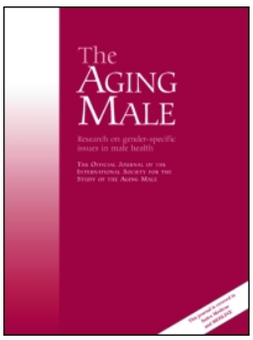

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Glimpses into the molecular pathogenesis of Peyronie's disease

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ABSTRACT

Peyronie's disease (PD) is a fibroproliferative disease of the penis. Since little is known about the molecular pathogenesis of PD, we compared the biochemical make-up of PD plaques with normal tunica albuginea to clarify pathological processes in the scarred tissue. Protein and mRNA levels were measured in plaques and in unaffected pieces of the tunica albuginea. We investigated the presence of myofibroblasts, the deposition of collagens, and some key elements of Wnt and YAP1 signaling at protein level. The expression of 45 genes, all related to collagen homeostasis and extracellular matrix proteins, was quantified. In plaques, more myofibroblasts were present, and we observed an activation of Wnt signaling and YAP1 signaling. Increased levels of the collagens types I and III confirm the fibrotic nature of plaques. The mRNA ratio of collagen types III, IV, and VI to type I was increased. The expression of lysyl hydroxylase 3 was higher, whereas a decreased expression level was seen for fibronectin and cathepsin K. The biochemical composition of plaques was different from unaffected tunica albuginea: the relative and absolute abundance of various extracellular matrix proteins were changed, as well as the quality of collagen and the level of the collagen-degrading enzyme cathepsin K.

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Penile induration; collagen; procollagen-lysine; 2-oxoglutarate 5-dioxygenase; Wnt signaling pathway; beta catenin; fibronectins; YAP1 (Yes-associated) protein; human; cathepsin K

Introduction

Peyronie's disease (PD) is a benign fibroproliferative disorder in which scar tissue, called "plaque", forms in the tunica albuginea of the penis. Bending and shortening eventually may lead to discomfort during penetration or even incapability of having sexual intercourse [1]. The prevalence varies from 0.4% to 8.9% and might be higher due to underreporting caused by embarrassment [2]. Treatment options depend on the phase of the disease. Nonsurgical treatment options vary from oral medication to intralesional injections with *Clostridium Histolyticum* collagenase [3–5], but up till now surgical management is the standard in patients with stable disease having serious difficulties with intercourse [6]. Little is known about the molecular pathogenesis of PD [7].

The epidemiology, pathogenesis, and genetic background of PD show similarities with Dupuytren's disease (DD), a fibromatosis of the hand in which nodules and cords are formed causing the fingers to bend toward the palm of the hand, leading to manual disabilities. A significant percentage of patients with PD have concomitant DD. Our research group found 22% overlap in prevalence [8], while other studies showed concomitance of 21 and 39% [9,10]. In PD and DD, older Caucasian males are more often affected and diabetes mellitus is suggested to be a risk factor [11,12]. Although we now know that DD is a polygenetic disease, for long PD and DD have been thought to have autosomal dominant inheritance patterns, but causative genes have never been identified across the whole DD and PD population [13–16].

Both PD and DD are characterized by fibrosis, a combination of contraction and matrix deposition caused by uncontrolled myofibroblast activity [17]. The development of myofibroblasts in general depends on a number of different environmental cues, including tension in the matrix and exposure to a variety of different mediators, such as transforming growth factor-β1 [18]. Myofibroblasts make up a significant percentage of cells in both PD plaques and DD palmar nodules [19,20]. In genome-wide association studies

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(GWAS) on DD, a total of 26 different loci were found to be associated with the disorder, many of which were linked to Wnt-related genes [13,14]. In PD, the Wnt pathway seems to be affected as well [21], and new data have confirmed the role of Wnt in DD [22,23].

Although the abundance of collagen is one of the main pathological events in PD and DD, only a limited amount of information is available regarding the biochemical make-up of the affected tissues in terms of types and post-translational modifications of collagen. This is remarkable, as the functioning of connective tissues is not only regulated by collagen abundance but also by the intricate relationships between collagen molecules, their interactions with other extracellular matrix components, and the subsequent network produced by the supramolecular assemblages.

In a recently published guideline on PD, one of the major conclusions was that a greater understanding of the pathophysiology is necessary to provide better diagnostic and therapeutic options for PD patients [24].

The aim of this study is to describe the expression of different genes and proteins, some of which are already known to be dysregulated in DD, to fill in a part of this puzzle.

