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Identification and characterization of human xylosyltransferase II promoter single nucleotide variants

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ABSTRACT

The human isoenzymes xylosyltransferase-I and -II (XT-I, XT-II) catalyze the rate-limiting step in proteoglycan biosynthesis. Therefore, serum XT activity, mainly representing XT-II activity, displays a powerful biomarker to quantify the actual proteoglycan synthesis rate. Serum XT activity is increased up to 44% in disorders which are characterized by an altered proteoglycan metabolism, whereby underlying regulatory mechanisms remain unclear. The aim of this study was to investigate new regulatory pathways by identifying and characterizing naturally occurring *XYLT2* promoter sequence variants as well as their potential influence on promoter activity and serum XT activity.

XYLT2 promoter single nucleotide variants (SNVs) were identified and genotyped in the genomic DNA of 100 healthy blood donors by promoter amplification and sequencing or restriction fragment length polymorphism analysis. The SNVs were characterized by an *in silico* analysis considering genetic linkage and transcription factor binding sites (TBSs). The influence of SNVs on promoter activity and serum XT activity was determined by dual luciferase reporter assay and HPLC-ESI mass spectrometry.

Allele frequencies of seven *XYLT2* promoter sequence variants identified were investigated. *In silico* analyses revealed a strong genetic linkage of SNVs c.-80delG and c.-188G > A, c.-80delG and c.-1443G > A, as well as c.-188G > A and c.-1443G > A. However, despite the generation of several SNV-associated changes in TBSs *in silico*, *XYLT2* promoter SNVs did not significantly affect promoter activity. Serum XT activities of SNV carriers deviated up to 8% from the wild-type, whereby the differences were also not statistically significant.

This is the first study which identifies, genotypes and characterizes *XYLT2* promoter SNVs. Our results reveal a weak genetic heterogeneity and a strong conservation of the human *XYLT2* promoter region. Since the SNVs detected could be excluded as causatives for strong interindividual variabilities in serum XT activity, our data provide increasing evidence that XT-II activity is obviously regulated by hitherto unknown complex genetic pathways, such as cis- or trans-acting enhancers, silencers or miRNAs.

1. Introduction

Two isoenzymes of xylosyltransferases (XT-I and -II, EC 2.4.2.26) catalyze the xylosylation of proteoglycan core proteins in humans. This is the rate-limiting step in the attachment of glycosaminoglycan chains contributing to proteoglycan maturation [1]. Proteoglycans represent a highly diverse family of biomolecules of the extracellular matrix (ECM). They exert influence on a vast number of signal transduction pathways, and physiological and pathological processes, for example, cell adhesion, cell proliferation, cartilage hydration or permeability of the glomerular basement membrane [2,3]. Both simultaneously expressed XTs are actively secreted from

Abbreviations: ECM, extracellular matrix; LD, linkage disequilibrium; RFLP, restriction fragment length polymorphism analysis; SNV, single nucleotide variant; TBS, transcription factor binding site; XT, xylosyltransferase.

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the golgi in the ECM and show isoform-specific tissue expression patterns [4,5]. Interestingly, XT-I and –II exhibit marginal differences in their biochemical characteristics so that their co-expression has not yet been completely elucidated [4,6–8].

The determination of human serum XT activity displays a noninvasive, readily accessible method to monitor the proteoglycan turnover rate in body fluids. Hence, serum XT activity reflects ECM remodeling. Increased serum XT activity is associated with abnormal proteoglycan accumulation and was formerly defined as a biomarker for liver fibrosis, systemic sclerosis, osteoarthritis and pseudoxanthoma elasticum [9–12].

Nevertheless, the underlying regulatory mechanisms for variabilities in serum XT activity occurring in terms of various ECM-associated disorders remain unclear. Until today, several cellular XT-I-regulating proteins and transcription factors, such as transforming growth factor-β1, interleukin-1β, SP1 or AP1, have been identified [13–15]. By contrast, little is known about the regulation of XT-II. While SP1 and SP3 transcription factors control the basal *XYLT2* expression, no further regulatory factors could be registered [16]. In addition, several single nucleotide variants (SNVs) in the coding region of the *XYLT* genes were identified as genetic cofactors or risk factors for diseases, such as osteoarthritis or abdominal aortic aneurysms [11,17].

