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Haploinsufficiency of *TNXB* Is Associated with Hypermobility Type of Ehlers-Danlos Syndrome

To the Editor:
Ehlers-Danlos syndrome (EDS) is a heterogeneous group of heritable connective-tissue disorders, generally affecting skin, joints, and blood vessels. The most recent classification recognizes six subtypes (Beighton et al. 1998), of which the hypermobility type (HT-EDS [formerly EDS type III] [MIM 130020]) is the most common. This type of EDS is similar to benign joint hypermobility syndrome (BJHS), and both are often considered to represent the same hyperlaxity syndrome, since no clear clinical distinction can be made (Grahame 1999). Although various causative genes have been found in all other types of EDS, the genetic basis of HT-EDS or BJHS remains unexplained (Steinmann et al. 2002). One family has been described that has a missense mutation in *COL3A1* (Narcisi et al. 1994), resulting in a phenotype that resembles HT-EDS,

without obvious vascular complications. Mutations in *COL3A1* generally result in the severe vascular type of EDS (MIM 130050). To our knowledge, no other cases of *COL3A1* mutations in HT-EDS have been reported.

Recently, we showed that deficiency of the extracellular-matrix protein tenascin-X (TNX), encoded by the *TNXB* gene, causes a new type of recessively inherited EDS (Schalkwijk et al. 2001). Patients with complete deficiency of TNX showed marked joint hypermobility, skin hyperextensibility, and easy bruising. The absence of atrophic scars and recessive inheritance distinguishes TNX deficiency from the classical type of EDS. In our initial report (Schalkwijk et al. 2001), only a few heterozygous family members were available for examination. Here, we have examined all 20 heterozygous family members (individuals from families A–D in table 1) who were available for further study, regardless of clinical symptoms; in all of these individuals, we have found significantly reduced serum TNX levels ($56\% \pm 6\%$ vs. $100\% \pm 14\%$ in the control population; $P < .001$, by Student's t test) (fig. 1*f*), and, in 17 of them, we have confirmed heterozygosity for a truncating *TNXB* mutation (table 1). Clinical examination revealed generalized joint hypermobility in nine family members (45%), using the Beighton score (Beighton et al. 1973), for HT-EDS, or the Brighton criteria (Grahame et al. 2000), for BJHS (table 1 and fig. 1*e*). Skin hyperextensibility and easy bruising, frequently seen in the individuals with complete TNX deficiency, were absent. A number of patients with haploinsufficiency had recurring joint dislocations and chronic joint pain, as are seen in HT-EDS and BJHS. Only four family members carrying two normal *TNXB* alleles were available for study, of whom none had hypermobility. The local medical ethics committee (CMO Regio Arnhem-Nijmegen) approved the study protocol, and informed consent was obtained from all patients.

A striking finding is that 0 of the 6 males with haploinsufficiency fulfilled the clinical criteria for HT-EDS or BJHS, whereas 9 of 14 (64%) females were positive. This finding is in accordance with previous population-based studies that show a female preponderance in joint hypermobility syndromes (Larsson et al. 1987; Rikken-Bultman et al. 1997). In a control group of 30 unaffected females of the same age as the females with haploinsufficiency in the present study, we found no individuals with a Beighton score >4. This indicates that the prevalence of generalized joint hypermobility in a population of females with haploinsufficiency is significantly higher than in a control population ($P < .001$, by χ^2 test). No sex differences in serum TNX levels in unaffected individuals and individuals with haploinsufficiency were found (not shown).

Because our observations in families carrying previously described *TNXB* mutations suggested an association between *TNXB* haploinsufficiency and joint

Table 1

Clinical and Molecular Findings in Individuals with *TNXB* Haploinsufficiency/Reduced Serum TNX Levels

Individual[a] (Sex)	Year of Birth	Mutation	Beighton Score	TNX Level (% of Control)	Clinical Feature(s)
AIII2 (F)	1961	[GT44906] ins	5/9	52	Velvety skin, piezogenic papules, back pain
AIII3 (M)	1964	[GT44906] ins	4/9	57	Loss of pliancy, Raynaud phenomenon
AIII6 (F)	1974	[GT44906] ins	6/9	59	Velvety skin, Raynaud phenomenon
AIII7 (F)	1977	[GT44906] ins	5/9	52	Striae
AIII8 (F)	1981	[GT44906] ins	5/9	50	Ankle sprains, knee pain
AIII9 (F)	1984	[GT44906] ins	5/9	68	Velvety skin
BI4 (M)	1931	Unknown	0/9	56	Multiple ankle sprains
BIII1 (F)	1976	Unknown	5/9	65	
BIII2 (F)	1979	Unknown	5/9	62	Wheelchair dependent, joint pain
BIII3 (F)	1981	30-kb del	5/9	62	Joint pain
CI1 (M)	1919	[AA56063] del	0/9	55	
CI5 (F)	1924	[AA56063] del	ND	54	
CII1 (F)	1944	[AA56063] del	1/9	46	
CIII3 (F)	1978	[AA56063] del	3/9	45	Piezogenic papules, lymphedema
CIII4 (F)	1980	[AA56063] del	6/9	53	Velvety skin, multiple (sub)luxations
CIII5 (M)	1981	[AA56063] del	0/9	57	
DI1 (M)	1919	30-kb del	0/9	53	Velvety skin
DI2 (F)	1931	30-kb del	0/9	54	Multiple fractures
DII1 (F)	1962	30-kb del	2/9	59	
DII2 (M)	1964	30-kb del	2/9	61	Piezogenic papules
E (F)	1961	30-kb del	3/9	58	Chronic joint pain, multiple (sub)luxations, wheelchair dependent, HT-EDS/BJHS according to Brighton criteria
F (F)	1970	[AA56063] del	6/9	52	
G1 (F)	1972	Unknown	6/9	56	Luxations, velvety skin, piezogenic papules
G2 (F)	1977	Unknown	6/9	61	Chronic pain, subluxations of multiple joints
H (F)	1961	Unknown	2/9	64	Wheelchair dependent, chronic musculoskeletal pain, shoulder luxations, HT-EDS/BJHS according to Brighton criteria
I (F)	1954	Unknown	ND	65	Diagnosis of HT-EDS
J (F)	1974	Unknown	ND	54	Diagnosis of HT-EDS

