

Children with vesico-ureteric reflux have joint hypermobility and occasional *TNXB* sequence variants

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Abstract

Introduction: To consider alternative mechanisms that give rise to a refluxing uretero-vesical junction (UVJ), we hypothesized that children with a common heritable urinary tract defect, vesico-ureteric reflux (VUR), may have a defect in the extracellular matrix composition of the UVJ and other tissues that would be revealed by assessment of the peripheral joints. Hypermobility joints can arise from defects in the extracellular matrix within the joint capsule that affect proteins, including Tenascin XB.

Methods: We performed an observational study of children with familial and non-familial VUR to determine the prevalence of joint hypermobility, renal scarring, and DNA sequence variants in *Tenascin XB*.

Results: The majority of children, 27/44, exhibited joint hypermobility using the Beighton scoring system. This included 15/26 girls (57.6%) and 12/18 boys (66.6%), which is a significantly higher prevalence for both sexes when compared to population controls ($p < 0.005$). We found no association between joint hypermobility and renal scarring. Seven of 49 children harbored rare pathogenic sequence variants in *TNXB*, and two also exhibited joint hypermobility. No sequence variants in *TNXB* were identified in 25/27 children with VUR and joint hypermobility. Due to the observational design of the study, there was missing data for joint hypermobility scores in six children and for DMSA scans in 17 children.

Conclusions: We observed a high prevalence of VUR and joint hypermobility in children followed within a tertiary care pediatric urology clinic. While mutations in *TNXB* have been reported in families with VUR and joint hypermobility, we identified only two children with these phenotypes and pathogenic variants in *TNXB*. We, therefore, speculate that VUR and joint hypermobility may be due to mutations in other extracellular matrix genes.

Introduction

Vesico-ureteric reflux (VUR) is a congenital defect of the uretero-vesical junction (UVJ) that results in the retrograde flow of urine from the bladder towards the kidneys. The ability of the UVJ to occlude the ureteral orifice requires adequate musculature within the intravesical portion of the ureter and sufficient collagen fibers to maintain its tensile strength.^{1,2} VUR affects a variable number of children ranging from 1-25 % of the population and may result in recurrent pyelonephritis that can produce scars with loss of function.^{3,4,5} Renal scarring associated with VUR continues to be a relatively common cause of chronic kidney disease in children.

To consider alternative mechanisms that give rise to a refluxing uretero-vesical junction (UVJ), we hypothesized that children with a common heritable urinary tract defect, vesico-ureteric reflux (VUR), may have a defect in the extracellular matrix composition of the UVJ and other tissues that would be revealed by assessment of the peripheral joints. Several papers have reported that children with primary VUR are at higher risk for generalized joint hypermobility or “double jointedness” which raises the intriguing possibility that there are common defects in the extracellular matrix of the joint capsules and the UVJ that result in hypermobile joints and a refluxing UVJ.^{6,7} Indeed, we discovered two families with dominantly inherited VUR and joint hypermobility and found that these phenotypes mapped to a locus on chromosome 6. Using whole exome sequencing, we determined that each family harbored a heterozygous mutation in a gene that encodes an extracellular matrix glycoprotein known as *Tenascin XB*.^{8,9} *Tenascin XB* is a large glycoprotein within the extracellular matrix that interacts with the fibrillary collagens, the fibril-associated collagens, and the proteoglycan, decorin.¹⁰ The protein consists of the N-terminal oligomerization domain, the epidermal growth factor (EGF)-like repeats, the fibronectin (FN) type III repeats, and a fibrinogen (FBG)-like domain at the C-terminus.¹⁰ Autosomal recessive mutations in *Tenascin XB* have been reported in individuals with classical-like Ehlers-

Danlos syndrome that encompasses skin hyperextensibility, skin ecchymoses, and joint hypermobility, typically from large deletions or truncating mutations.¹¹ We tested children with both familial and non-familial primary VUR for joint hypermobility and demonstrate that there is a high prevalence of joint hypermobility that surpasses the population prevalence. We did not find that children with VUR and joint hypermobility were more likely to have recurrent urinary tract infections, nor did they exhibit more renal scars. Children with VUR were sequenced to determine if they had mutations in *Tenascin XB*. While we did identify pathogenic variants in 7/49 children with VUR, only two of them exhibited joint hypermobility. This suggests that the association between VUR and joint hypermobility may be due to mutations in other genes that are relevant for the formation of the extracellular matrix in these tissues.