Since Condac et al. recently reported that XT-II is the predominant isoenzyme in the serum of mice and men under physiological conditions [18], we focused on XT-II in this study. More precisely, we aimed at revealing hitherto unconsidered regulation pathways of XT-II by examining an X*YLT2* promoter sequence analysis for the first time. Promoter regions convert intra- and extracellular signals in aberrant gene expression patterns and, therefore, display the most important regulatory DNA elements [19]. Thus, the aim of this study was to analyze the occurrence of putative *XYLT2* promoter sequence variants as well as their influence on transcriptional *XYLT2* regulation and serum XT activity. To avoid any pathological influence on serum XT activity, we focused on the *XYLT2* promoter analysis of healthy blood donors.

2. Materials and methods

2.1. Study subjects

Ethylenediaminetetraacetic acid (EDTA) plasma and serum samples of 100 healthy blood donors (50% males, 18–60 years of age, mean age ± SD: 36.2 ± 13.5 years) were collected. The experimental design was approved by the local ethics committee (Medical Faculty, Ruhr-Universität Bochum, Bad Oeynhausen, Germany) and all blood donors gave their informed consent.

2.2. Promoter amplification

After plasma centrifugation, genomic DNA was extracted from 200 μL EDTA blood leukocytes using the Nucleo Spin Blood Kit (Macherey–Nagel, Düren, GER). Referring to the current *XYLT2* reference sequence (GenBank Accession Number NG_012175.1), the promoter region was divided into six overlapping fragments F (c.-2720 to c.-2050), E (c.-2078 to c.-1506), D (c.-1656 to c.-1247), C (c.-1294 to c.-689), B (c.-825 to c.-354) and A (c.-402 to c.+107). The amplification was performed by an initial denaturation step at 95 °C (15 min) followed by 35 cycles (denaturation at 95 °C for 1 min, annealing at optimal annealing temperature (T_A) for 1 min and elongation at 72 °C for 1 min) and final elongation at 72 °C for 15 min. Primer sequences and T_A are listed in Table 1. The composition of the reaction mixture was described previously [20]. PCR products were visualized by UV light after agarose gel electrophoresis and intercalation of ethidium bromide.

2.3. Restriction fragment length polymorphism analysis (RFLP)

Analysis of the allele frequencies of SNVs c.-664T > G and c.-1044G > A was performed by RFLP. A restriction site was inserted during the amplification of DNA using specific primers (Table 1). The PCR products were digested overnight with *MseI* or *BsrI* (NEB, Frankfurt, GER), respectively, according to the manufacturer's instructions. Electrophoretic fragment analysis was used for genotyping.

2.4. Sequencing

Sequencing was performed to genotype SNVs c.-80delG, c.-84G > C, c.-188G > A, c.-1443G > A and c.-2448T > C, as well as to verify site-directed mutagenesis of promoter plasmids. Methods were described previously [20].

2.5. Site-directed mutagenesis

In order to analyze the influence of SNVs on promoter activity, pGL4.10 luciferase reporter vectors containing the complete *XYLT2* promoter region (c.-2568 to c.+2) or the most active region (c.-263 to c.+2) [16] were used. The SNVs were inserted using the QuikChange site-directed mutagenesis kit (Agilent, Santa Clara, USA). Site-directed mutagenesis was applied according to the manufacturer's instructions (primers are listed in Table 1). Plasmid DNA was purified using the QIAprep Spin miniprep kit (Qiagen, Hilden, GER). Successful insertion of *XYLT2* SNVs was checked by plasmid sequencing using vector-specific primers (Table 1).