[a] Pedigrees for families A–D are depicted in figure 1. Patients E–J were identified in a cohort with HT-EDS. G2 is a sister of G1 and was identified independently of the screening of the 80 patients with HT-EDS/BJHS. We examined all patients available for study except individuals I and J.

[b] ND = not determined.

hypermobility, we wondered about the prevalence of *TNXB* haploinsufficiency in patients with HT-EDS. We measured serum TNX levels (by ELISA) in an unselected cohort of 80 patients with HT-EDS who were recruited through the Dutch organization for patients with EDS. All patients were diagnosed with HT-EDS by a medical specialist, and ~90% were female. Although the mean serum TNX level was not different in the cohort with HT-EDS overall (99.4% ± 19.7%) (fig. 1*f*), six of these patients (7.5% [all female]) had serum TNX levels >2.5 SDs (65%) below the mean for unaffected individuals. On the basis of the normal distribution of serum TNX levels, only 0.6% of individuals would be expected to have such low serum TNX levels, which is significantly less than the frequency found in the population with HT-EDS described in the present study ($P < .001$, by Fisher's exact test).

Clinically, patients with reduced TNX levels showed hypermobile joints, often associated with joint subluxations and chronic musculoskeletal pain (table 1). The clinical findings in these patients differ from those with complete TNX deficiency. Patients with haploinsufficiency do not have skin hyperextensibility and lack the easy bruising seen in patients with TNX deficiency. In addition, *TNXB* haploinsufficiency is expected to be an autosomal dominant trait, which is in accordance with the observed mode of inheritance of HT-EDS and BJHS.