Methods

Clinical phenotyping and joint hypermobility testing

We previously collected DNA samples from a large cohort of 250 children with primary VUR from pediatric urology clinics.¹² Within the cohort, there were familial cases and the majority of these were identified by sibling screening. The children were recruited at the time of diagnosis and had a mean age of 18 months at the time of enrollment. The Beighton scoring system assesses joint hypermobility in children and is validated for use in children greater than or equal to 6 years of age.¹³ The scoring system uses a 9 point scale to assess the 5th fingers, the thumbs, the elbows, the knees and the spine for joint hypermobility. Joint hypermobility is defined by a score greater than or equal to 6/9 as per the guidelines established by the International Consortium of Ehlers-Danlos Syndrome.¹⁴ For this study, we re-approached children greater than or equal to 6 years of age who presented for routine followups with their treating urologist and fifty of the 250 children were eligible by age for joint hypermobility testing. Of the fifty children, forty-four consented to joint hypermobility testing, forty-nine agreed to submit DNA samples for sequencing and thirty-three underwent DMSA scans as part of routine clinical care (Table 1). The children and their parents were recruited in accordance with the research ethics board at the McGill University Health Centre (Protocol #1389) after obtaining informed consent. All children were tested for joint hypermobility by one investigator (IRG) who was blinded to their VUR status. The grade of VUR was defined by the initial VCUG. In cases of bilateral VUR, grade was defined by the highest grade reported on either side. Nationality was determined by asking the parents their country of origin. Within the subset of fifty children, thirty-three underwent DMSA scans as requested by the treating pediatric urologist as part of routine clinical care. DMSA scans were performed at least 6 months after a urinary tract infection in the presence of recurrent febrile urinary tract infections, severe VUR (grade IV or V) and/or renal abnormalities on ultrasound. Renal scintigraphy was performed using either Tc99m-DMSA or Tc99m-Glucoheptonate and the presence of renal scarring was defined using RIVUR guidelines.¹⁵ Interpretation of the renal scan was performed by an experienced nuclear medicine

physician (ST) who was blinded to the patient's Beighton score and *Tenascin XB* sequence, but not to the presence of VUR. Forty-nine patients consented to DNA sequencing and submitted saliva samples that were used for DNA extraction (Oragene DNA).

Sequencing studies of children with VUR

We used 41 primer pairs to sequence all exons,^{9,16} including a pair used for long-range PCR to cover the duplicated region of *TNXB* (exons 32-44) in 49 children with VUR. Primer sequences are provided (Supplementary Table 1). Sequenced reads were mapped to the hg19 reference genome using the PHRAP algorithm of the PHRED/PHRAP/Consed software.¹⁷ Variants were determined using Atlas-SNP2 and the PHRED-base caller to detect single nucleotide variants. Variants were annotated using 1000 genome database,¹⁸ ExAC,¹⁹ dbSNP,²⁰ RefSeq,²¹ HSF3.1,²² and dbNSFP.²³ Variants not reported in these databases were considered novel. Variants with a minor allele frequency (MAF) of $\leq 1\%$ when compared to the frequency in ethnically matched populations were considered as rare variants. The predicted effects of rare variants were determined using the PROVEAN,²⁴ SIFT,²⁵ and Polyphen2²⁶ algorithms. Clustal Omega alignment tool (The European Bioinformatics Institute, EMBL-EBI) was used for the phylogenetic analysis. Pathogenic coding variants were defined as such if 2 or more *in silico* prediction formulae reported they were deleterious.

Statistical analysis

The R-squared statistic was used to assess the relationship between the grade of VUR and the Beighton score. Chi-square analysis was used to compare the proportion of children with VUR and joint hypermobility who had renal scars on DMSA scans versus those without joint hypermobility and/or recurrent urinary tract infections.