2.6. Cell culture and transient transfection

HepG2 cells (ATCC, human hepatoma cells) were routinely grown in Dulbecco's Modified Eagle's medium (DMEM; Life Technologies, San Diego, USA), supplemented with 10% FCS (Pan biotech, Aidenbach, GER) and 1% antibiotic/antimycotic solution (Pan biotech, Aidenbach, GER). For transfection, 180,000 cells were seeded in triplicate in six-well culture dishes and incubated for 24 h. The next day, 188 μL medium (without any supplements) was mixed with 12 μL FuGene 6 transfection reagent (Promega, Mannheim, GER) and incubated at room temperature for 5 min. After the addition of 1 μg of the appropriate pGL4.10 plasmid and 10 ng of pGL4.74, the reaction mixture was incubated for 30 min and applied to the cells (serum-free medium). After 24 h, the cell culture medium was replaced with DMEM supplemented with 10% FCS and 1% AB/AM. The next day, the cells were harvested and the promoter activity was analyzed.

2.7. Dual luciferase reporter assay

Cellular luciferase activity was assayed with the Dual Luciferase Reporter assay system (Promega, Mannheim, GER) on a Lumat LB9705 luminometer (Berthold Technologies, Gütersloh, GER), according to the manufacturer's instructions. In order to calculate relative luciferase activity, *firefly* luciferase activity (encoded by the pGL4.10 vector construct) was measured in each sample and normalized to *renilla* luciferase activity (encoded by cotransfected pGL4.74 vector) as described previously [20].

Table 1
Primers used for amplification, sequencing, restriction fragment length polymorphism analysis (RFLP) and site-directed mutagenesis (SDM). Mutated bases are marked in bold.

Application		Primer sequence (5′ → 3′)	T_A [°C]
PCR and sequencing	Fragment A	GCTTACCCGCGTGGGACTGA	61.2
		CTGAAGCTCCACACTACCAG	
	Fragment B	AGCAGCATGTGTCGAGGTCT	65.9
		GTCTGCGGCACAACCTCTCT	
	Fragment C	CCTCCATCATGGCCTTCTAA	58.8
		GATGCTGATGCCTTTGCTCT	
	Fragment D	TCTTGGCAGGTAGAAGTC	54.0
		GACGTTAGGAACCACTACAG	
	Fragment E	AGACCATCCTGGCTAACAC	58.8
		GTTTCCGACGACAGTTTG	
	Fragment F	GGAGACAGAGGGAAACAATG	58.8
		GGGTTTCACCGTGTTAGC	
Plasmid sequencing		CTAGCAAAATAGGCTGTCCC	
		CTTAATGTTTTTGGCATCTTCCA	
RFLP	c.-664T > G	GGAGTCTCTTGGAGGTCTTGTTA	
		AACAATCTCTTCCCACTCCC	
	c.-1044G > A	GCTGTCCCTAACATTTGTTTGAGAAAC	
		ATGGATGGGGAAAAGGACAC	
SDM	c.-80delG	CCTAGGCGTGGAGGAGGGGGGCGGCTCAGCCCCGCGCCCGTG	
		CACGGGCGCGGGGCTGAGCCGCCCCCCTCCTCCACGCCTAGG	
	c.-84G > C	CCTCCCTAGGCGTGGAGGAGG**C**GGGGCGGCTCAGCCCCGCGC	
		GCGCGGGGCTGAGCCGCCCC**G**CCTCCTCCACGCCTAGGGAGG	
	c.-188G > A	ACTGAAGGTGTTGCGAGCCCC**A**GCTCCACCCCTAGCCTGCGG	
		CCGCAGGCTAGGGGTGGAGC**T**GGGGCTCGCAACACCTTCAGT	
	c.-664T > G	GAGGGGCACTAAAAATCTGTT**G**TATCAAGACCTCCAAGAGAC	
		GTCTCTTGGAGGTCTTGATA**C**AACAGATTTTTAGTGCCCCTC	
	c.-1044G > A	GCGGTGCTCATTGAACACGCC**A**GGTTCTCAAACAAATGTTAG	
		CTAACATTTGTTTGAGAACC**T**GGCGTGTTCAATGAGCACCGC	
	c.-1443G > A	AATGATTACAAATGCTAAAGC**A**CAGAAGACGATTAAAGGAGG	
		CCTCCTTTAATCGTCTTCTG**T**GCTTTAGCATTTGTAATCATT	
	c.-2448T > C	CAGTGGGCTGGGCTCGGCGGC**C**CATGCTTGTAATCCCAGCAC	
		GTGCTGGGATTACAAGCATG**G**GCCGCCGAGCCCAGCCCACTG	

