Mini Review

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Tenascin-X, Congenital Adrenal Hyperplasia, and the CAH-X Syndrome

Walter L. Miller[a] Deborah P. Merke[b]

[a]Department of Pediatrics, Center for Reproductive Sciences, and Institute of Human Genetics, University of California, San Francisco, CA, USA; [b]National Institutes of Health Clinical Center and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Bethesda, MD, USA

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Abstract
Mutations of the *CYP21A2* gene encoding adrenal 21-hydroxylase cause congenital adrenal hyperplasia (CAH). The *CYP21A2* gene is partially overlapped by the *TNXB* gene, which encodes an extracellular matrix protein called Tenascin-X (TNX). Mutations affecting both alleles of *TNXB* cause a severe, autosomal recessive form of Ehlers-Danlos syndrome (EDS). Rarely, patients with severe, salt-wasting CAH have deletions of *CYP21A2* that extend into *TNXB*, resulting in a "contiguous gene syndrome" consisting of CAH and EDS. Heterozygosity for *TNXB* mutations causing haploinsufficiency of TNX may be associated with the mild "hypermobility form" of EDS, which principally affects small and large joints. Studies of patients with salt-wasting CAH found that up to 10% had clinical features of EDS, associated joint hypermobility, haploinsufficiency of TNX and heterozygosity for *TNXB* mutations, now called "CAH-X." These patients have joint hypermobility and a spectrum of other comorbidities associated with their connective tissue disorder, including chronic arthralgia, joint subluxations, hernias, and cardiac defects. Other disorders are beginning to be associated with TNX deficiency, including familial vesicoureteral reflux and neurologic disorders. Further work is needed to delineate the full spectrum of TNX-deficient disorders, with and without associated CAH.

Introduction

Congenital adrenal hyperplasia (CAH) caused by 21-hydroxylase deficiency is one of the most common disorders of the adrenal gland and is familiar to most endocrinologists. In "classic CAH," the adrenal cannot produce cortisol adequately and overproduces androgens and androgen precursors resulting in prenatal virilization of female fetuses, whereas in "nonclassic CAH" cortisol production is essentially normal and adrenal hyperandrogenism is minimal or mild. Classic CAH is further subdivided into "salt-wasting CAH," a severe, potentially

Walter L. Miller, MD, Distinguished Professor of Pediatrics, Emeritus
Center for Reproductive Sciences
University of California, San Francisco
San Francisco, CA 94143–0556 (USA)
E-Mail wlmlab@ucsf.edu

Deborah P. Merke, MD, MS
Senior Investigator, National Institutes of Health Clinical Center
10 Center Drive, Room 1-2740
Bethesda, MD 20892-1932 (USA)
E-Mail dmerke@nih.gov

KARGER

E-Mail karger@karger.com
www.karger.com/hrp

life-threatening disorder in which the synthesis of both cortisol and aldosterone are impaired, and "simple virilizing CAH" in which there is no salt loss, but there is hyperandrogenism and impaired cortisol synthesis. All CAH cases are caused by mutations in the single functional adrenal 21-hydroxylase gene, *CYP21A2*; the 3 "forms" of CAH simply reflect the degree to which the *CYP21A2* gene is impaired, and represent convenient clinical pictures in a continuous spectrum of disease severity. CAH is a complicated disorder that is fairly well understood and requires intensive management [1]; this review focusses on a newly described variant called "CAH-X" in which CAH is accompanied by defects in the overlapping gene for Tenascin-X (TNX).

21-Hydroxylase Genes

Although small amounts of 21-hydroxylase activity (especially in the synthesis of aldosterone) can be catalyzed by some hepatic enzymes [2], only deficiency of the adrenal 21-hydroxylase causes CAH. Adrenal 21-hydroxylase activity is catalyzed by an enzyme called P450c21 or CYP21. The gene encoding CYP21 lies in the middle of the human leukocyte antigen (HLA) locus on chromosome 6p21.33, hence CAH is closely linked to specific HLA types [3]. The region containing the *CYP21* genes is duplicated and contains several other very closely linked (sometimes overlapping) genes, thus this locus is among the most complex in the human genome [4, 5]. The duplicated 30-kb units contain the functional *CYP21A2* gene that encodes adrenal 21-hydroxylase and the nonfunctional *CYP21A1P* pseudogene duplicated in tandem with the *C4A* and *C4B* genes that encode the fourth component of serum complement [6–8]. In some literature, the functional, centromeric, *CYP21A2* gene is termed 21B (because it is in the "B unit") and the nonfunctional, telomeric *CYP21A1P* gene is termed 21A. Somewhat surprisingly, the *CYP21A1P* pseudogene is transcribed, but the resultant RNAs do not encode protein [9, 10]. The *CYP21A2* and *CYP21A1P* genes are about 3.4 kb long, are divided into 10 exons, and differ in only 87 or 88 bases [11–13]. This high degree of sequence similarity indicates that these two genes are evolving in tandem through intergenic exchange of DNA. This happens because the HLA locus has a very high rate of genetic recombination. Thus, 99% of mutations causing CAH derive either from gene conversion events where some or all of the *CYP21A1P* pseudogene replaces the corresponding area of the *CYP21A2* gene, or from gene deletions where homologous recombination excises the functional gene. This high rate of genetic recombination explains why 21-hydroxylase deficiency is so much more common than deficiencies of other steroidogenic enzymes. The *CYP21* genes of most, but not all mammals are duplicated similarly, albeit with different duplication boundaries; however, while only the *CYP21A2* gene functions in human beings, only the *cyp21a1* gene corresponding to *CYP21A1P* functions in mice [14, 15] and both genes function in cattle [16, 17]. Sequencing of the gene duplication boundaries show that the human locus duplicated after mammalian speciation [18], consistent with data that indicate that some mammals have single copies of this locus [19].

Other Genes in the 21-Hydroxylase Locus

Several other genes lie in the *CYP21* gene locus, and several adrenal-specific transcripts have been identified that lack known functions (Fig. 1). The best-studied of these neighboring genes are the tandemly duplicated *C4A* and *C4B* genes that encode the two isoforms of C4, the fourth component of serum complement. The C4B protein has >99% amino acid sequence identity with C4A but has more hemolytic activity [20]. The *C4A* and *C4B* genes are ~22 kb long, although C4B may be only 16 kb long on some alleles due to a deletion within one intron [21]. In most regions of the genome, genes are separated by long segments of intergenic DNA, but in the *CYP21* locus the 3′ ends of the *C4A* and *C4B* genes are only 2,466 bp upstream from the transcriptional start sites of the *CYP21A1P* and *CYP21A2* genes, respectively. The *C4* and *CYP21* genes have remained in close physical association throughout evolution because the promoter sequences responsible for the adrenal-specific expression of the mouse [22] and human [23] *CYP21* genes lie far upstream within intron 35 of the *C4* genes. Just upstream from *C4A* is a gene now called *STK19*; it was originally called G11 [24] or RP [25], but the name *STK19* is now accepted because it encodes a serine/threonine kinase [26]. The STK19 protein is localized to the nucleus, suggesting a role in gene transcription, but its function remains unknown. There is no homologue of *STK19* immediately upstream from the *C4B* gene because much of the coding DNA in this region was lost during the duplication of the *C4/CYP21/TNX* locus, so that only 914 bases of the 3′ end of the gene lie upstream from C4B [24, 25].

