP5 in the ductal system of the human pancreas. *Gut* 52, 1008-1016.
- Cohen N.P., Foster R.J. and Mow V.C. (1998). Composition and dynamics of articular cartilage: structure, function, and maintaining healthy state. *J. Orthop. Sports Phys. Ther.* 28, 203-215.
- Devuyt O., Burrow C.R., Smith B.L., Agre P., Knepper M.A. and Wilson P.D. (1996). Expression of aquaporins-1 and -2 during nephrogenesis and in autosomal dominant polycystic kidney disease. *Am. J. Physiol.* 271, F169-F183.
- Edwards J.C.W. (1999). The biology of synovial cells. In: *Biology of the synovial joint*. Archer C.W., Caterson B., Benjamin M. and Ralphs J.R. (eds). Harwood Academic publishers. Amsterdam. pp 225-233.
- Guilak F. (1995). Compression-induced changes in the shape and volume of the chondrocyte nucleus. *J. Biomech.* 28, 1529-1542.
- Guilak F. (2000). The deformation behavior and viscoelastic properties of chondrocytes in articular cartilage. *Biorheology* 37, 27-44.
- Guilak F., Erickson G.R. and Ting-Beall H.P. (2002). The effects of osmotic stress on the viscoelastic and physical properties of articular chondrocytes. *Biophys. J.* 82, 720-727.
- Guilak F., Ratcliffe A. and Mow V.C. (1995). Chondrocyte deformation and local tissue strain in articular cartilage: a confocal microscopy study. *J. Orthop Res.* 13, 410-421.
- Hurley P.T., Ferguson C.J., Kwon T.H., Andersen M.L., Norman A.G., Steward M.C., Nielsen S. and Case R.M. (2001). Expression and immunolocalization of aquaporin water channels in rat exocrine pancreas. *Am. J. Physiol. Gastrointest. Liver Physiol.* 280, G701-G709.
- Ma T., Jayaraman S., Wang K.S., Song Y., Yang B., Li J., Bastidas J.A. and Verkman A.S. (2001) Defective dietary fat processing in transgenic mice lacking aquaporin-1 water channels. *Am. J. Physiol. Cell. Physiol.* 280, C126-C134.
- Mankin H.J., Dorfman H., Lippiello L. and Zarins A. (1971). Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic hips. II Correlation of morphology with biochemical and metabolic data. *J. Bone Joint Surg.* 53A, 523-537.
- Maunsbach A.B., Marples D., Chin E., Ning G., Bondy C., Agre P. and Nielsen S. (1997). Aquaporin-1 water channel expression in human kidney. *J. Am. Soc. Nephrol.* 8, 1-14
- Mobasheri A. and Marples D. (2004). Expression of the AQP-1 water channel in normal human tissues: a semiquantitative study using tissue microarray technology. *Am. J. Physiol. Cell. Physiol.* 286, C529-C537.
- Mobasheri A., Mobasheri R., Francis M.J.O., Trujillo E., Alvarez de la Rosa D. and Martín-Vasallo P. (1998). Ion transport in chondrocytes: membrane transporters involved in intracellular ion homeostasis and the regulation of cell volume, free  $[Ca^{2+}]$  and pH. *Histol. Histopathol.* 13, 893-910.
- Mobasheri A., Carter S.D., Martín-Vasallo P. and Shakibaei M. (2002a). Integrins and stretch activated ion channels: putative components of functional cell surface mechanoreceptors in articular chondrocytes. *Cell Biol. Int.* 26, 1-18.
- Mobasheri A., Neama G., Bell S., Richardson S. and Carter S.D. (2002b). Human articular chondrocytes express three facilitative glucose transporter isoforms: GLUT1, GLUT3 and GLUT9. *Cell Biol. Int.* 26, 297-300.
- O'Hara B.P., Urban J.P. and Maroudas A. (1990). Influence of cyclic loading on the nutrition of articular cartilage. *Ann. Rheum. Dis.* 49, 536-539.
- Poli A., Scott D., Bertin K., Miserocchi G., Mason R.M. and Levick J.R. (2001) Influence of actin cytoskeleton on intra-articular and interstitial fluid pressures in synovial joints. *Microvasc. Res.* 62, 293-305.
- Richardson S., Neama G., Phillips T., Bell S., Carter S.D., Moley K.H., Moley J.F., Vannucci S.J. and Mobasheri A. (2003). Molecular characterization and partial cDNA cloning of facilitative glucose transporters expressed in human articular chondrocytes; stimulation of 2-deoxyglucose uptake by IGF-I and elevated MMP-2 secretion by glucose deprivation. *Osteoarthritis Cartilage* 11, 92-101.
- Shakibaei M., John T., De Souza P., Rahmzadeh R. and Merker H.J. (1999). Signal transduction by  $\beta 1$ -integrin receptors in human chondrocytes in vitro: collaboration with the insulin-like growth factor-I receptor. *Biochem. J.* 342, 615-623.
- Shikhman A.R., Brinson D., Valbracht J. and Lotz M. (2001). Cytokine regulation of facilitated glucose transport in human articular chondrocytes. *J. Immunol.* 167, 7001-7008.
- Song Y., Jayaraman S., Yang B., Matthay M.A. and Verkman A.S. (2001). Role of aquaporin water channels in airway fluid transport, humidification, and surface liquid hydration. *J. Gen. Physiol.* 117, 573-582.
- Speake T., Freeman L.J. and Brown P.D. (2003). Expression of aquaporin 1 and aquaporin 4 water channels in rat choroid plexus. *Biochim. Biophys. Acta.* 1609, 80-86.
- Trujillo E., Marin R., Masrani B., Marples D., Martín-Vasallo P. and Mobasheri A. (2000). Aquaporin water channels in porcine and human articular cartilage; differential expression in pathologies of

*AQP1 in chondrocytes and synoviocytes*

human cartilage. *Int. J. Exp. Pathol.* 81, A37 (abstract).  
Wallis W.J., Simkin P.A. and Nelp W.B. (1987). Protein traffic in human synovial effusions. *Arthritis Rheum.* 30, 57-63.  
Wang W., Hart P.S., Piesco N.P., Lu X., Gorry M.C. and Hart T.C.

(2003). Aquaporin expression in developing human teeth and selected orofacial tissues. *Calcif. Tissue Int.* 72, 222-227.

Accepted November 28, 2003