2.8. XT activity assay

Determination of serum XT activity was performed by high-performance liquid chromatography electrospray ionization tandem mass spectrometry, as described previously [21]. The method used a synthetic peptide Biotin–NH–QEEEGSGGGQKK(fluorescein)-$CONH_2$ as the acceptor protein.

2.9. In silico analysis

In silico analysis of TBSs was carried out using the Genomatix online software suite, while sequence alignments were performed with DNASTAR (DNASTAR, Inc., Madison, WI, USA). Evaluation of LD maps and haplotype frequencies was carried out using Haploview 4.0 (Broad Institute, Cambridge, MA, USA) [22]. Blocks were defined according to the "solid spine of LD" setting in the software.

2.10. Statistics

Experimental data were analyzed by Mann–Whitney *U* Test using GraphPad Prism 5.0 (GraphPad Software, San Diego, USA). P values less than 0.05 were considered statistically significant. An χ^2-test was performed to examine whether genotype distributions fit into the Hardy–Weinberg equilibrium.

3. Results

3.1. Identification and characterization of XYLT2 promoter SNVs

An initial sequence analysis of the entire *XYLT2* promoter region of ten healthy blood donors was performed to find out whether naturally occurring *XYLT2* promoter SNVs exist and exert influence on promoter activity and serum XT activity. Seven *XYLT2* promoter SNVs were detected and genotyped in a study population of 100 healthy blood donors. Based on their description in the 1000 genomes project, rs numbers were deposited for all SNVs except for c.-80delG. Detected allele frequencies of polymorphic variants c.-80delG, c.-84G > C, c.-188G > A, c.-664T > G, c.-1044G > A, c.-1443 G > A and c.-2448T > C, all conforming to the Hardy–Weinberg equilibrium, are listed in Table 2.

Haplotype analysis defined one LD (linkage disequilibrium) block in the *XYLT2* promoter region, as well as 18 haplotypes (Table 3). In addition, *in silico* analyses revealed a strong genetic linkage (Fig. 1) between c.-80delG and c.-188G > A ($D' = 1.0$, $r^2 = 0.95$), c.-80delG and c.1443G > A ($D' = 0.94$, $r^2 = 0.86$), and c.-188G > A and c.-1443G > A ($D' = 0.92$, $r^2 = 0.86$).

Table 2
Allele frequencies of *XYLT2* promoter SNVs detected in healthy blood donors.

SNV	rs ID	Allele	Frequency
c.-80delG	–	G	141/200 (0.705)
		–	59/200 (0.295)
c.-84G > C	rs9912067	G	164/200 (0.820)
		C	36/200 (0.180)
c.-188G > A	rs115632911	G	143/200 (0.715)
		A	57/200 (0.285)
c.-664T > G	rs2526542	T	92/200 (0.460)
		G	108/200 (0.540)
c.-1044G > A	rs76924269	G	179/200 (0.895)
		A	21/200 (0.105)
c.-1443G > A	rs11079913	G	143/200 (0.715)
		A	57/200 (0.285)
c.-2448T > C	rs35664950	T	144/200 (0.720)
		C	56/200 (0.280)

Table 3
XYLT2 promoter haplotypes.

