# Increased and localized accumulation of chondroitin sulphate proteoglycans in the hyperplastic human prostate

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## OBJECTIVE

To analyse by immunohistochemistry the expression of chondroitin sulphate (CS) (detected in the hyperplastic prostate and possibly affecting the proliferation of prostate cells) in benign prostatic hyperplasia (BPH) to determine its distribution and location.

## MATERIALS AND METHODS

Samples of BPH were obtained from 11 patients (aged 58–83 years) and controls consisting of the transitional zone of five prostates from young men aged 19–27 years. Tissue sections were labelled with antibodies against CS, perlecan, type IV collagen, laminin, and smooth muscle cell (SMC) $\alpha$-actin. The amount of CS immunostaining was estimated by semi-quantitative scoring and correlated with prostate-specific antigen (PSA) level and prostate size.

## RESULTS

The anti-CS antibody faintly stained the stroma of normal prostates, but in BPH samples the staining was intense and concentrated around acini, including the periphery of adjacent SMCs. This staining pattern was totally absent in the normal samples. Type IV collagen, perlecan and laminin were homogeneously distributed in the whole stroma of both normal and BPH samples. There was no significant correlation between intensity of CS staining and either PSA or prostate size.

## CONCLUSIONS

The expression of CS proteoglycans is increased in BPH, where they co-locate with basement membranes of the acinar epithelium and of peri-acinar SMCs. This enhanced expression is specific for these proteoglycans, as other basement membrane components are unaffected, and this may result from the regulatory effects of local factors that are active in BPH.

## KEYWORDS

prostate, BPH, adenoma, connective tissue, smooth muscle, chondroitin sulphate proteoglycans

## INTRODUCTION

BPH is one of the commonest urological diseases of men aged ≥50 years [1], and is one of the most prevalent disorders of elderly man, but little is known about its causes, especially at the molecular level. BPH is characterized by a marked proliferation of epithelial and stromal components [2], and several studies now indicate that this growth is androgen-dependent and regulated by several factors, notably TGF-$\beta$, basic fibroblast growth factor (FGF), FGF7, and platelet-derived growth factor (PDGF) [3–6].

Proteoglycans are a heterogeneous and ubiquitous group of glycoconjugates comprising a protein core linked to variable amounts of glycosaminoglycan (GAG) side chains [7]. Although many proteoglycans have been detected in the prostate, not all of them are directly involved in BPH, especially as it relates to stromal modifications and remodelling. For example, heparin is found in mast cell granules only, and although these cells are present in the prostate, their number in the hyperplastic area is not significantly different from that in normal regions of the gland [8]. Keratan sulphate is found in the prostate as epithelial secretory granules and luminal corpora amylacea exclusively [9], and is thus more closely associated with prostate exocrine function.

However, recently the expression of several genes was shown to be specifically associated with BPH, including genes for extracellular molecules such as chondroitin sulphate (CS) proteoglycans and factors that promote their synthesis [5]. CS proteoglycans include well-known molecular species such as versican, biglycan, aggrecan, lumican, bamacan and decorin, to which a variable number of CS GAG chains is attached [7]. These molecules are widespread in all tissues, have various biological functions, and are involved in the regulation of cell proliferation in prostate cancer [10,11]. Also, biochemical studies show that tissue concentration of CS GAG chains is increased in BPH [12–14], which suggests that CS proteoglycans are associated with the proliferative responses that occur in this disorder. Little is known, however, about the location and distribution of these molecules in the hyperplastic prostate.

The hyperplastic tissue in BPH is derived mostly from the transitional zone of the gland [15], and recent data indicate that there are important histological [16] and functional [17,18] heterogeneities among the different regions of the prostate. If not considered, such heterogeneities may lead to misinterpretations when assessing changes in an altered prostatic tissue [16]. Therefore the transitional zone is the appropriate control for analysing modifications in hyperplastic tissue. However, the available studies on CS proteoglycans in BPH have used as controls either other prostate regions [12] or do not specify their anatomical location [13,14].



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The objective of the present study was to investigate the distribution of CS proteoglycans in the human hyperplastic prostate. We used immunohistochemical techniques to label the CS chains, and not the protein core, so that all CS-bearing proteoglycans could be detected, and the controls consisted of the transitional zone of normal prostates.