Results

Children with VUR have joint hypermobility

We previously recruited a cohort of children with primary vesico-ureteric reflux¹² and fifty were eligible for joint hypermobility testing using the Beighton scoring system. Of the fifty children, forty-four consented to joint hypermobility testing, forty-nine agreed to submit DNA samples for sequencing and thirty-three underwent DMSA scans as part of routine clinical care (Table 1). Fifteen of the 26 girls that were tested, 57.6%, exhibited joint hypermobility, while 12 of the 18 boys that were tested, 66.6%, exhibited joint hypermobility (Table 1 & 2) that was not significantly different ($\chi^2=0.3613$, $P=0.54$). This observation is of interest because in most studies, girls are more hypermobile than boys,^{27, 28, 29} but in our cohort, boys were especially hypermobile. There was a significantly higher proportion of girls and boys with VUR and joint hypermobility when compared to age-matched girls (91/516, 26%) and boys (67/530, 18%) in the general population ($Z=3.45$, $P=0.0005$ for girls; $Z=5.04$, $P<0.00001$, for boys using population proportions).²⁹ Two children had bladder diverticula in addition to VUR: one had confirmed

joint hypermobility with a Beighton score of 9, while the other did not. The parents self-reported their nationality and the majority of the children, 36/50, were defined as European, Non-Finnish using the categories as listed in the ExAc Browser.¹⁹ Within the cohort, 31 children were diagnosed with VUR because they presented with a urinary tract infection (19 females, 12 males), and most of these children (24/31) went on to experience recurrent urinary tract infections (Table 1 & 2). The remaining 19 children that did not present with a urinary tract infection were diagnosed because of an abnormal antenatal ultrasound that prompted further postnatal evaluation including a voiding cystourethrogram. Children with VUR and joint hypermobility were not more likely to present with a urinary tract infection or to experience recurrent urinary tract infections compared to children without joint hypermobility ($\chi^2=0.017$, $P=0.89$). Similarly, there was no relationship between the grade of VUR and the Beighton score ($r^2=0.02$, $P=0.3511$).

Twenty-eight of the 44 children who underwent joint hypermobility testing had a DMSA scan as part of routine clinical care. While a large proportion, 16/28, had VUR, joint hypermobility and renal scars on DMSA scan, this was not statistically significant when compared to 9/28 children with VUR and renal scars who did not exhibit joint hypermobility ($\chi^2= 0.0083$, $P=0.92$).

***TNXB* sequence variants in children with VUR and joint hypermobility**

We previously reported an association between heterozygous sequence variants in *Tenascin XB* and familial VUR with joint hypermobility.^{8, 9} We performed Sanger sequencing for this gene in 49 children with familial (8/49) and non-familial (41/49) VUR. Of the familial cases of VUR, in only one, was there a family history of joint hypermobility, and this child did not harbour a pathogenic variant in *TNXB* (Patient 22, Table 1). Overall, a total of 100 sequencing variants in *TNXB* were identified in 49 children. Of the 100 variants, 66 were exonic variants: 25 synonymous and 41 nonsynonymous. Amongst the nonsynonymous variants, 9 were defined as rare. The remaining 34 variants were intronic and 10 were splice variants. Two of the 10 splice variants were identified as rare. The eleven variants, 9 exonic and 2 intronic, were inherited as heterozygous alleles in children with non-familial VUR. The variants were analysed using prediction software as shown in Table 3. None of the patients sequenced had more than one variant. Five patients harbor pathogenic exonic variants based on deleterious scores from two or more prediction software: G878D, G1163E, P2217T, R3721W, and R4173Q (Table 3). All of the variants with the exception of the R4173Q variant affect one of the fibronectin type III (FNIII) repeat domains (Fig.1). Amongst the 5 children, 3 are female and 2 are male and 2 have high grade VUR, grade 4 or higher (Patients 12 & 29). Four of the 5 children are not hypermobile: Patient 5, Patient 12, Patient 29 and Patient 48. Only one individual, Patient 20, is hypermobile: she has low grade VUR, grade 2, and harbors a novel variant that is predicted to be deleterious, R3721W. This variant affects exon 33 that encodes a FNIII repeat (Fig.1). Interestingly, amongst the 5 children, she is the only one with renal scars in spite of a mild grade of VUR. Two rare intronic variants were identified that are predicted to affect a splice acceptor

site. One male patient harbors a rare 2 base pair intronic variant that is predicted to affect a splice acceptor site (Patient 27). He has high grade VUR, joint hypermobility, and renal scars. A second patient, Patient 47, harbors a rare intronic variant, but she is not hypermobile (Table 1 and Table 3).