The *TNXA* and *TNXB* genes lie on the opposite strand of DNA from the *C4* and *CYP21* genes and hence have the

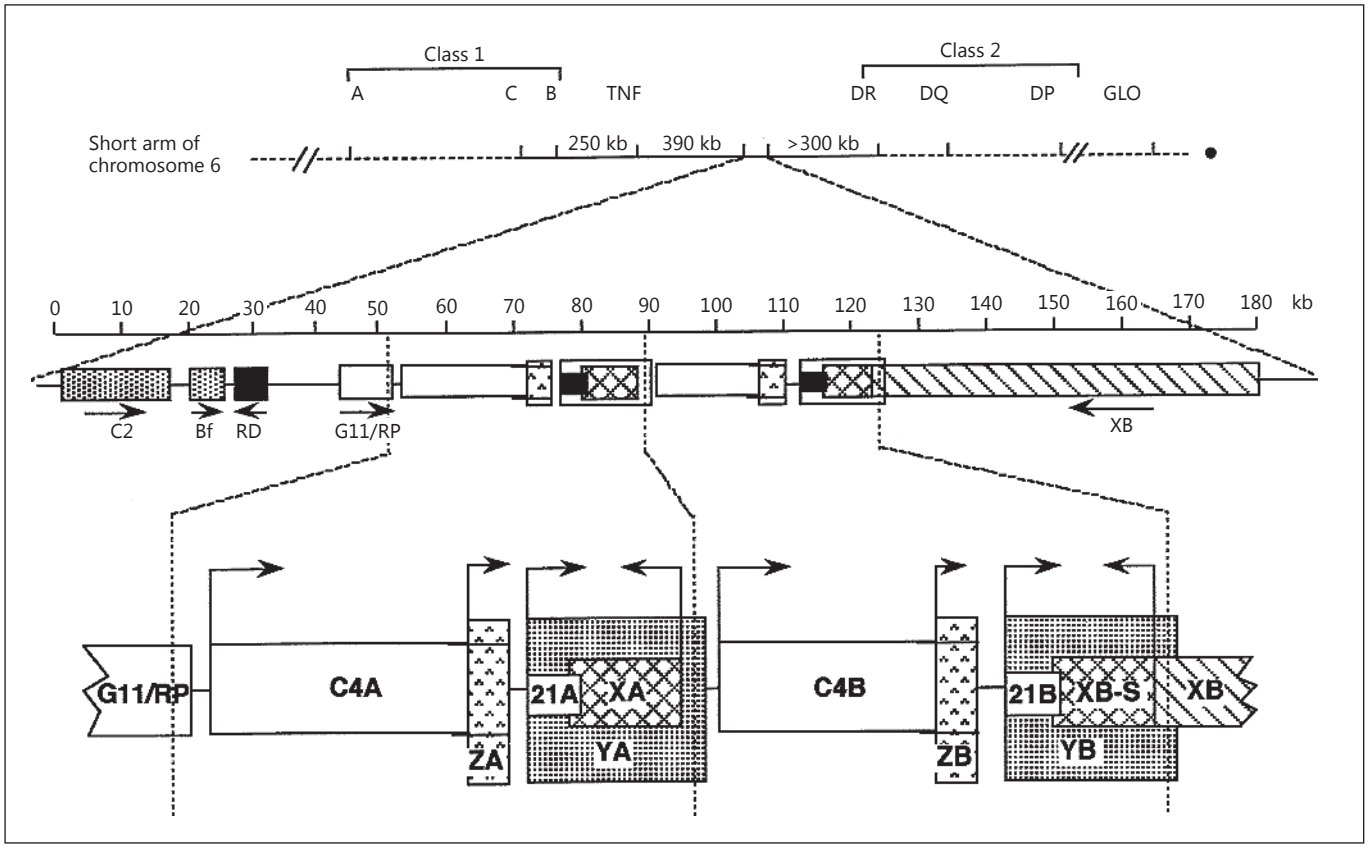

Fig. 1. The 21-hydroxylase gene locus. Top: diagram of the short arm of chromosome 6, showing the HLA class 1 and class 2 regions; the telomere is to the left and the centromere to the right. Middle: enlarged view of ~180 kb showing the class 3 region on chromosome 6p21.33. C2, complement factor C2; Bf, properdin factor Bf; RD (now known as *NEFLE*), negative elongation factor subunit E; G11/RP (now known as *STK19*), serine/threonine kinase 19. The arrows indicate transcriptional orientation. Bottom: the duplicated 30-kb "A" and "B" units: C4A and C4B, genes for complement component 4; 21A, the inactive *CYP21A1P* pseudogene; 21B, the active *CYP21A2* gene; XA, YA, and YB, adrenal transcripts that lack open reading frames; XB, the *TNXB* gene for TNX; XB-S, a short, adrenal-specific form of TNX of unknown function; ZA and ZB, adrenal-specific transcripts with open reading frames arising from promoters within the C4 genes; the ZA and ZB promoters are essential components of the *CYP21A1P* and *CYP21A2* promoters. The vertical dotted lines designate the boundaries of the genetic duplication event that resulted in the A and B regions. Copyright W.L. Miller.

opposite transcriptional orientation. These genes partially overlap the 3′ ends of the CYP21 genes: the last exon of *TNXA* and *TNXB* lies within the 3′ untranslated region of exon 10 in *CYP21A1P* and *CYP21A2*, respectively [27], and contains fibronectin type III repeats [28]. The *TNXA* gene was truncated during the duplication of the ancestral *C4/CYP21/TNX* genetic unit, but nevertheless it is transcribed in the adrenal [18]. Immediately upstream of *TNXB* lies a gene called *CREB-RP*, which encodes a protein related to the CREB (cyclic AMP response element-binding protein) transcription factor [29]. Transcription of *TNXB* is initiated from multiple start sites in or near *CREB-RP* [30, 31]. Thus, *TNXB* is unique in having both ends overlapping other genes. The *TNXB* gene is very large, spanning 68.2 kb of DNA and includes 43 exons encoding a 12-kb mRNA that encodes the extracellular matrix protein, TNX [30, 32]. The *TNXB* gene also encodes a truncated 74-kDa form of TNX, called XB-S (XB-Short), which is identical to the carboxy-terminal 673 amino acids of TNX, arises from an intergenic promoter and is expressed uniquely in the adrenal [33]. Expression of XB-S is induced by hypoxia [34], and proteomics studies indicate that it is associated with mitotic motor kinesin Eg5, but the precise function of XB-S remains unclear [35]. In addition, RNA transcripts termed YA and YB arise from the *CYP21A1P* and *CYP21A2* promoters, re-

spectively, but do not encode protein [9]. Transcripts having an open reading frame, termed ZA and ZB, arise from a promoter element within intron 35 of the C4 genes, but it is not clear whether or not they encode protein [36].