Materials and methods

Primary tissues

Fibrotic parts, plaques, as well as unaffected pieces of the tunica albuginea from PD patients were obtained during surgery according to Nesbit. Nesbit plication is one type of procedure to suture (plicate) the side of the penis that doesn't have scar tissue [25]. All procedures were performed by the same urologist. Plaques were considered affected, and the unaffected tunica albuginea was considered control tissue. Tissue samples were collected after informed written consent and approval of the Medical Ethics Committee (METc) of the University Medical Center Groningen (2007/067), in accordance with the Declaration of Helsinki.

Immunohistochemistry

The Tissue-Tek embedded tissues were cut into 5 μm cryosections and placed onto microscope slides (Starfrost, Waldemar Knittel GmbH, Braunschweig, Germany). Sections were fixed for 10 min with acetone (stainings for α-SMA, Wnt2, Wnt4, Wnt5a, Wnt7b, WISP1, and YAP1), or 4% paraformaldehyde in phosphate buffered saline with permeabilization using 0.1% Triton X (stainings for collagen type I, collagen type III, and β-catenin). The sections were incubated with a primary antibody for 60 min at room temperature, with 1% bovine serum albumin (antibody information: see Table 1). Endogenous peroxidases were blocked using 0.1% H_2O_2 during 15 min. For all biotinylated secondary antibodies, avidin and biotin were blocked for 15 min using the Biotin Blocking System (Dako, Glosstrup, Denmark). Secondary antibodies (Dako and Southern Biotech, Birmingham, AL) diluted 1:100 with 2% human serum, were incubated during 30 min. The stainings were visualized using Immpact DAB (Vector Laboratories, Burlingame, CA) for 5 min. Hematoxylin (Merck, Darmstadt, Germany) was used as counterstaining. Slides were dehydrated and mounted in Vectamount (Vector Laboratories, Burlingame, CA).

Table 1. Primary antibodies used for immunohistochemical staining.

Protein	Manufacturer	Cat. no.	Dilution
α-SMA	Dako	M0851	1:50
β-catenin	BD Biosciences	610153	1:100
Collagen type I	Abcam	ab6308	1:5000
Collagen type III	Abcam	ab6310	1:5000
WISP1	Abcam	ab10737	1:50
Wnt2	R&D Systems	AF3464	1:50
Wnt4	Sigma-Aldrich	HPA011397	1:50
Wnt5a	Abcam	ab72583	1:100
Wnt7b	Sigma	SAB2104506	1:200
YAP1	GeneTex	GTX129151	1:100

Quantification of stainings

Stainings were analyzed using a Leica DM 2000 microscope (Leica, Wetzlar, Germany). For quantification, five representative photomicrographs (40× magnification) were taken per tissue section, and analyzed using Nuance 3.0 software (PerkinElmer, Waltham, MA), allowing detection of a specific signal without interfering background noise. For each specific staining, stained areas were quantified as μm^2/high-power field.

Quantitative RT-PCR

Snap frozen tissues were placed in Tissue-Tek (Sakura, Zoeterwoude, The Netherlands) and cut into 10 μm cryosections, up to 30 mg of tissue per sample. RNA extraction was performed using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. RNA was quantified using a NanoDrop-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Because of low RNA concentrations, mRNA was quantified with a custom-made micro-fluidic card-based low-density array containing 45 extracellular matrix proteins and collagen processing-related genes, and three

household genes. Complementary DNA was synthesized from RNA using the RevertAid First Strand cDNA Synthesis Kit (ThermoFisher, Waltham, MA). Up to 100 ng of cDNA was diluted in distilled water and mixed with TaqMan PCR Master Mix (ThermoFisher, Waltham, MA). This mix was added to the 384-well TaqMan Microfluidic Card (ThermoFisher, Waltham, MA). Plates were run on the ViiA 7 Real-Time PCR system (Applied Biosystems, Foster City, CA). The relative amount of product was calculated using the ΔΔCt method, normalizing for the averaged expression of the most stable household gene *ACTB*, coding for the β-actin protein, and related to the control tissue. Patients were removed from the analysis if there was no detectable expression in both plaque and tunica albuginea. Table 2 shows numbers of included patients per gene.

Statistics

Statistical analysis was performed using GraphPad Prism version 7 (GraphPad Software, La Jolla, CA). A Wilcoxon-signed rank test was used to determine whether there were differences in ranks between both groups. p values $<.05$ were considered to be statistically significant. Graphs show means per group, with error bars representing standard errors of the mean. Significances are shown as $*(p < .05)$, $**(p < .01)$, and $***(p < .001)$.