## MATERIALS AND METHODS

Controls samples consisted of the transitional zone [15] of five normal prostates from men (mean age 24.3 years, SD 2.1, range 19–27) who had died in accidents. The prostates were obtained during necropsy, carried out within 8 h after death. BPH samples were obtained from 11 patients (mean age 69.7 years, SD 8.9, range 58–83) who had undergone open prostatectomy and whose diagnosis was confirmed by histopathological analysis. Immediately after excision during necropsy or surgery the prostate samples were either fixed in 10% buffered formalin or placed in embedding compound (OCT, Tissue Tek, Torrance, CA, USA) and frozen at −80 °C.

### IMMUNOLABELLING

Formalin-fixed, paraffin-embedded samples were assessed by immunohistochemistry using a sensitive peroxidase-streptavidin method, as previously described [19]; 5-μm sections were labelled using monoclonal antibodies against CS (CS-56, Sigma Chem. Co., St Louis, MO, USA), type IV collagen (MS-448-S0, Lab Vision, Fremont, CA, USA), laminin (25011/1965, Institut Pasteur, Lyon, France), and smooth muscle cell (SMC) α-actin (Sigma A-2547). For immunofluorescence frozen samples were cryostat-sectioned at 5–7 μm and fixed in 100% methanol at −20 °C for 10 min. After washing in PBS sections were incubated with a primary monoclonal antibody to perlecan (Lab Vision RT-794-P0) at a dilution of 1 : 50 in PBS, for 1 h and in a humid chamber at room temperature, in accordance with the manufacturer's recommendation. Washing in PBS was followed by incubation with fluorescein-isothiocyanate-labelled secondary antibody, at a dilution of 1 : 100 in PBS, for 1 h. Slides were then covered with Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA) and visualized using fluorescence microscopy. Images were acquired on a Zeiss Axioplan 2 microscope equipped with a MD80 camera.

### SEMI-QUANTITATIVE SCORING OF IMMUNOSTAINING

The approximate amount of periacinar CS was estimated semi-quantitatively from the intensity of the immunostaining as determined by visual examination carried out by two observers. Six random slides were examined per prostate sample, assigning a mean score to each sample which varied from zero (no staining) to +4 (maximum staining). The final patient's score reported is the mean of the scores of the two observers. To avoid differences in staining intensity among sections caused by technical variations, all slides used in these semi-quantitative analyses were labelled as a single batch and using the same reagents.

### STATISTICS

The statistical association between the final immunohistochemical score and either the serum level of PSA or prostate size (as determined by TRUS) was investigated by linear regression analysis followed by a *t*-test for the correlation coefficient [20], and considered significant at $P < 0.05$.

## RESULTS

The anti-CS antibody had little or no reaction with sections from the transitional zone of the normal prostate (Fig. 1a). When there was a reaction the staining was notably faint, and diffusely and homogeneously distributed throughout the stroma. However, in BPH samples there was markedly intense labelling (Fig. 1b–d). In addition, although faint staining was apparent in the more interstitial stroma in a few cases, the prevailing pattern was a localized and largely peri-acinar staining. Assessing prostate sections at higher magnification showed that the labelling was at the periphery of the epithelial layer and around fusiform cells in the peri-acinar region (Fig. 1d).

The stromal extracellular matrix changes were further investigated by immunolabelling prostate sections with antibodies against perlecan, type IV collagen and laminin. In contrast to the results for the anti-CS antibody, the labelling of perlecan in normal transitional zone (Fig. 2a) was comparable with that of BPH (Fig. 2b), i.e. the intensities of the fluorescence in the peri-acinar region and in the more interstitial stroma were similar. However, immunostaining of prostate samples with antibodies against type IV collagen (Fig. 2c,d) and laminin (not shown) gave the same results as those of perlecan, i.e. both stained the whole stroma homogeneously and there were no differences between controls (Fig. 2c) and BPH (Fig. 2d).

The cellular composition of the stroma was analysed by immunostaining prostate sections with antibodies against SMC α-actin. Both in the transitional zone of the normal prostate (Fig. 3a) and in BPH samples (Fig. 3b), cells positive to this antibody were the predominant cell type. Moreover, in both cases the cell density was homogeneous throughout the stroma, with no signs of localized, denser aggregations.

To determine whether the conspicuous CS staining in BPH was associated with clinical variables normally used for evaluating BPH, correlation analysis was used to assess the amount of staining and either PSA serum level (Fig. 4a) or prostate size (Fig. 4b); neither PSA nor prostate size were significantly associated with the amount of peri-acinar CS staining.