Tenascin-X

The tenascins are a widely expressed family of extracellular matrix proteins. Their functions typically oppose those of fibronectin and are largely associated with antiadhesive effects but extend beyond cellular architecture [37]. There are 4 mammalian tenascins: according to the nomenclature first proposed by Bristow et al. [32], these are now called tenascin-C (TNC, formerly called cytotactin) [38, 39], tenascin-R (TNR, formerly called "restrictin" or "janusin") [40, 41], tenascin-X (TNX), and tenascin-W (TNW, also called tenascin-N) [42, 43]. The tenascin proteins are characterized structurally from N-terminus to C-terminus by: (i) an N-terminus with 7-amino-acid repeats flanked by cysteine residues (TNX has 4 such repeats); (ii) a series of repeats that resemble epidermal growth factor (TNX has 18.5); (iii) a stretch of fibronectin type III repeats that vary in number as a result of alternative RNA splicing (TNX has 33); and (iv) a large C-terminal domain structurally related to fibrinogen [32, 44]. The N-terminal heptad repeats mediate oligomerization; TNX [45] and TNR [46] form homo-trimers; TNC [44] and TNW form homo-hexamers. Each TNX monomer is 4,267 amino acids long with a mass of about 450 kDa; TNX is variably glycosylated and forms trimers whose masses approach 1.4 million daltons; TNX is expressed in most tissues, especially connective tissues [47].

Identification of a CAH patient with a "contiguous gene syndrome" comprising a deletion of both the *CYP21A2* and *TNXB* genes demonstrated that TNX deficiency results in Ehlers-Danlos syndrome (EDS) [48]. The causative role of TNX deficiency in connective tissue disorders is confirmed in *tnx* knockout mice [49]; it is noteworthy that mouse knockouts of other tenascins do not yield identifiable phenotypes [50–52]. EDS is typically caused by autosomal dominant mutations in collagen, but recessive forms can be caused by mutations in genes for collagen-modifying factors, such as TNX, which is associated with and stabilizes collagen fibrils [45, 53]. TNX deficiency causes a clinically distinct, more severe form of EDS, either with or without associated CAH [54–56]. Heterozygosity for severe *TNXB* mutations causing haploinsufficiency for TNX may cause "hypermobility type EDS," characterized by joint hypermobility, recurring joint dislocations, and joint pain. Among 20 obligate heterozygotes for a severely defective *TNXB* allele, 9 of 14 females but no males had hypermobility EDS [57]. The diagnosis of TNX-deficient EDS or TNX haploinsufficiency is facilitated by the existence of a 140-kDa proteolytic fragment of TNX in normal serum, but not in the sera of TNX-deficient patients; this 140-kDa fragment of TNX can be measured easily [54, 58].

Beyond classical EDS, TNX is important in development as it promotes epithelial-mesenchymal transitions via TGF-β [59], and may be associated with tumor invasion [60, 61]. TNX deficiency has been associated with primary myopathy [62, 63], recurrent gastrointestinal perforation [64], and missense mutations in TNX have been found in vesicoureteral reflux [65, 66]. TNX, which is expressed in the leptomeninges and choroid plexus [67, 68], may play a role in brain and behavior. Single nucleotide polymorphisms in *TNXB* are associated with schizophrenia [69, 70], and *tnxb* knockout mice have increased anxiety, improved memory, and higher sensorimotor coordination than control animals [71].

CAH-X

The initial report of TNX deficiency was in a single patient with CAH and EDS [48]. To evaluate whether isolated TNX deficiency was associated with EDS, Schalkwijk et al. [54] evaluated 151 patients with EDS of unknown etiology and found that 5 patients had TNX deficiency, one of whom had CAH. These initial studies of TNX deficiency were consistent with an autosomal recessive pattern of inheritance, and the 2 patients with concomitant CAH had 30-kb deletions involving both the *CYP21A2* and *TNXB* genes [48, 54]. Relatives who were heterozygous for *TNXB* mutations were either asymptomatic or had mild hypermobility-type EDS of unknown clinical importance [57].

The first evaluation of the potential clinical implications of *TNXB* heterozygosity in CAH patients was performed in an ongoing observational study of CAH at the National Institutes of Health Clinical Center. In a prospective study, 193 consecutive unrelated patients with CAH were evaluated clinically for manifestations of EDS and genetically for *TNXB* mutations. Heterozygosity for a *TNXB* deletion was present in 7% of CAH patients; these CAH patients were more likely than age- and sex-matched CAH patients with normal *TNXB* to have joint hypermobility, chronic joint pain, multiple joint disloca-