Haplotype	c.-80	c.-84	c.-188	c.-664	c.-1044	c.-1443	c.-2448	Allele frequency (n = 200)
A	G	G	G	G	G	G	G	101 (0.503)
B	A	G	A	T	G	A	A	48 (0.239)
C	G	C	G	T	A	G	G	18 (0.091)
D	G	C	G	T	G	G	G	10 (0.050)
E	A	G	A	T	G	A	G	6 (0.031)
F	G	G	G	T	G	G	G	3 (0.015)
G	G	C	G	G	G	G	G	2 (0.010)
H	G	C	G	T	G	G	A	2 (0.010)
I	A	G	A	G	G	G	G	1 (0.006)
J	G	G	G	G	G	G	A	1 (0.006)
K	G	C	G	G	G	G	A	1 (0.005)
L	G	C	G	G	A	G	G	1 (0.005)
M	G	G	G	T	G	A	A	1 (0.005)
N	G	G	G	G	G	A	G	1 (0.005)
O	A	G	A	T	G	G	A	1 (0.005)
P	A	C	G	T	A	G	A	1 (0.005)
Q	A	G	G	T	G	A	A	1 (0.005)
R	A	C	A	T	A	G	G	1 (0.004)

3.2. Analysis of the influence of XYLT2 promoter SNVs on promoter activity

A comprehensive promoter activity analysis was performed by inserting the SNVs identified into *XYLT2* promoter reporter gene constructs, which had been cloned previously [16]. Although nucleotide alterations according to c.-80delG and c.-84G > C were associated with changes in putative TBSs regulating XT or fibrotic remodeling, site-directed mutagenesis was not accompanied by any change in promoter activity (Table 4; Fig. 2). *In silico*, c.-80delG entails the loss of three TBSs (early growth response-1: EGR1, Krueppel-like factor 15: KLF15, Wilm's tumor suppressor: WTS), while c.-84G > C causes the formation of one EGR1-TBS as well as the disappearance of TBSs for WTS, KLF15 and SP4 (Table 4).

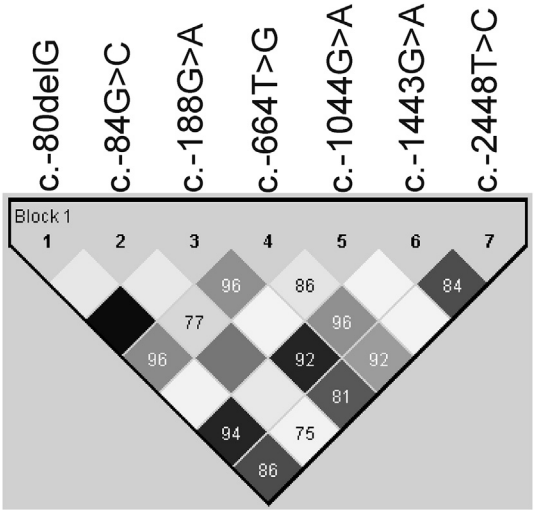

Fig. 1. LD (linkage disequilibrium) structure of the *XYLT2* promoter region. Calculations are based on the setting "spine of LD" of the Haploview software, which is based on each end marker of a block having a D′ value of >0.8. Color gradation from white to black represents strength of correlation between two SNVs (no LD: white, $r^2 = 0$; perfect LD: black, $r^2 = 1$). Numbers indicate values of parameter D'.

Table 4
Changes in transcription factor-binding sites in the *XYLT2* promoter due to SNVs. The SNVs are marked in bold and binding sites are underlined.

SNV	Strand	Wild-type	Variation	Transcription factor family
c.-80delG	+	A<u>GGGGGG**G**CG</u>	AGGGGGGCG	*Wilm's tumor suppressor* (EGRF)
	+	A<u>GGGGGG**G**CG</u>	AGGGGGGCG	*Early growth response-1* (EGRF)
	+	G<u>GGGGG**G**CGG</u>	GGGGGGCGG	*Krueppel-like factor-15* (KLFS)
c.-84G > C	+	A<u>GGG**G**GGGCG</u>	AGGG**C**GGGCG	*Wilm's tumor suppressor* (EGRF)
	+	GA<u>GGG**G**GGGC</u>	GAGGG**C**GGGC	*Krueppel-like factor-15* (KLFS)
	+	G<u>GG**G**GGGCGG</u>	GGG**C**GGGCGG	*SP4* (SP1F)
	+	AGGAGG**G**GGG	<u>AGGAGG**C**GG</u>G	*Early growth response-1* (EGRF)