Discussion

While VUR remains a common pediatric disorder, the ability for clinicians to determine who will develop recurrent urinary tract infections, who will develop renal scarring, and how these patients should be managed remains unclear. An additional challenge is the fact that VUR is both genetically^{30, 31, 32, 33, 34} and phenotypically heterogeneous. The purpose of this paper was to ascertain if VUR with joint hypermobility is a common phenotype and to determine if children with VUR and joint hypermobility might be at higher risk for recurrent urinary tract infections and/or renal scars. We also sought to determine if children with VUR and joint hypermobility would be more likely to harbour coding DNA sequence variants in an extracellular matrix gene known as *Tenascin XB* that has been reported in familial cases of VUR and joint hypermobility. We tested 44 children with VUR for joint hypermobility and determined that the majority exhibited joint hypermobility using the Beighton scoring system. We did not observe an association between VUR, joint hypermobility and recurrent urinary tract infections nor did we observe an association between VUR, joint hypermobility and renal scars, but this may be due to the sample sizes that were assessed. We identified rare heterozygous variants in *TNXB* in 7/49 children with VUR of which 5 are predicted to be pathogenic exonic variants and 2 are predicted to affect a splice acceptor site. Amongst the 7 children, 2 exhibit joint hypermobility and they both have renal scars. Importantly, 25/27 children who exhibited VUR and joint hypermobility did not have DNA sequence variants in *TNXB*, suggesting that either other genes implicated in the composition of the extracellular matrix or environmental factors might explain the co-occurrence of these phenotypes.

Our results support the findings of van Eerde *et al.*,⁶ but demonstrate that an even higher percentage of children with VUR, 66.6 % of boys and 57.6 % of girls exhibit joint hypermobility using cutoffs of ≥ 6 for pre-pubertal children and ≥ 5 for pubertal adolescents as reported by the International Consortium on the Ehlers-Danlos Syndromes.¹⁴ Our study did not include age and gender-matched controls without VUR, which ideally would have included children ≥ 6 years of age with a normal voiding cystourethrogram. Given the radiation exposure and the need for urethral catheterization, very few children undergo a voiding cystourethrogram unless there is a high suspicion of high grade VUR. Therefore, we compared the prevalence of joint hypermobility in our VUR cohort to a population of Italian school-age children with a mean age of 10.8 years in which the prevalence of joint hypermobility was reported to be 26 % in girls and 18 % in boys using the same cutoff criteria as our study.²⁹ Other population estimates of joint hypermobility using the Beighton score and/or the Bulbena score report that 18-26% of girls and 12-18% of

boys exhibit joint hypermobility.^{35, 36, 27, 28} Taken together, it appears that joint hypermobility is more frequently observed in children with VUR.

The majority of the children, 31/50, reported at least one urinary tract infection, while 24/31 experienced recurrent urinary tract infections. While we did not formally assess for bowel and bladder dysfunction, it is possible that in addition to VUR, these children had concurrent bowel and bladder dysfunction as a risk factor for urinary tract infection. This is of interest because De Kort *et al.*, have reported that children with generalized joint hypermobility are more likely to have lower urinary tract dysfunction including chronic constipation, urinary incontinence, and urinary tract infections.³⁷ Therefore, we cannot exclude the possibility that children with VUR and joint hypermobility may also be at risk for bladder sphincter dysfunction possibly due to a defect in the composition of the extracellular matrix within the bladder sphincter.

Fibrillary collagens, fibrillins, elastins, glycoproteins and proteoglycans form the extracellular matrix within the joint capsule and the surrounding ligaments as well as within the UVJ and bladder. Indeed, mutations in the genes that encode these proteins result in joint hypermobility in hereditary connective tissue disorders including Cutis laxa, and Ehlers-Danlos syndrome. VUR and bladder diverticula are reported in these disorders although the penetrance of these urinary tract phenotypes has not been systematically examined.³⁸ This suggests that a portion of children with VUR may have a global defect in the composition of the extracellular matrix that perturbs joint capsules and surrounding ligaments as well as the UVJ and the integrity of the bladder wall.