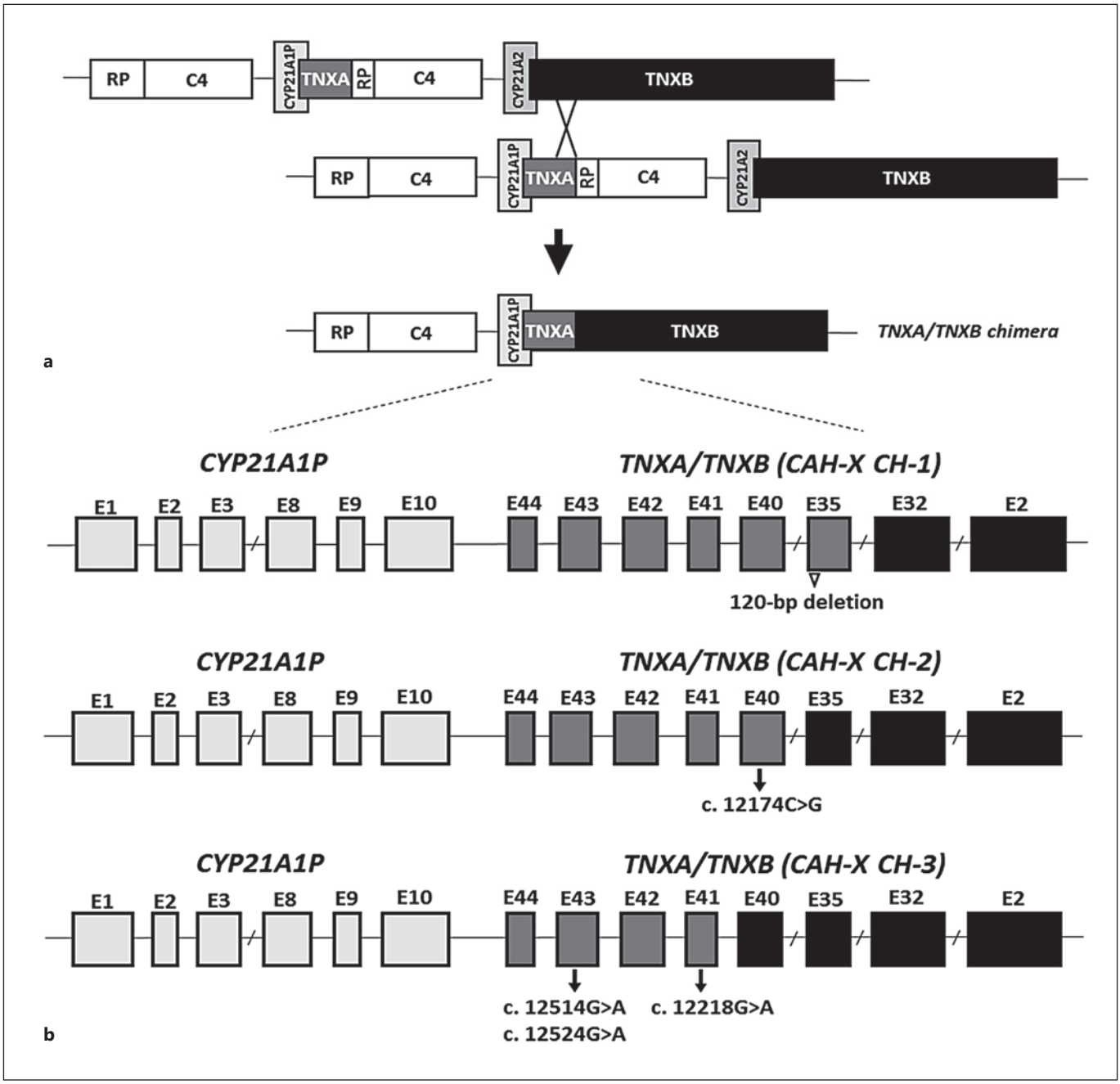

Fig. 2. Schematic diagram of the *TNXA/TNXB* chimeric genes. Most common is a bimodular *RP-C4-CYP21-TNX* region. *TNXB* encodes the active tenascin-X gene (black) and *TNXA* encodes the corresponding pseudogene (dark gray). *CYP21A2* encodes the active 21-hydroxylase gene (medium gray) and *CYP21A1P* encodes the corresponding pseudogene (light gray). **a** Formation of a *TNXA/TNXB* chimeric gene due to misalignment during meiosis resulting in deletion of the *CYP21A2* gene. **b** A schematic of exons (rectangles) of representative *TNXA/TNXB* chimera. *TNXA/TNXB* chimeric genes have been classified into 3 types (CH-1 to CH-3) based on the junction site location. The 120-bp deletion at the boundary of exon 35 and intron 35 of *TNXB* is shown by an open triangle and present in CAH-X CH-1. The c.12174C>G pseudogene variant in exon 40 identifies CAH-X CH-2, and CAH-X CH-3 is characterized by a cluster of 3 pseudogene variants: c.12218G>A in exon 41, and c.12514 G>A, and c.12524 G>A in exon 43. Adapted from Morissette et al. [78], with permission.

Table 1. Clinical characteristics of patients with CAH-X

	Monoallelic CAH-X CH-1[a] (n = 14)	Monoallelic CAH-X CH-2[b] (n = 10)	Biallelic CAH-X[c] (n = 5)
Age, years	18.1±8.3 (8–32)	20.8±16.4 (2–45)	24.0±7.4 (14–32)
Females	8 (57.1)	6 (60.0)	1 (20.0)
Musculoskelatal			
Generalized hypermobility[d]	7 (50.0)	10 (100.0)	5 (100.0)
Subluxations	5 (35.7)	4 (40.0)	2 (40.0)
Chronic arthralgia	5 (35.7)	4 (40.0)	2 (40.0)
Chronic tendonitis, bursitis or fasciitis	2 (11.8)	3 (30.0)	2 (40.0)
Dermatologic			
Hyperextensible skin	0	4 (40.0)	5 (100.0)
Wide scars	3 (21.4)	2 (20.0)	2 (40.0)
Easy bruising	5 (35.7)	4 (40.0)	5 (100.0)
Poor wound healing	0	0	2 (40.0)
Gastrointestinal			
Chronic disorder[e]	1 (7.1)	4 (40.0)	1 (20.0)
Hernia or rectal prolapse	0	3 (30.0)	2 (40.0)
Cardiac			
Congenital defect[f]	3 (21.4)	3 (30.0)	0
Chamber enlargement	2 (11.8)	0	2 (40.0)
Enlarged aortic root	0	2 (20.0)	0

Figures indicate mean ± SD (range) or n (%). [a] In Merke et al. [72]. [b] In Morissette et al. [78]. [c] In Burch et al. [48]; Schalkwijk et al. [54]; Chen et al. [79]. [d] Generalized hypermobility defined as a Beighton score of 5 of 9 or greater for children and of 4 of 9 or greater for postpubertal adolescents and adults. [e] Chronic disorder includes gastroesophageal reflux or irritable bowel syndrome. [f] Congenital heart defect includes structural valve abnormality, left ventricular diverticulum, and patent foramen ovale.

tions, and a structural cardiac valve abnormality by echocardiography [72]. Six of 13 probands had a cardiac abnormality including the rare finding of a quadricuspid aortic valve [73], a left ventricular diverticulum and an elongated anterior mitral valve leaflet [72]. Six parents, representing relatives who did not have CAH but were heterozygous for a *TNXB* deletion, were also evaluated. Similarly to earlier findings with relatives of autosomal recessive TNX-deficient EDS patients, parents displayed variable symptoms ranging from no manifestations of EDS to hypermobility-type EDS, 2 with cardiac findings (dilated aortic root and redundant anterior mitral leaflet) [72]. In general, carrying a contiguous deletion of the *CYP21A2* and *TNXB* genes resulted in a more severe EDS phenotype in CAH patients than in their CAH-unaffected relatives. As a result of this study, the term CAH-X was coined to describe the subset of CAH patients who display an EDS phenotype due to the monoallelic presence of a *CYP21A2* deletion extending into the *TNXB* gene.

The study of CAH-X has provided insight into the recombination events that occur in the class III region of the MHC locus. This region of the genome is predisposed to genetic recombination. Because both the *CYP21A2* and *TNXB* genes have corresponding homologous pseudogenes (*CYP21A1P* and *TNXA*), misalignment may occur during meiosis. Approximately 20% of deleterious *CYP21A2* mutations are 30-kb gene deletions that result in *CYP21A1P/CYP21A2* chimeric genes [74–76]. Nine types of *CYP21A1P/CYP21A2* chimeras have been identified and named chronologically CH-1 to CH-9 following determination of the junction sites [77]. Similarly, chimeric recombination occurs between *TNXB* and *TNXA* (Fig. 2a). Such recombination events delete *CYP21A2*, and therefore also represent an allele causing CAH. The contiguous gene deletion described in the first studies of *TNXB* in EDS [48, 54] and CAH populations [72] was a *TNXA/TNXB* chimeric gene [48] which was later termed *TNXA/TNXB* CH-1 or CAH-X CH-1, in keeping with *CYP21A1P/CYP21A2* terminology [78]. CAH-X CH-1 retains a 120-bp deletion derived from the *TNXA* pseudogene and renders the gene nonfunctional, resulting in reduced dermal and serum