Results

Since plaques are of a fibrotic origin, and thus should contain more α-SMA-positive myofibroblasts and more collagen than control tissue, we first checked whether our samples originate from affected (plaque) and

Table 2. Relative gene expression to ACTB (β-actin), both in healthy tunica albuginea (control) and affected plaque.

Category	Gene symbol	*N*	Mean plaque	Mean control	Fold change plaque/control	*p* Value
Collagens	COL1A1	7	0.5984	2.5477	0.235	.0313
	COL1A2	7	0.6007	2.3743	0.253	.0156
	COL3A1	7	0.3012	0.5410	0.557	n.s.
	COL4A1	7	0.1098	0.1243	0.883	n.s.
	COL5A1	7	0.0193	0.0426	0.454	n.s.
	COL6A1	7	0.5056	0.9369	0.540	n.s.
Other extracellular matrix proteins	FN1	7	0.3425	0.8087	0.424	.0469
	ELN	7	0.0261	0.0296	0.881	n.s.
	DCN	7	0.0134	0.0555	0.242	n.s.
	BGN	7	0.0902	0.1327	0.680	n.s.
	FMOD	7	0.0504	0.1967	0.256	n.s.
Lysyl hydroxylases	PLOD1	7	0.0035	0.0039	0.908	n.s.
	PLOD2	5	0.0016	0.0040	0.409	n.s.
	PLOD3	7	0.0037	0.0014	2.653	.0156
Prolyl hydroxylases	P4HA1	7	0.0019	0.0057	0.331	n.s.
	P4HA2	0	N/A	N/A	N/A	N/A
	P4HA3	7	0.0023	0.0178	0.129	.0313
	P4HB	7	0.0500	0.1060	0.472	n.s.
	LEPRE1	6	0.0039	0.0066	0.587	n.s.
	LEPREL1	5	0.0082	0.0109	0.755	n.s.
	LEPREL2	7	0.0073	0.0168	0.434	n.s.
Lysyl oxidases	LOX	5	0.0035	0.0039	0.891	n.s.
	LOXL1	7	0.0186	0.0357	0.521	n.s.
	LOXL2	4	0.0216	0.0298	0.726	n.s.
	LOXL3	5	0.0045	0.0030	1.517	n.s.
	LOXL4	5	0.0093	0.0139	0.673	n.s.
Procollagen propeptidases and enhancers	ADAMTS2	6	0.0252	0.0550	0.459	n.s.
	ADAMTS3	0	N/A	N/A	N/A	N/A
	ADAMTS14	1	N/A	N/A	N/A	N/A
	BMP1	7	0.0108	0.0203	0.535	n.s.
	PCOLCE1	7	0.0505	0.1152	0.438	n.s.
	PCOLCE2	7	0.0480	0.2391	0.201	n.s.
Collagen folding, glycosylation	SERPINH1	7	0.0192	0.0492	0.390	n.s.
	COLGALT1	3	0.0002	0.0001	4.829	n.s.
Collagen receptors	DDR1	7	0.0119	0.0193	0.618	n.s.
	DDR2	6	0.0122	0.0280	0.436	n.s.
	MRC2	6	0.0209	0.0698	0.299	n.s.
Collagen degradation	MMP1	0	N/A	N/A	N/A	N/A
	MMP13	0	N/A	N/A	N/A	N/A
	MMP14	7	0.1386	0.2620	0.529	n.s.
	TIMP1	7	0.4191	1.3028	0.322	n.s.
	CTSK	7	0.0182	0.1462	0.125	.0313
Others	FKBP10	7	0.0075	0.0123	0.612	n.s.
	SLC39A13	7	0.0085	0.0175	0.485	n.s.

N: number of patients included in the analysis per gene. *p* values as determined by the Wilcoxon paired rank test. N/A: not applicable; n.s.: not significant.