## DISCUSSION

Immunostaining prostate samples with an anti-CS antibody showed that CS proteoglycans are a minor component in the stroma of the normal transitional zone. However, in BPH the staining was markedly greater, which is in line with biochemical results showing overall greater concentrations of CS in the hyperplastic tissue [12–14]. Moreover, the staining was distributed mostly around acini, which corresponds to the location of epithelial and SMC basement membranes. In the normal prostate this staining pattern was totally absent.

Although these findings suggest a higher concentration of CS proteoglycans at a specific location, the possibility cannot be excluded that this might be caused by an overall thickening of acinar and SMC basement membranes, rather than an enhanced expression of these molecules. Indeed, epithelial or vascular basement membranes thicken in several pathological and degenerative conditions, e.g. asthma [21], Alport disease [22], diabetic nephropathy [23], and ageing [24]. Thus, to test this hypothesis,

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*FIG. 1. Immunohistochemical staining of CS in prostate samples. In the transitional zone of the normal prostate (a), the staining is very faint and diffuse in the stroma (asterisk). However, hyperplastic prostate (b,c) shows a marked increase in staining, which is mainly peri-acinar (arrowheads), with only a faint staining in the stroma (asterisk). Higher magnification (d) shows that the staining is close to the acinar epithelium and around fusiform cells in the peri-acinar stroma (arrowheads). A, acinus. (a,b,c, × 100; d × 400).*