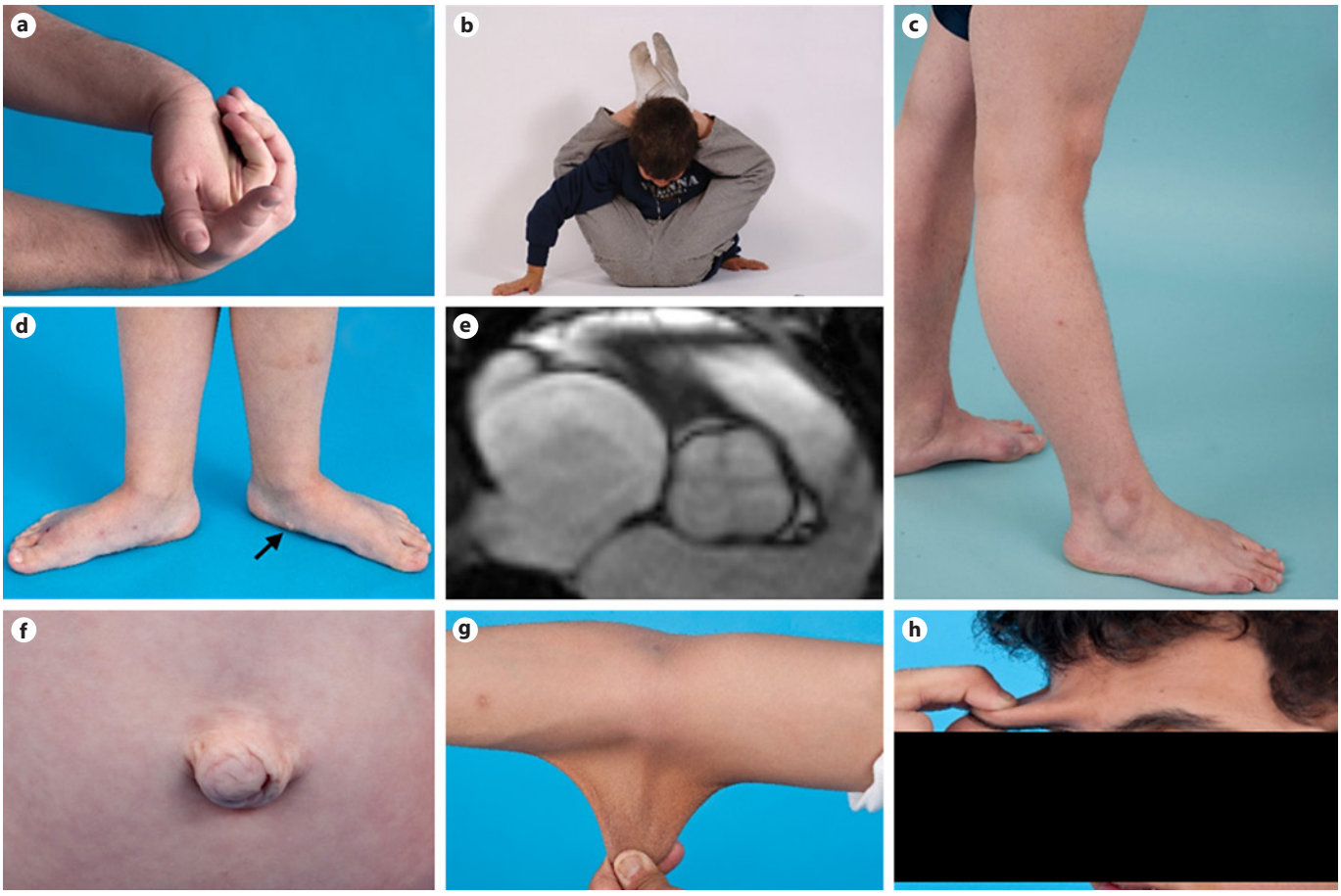

Fig. 3. Clinical manifestations of CAH-X. Patients with CAH-X due to heterozygosity of a *TNXA/TNXB* chimera commonly have hypermobility of small joints (**a**) and large joints (**b**, **c**). Pes planus and piezogenic papules (arrow) (**d**) are frequently observed. Approximately 25% of patients have a congenital cardiac defect. **e** A quadricuspic aortic valve in a patient heterozygous for CAH-X CH-1 [73]. Hernias are most often observed in patients heterozygous for CAH-X CH-2 (**f**), or biallelic for CAH-X. Hyperextensible skin is observed with CAH-X CH-2, and most severe with biallelic CAH-X (**g**, **h**). **c** and **f** are from Morissette et al. [78], with permission.

TNX expression supporting a haploinsufficent mechanism [54, 72].

After the initial description of CAH-X in a large cohort of patients with CAH, novel *TNXA/TNXB* chimeras, named CAH-X CH-2 and CAH-X CH-3, were identified [78, 79]. CAH-X CH-2 is characterized by the variant c.12174C>G (p.C4058W) derived from the *TNXA* pseudogene [78], and CAH-X CH-3 is characterized by a cluster of 3 closely linked mutations (p.R4073H, p.D4172N, and p.S4175N), also derived from the *TNXA* pseudogene [79] (Fig. 2b). Unlike CAH-X CH-1, both CAH-X CH-2 and CAH-X CH-3 cause structural changes in the TNX protein. CH-2 (p.C4058W) deletes a cysteine that forms a disulfide bond, and computational studies show that it alters protein structure [78]. CH-3 changes three residues (p.R4073H, p.D4172N, and p.S4175N). The p.R4073H change is predicted to reduce protein folding energy by interfering with a cation-pi interaction between p.R4073 and p.F4080 [79]. The changes p.D4172N and p.S4175N are not predicted to significantly affect the folding energies in the models, but computational analysis is imperfect and future experimental verification is needed. Because CAH-X CH-2 and CH-3 produce altered proteins, rather than reducing TNX expression, we no longer use the term "haploinsufficiency" in describing the monoallelic presence of a *TNXA/TNXB* chimera. Similarly, the term "autosomal recessive" is not used to describe patients with CAH-X who have *TNXB* disease causing mutations on both alleles because "autosomal recessive" implies that having one affected allele

does not result in a clinical phenotype. This is not the case with CAH-X.

To date, 24 patients (19 families) with monoallelic CAH-X [72, 78] and 5 patients (5 families) with biallelic CAH-X [48, 54, 79] have been described. Approximately 10% of patients with CAH due to 21-hydroxylase deficiency are now estimated to be affected by CAH-X [78]. Overall, CAH-X patients have generalized joint hypermobility, subluxations, chronic arthralgia and about 25% have cardiac abnormalities (Table 1; Fig. 3). CAH-X CH-2 causes a more severe phenotype than CAH-X CH-1, characterized by greater skin and joint involvement [78] (Table 1). Patients heterozygous for CAH-X CH-1 have normal skin, while 40% of CAH-X CH-2 patients have skin laxity. Gastrointestinal disorders, such as chronic gastroesophageal reflux and irritable bowel syndrome, and hernia or rectal prolapse are more commonly found in patients heterozygous for CAH-X CH-2 than CAH-X CH-1 (Table 1). Other clinical findings in monoallelic CAH-X include bifid uvula (n = 4), scoliosis (n = 3), pectus excavatum (n = 1), and early-onset osteoarthritis (n = 1). CAH-X CH-3 has only been described in biallelic CAH-X, so the CAH phenotype associated with heterozygosity for CAH-X CH-3 is unknown. Once again, relatives of CAH-X patients who carry a CAH-X CH-2 or CAH-X CH-3 allele but do not have CAH had a milder phenotype than CAH-X patients, although the majority of relatives had hypermobile joints and 2 had cardiac findings (atrial septum aneurysm with patent foramen ovale, and chamber enlargement) [78, 79].

All patients with biallelic CAH-X show severe skin hyperextensibility (Fig. 3g, h), and significant joint hypermobility, and 2 (19-year-old male, 26–year-old male) had delayed wound healing [48, 79]. Other EDS manifestations in CAH-X biallelic patients include multiple joint dislocations, chronic arthralgia, chronic tendonitis and/or bursitis, rectal prolapse, severe gastroesophageal reflux, and cardiac abnormalities (ventricular enlargement in 2). Biallelic CAH-X patients appear to have a more severe phenotype than biallelic TNX-deficient-type EDS patients without CAH who have normal wound healing [54, 56, 80]. *TNXA/TNXB* chimeras may lead to a more severe phenotype than *TNXB* missense or nonsense mutations, but the hormonal factors characteristic of CAH including chronic glucocorticoid treatment may also influence phenotype.