To pursue the association of VUR with joint hypermobility, we performed candidate gene sequencing for *Tenascin XB*, which is a large glycoprotein within the extracellular matrix that interacts with the fibrillary collagens, the fibril-associated collagens, and the proteoglycan, decorin.¹⁰ Autosomal recessive mutations in *Tenascin XB* have been reported in individuals with classical-like Ehlers-Danlos syndrome that encompasses skin hyperextensibility, skin ecchymoses, and joint hypermobility, typically from large deletions or truncating mutations.^{16, 11} While the genetic basis of autosomal dominant joint hypermobility is defined as unknown in the most recent EDS guidelines,¹⁴ we previously identified heterozygous mutations in *TNXB* in 5 families with VUR and joint hypermobility.^{8, 9} Within the TNX protein, there are at least 32 fibronectin (FN) type III repeats that are postulated to be important for facilitating the extensibility of the TNX protein itself which imparts elasticity to the extracellular matrix.³⁹ Most of the missense mutations identified to date in patients with VUR and joint hypermobility, including our study, localize to the FNIII repeats.^{8, 9} In this current study, we sequenced 49 children for *TNXB* in which the majority, 41, were sporadic cases of VUR, while the other 8 cases had a positive family history of VUR. While none of the familial cases had pathogenic variants in *TNXB*, 7 children with sporadic VUR did harbor pathogenic variants. One child with sporadic VUR and joint hypermobility harbored a novel variant in *TNXB*, R3721W, which is

evolutionarily conserved and affects the FNIII repeat. Another child with VUR and joint hypermobility had a rare intronic variant that is predicted to affect a splice site. There were 5 other children with VUR and putative pathogenic variants in *TNXB*, but none of these children fulfilled criteria for joint hypermobility. Combining all of the *TNXB* sequencing studies,^{8, 9} a total of 5/63 families, 8%, exhibit a mutation in *TNXB*, and two families have both VUR and joint hypermobility. Amongst the cases of sporadic or non-familial VUR, 9/96, 9.3%, harbor a mutation in *TNXB*, and of these, only three have both joint hypermobility and VUR. The *TNXB* protein is strongly expressed in the uroepithelium of the UVJ in children with VUR and without VUR.⁹ We speculate that heterozygous mutations in *TNXB* affect collagen deposition within the uroepithelial layer and may reduce the tensile strength of the UVJ and the joint capsule predisposing to VUR and to joint hypermobility.

There are limitations to this study. It was an observational study of children seen in a tertiary care centre in pediatric urology clinics. This factor explains the large number of severe cases of VUR within the cohort. We do not know if children with mild forms of VUR also exhibit a high prevalence of joint hypermobility. Another limitation of the study is the fact that the diagnosis of VUR is usually made at the age of 2 years, while the Beighton scoring system has been validated for use in children ≥ 6 years. Therefore, some children submitted DNA samples at the time of diagnosis, but did not return for their follow-up appointment at the age of 6 years when joint hypermobility testing was performed. This accounts for the 6 children who did not undergo joint hypermobility testing. Another limitation is the fact that DMSA scans were requested as per clinical indications, so there was missing data on a total of 17 of the 50 children. It would have been informative to perform DMSA scans on all of the children to ascertain if there is a relationship between VUR and renal scarring, but this would require a different study design in which we would need to convince parents to permit their children to undergo a DMSA scan even when not deemed to be clinically indicated.

Conclusions

In summary, children with VUR have a higher prevalence of joint hypermobility than age-matched population controls, suggesting there is a common mechanism that gives rise to both defects. From sequencing studies, it appears that mutations in *TNXB* do not explain most cases of VUR and joint hypermobility. This suggests that the association between VUR and joint hypermobility may be due to mutations in other genes that are relevant for the formation of the extracellular matrix in these tissues.