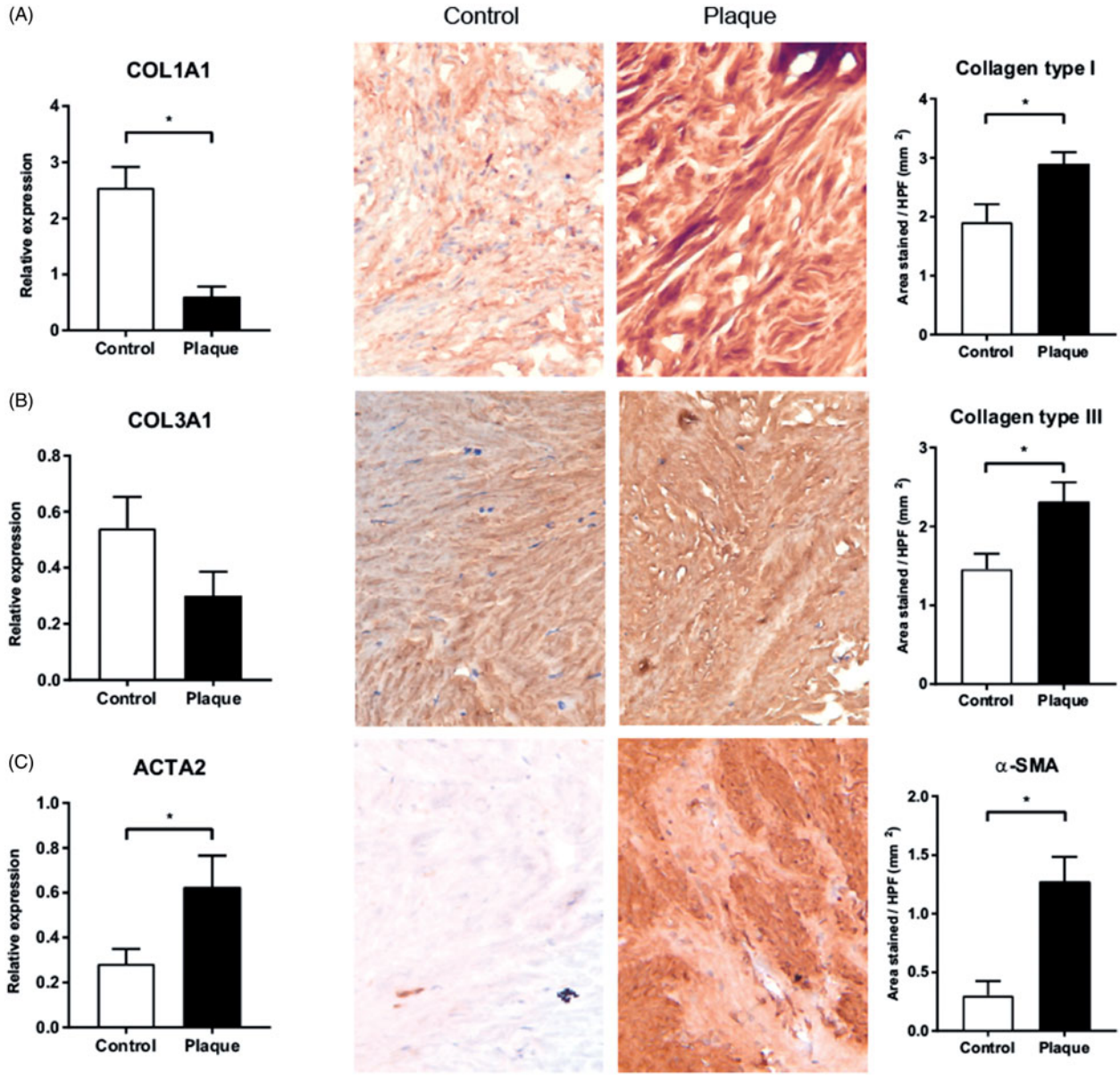

Figure 1. Relative gene expression to *ACTB* (β-actin), and representative pictures with quantification of immunohistochemical staining, both in tunica albuginea (control) and plaque. (A) *COL1A1* gene and corresponding protein collagen type I. (B) *COL3A1* gene and corresponding protein collagen type III. (C) *ACTA2* gene and corresponding protein α-SMA. $^{*}p < .05$ as determined by the Wilcoxon paired rank test. HPF: high-power field.

unaffected (control) tissue, as a quality control. Indeed, a significant increase in expression of α-SMA was seen (both at protein level and mRNA level) in the affected tissues, as was the case with protein levels of collagen type I and collagen type III (Figure 1).

In our stainings, we found that Wnt4 was upregulated in plaques (Figure 2). Staining for β-catenin, a downstream target of the Wnt pathway, also revealed significantly higher levels (Figure 2). Staining of YAP1 showed a much higher intensity in affected tissues than in unaffected tissues (Figure 2).

The only difference we found in collagen-related enzymes was the higher expression of *PLOD3 in affected tissue*, the enzyme that converts triple helical Lys into Hyl and that can subsequently add an additional sugar to a modified Hyl [26], and a lower expression of the prolyl hydroxylase *P4HA3*.