3.3. Analysis of the influence of XYLT2 promoter SNVs on serum XT activity

Serum XT activities of all healthy blood donors genotyped were quantified and divided into three groups according to the genotype (wild-type, heterozygous, homozygous) of the *XYLT2* promoter SNVs, respectively. In compliance with former studies, the serum XT activity of men was significantly increased in comparison to the serum XT activity of women. Nevertheless, the serum XT activity did not reflect any genotype-associated changes concerning *XYLT2* promoter SNVs (Fig. 3). The greatest deviation between the XT activity of heterozygous or homozygous SNV carriers and XT activity of SNV wild-type carriers was 8% (comparison of c.-84G/C with c.-84G (males)). No statistic evaluation was realizable due to the small sample size of male homozygous carriers for SNVs c.-84C and c.-1044A.

4. Discussion

Abnormal serum XT activity is not only associated with fibrotic diseases, but also with infertility or diabetes [23–25]. Consequently, it is of great scientific interest to uncover XT-regulating mechanisms. The elucidation of every regulatory pathway offers the opportunity to interfere with the according pathological

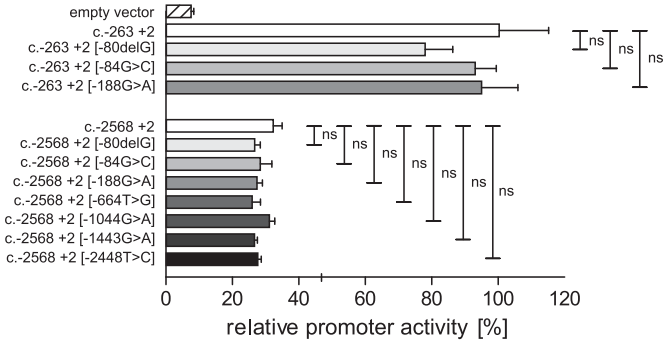

Fig. 2. Influence of *XYLT2* promoter SNVs on promoter activity. Changes in relative promoter activity of pGL4.10 *XYLT2* promoter cloning constructs c.-2568 to c.+2 (full-length construct) or c.-263 to c.+2 (most active construct) were determined in response to SNV-associated base exchanges by site-directed mutagenesis. Promoter activities are expressed relative to the activity of construct c.-263 to c.+2, which was defined as 100%. Plasmids were transfected into HepG2 cells and promoter activity was determined in lysates by dual luciferase reporter assay. Values are means ± SEM of triplicates from at least two or three independent experiments. ns: not significant (Mann–Whitney *U* test).