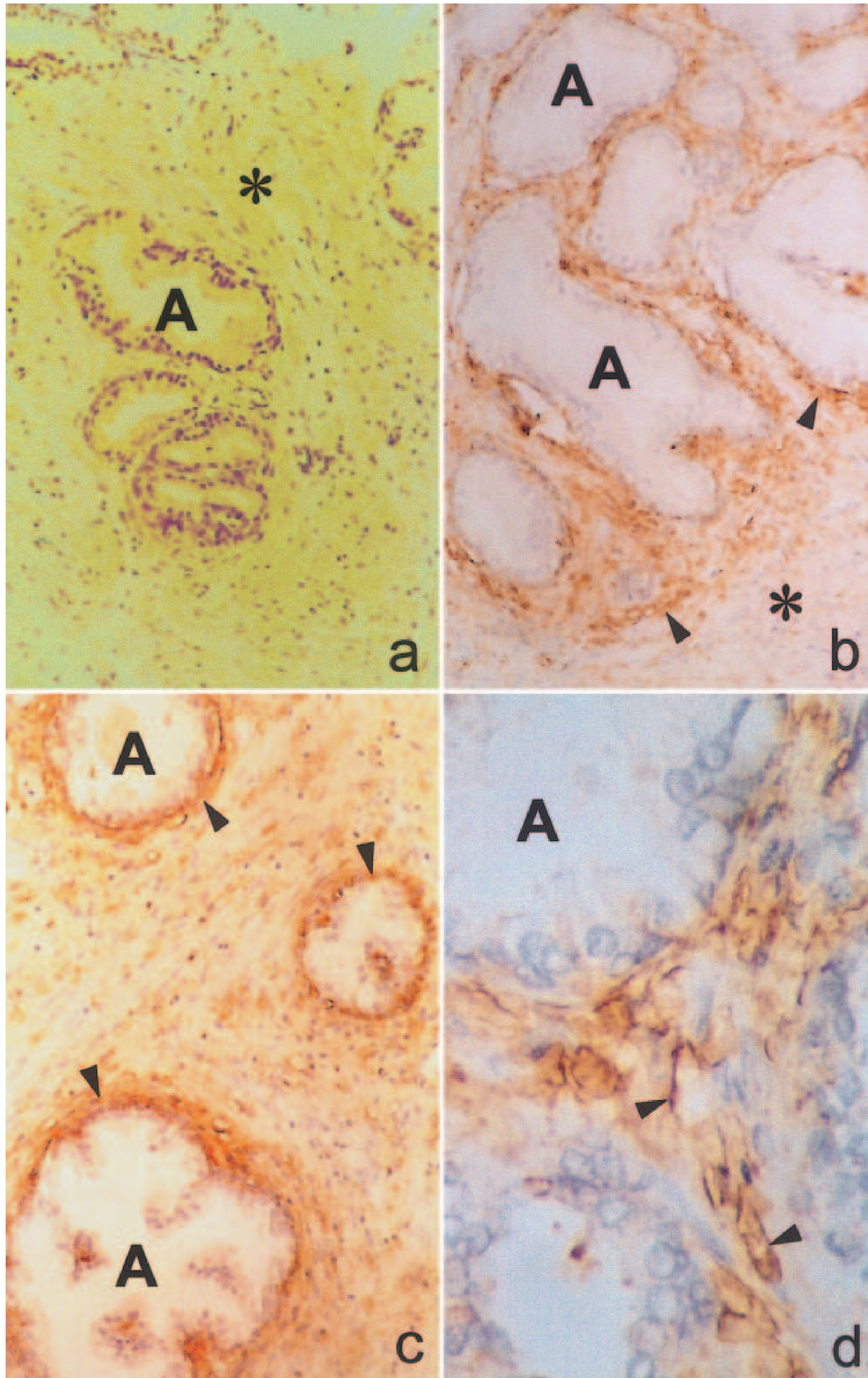




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*FIG. 2. Immunolabelling of perlecan (a,b) and type IV collagen (c,d) in prostate samples. In the transitional zone of the normal prostate, the labelling for perlecan (a) or type IV collagen (c) is homogeneously distributed throughout the stroma (asterisk), including the region around acini (A). The labelling pattern was essentially the same in the hyperplastic prostate, both for perlecan (b) and type IV collagen (d). V and arrowhead, blood vessels. (a,b,d, ×100; c ×200).*