The low density array (Table 2 and Figure 3) provided some other information on genes that possibly play a role in fibrosis. No changes in expression were seen for the extracellular matrix proteins collagen type IV (*COL4A1*), collagen type V (*COL5A1*), collagen type VI (*COL6A1*), elastin (*ELN*), decorin (*DCN*), biglycan (*BGN*), and fibromodulin (*FMOD*), the collagen receptors *DDR1*, *DDR2,* and *MRC2*, the membrane-bound collagenase *MMP14*, or the collagenase inhibitor

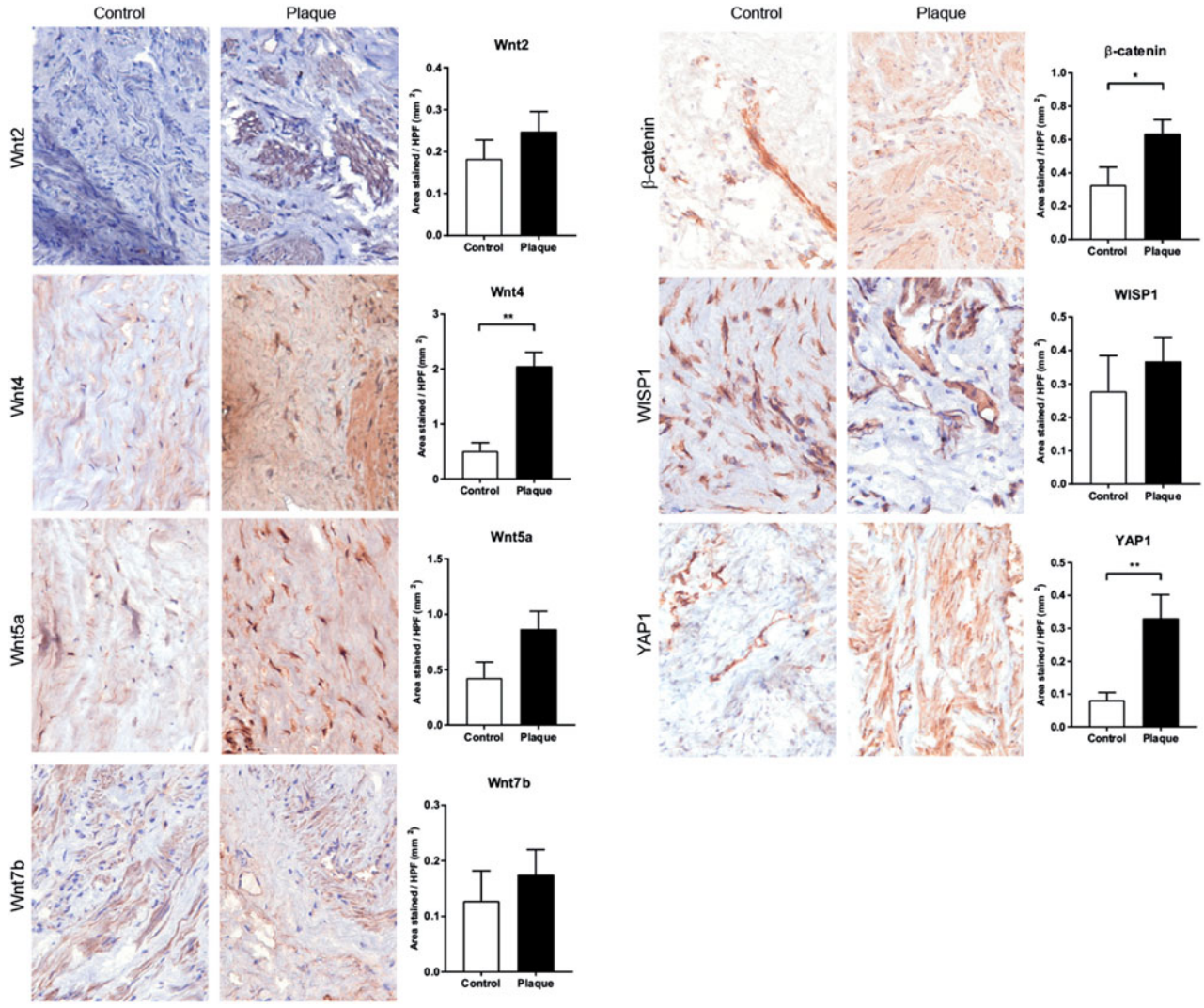

Figure 2. Representative pictures and quantification of immunohistochemical staining, both in tunica albuginea (control) and plaque. The following proteins were tested: Wnt2; Wnt4; Wnt5a; Wnt7b; β-catenin; WISP1; YAP1. $^{*}p < .05$, $^{**}p < .01$ as determined by the Wilcoxon paired rank test. HPF: high-power field.