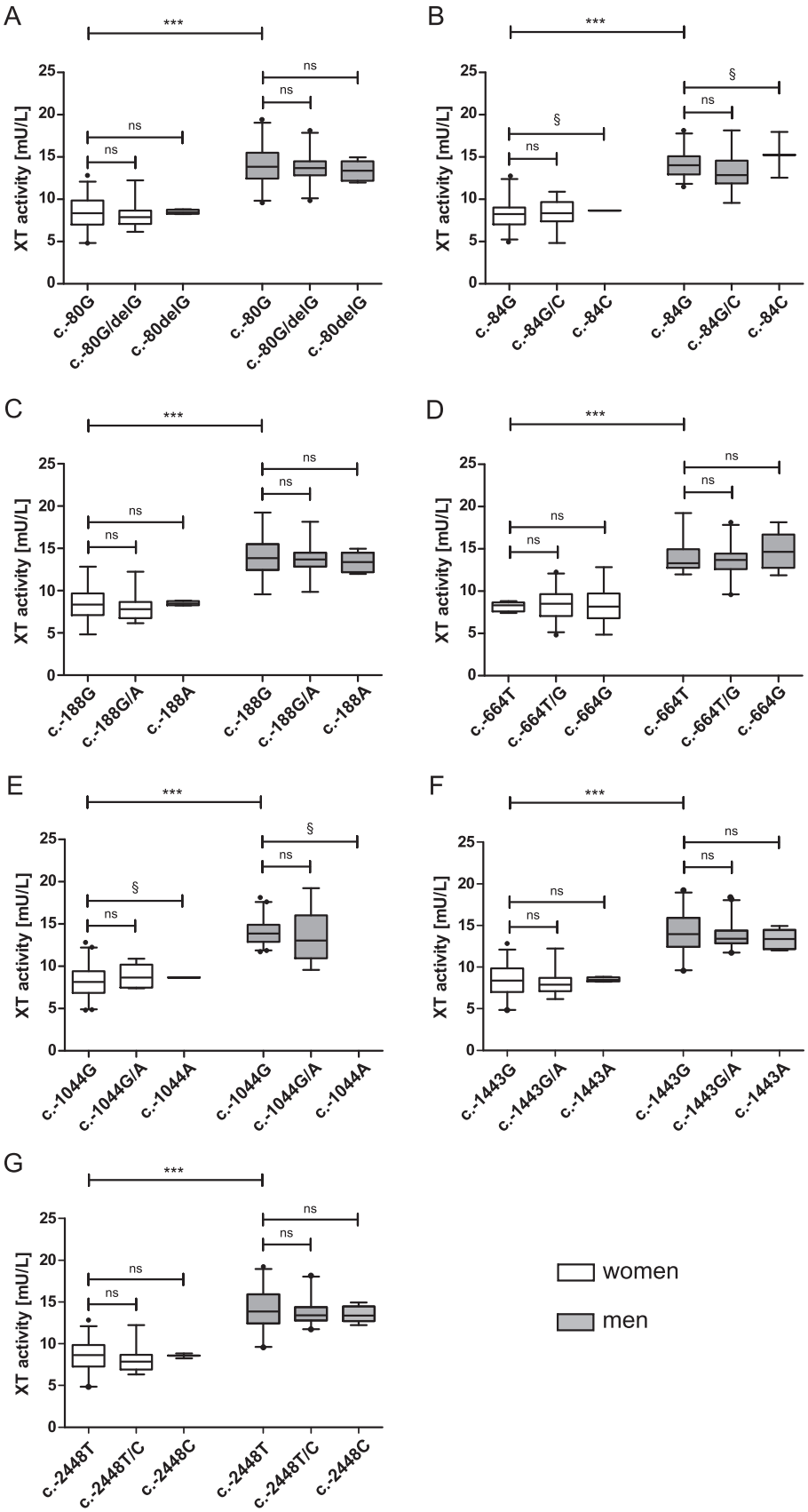

Fig. 3. Influence of *XYLT2* promoter SNVs on serum XT activity (n = 100). Differences in serum XT activity depending on genotype and gender (white bars: women; grey bars: men) are indicated. Boxes show the 25th to 75th percentiles. Horizontal lines in the boxes show the median, while vertical whiskers show the 5th to 95th percentile. ns: not significant; §: no statistics (Mann–Whitney *U* test).

process and to develop a therapeutic strategy. Since serum XT activity of *XYLT2* knock-out mice is reduced to 1% of wild-type serum XT activity, XT-II was described as the predominant serum isoform in the physiological state. This observation is transferable to the composition of human serum, as could be shown by exploiting isoenzyme-specific substrate affinities [18]. Therefore, focusing on XT-II might be a key to understanding and controlling serum XT activity regulation. Here, we examined whether the identification and characterization of genetic *XYLT2* promoter SNVs might provide new insights into the transcriptional regulation of serum XT activity.