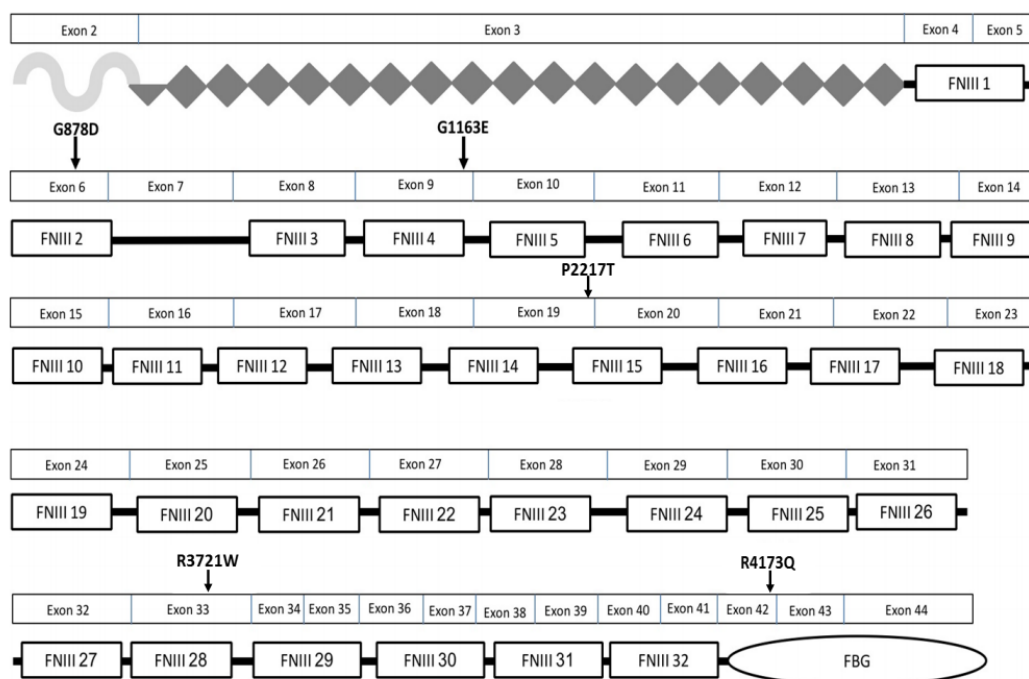
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Figure and Tables

Fig. 1. Location of pathogenic rare *TNXB* sequence variants within the TNXB protein domains. Five heterozygous *TNXB* missense variations were identified in the 49 children with vesico-ureteric reflux who underwent sequencing and their corresponding amino acid changes and locations within the protein are shown. The majority of the variants affect the fibronectin type III (FNIII) modules that are implicated in binding to decorin and in mediating extensibility of the TNX protein which imparts elasticity to the tissue. One amino acid variant, R4173Q, affects the fibrinogen-like (FBG) domain which directly binds to collagen fibrils and promotes the formation of collagen fibrils. The symbol under exon 2 depicts the N-terminal oligomerization domain. The diamonds depict the EGF-like repeats. The rectangular boxes depict the FNIII repeats, and the oval depicts the fibrinogen-like domain (FBG).



Patient ID	Population	Sex	Beighton score	UTI at VUR diagnosis	Recurrent UTI	VUR grade	Family history	Scarring
1	Other	F	4	No	No	3	VUR	NA
2	European (Non-Finnish)	M	6	No	No	3	No	NA
3	South Asian	M	2	Yes	Yes	5	No	Yes
4	Other	F	8	Yes	Yes	3	HM	Yes
5	European (Non-Finnish)	M	5	Yes	Yes	2	No	NA
6	Other	M	Not tested	Yes	Yes	3	No	Yes
7	Other	F	4	Yes	Yes	5	No	Yes
8	European (Non-Finnish)	M	2	Yes	No	2	No	NA
9	Other	M	6	No	Yes	3	No	Yes
10	European (Non-Finnish)	F	6	No	Yes	4	No	NA
11	European (Non-Finnish)	F	7	No	No	2	VUR	NA
12	European (Non-Finnish)	M	5	Yes	Yes	5	No	Yes
13	European (Non-Finnish)	F	9	Yes	Yes	4	HM	Yes
14	European (Non-Finnish)	F	6	No	Yes	2	HM	Yes
15	European (Non-Finnish)	F	8	Yes	Yes	4	HM	Yes
16	European (Non-Finnish)	F	6	Yes	Yes	3	No	Yes
17	European (Non-Finnish)	F	5	Yes	Yes	2	VUR	Yes
18	European (Non-Finnish)	F	9	Yes	No	3	VUR	NA
19	European (Non-Finnish)	F	Not tested	Yes	No	3	No	NA
20	European (Non-Finnish)	F	8	No	No	2	No	No
21	European (Non-Finnish)	M	3	Yes	No	4	HM	Yes
22	European (Non-Finnish)	F	6	Yes	Yes	5	VUR, HM	NA
23	European (Non-Finnish)	M	9	Yes	Yes	2	No	NA
24	European (Non-Finnish)	M	Not tested	Yes	Yes	5	No	Yes