TIMP1. However, a downregulation was seen in plaques with regard to fibronectin (*FN1*) and cathepsin K (*CTSK*). Also, a downregulation was observed for the two genes encoding for collagen type I, namely *COL1A1,* and *COL1A2*.

Discussion

We found an increased expression of Wnt4, β-catenin and YAP1 in plaques, and observed changes related to collagen quantity and quality. Part of our findings coincide with recent observations in DD [27–29], underscoring a certain relationship between both disease states. We obtained plaque and unaffected tissue from the tunica albuginea of the same patient, and by doing so, we excluded inter-individual variations.

First, to check whether the sampling was correct, we determined the expression and staining of α-SMA (encoded by the *ACTA2* gene), which is a marker of myofibroblasts, the cells that play a key role in fibrosis. In line with previous data [30,31], we found increased expression and staining, confirming the adequacy of the sampling method. Recently, the transcription factor YAP1 has been found to be involved in fibrosis [32–34], and there is a molecular cross-talk between the Wnt pathway and YAP1 [35]. We also stained for YAP1 and found it highly upregulated as well. As expected, we also measured an increased staining of β-catenin in plaques. Although it has been reported that fibroblasts derived from plaque and normal tunica albuginea markedly differ in β-catenin expression [31,36], this is the first report that shows β-catenin staining *in vivo* in affected tissue. Since the expression of β-catenin is mainly a result of the Wnt signaling pathway, we also stained for Wnt2, Wnt4, Wnt5a, Wnt7b and WISP1, and found that Wnt4 was

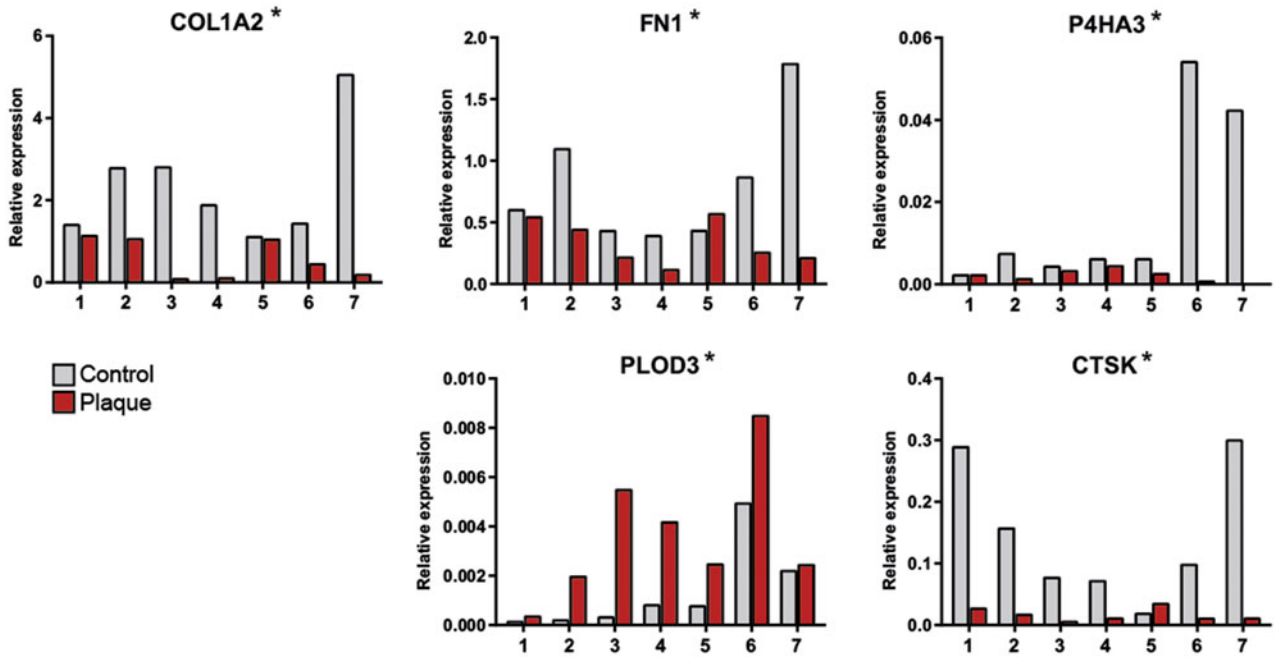

Figure 3. Relative gene expression to *ACTB* (β-actin), both in tunica albuginea (control) and affected plaque from seven patients with PD. The following genes are shown: *COL1A2*; *FN1*; *PLOD3*; *P4HA3*; *CTSK*) $^{*}p < .05$ as determined by the Wilcoxon paired rank test.

highly upregulated. The accumulation of β-catenin in plaques is likely to be connected to an (over)activated Wnt signaling pathway, a pathway that is known to be involved both in DD and PD [13,21–23]. Unlike in DD nodules [27,28], we did not find an upregulation of Wnt7b or WISP1 in PD plaques as compared to patient-matched control tissue. A downregulation of the previously described Wnt2 [27] could also not be confirmed in plaques. However, we observed a higher expression of Wnt4 and β-catenin, of which the latter corresponds to previous immunohistochemical findings in DD [27]. Furthermore, Wnt4 is one of the genes that was connected to DD in the GWAS by Dolmans et al. [13].