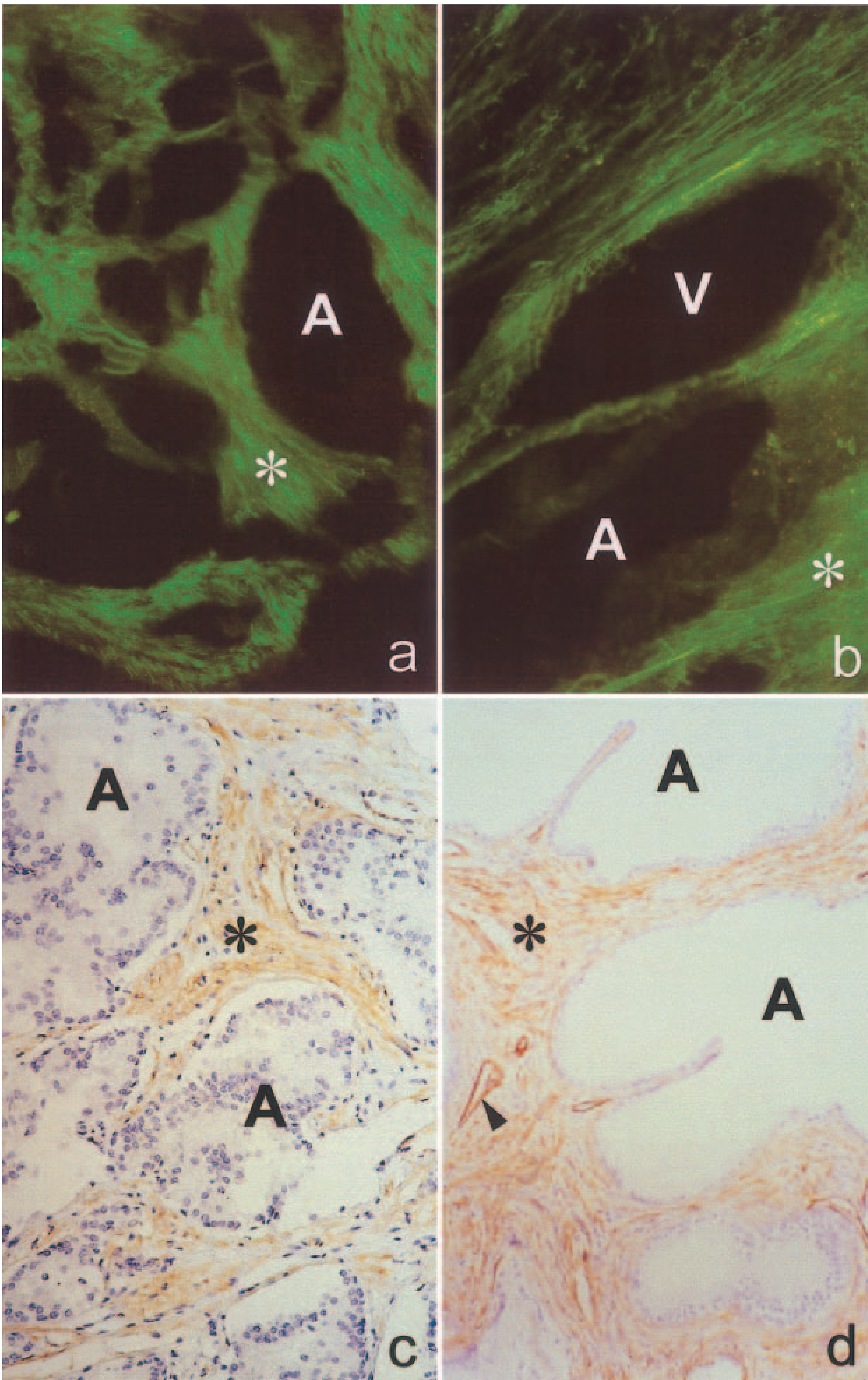
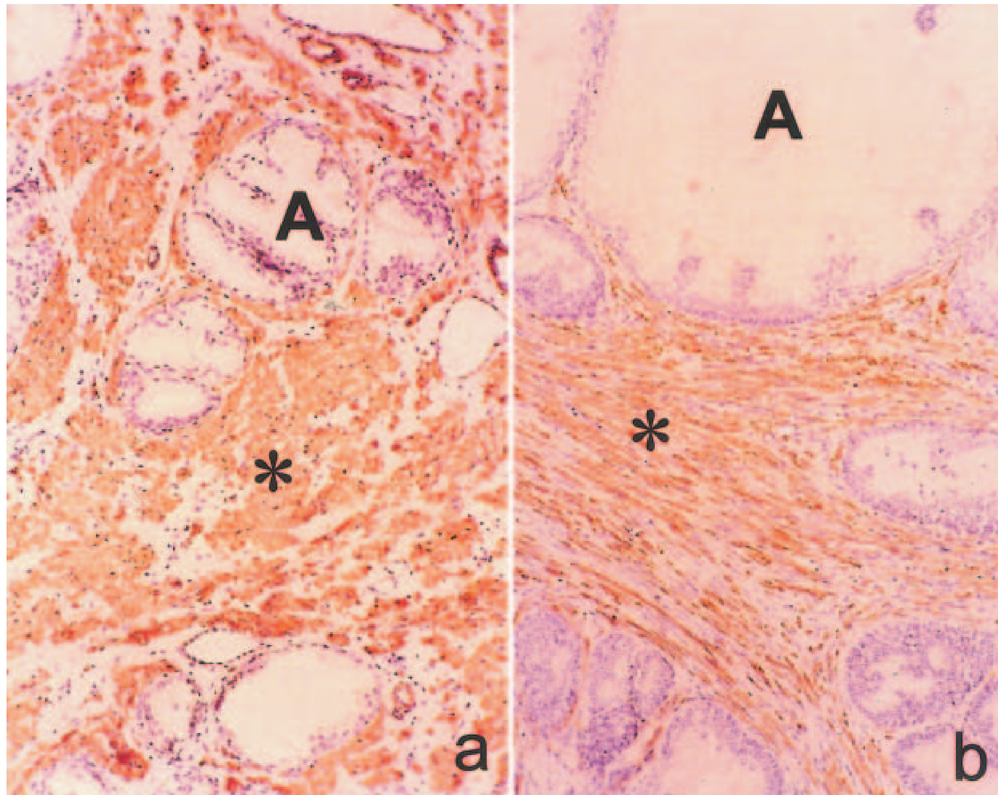


*FIG. 3. Immunohistochemical staining of SMC α-actin in prostate samples. Antibody-positive cells are evenly distributed in the whole stroma (asterisk), both in the transitional zone of the normal prostate (a) and in the hyperplastic prostate (b). A, acinus. ×100.*




sections were labelled for other basement membrane components; the results showed that type IV collagen, perlecan and laminin had identical staining patterns in both the normal and hyperplastic prostate. Alternatively, and as SMCs have a basement membrane [25], the more intense CS labelling might result from a higher density of these cells around acini. To investigate this the normal and BPH prostate sections were stained with an anti-SMC α-actin antibody; SMCs were homogeneously distributed in the stroma, with no signs of a denser aggregation at the periphery of acini. Altogether, these results indicate that the more intense staining of CS around acini corresponds to an increased expression of this molecule in the peri-acinar region of the hyperplastic prostate. Interestingly, this distribution of CS also occurs in the normal prostate of both young and old guinea pigs [26].