25	South Asian	M	7	Yes	Yes	4	No	Yes
26	Other	F	Not tested	No	No	5	No	No
27	Other	M	6	No	No	4	No	Yes
28	European (Non-Finnish)	M	6	Yes	No	3	No	Yes
29	European (Non-Finnish)	F	4	No	No	4	No	NA
30	Other	M	6	Yes	Yes	5	No	Yes
31	European (Non-Finnish)	F	4	No	Yes	3	No	NA
32	European (Non-Finnish)	F	Not tested	Yes	No	5	VUR	Yes
33	European (Non-Finnish)	F	4	Yes	Yes	5	HM	No
34	Other	M	8	Yes	Yes	3	No	Yes
35	European (Non-Finnish)	F	6	No	Yes	1	No	NA
36	European (Non-Finnish)	M	6	No	No	5	VUR	Yes
37	European (Non-Finnish)	F	8	Yes	Yes	3	HM	No
38	European (Non-Finnish)	F	2	Yes	Yes	2	No	Yes
39	European (Non-Finnish)	F	3	Yes	Yes	2	No	NA
40	Other	M	Not tested	No	No	4	No	No
41	European (Non-Finnish)	F	4	No	No	3	No	NA
42	European (Non-Finnish)	M	2	No	No	4	VUR	Yes
43	European (Non-Finnish)	F	9	Yes	No	2	HM	NA
44	European (Non-Finnish)	M	6	No	No	3	No	NA
45	European (Non-Finnish)	F	9	Yes	Yes	2	HM	Yes
46	South Asian	M	7	No	Yes	3	No	Yes
47	South Asian	F	4	Yes	Yes	3	No	Yes
48	European (Non-Finnish)	F	4	Yes	Yes	3	No	Yes
49	European (Non-Finnish)	M	7	No	Yes	5	No	Yes
50	European (Non-Finnish)	F	6	Yes	Yes	5	HM	Yes

F: female; HM: hypermobility; M: male; UTI: urinary tract infection; VUR: vesico-ureteric reflux.

Sex	
Male	21
Female	29
HM	
HM	27 (15F, 12M)
No HM	17 (11F, 6M)
Not tested	6 (3F, 3M)
UTI at diagnosis	
Yes	31 (12F, 19M)
No	19 (10F, 9M)
VUR grade	
1	1
2	11
3	17
4	9
5	12
Family history (no. of index cases)	
VUR	7
HM	10
VUR and HM	1
Scarring	
Renal scarring	28
No scarring	5
No DMSA	17

F: female; HM: hypermobility; M: male; UTI: urinary tract infection; VUR: vesico-ureteric reflux.

Patient ID	Hypermobility status	Mutation	MAF	SIFT	Provean	Polyphen2	Predicted effect
3	Not hypermobile	G3174R	0.0031	0.059	-4.12*	0.033	Neutral
5	Not hypermobile	R4171Q	Novel	0.027*	-2.91*	0.999*	Deleterious
9	Hypermobile	E2652K	0.0011	0.206	-1.98	0.926*	Neutral
12	Not hypermobile	G1163E	0.0012	0.037*	-4.19*	0.954*	Deleterious
13	Hypermobile	S1830R	0.0009	0.071	-1.66	0.039	Neutral
20	Hypermobile	R3719W	Novel	0.011*	-5.18*	1.00*	Deleterious
29	Not hypermobile	G878D	0.0017	0.151	-3.07*	1.00*	Deleterious
46	Hypermobile	G3161A	0.0079	0.242	-1.09	0.027	Neutral
48	Not hypermobile	P2217T	0.0023	0.004*	-2.78*	0.204	Deleterious
27	Hypermobile	Intronic (Chr6:32035762-32035763, CT>TG)	0.004	N/A	N/A	N/A	Possible effect on splicing
47	Not hypermobile	Intronic (Chr6:32015793, G>A)	0.0018	N/A	N/A	N/A	Possible effect on splicing

Joint hypermobility status was determined using the Beighton scoring system. A score of $\geq 6/9$ was defined as joint hypermobility. Exonic or coding variants were defined as pathogenic based on deleterious scores using two or more *in silico* prediction software and are indicated with a superscript asterisk symbol. SIFT scores range from 0–1.0, with 0 most damaging. Provean scores < -2.5 are deleterious and > -2.5 are considered neutral. Polyphen2 scores range from 0–1.0, with 1.0 most damaging.