The hallmark of fibrosis is an accumulation of collagen. The most abundant collagen type, being collagen type I, is indeed increased in plaques as revealed by immunohistochemistry. Remarkably, mRNA levels of collagen type I coding genes *COL1A1* and *COL1A2* were invariably decreased. Since surgery in PD is only performed in a stable stage of the disease, in our hospital not earlier than 12 months after the first symptoms of PD, all examined subjects had plaques for quite a long time. Possibly, this might have resulted in a negative feedback against excess collagen type I expression.

It is widely agreed that there is an increase in the amount of collagen type III in affected tissues of patients with DD, giving a higher collagen type III to type I protein ratio [37–42]. Up till now, there was no information available about the collagen type III to type I ratio in PD. Using immunohistochemistry we showed that collagen type I and collagen type III are both increased in plaque compared to control tissue. Unfortunately, immunohistochemistry data cannot be used to quantify the respective collagen ratios. However, mRNA levels of the same and additional tissues showed that the ratio of *COL3A1* (the gene coding for collagen type III) to *COL1A1* or *COL1A2* is increased in plaques compared to control tissues, making it likely that a higher collagen type III: type I protein ratio will also be found in PD. Interestingly, mRNA of the three other collagen types that we measured, namely *COL4A1*, *COL5A1,* and *COL6A1*, also showed an increased ratio towards *COL1A1*. An increased level of collagen type IV and VI expression normally is seen in fibrotic disorders, and was thus expected in PD. The ratio of *COL1A1* to *COL1A2* was not significantly different between affected and unaffected tissues, which is interesting as in fibrotic tissues an increased amount of homotrimeric $[\alpha1(I)]_3$ collagen often is seen. Such homotrimers are difficult to degrade [43], thereby contributing to an accumulation of collagen. In PD, these homotrimers are not expected to be increased, based on the *COL1A1* to *COL1A2* ratio.

Collagen types I, III, and V are fibrillar collagens and can form heterotypic fibrils, i.e. molecules of all three collagen types are found to be associated in a single

fibril [44]. An inverse relationship has been found between fibril diameter and the amount of collagen type III or V towards collagen type I [45]. Thus, an increased amount of collagen type III or V at the expense of collagen type I results in thinner collagen fibrils. Keeping in mind that we observed an increased *COL3A1* and *COL5A1* mRNA ratio towards *COL1A1* in affected plaques, compared to healthy tunica albuginea, one would expect thinner collagen fibrils in plaques. We have not investigated this, but thinner collagen fibrils have indeed been observed in PD [46]. The same applied for an increased amount of collagen type V protein, which is in agreement with our mRNA data.

Collagen synthesis is a complex process, and many enzymes are involved. Chaperones assist in folding of the procollagen molecules and propeptidases cleave off the propeptides to convert procollagen into collagen. Conversion of proline into 3-hydroxyproline or 4-hydroxyproline is catalyzed by prolyl hydroxylases, conversion of lysine (Lys) into 4-hydroxylysine (Hyl) by lysyl hydroxylases, and cross-linking is initiated by lysyl oxidases [47]. We used a low-density array (Table 2 and Figure 3) to quantify the expression of these enzymes, to clarify whether there were possible aberrations in (pro)collagen processing. An important post-translational modification of collagen is the conversion of triple helical lysine (Lys) into hydroxylysine (Hyl), and the addition of sugars to Hyl, resulting in the glycosylated residues galactosylhydroxylysine (Gal–Hyl) and glucosylgalactosylhydroxylysine (Glc–Gal–Hyl) [26]. It has now been established that the conversion of triple helical Lys into Hyl is catalyzed by lysyl hydroxylase 1 (encoded by *PLOD1*) and lysyl hydroxylase 3 (encoded by *PLOD*3), and that the formation of Glc–Gal–Hyl (but not Gal-Hyl) is catalyzed by lysyl hydroxylase 3 [26]. We have observed no differences in mRNA levels of *PLOD1* between plaque and control tissue, but there was a major increase in mRNA levels of *PLOD3* in plaque tissue. Therefore, an overhydroxylation of Lys in PD tissue is expected, as well as increased levels of Glc–Gal–Hyl. A Lys overhydroxylation and a Hyl overglycosylation have been reported for affected DD tissues [37,40,41].