Most previously reported *TNXB* mutations causing EDS are located in the region encoding the EGF-like repeats or the fibronectin type III domain of TNX, while *TNXA/TNXB* chimeras either interfere with TNX production (CAH-X CH-1) or affect the fibrinogen-like domain (CAH-X CH-2, CAH-X CH-3). The junction site of a *TNXA/TNXB* chimera could be anywhere between exons 32 and 44, the homologous region between *TNXA* and *TNXB*. Unlike *CYP21A2* and *CYP21A1P*, variations in the sequences of *TNXB* and *TNXA* are not well characterized and novel chimeras may yet exist. Chimeric junction sites can be clinically meaningful. Two rare *CYP21A1P/CYP21A2* chimeras (CH-4 and CH-9) result in a milder CAH phenotype than other *CYP21A1P/CYP21A2* chimeras [77, 81]. Mutation-specific effects on TNX protein expression have been described between CAH-X chimeras and might occur across the spectrum of *TNXB* mutations. Further studies are needed, as variations within the *TNXA* and *TNXB* genes have not been investigated in detail.

Extensive studies of the *CYP21A2* gene led to the discovery of *TNXB* and have expanded our understanding of the spectrum of TNX-related disorders. The study of CAH-X led to the discovery of different *TNXA/TNXB* chimeras; 3 have been identified to date. The TNX protein interacts with TGF-β [59], but the precise molecular mechanisms have yet to be determined. The identification of the CAH-X syndrome and additional *TNXB*-related diseases promise to expand our understanding of this widely expressed extracellular matrix protein in various disease processes.

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Disclosure Statement

The authors have no conflicts of interest to declare.

References

1 Speiser PW, Azziz R, Baskin LS, Ghizzoni L, Hensle TW, Merke DP et al; Endocrine Society. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010 Sep;95(9):4133–60.

2 Gomes LG, Huang N, Agrawal V, Mendonça BB, Bachega TA, Miller WL. Extraadrenal 21-hydroxylation by CYP2C19 and CYP3A4: effect on 21-hydroxylase deficiency. J Clin Endocrinol Metab. 2009 Jan;94(1):89–95.

3 Dupont B, Oberfield SE, Smithwick EM, Lee TD, Levine LS. Close genetic linkage between HLA and congenital adrenal hyperplasia (21-hydroxylase deficiency). Lancet. 1977 Dec;2(8052-8053):1309–12.
4 Morel Y, Miller WL. Clinical and molecular genetics of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Adv Hum Genet. 1991;20:1–68.
5 Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev. 2011 Feb;32(1):81–151.
6 Amor M, Tosi M, Duponchel C, Steinmetz M, Meo T. Liver mRNA probes disclose two cytochrome P-450 genes duplicated in tandem with the complement C4 loci of the mouse H-2S region. Proc Natl Acad Sci USA. 1985 Jul;82(13):4453–7.
7 Carroll MC, Campbell RD, Porter RR. Mapping of steroid 21-hydroxylase genes adjacent to complement component C4 genes in HLA, the major histocompatibility complex in man. Proc Natl Acad Sci USA. 1985 Jan; 82(2):521–5.
8 White PC, Grossberger D, Onufer BJ, Chaplin DD, New MI, Dupont B et al. Two genes encoding steroid 21-hydroxylase are located near the genes encoding the fourth component of complement in man. Proc Natl Acad Sci USA. 1985 Feb;82(4):1089–93.
9 Bristow J, Gitelman SE, Tee MK, Staels B, Miller WL. Abundant adrenal-specific transcription of the human P450c21A "pseudogene". J Biol Chem. 1993 Jun;268(17):12919–24.
10 Chang SF, Chung BC. Difference in transcriptional activity of two homologous CYP21A genes. Mol Endocrinol. 1995 Oct;9(10):1330–6.
11 Higashi Y, Yoshioka H, Yamane M, Gotoh O, Fujii-Kuriyama Y. Complete nucleotide sequence of two steroid 21-hydroxylase genes tandemly arranged in human chromosome: a pseudogene and a genuine gene. Proc Natl Acad Sci USA. 1986 May;83(9):2841–5.
12 White PC, New MI, Dupont B. Structure of human steroid 21-hydroxylase genes. Proc Natl Acad Sci USA. 1986 Jul;83(14):5111–5.
13 Rodrigues NR, Dunham I, Yu CY, Carroll MC, Porter RR, Campbell RD. Molecular characterization of the HLA-linked steroid 21-hydroxylase B gene from an individual with congenital adrenal hyperplasia. EMBO J. 1987 Jun;6(6):1653–61.
14 Parker KL, Chaplin DD, Wong M, Seidman JG, Smith JA, Schimmer BP. Expression of murine 21-hydroxylase in mouse adrenal glands and in transfected Y1 adrenocortical tumor cells. Proc Natl Acad Sci USA. 1985 Dec;82(23):7860–4.
15 Chaplin DD, Galbraith LJ, Seidman JG, White PC, Parker KL. Nucleotide sequence analysis of murine 21-hydroxylase genes: mutations affecting gene expression. Proc Natl Acad Sci USA. 1986 Dec;83(24):9601–5.
16 Chung BC, Matteson KJ, Miller WL. Structure of a bovine gene for P-450c21 (steroid 21-hydroxylase) defines a novel cytochrome P-450 gene family. Proc Natl Acad Sci USA. 1986 Jun;83(12):4243–7.
17 John ME, Okamura T, Dee A, Adler B, John MC, White PC et al. Bovine steroid 21-hydroxylase: regulation of biosynthesis. Biochemistry. 1986 May;25(10):2846–53.