Until today, the sole focus regarding the influence of genetic variants on serum XT activity was on analyzing the *XYLT1* promoter region [20] as well as the coding regions of *XYLT1* and *XYLT2*. As the *XYLT2* exons were sequenced with primers binding to flanking regions, Schön et al. also detected two SNVs, c.-86delG and c.-84G > A, located in the *XYLT2* promoter region. Allele frequencies were not analyzed in a cohort of healthy blood donors, but it could be shown that c.-84G > A underlies an allele frequency of 37% in osteoarthritis patients [11]. Therefore, we performed the first *XYLT2* promoter sequence analysis in healthy probands in this study. We detected seven *XYLT2* promoter sequence variants (c.-80delG, c.-84G > C, c.-188G > A, c.-664T > G, c.-1044G > A, c.-1443G > A and c.-2448T > C). Due to the sequential accumulation of seven guanine residues, the deletion which was observed at position c.-80 is identical to the SNV c.-86delG identified previously. The SNV at position c.-84G was also detected by Schön et al., even though we only registered a replacement of guanine to cytosine instead of guanine to adenine. All the *XYLT2* promoter SNVs identified in our study, except for the SNV c.-80delG, were recently mentioned by the 1000 genomes project [26]. The allele frequencies given of this large-scale sequencing network concur with the allele frequencies determined here [27]. This observation demonstrates that our study cohort is representative to analyze population genomics.

The identification of only seven SNVs in the whole *XYLT2* promoter region comprising approximately 2500 bp implies a strong conservation of this genomic region as well as the evolutionary essentiality of XT-II in diverse physiological processes. This issue is also reflected by the absence of causal coding SNVs in the *XYLT2* gene. By contrast, six mutations in the *XYLT1* gene eliciting ossification disorders were described recently [28,29].

The characterization of the SNVs identified included the analysis of their influence on promoter activity, serum XT activity and an *in silico* analysis. Surprisingly, no associations of SNVs with changes in promoter activity were obtained. Although the *in silico* analysis revealed SNV-dependent changes in the *XYLT2* promoter TBS profile, these changes apparently do not exert influence. Electrophoretic mobility shift assays may uncover TBS occupation *in vivo*. *In silico* analysis also yielded a strong genetic linkage between c.-80delG and c.-84G > C, c.-80delG and c.1443G > A, and c.-188G > A and c.-1443G > A. Whether resulting *XYLT2* promoter haplotypes are associated with pathological ECM remodeling should be examined in subsequent studies. Analogously, Hendig et al. have already described the association of a *XYLT2* haplotype with diabetic nephropathy [30].

Moreover, serum XT activity was independent of the genotype of *XYLT2* promoter SNVs. Generally, the serum XT activity of men was higher than that of women. The discrepancy described might have a bearing on hormonal regulation, but has not yet been studied. Differences in serum XT activity between wild-type and SNV carriers varied from 1% to 8%. In comparison with pathological serum XT activity variabilities comprising up to 44%, 14%, 38% or 21% in systemic sclerosis, liver fibrosis, diabetes or osteoarthritis, respectively, SNV-associated changes in serum XT activity do not represent relevant regulatory risk factors [9–11,23]. Nevertheless, the fact that study populations of male homozygous carriers for SNVs c.-84G > C and c.-1044G > A were too small to perform a statistic evaluation should be taken into consideration. Hence, we cannot exclude a significant regulatory influence of these genotypes. Besides, it can be assumed that SNVs which increase serum XT activity are accompanied by an evolutionary disadvantage, so that their frequency might be very small although their influence on serum XT activity is significant.

In summary, this is the first study examining genetic variability of the *XYLT2* promoter. Since only seven naturally occurring and partially linked SNVs were detected, the genomic region underlies a strong conservation pointing to an important physiological function of XT-II. The SNVs detected could be excluded as causatives for strong interindividual variabilities in serum XT activity. Hence, our data provide increasing evidence that XT-II activity is regulated by hitherto unknown complex genetic pathways, such as cis- or trans-acting enhancers, silencers or miRNAs. Based on our findings, future studies should also clarify the putative capacity of the SNVs identified to represent risk factors for abnormal ECM remodeling.

Conflict of interest

None.

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Transparency document

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