There is a large number of other post-translational modifications of collagen catalyzed by enzymes, such as 4-prolyl hydroxylation (catalyzed by *P4HA1*, *P4HA3*, *P4HB*, and *P4HA2* was not measurable), 3-prolyl hydroxylation (catalyzed by *LEPRE1*, *LEPREL1*, and *LEPREL2*), and the amount and type of cross-linking (catalyzed by *FKBP10*, *PLOD2*, *LOX*, *LOXL1*, *LOXL2*, *LOXL3*, and *LOXL4*). None of mentioned enzymes shows a consistent up- or downregulation in plaques compared to the samples out of normal tunica. The same applied for the collagen receptors *DDR1*, *DDR2* and *MRC2* (= Endo180), for the enzymes involved in the processing of the propeptides of procollagen (*ADAMTS2*, *BMP1*, *PCOLCE1*, *PCOLCE2*; *ADAMTS3*, and *ADAMTS14* were not measurable), and for the extracellular matrix proteins, elastin, decorin, biglycan, and fibromodulin. A significantly decreased expression of fibronectin was seen in plaques. As the main constituents in fibrotic tissues are collagen and fibronectin, the decreased expression of fibronectin will contribute to a different composition of the extracellular matrix in plaque. Differences in fibronectin mRNA expression have also been observed between the cord and the nodule in DD [29], whereas no differences were seen in mRNA levels among elastin, decorin, biglycan, and fibromodulin, which is similar to what we observed in PD.

An interesting observation is that the expression of the enzyme cathepsin K is significantly downregulated in plaque. Cathepsin K can effectively degrade collagen that is cross-linked by means of hydroxyallysine cross-links. This particular type of cross-link gives rise to collagen that is difficult to degrade by regular collagenases, for example MMP1 and MMP8, the genes coding for Matrix Metallopeptidase 1 and 8 [48]. Hydroxyallysine cross-links such as pyridinolines are increased in fibrotic disorders in a wide variety of tissues (e.g., skin, liver, kidney, DD, and myocard) [49,50] and it is likely that this is also the case in PD. The low expression of cathepsin K may contribute to the collagen accumulation in the plaque. In DD, differences in cathepsin K expression are seen between cords and nodules as well.

Conclusion

Using immunohistochemical studies, we have shown that in PD plaques contains increased amounts of collagen types I and III, higher numbers of myofibroblasts, and increased levels of β-catenin, Wnt4, and YAP1 (but normal levels of Wnt2, Wnt5a, Wnt7b, and WISP1). The mRNA tissue expression levels indicate differences in ratios between the different fibrillar collagen types (in favor of collagen types III and V compared to collagen type I), which is in agreement with the thinner collagen fibrils as previously observed in PD. The fibrotic nature of the plaque is confirmed by the increased mRNA levels of collagen types IV and VI, and fibronectin. Not only the quantity of collagen is affected but also the quality of the collagen, as concluded from the increased mRNA levels of *PLOD3*.

Differences in extracellular matrix composition and post-translational modifications all contribute to aberrations in supramolecular assemblages. The low mRNA level of cathepsin K is also noteworthy, as this enzyme is capable of effectively degrading pyridinoline cross-linked collagen. The lower levels of cathepsin K may further contribute to the consistent collagen accumulation in the plaque. Clearly, the homeostasis of the extracellular matrix is compromised, and should be targeted in future treatments.

Disclosure statement

No potential conflict of interest was reported by the authors.

Data availability statement

The data that support the findings of this study are available from the corresponding author, EJtD, upon reasonable request.

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Notes on contributors

Evert-Jan P. M. ten Dam is a resident in Plastic Surgery and a PhD student in Medical Biology regarding fibromatosis of the hand (Dupuytren's disease) and the penis (Peyronie's disease)

Mels F. van Driel is a urologist, specialized in andrology and sexology. He wrote multiple books on male sexology.

Igle Jan de Jong is the head of the Department of Urology and has expertise in oncology, reconstructive urology, and molecular imaging.

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Ruud A. Bank is the head of the Matrix group at the Medical Biology Department. Main interests are fibrosis, foreign body reactions, tissue engineering of cartilage, the role of fibroblasts and macrophages in tissue repair, and the role of mesenchymal stem cells in tissue repair.

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