18 Gitelman SE, Bristow J, Miller WL. Mechanism and consequences of the duplication of the human C4/P450c21/gene X locus. Mol Cell Biol. 1992 Jul;12(7):3313–4.
19 Geffrotin C, Chardon P, de Andres-Cara DF, Feil R, Renard C, Vaiman M. The swine steroid 21-hydroxylase gene (CYP21): cloning and mapping within the swine leucocyte antigen complex. Anim Genet. 1990;21(1):1–13.
20 Law SK, Dodds AW, Porter RR. A comparison of the properties of two classes, C4A and C4B, of the human complement component C4. EMBO J. 1984 Aug;3(8):1819–23.
21 Yu CY, Belt KT, Giles CM, Campbell RD, Porter RR. Structural basis of the polymorphism of human complement components C4A and C4B: gene size, reactivity and antigenicity. EMBO J. 1986 Nov;5(11):2873–81.
22 Milstone DS, Shaw SK, Parker KL, Szyf M, Seidman JG. An element regulating adrenal-specific steroid 21-hydroxylase expression is located within the slp gene. J Biol Chem. 1992 Oct;267(30):21924–7.
23 Wijesuriya SD, Zhang G, Dardis A, Miller WL. Transcriptional regulatory elements of the human gene for cytochrome P450c21 (steroid 21-hydroxylase) lie within intron 35 of the linked C4B gene. J Biol Chem. 1999 Dec; 274(53):38097–106.
24 Sargent CA, Anderson MJ, Hsieh SL, Kendall E, Gomez-Escobar N, Campbell RD. Characterisation of the novel gene G11 lying adjacent to the complement C4A gene in the human major histocompatibility complex. Hum Mol Genet. 1994 Mar;3(3):481–8.
25 Shen L, Wu LC, Sanlioglu S, Chen R, Mendoza AR, Dangel AW et al. Structure and genetics of the partially duplicated gene RP located immediately upstream of the complement C4A and the C4B genes in the HLA class III region. Molecular cloning, exon-intron structure, composite retroposon, and breakpoint of gene duplication. J Biol Chem. 1994 Mar;269(11):8466–76.
26 Gomez-Escobar N, Chou CF, Lin WW, Hsieh SL, Campbell RD. The G11 gene located in the major histocompatibility complex encodes a novel nuclear serine/threonine protein kinase. J Biol Chem. 1998 Nov;273(47):30954–60.
27 Morel Y, Bristow J, Gitelman SE, Miller WL. Transcript encoded on the opposite strand of the human steroid 21-hydroxylase/complement component C4 gene locus. Proc Natl Acad Sci USA. 1989 Sep;86(17):6582–6.
28 Matsumoto K, Arai M, Ishihara N, Ando A, Inoko H, Ikemura T. Cluster of fibronectin type III repeats found in the human major histocompatibility complex class III region shows the highest homology with the repeats in an extracellular matrix protein, tenascin. Genomics. 1992 Mar;12(3):485–91.
29 Min J, Shukla H, Kozono H, Bronson SK, Weissman SM, Chaplin DD. A novel Creb family gene telomeric of HLA-DRA in the HLA complex. Genomics. 1995 Nov;30(2): 149–56.
30 Speek M, Barry F, Miller WL. Alternate promoters and alternate splicing of human Tenascin-X, a gene with 5′ and 3′ ends buried in other genes. Hum Mol Genet. 1996 Nov; 5(11):1749–58.
31 Wijesuriya SD, Bristow J, Miller WL. Localization and analysis of the principal promoter for human Tenascin-X. Genomics. 2002 Oct; 80(4):443–52.
32 Bristow J, Tee MK, Gitelman SE, Mellon SH, Miller WL. Tenascin-X: a novel extracellular matrix protein encoded by the human XB gene overlapping P450c21B. J Cell Biol. 1993 Jul;122(1):265–78.
33 Tee MK, Thomson AA, Bristow J, Miller WL. Sequences promoting the transcription of the human XA gene overlapping P450c21A correctly predict the presence of a novel, adrenal-specific, truncated form of Tenascin-X. Genomics. 1995 Jul;28(2):171–8.
34 Kato A, Endo T, Abiko S, Ariga H, Matsumoto K. Induction of truncated form of Tenascin-X (XB-S) through dissociation of HDAC1 from SP-1/HDAC1 complex in response to hypoxic conditions. Exp Cell Res. 2008 Aug;314(14):2661–73.
35 Endo T, Ariga H, Matsumoto K. Truncated form of Tenascin-X, XB-S, interacts with mitotic motor kinesin Eg5. Mol Cell Biochem. 2009 Jan;320(1-2):53–66.
36 Tee MK, Babalola GO, Aza-Blanc P, Speek M, Gitelman SE, Miller WL. A promoter within intron 35 of the human C4A gene initiates abundant adrenal-specific transcription of a 1 kb RNA: location of a cryptic CYP21 promoter element? Hum Mol Genet. 1995 Nov;4(11): 2109–16.
37 Valcourt U, Alcaraz LB, Exposito JY, Lethias C, Bartholin L. Tenascin-X: beyond the architectural function. Cell Adh Migr. 2015;9(1–2):154–65.
38 Grumet M, Hoffman S, Crossin KL, Edelman GM. Cytotactin, an extracellular matrix protein of neural and non-neural tissues that mediates glia-neuron interaction. Proc Natl Acad Sci USA. 1985 Dec;82(23):8075–9.
39 Pearson CA, Pearson D, Shibahara S, Hofsteenge J, Chiquet-Ehrismann R. Tenascin: cDNA cloning and induction by TGF-beta. EMBO J. 1988 Oct;7(10):2977–82.
40 Rathjen FG, Wolff JM, Chiquet-Ehrismann R. Restrictin: a chick neural extracellular matrix protein involved in cell attachment co-purifies with the cell recognition molecule F11. Development. 1991 Sep;113(1):151–64.