Because GAG chains such as CS are normally covalently linked to a protein core to form a proteoglycan molecule, and because GAG chain length can vary appreciably when cells are under certain stimuli [27], the greater amount of CS close to acini could be a result of an increased expression of proteoglycan core proteins and/or enhanced GAG chain elongation. The immunohistochemical results do not differentiate between these alternatives. However, factors that are active in BPH and involved in prostate cell proliferation, e.g. FGF, TGF-β, PDGF, and protein kinase C activation [3–6], also up-regulate the message for CS proteoglycans [28] and promote CS chain elongation [27]. Thus, the peri-acinar staining of CS in BPH should reflect an extracellular matrix that is enriched in CS proteoglycan monomers, which in turn have longer GAG side chains. Furthermore, the lack of correlation between the intensity of CS staining and the clinical variables implies that the factors determining ultimate prostate size in BPH do not significantly affect the expression of these CS proteoglycans.

Even though CS-bearing proteoglycans such as versican and biglycan are more typically distributed in the interstitial stroma, including that of the normal prostate [5,26,29], a CS proteoglycan (bamacan) was detected as a constituent of basement membranes [30,31]. This proteoglycan has been implicated in the regulation of cell growth and transformation [31,32], and its expression is increased in degenerative disorders [33,34]. As the CS immunostaining in the present BPH samples had a characteristic basement membrane location, both under epithelial cells and around SMCs, it is a reasonable presumption that the expression of bamacan is enhanced in the hyperplastic prostate, and as such it should be involved in its proliferation. It can also be inferred from these results that the greater expression of peri-acinar CS proteoglycans is specific, as other basement membrane components, including the heparan sulphate proteoglycan perlecan, were unchanged in BPH. This can be explained by the effect of regulatory factors present in hyperplastic tissue, e.g. TGF-β, basic FGF and PDGF [3–6], which are know to selectively affect the expression of certain proteoglycans [27,35].

That only SMCs close to the acinus and their surrounding stroma show enhanced CS staining suggests the involvement of a



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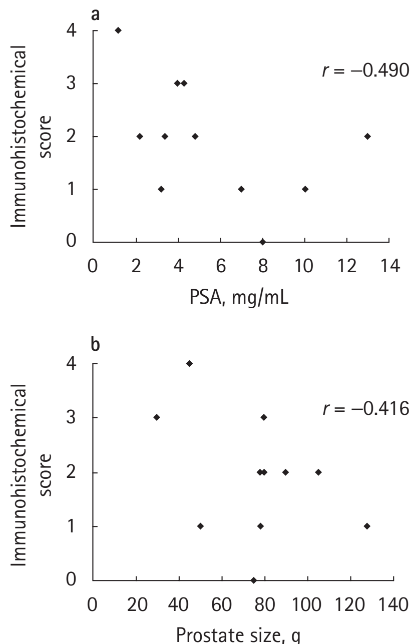


*FIG. 4. Correlation analysis for the amount of peri-acinar CS immunostaining in hyperplastic prostate samples. This was determined semi-quantitatively as a immunohistochemical score on histological sections and was correlated with PSA serum levels (a) and prostate size (b). A* t*-test for the correlation coefficient showed that in both cases there is no significant correlation.*

regulatory role by acinar epithelial cells. Indeed, epithelial cells from BPH tissue, both *in vitro* and *in vivo*, over-express several growth factors and cytokines that affect adjacent cells [36,37], so that autocrine and paracrine stimulatory pathways may underlie the enhanced expression of peri-acinar CS proteoglycans.

In summary, the expression of CS proteoglycans is increased in BPH, where they co-locate with basement membranes of the acinar epithelium and of peri-acinar SMCs. This enhanced expression is specific for these proteoglycans, as other basement membrane components are unaffected, and may result from the regulatory effects of local factors that are active in BPH.

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Abbreviations: **CS**, chondroitin sulphate; **FGF**, fibroblast growth factor; **PDGF**, platelet-derived growth factor; **GAG**, glycosaminoglycan; **SMC**, smooth muscle cell.


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