On screening for the presence of a 30-kb deletion described previously (Burch et al. 1997; Schalkwijk et al. 2001), we found that this deletion was present in one of these six patients. The 30-kb deletion creates a fusion gene of *TNXB* and *XA,* a partial duplicate of *TNXB*. The *XA* gene has an internal deletion that truncates its ORF, rendering *XA* and the fusion gene nonfunctional (Gitelman et al. 1992). The deleted allele also lacks *CYP21,* so this individual is also a carrier for congenital adrenal hyperplasia. Subsequently, we PCR amplified and directly sequenced the coding regions and the intron-exon boundaries of *TNXB* in the other five patients presumed to have haploinsufficiency (for primers used, see Schalkwijk et al. 2001). One patient (individual F in table 1) was heterozygous
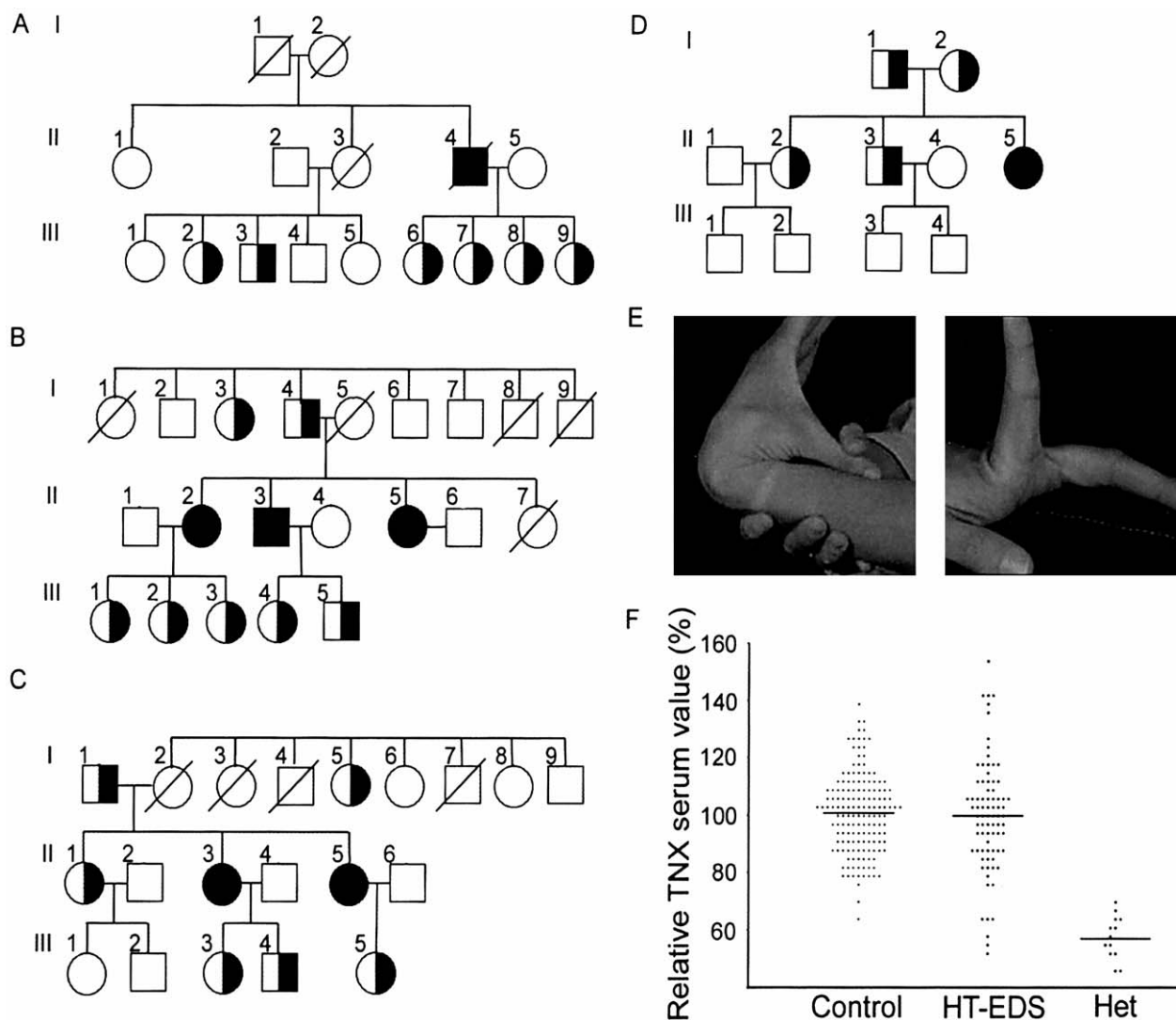
Figure 1 *TNXB* haploinsufficiency and generalized joint hypermobility. *a–d,* Pedigrees of families A–D (also see table 1). *e,* Joint hypermobility in individual III9 from family A. *f,* Distribution of serum TNX levels in control population, population with HT-EDS, and heterozygous (Het) population. Values are given as percentage of the control mean.

for a 2-bp deletion, [AA56063] del, in exon 8, resulting in a premature stop codon at the position of amino acid 1231. In the other four patients, we were unable to identify mutations in *TNXB*. These patients may have mutations, in regulatory sequences or in exons of the *TNXB* gene, that have not yet been identified, or they may represent the extreme in normal variation of TNX expression.

In conclusion, in the present study, we have reported a genetic defect associated with HT-EDS or BJHS. On the basis of the observed phenotype in patients with complete TNX deficiency and the high prevalence of generalized joint hypermobility in heterozygous females, this is

likely to be a causative relationship. Reduced TNX expression could disturb deposition of collagen (Mao et al. 2002) and the elastic fiber network (Burch et al. 1997), as has been shown for complete TNX deficiency, resulting in increased laxity of ligaments and tendons. *TNXB* haploinsufficiency is dominantly inherited and appears to produce clinical findings primarily in women, consistent with clinical descriptions of HT-EDS. Although we identified inactivating TNX mutations in only 2.5% of this cohort with HT-EDS, 7.5% had serum TNX levels low enough to affect collagen metabolism. The present study demonstrates that *TNXB* haploinsufficiency is associated with HT-EDS and suggests that locus heterogeneity exists for

this disorder, as it does for other types of EDS (Byers 1994).

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MANON C. ZWEERS,[1] JIM BRISTOW,[3]
PETER M. STEIJLEN,[1] WILLOW B. DEAN,[3]
BEN C. HAMEL,[2] MARISOL OTERO,[1]
MARTINA KUCHAREKOVA,[1] JAN B. BOEZEMAN,[1]
AND JOOST SCHALKWIJK[1]

*Departments of [1]Dermatology and [2]Human Genetics,
University Medical Center Nijmegen, Nijmegen,
The Netherlands; and [3]Department of Pediatrics,
University of California at San Francisco, San
Francisco*

Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www
.ncbi.nlm.nih.gov/Omim/ (for HT-EDS and vascular type of
EDS)

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Address for correspondence and reprints: Dr. Manon C. Zweers, Department of Dermatology, University Medical Center Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. E-mail: m.zweers@derma.umcn.nl