41 Schachner M, Taylor J, Bartsch U, Pesheva P. The perplexing multifunctionality of janusin, a tenascin-related molecule. Perspect Dev Neurobiol. 1994;2(1):33–41.
42 Neidhardt J, Fehr S, Kutsche M, Löhler J, Schachner M. Tenascin-N: characterization of a novel member of the tenascin family that mediates neurite repulsion from hippocampal explants. Mol Cell Neurosci. 2003 Jun;23(2):193–209.
43 Scherberich A, Tucker RP, Samandari E, Brown-Luedi M, Martin D, Chiquet-Ehrismann R. Murine tenascin-W: a novel mammalian tenascin expressed in kidney and at sites of bone and smooth muscle development. J Cell Sci. 2004 Feb;117(Pt 4):571–81.
44 Spring J, Beck K, Chiquet-Ehrismann R. Two contrary functions of tenascin: dissection of the active sites by recombinant tenascin fragments. Cell. 1989 Oct;59(2):325–34.
45 Lethias C, Carisey A, Comte J, Cluzel C, Exposito JY. A model of tenascin-X integration within the collagenous network. FEBS Lett. 2006 Nov;580(26):6281–5.
46 Nörenberg U, Wille H, Wolff JM, Frank R, Rathjen FG. The chicken neural extracellular matrix molecule restrictin: similarity with EGF-, fibronectin type III-, and fibrinogen-like motifs. Neuron. 1992 May;8(5):849–63.
47 Burch GH, Bedolli MA, McDonough S, Rosenthal SM, Bristow J. Embryonic expression of tenascin-X suggests a role in limb, muscle, and heart development. Dev Dyn. 1995 Aug;203(4):491–504.
48 Burch GH, Gong Y, Liu W, Dettman RW, Curry CJ, Smith L et al. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. Nat Genet. 1997 Sep;17(1):104–8.
49 Mao JR, Taylor G, Dean WB, Wagner DR, Afzal V, Lotz JC et al. Tenascin-X deficiency mimics Ehlers-Danlos syndrome in mice through alteration of collagen deposition. Nat Genet. 2002 Apr;30(4):421–5.
50 Saga Y, Yagi T, Ikawa Y, Sakakura T, Aizawa S. Mice develop normally without tenascin. Genes Dev. 1992 Oct;6(10):1821–31.
51 Forsberg E, Hirsch E, Fröhlich L, Meyer M, Ekblom P, Aszodi A et al. Skin wounds and severed nerves heal normally in mice lacking tenascin-C. Proc Natl Acad Sci USA. 1996 Jun;93(13):6594–9.
52 Weber P, Bartsch U, Rasband MN, Czaniera R, Lang Y, Bluethmann H et al. Mice deficient for Tenascin-R display alterations of the extracellular matrix and decreased axonal conduction velocities in the CNS. J Neurosci. 1999 Jun;19(11):4245–62.
53 Elefteriou F, Exposito JY, Garrone R, Lethias C. Characterization of the bovine Tenascin-X. J Biol Chem. 1997 Sep;272(36):22866–74.
54 Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen IM et al. A recessive form of the Ehlers-Danlos syndrome caused by Tenascin-X deficiency. N Engl J Med. 2001 Oct;345(16):1167–75.
55 Lindor NM, Bristow J. Tenascin-X deficiency in autosomal recessive Ehlers-Danlos syndrome. Am J Med Genet A. 2005 May;135(1):75–80.
56 Demirdas S, Dulfer E, Robert L, Kempers M, van Beek D, Micha D et al. Recognizing the Tenascin-X deficient type of Ehlers-Danlos syndrome: a cross-sectional study in 17 patients. Clin Genet. 2017 Mar;91(3):411–25.
57 Zweers MC, Bristow J, Steijlen PM, Dean WB, Hamel BC, Otero M et al. Haploinsufficiency of TNXB is associated with hypermobility type of Ehlers-Danlos syndrome. Am J Hum Genet. 2003 Jul;73(1):214–7.
58 Yamada K, Watanabe A, Takeshita H, Matsumoto KI. A method for quantification of serum Tenascin-X by nano-LC/MS/MS. Clin Chim Acta. 2016 Aug;459:94–100.
59 Alcaraz LB, Exposito JY, Chuvin N, Pommier RM, Cluzel C, Martel S et al. Tenascin-X promotes epithelial-to-mesenchymal transition by activating latent TGF-β. J Cell Biol. 2014 May;205(3):409–28.
60 Matsumoto K, Takayama N, Ohnishi J, Ohnishi E, Shirayoshi Y, Nakatsuji N et al. Tumour invasion and metastasis are promoted in mice deficient in Tenascin-X. Genes Cells. 2001 Dec;6(12):1101–11.
61 Lévy P, Ripoche H, Laurendeau I, Lazar V, Ortonne N, Parfait B et al. Microarray-based identification of tenascin C and Tenascin XB, genes possibly involved in tumorigenesis associated with neurofibromatosis type 1. Clin Cancer Res. 2007 Jan;13(2 Pt 1):398–407.
62 Voermans NC, van Alfen N, Pillen S, Lammens M, Schalkwijk J, Zwarts MJ et al. Neuromuscular involvement in various types of Ehlers-Danlos syndrome. Ann Neurol. 2009 Jun;65(6):687–97.
63 Pénisson-Besnier I, Allamand V, Beurrier P, Martin L, Schalkwijk J, van Vlijmen-Willems I et al. Compound heterozygous mutations of the TNXB gene cause primary myopathy. Neuromuscul Disord. 2013 Aug;23(8):664–9.
64 Sakiyama T, Kubo A, Sasaki T, Yamada T, Yabe N, Matsumoto K et al. Recurrent gastrointestinal perforation in a patient with Ehlers-Danlos syndrome due to Tenascin-X deficiency. J Dermatol. 2015 May;42(5):511–4.
65 Gbadegesin RA, Brophy PD, Adeyemo A, Hall G, Gupta IR, Hains D et al. TNXB mutations can cause vesicoureteral reflux. J Am Soc Nephrol. 2013 Jul;24(8):1313–22.
66 Tokhmafshan F, Brophy PD, Gbadegesin RA, Gupta IR. Vesicoureteral reflux and the extracellular matrix connection. Pediatr Nephrol. 2017 Apr;32(4):565–76.
67 Geffrotin C, Garrido JJ, Tremet L, Vaiman M. Distinct tissue distribution in pigs of Tenascin-X and Tenascin-C transcripts. Eur J Biochem. 1995 Jul;231(1):83–92.
68 Imura K, Sato I. Identification of the novel localization of TenascinX in the monkey choroid plexus and comparison with the mouse. Eur J Histochem. 2009 Dec;53(4):225–31.
69 Wei J, Hemmings GP. TNXB locus may be a candidate gene predisposing to schizophrenia. Am J Med Genet B Neuropsychiatr Genet. 2004 Feb;125B(1):43–9.
70 Tochigi M, Zhang X, Ohashi J, Hibino H, Otowa T, Rogers M et al. Association study between the TNXB locus and schizophrenia in a Japanese population. Am J Med Genet B Neuropsychiatr Genet. 2007 Apr;144B(3):305–9.
71 Kawakami K, Matsumoto K. Behavioral alterations in mice lacking the gene for Tenascin-X. Biol Pharm Bull. 2011;34(4):590–3.
72 Merke DP, Chen W, Morissette R, Xu Z, Van Ryzin C, Sachdev V et al. Tenascin-X haploinsufficiency associated with Ehlers-Danlos syndrome in patients with congenital adrenal hyperplasia. J Clin Endocrinol Metab. 2013 Feb;98(2):E379–87.
73 Chen W, Kim MS, Shanbhag S, Arai A, Van-Ryzin C, McDonnell NB et al. The phenotypic spectrum of contiguous deletion of CYP21A2 and tenascin XB: quadricuspid aortic valve and other midline defects. Am J Med Genet A. 2009 Dec;149A(12):2803–8.
74 Vrzalová Z, Hrubá Z, Hrabincová ES, Vrábelová S, Votava F, Kolouškova S et al. Chimeric CYP21A1P/CYP21A2 genes identified in Czech patients with congenital adrenal hyperplasia. Eur J Med Genet. 2011 Mar-Apr;54(2):112–7.
75 Finkielstain GP, Chen W, Mehta SP, Fujimura FK, Hanna RM, Van Ryzin C et al. Comprehensive genetic analysis of 182 unrelated families with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab. 2011 Jan;96(1):E161–72.
76 Krone N, Braun A, Roscher AA, Knorr D, Schwarz HP. Predicting phenotype in steroid 21-hydroxylase deficiency? Comprehensive genotyping in 155 unrelated, well defined patients from southern Germany. J Clin Endocrinol Metab. 2000 Mar;85(3):1059–65.
77 Chen W, Xu Z, Sullivan A, Finkielstain GP, Van Ryzin C, Merke DP et al. Junction site analysis of chimeric CYP21A1P/CYP21A2 genes in 21-hydroxylase deficiency. Clin Chem. 2012 Feb;58(2):421–30.
78 Morissette R, Chen W, Perritt AF, Dreiling JL, Arai AE, Sachdev V et al. Broadening the Spectrum of Ehlers Danlos Syndrome in Patients With Congenital Adrenal Hyperplasia. J Clin Endocrinol Metab. 2015 Aug;100(8):E1143–52.
79 Chen W, Perritt AF, Morissette R, Dreiling JL, Bohn MF, Mallappa A et al. Ehlers-Danlos Syndrome Caused by Biallelic TNXB Variants in Patients with Congenital Adrenal Hyperplasia. Hum Mutat. 2016 Sep;37(9):893–7.
80 Hendriks AG, Voermans NC, Schalkwijk J, Hamel BC, van Rossum MM. Well-defined clinical presentation of Ehlers-Danlos syndrome in patients with Tenascin-X deficiency: a report of four cases. Clin Dysmorphol. 2012 Jan;21(1):15–8.
81 L'Allemand D, Tardy V, Grüters A, Schnabel D, Krude H, Morel Y. How a patient homozygous for a 30-kb deletion of the C4-CYP 21 genomic region can have a nonclassic form of 21-hydroxylase deficiency. J Clin Endocrinol Metab. 2000 Dec;85(12):4562–7.