Supplementary Table 1. Primers used for sequencing of <i>Tenascin XB</i>		
Exon	Forward primer (5'–3')	Reverse primer (5'–3')
3	ATGCCACAGTCGTCACCA	AGAGCAGAGCTGGGCTACAT
3	GCAATCGGTTCCAGTGTACC	GGTCGTTGCGTGTGCTTT
3	GCAGTCTTCCCCTGAGTAGC	GAATGCATTTGCGACACG
3	AGGCACACTCCTTGCACAC	GAGAACGGCGTGTGTGTTT
3	CCCTCTACACACACACTGG	GGAAGGCTACGTGAGTGAGG
3	CATGCTCTCCCTCCACTCTT	GTGCAAGGAGTGTGCCTGT
4	GCCATCTGGACTCAACCAAT	CTGAGTAAAAGGGGCTGTGG
5	GGCAGATTCCCTCTCTAGTCC	GAGATAAGGGGGATTGAGCA
6	CCAGAAGCATTTCAGAGGAGTC	TGGACTAGAGAGGGAATCTGC
7	CCAATAACCCAGCTCCTC	GGACTGGGGATTCTTTCTAGT
8	CCCAAAGCACTGAGAAAACC	ATCCAGGATGGAGTGAGGTG
9	CTGACACAGCCAGGGTATGA	CCTATGTGGGATTTGGCTTC
10	GGCAAATGAGCTGAGAAGG	TGTCAGGCTTCCCAGAAGTT
11	CTGGAGCAAGGAGAGCAACT	TTTCCATGGCTGTCATCTGT
12	GGAGGAGTAAAGGGGTCAGG	GGTGACAGCGAGACTCCATC
13	CAGGTGGACAAAGGGAAGAC	CCCCATCTCAGTTCACAGC
14	CTGGGGCCAAATAATGGTAA	GCAGTTCTGGGTTTTTCCAG
15	AAAGGGGCACAAGGAACTT	CCCAGTCTTCCAGAAACAGC
16	TTCTGAAGGCTTCTCCTCCTC	TTTCGATTGCTGACTGCTTG
17	ACCAAAGAGCAAGAGGGTGA	CTTTCAGATGGCTGGGAGAG
18	AGGAGATGCTGGAGGCTGTA	CCAGTCATAGCCTTGGCTTC
19	AGTGAAGGCACCAGCAGAA	CCTCAACACCTCCTTGCAG
20	ACCAAAGAGCAAGAGGGTGA	GCACCAGCATCCAGACTGT
21	GGTACCCATGAGGGAAAGGT	CCACGACGTAAGCACATCC
22	ACTGTGAGCCCCATCAAGAC	AGCAAAGCAAGTTGCCCTTA

23	ACCAAAGAGCAAGAGGGTGA	GGGCACTTTGTGTTTTGTGA
24	CATGGAAACGTGCAAAAGAA	CTTGAAGACCTGAGCACATCC
25	GTCAGTCCTCAGGGAAGTGG	AACAAAAGATGGCGAGGAGA
26	CGAAGACTGGAGAGACAGCA	CCTTCCTCACAAGACCCAAG
27	CCTGTTCTTGGGCACTTTGT	CCTCTGCAGTGGAGAAGGAG
28	AAGAGGTGCCAAGATCCAAA	CCAGTCATAGCCTTGGCTTC
29	ATCAGTGGGTGCTGAGGACT	GCCGCTAAGAAATGCTCACT
30	GAGGGACTCACTTTCGGAGTT	ATAGCAGCCCAGGAAGCTC
31	TTGTCTTCAGCCCAAATGC	CTCGATCACAGCAGGGAAG
32 (start of duplicated region)	GGCCAAGCCTGGAAGATAAA	GATTGGAGACAGAAGCACAC
33	CCAGGGAGAGAGGATGGAT	GTCCCCAGGAATGGAAGT
34, 35	GACCTAGTGCCTCAGCCA	GGCTCTCTCTACTCCGTG
36, 37, 38	ATGGGTGGGAGTTGAGAG	TGGAAGCTGAGCAGGTAG
38, 39, 40	TCTCCTCTTCTGCTTTCCC	CCCCATCAGTCTCCATGTC
40, 41, 42, 43	CAGGACCAGCACCATCTT	TTGAGGTTGGCGTAGTGG
43, 44	GCTGTCTCCTACCGAGGG	GCAGAGAAGGCTTCTCC
Long range primers for duplicated region	GTCTCTGCCCTGGGAATGA	TGTAAACACAGTGCTGCGA