Transgenic mice with inactive alleles for procollagen *N*-proteinase (ADAMTS-2) develop fragile skin and male sterility

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Transgenic mice were prepared with inactive alleles for procollagen *N*-proteinase (ADAMTS-2; where ADAMTS stands for **a** **d**isintegrin **a**nd **m**etalloproteinase with **t**hrombo**s**pondin repeats). Homozygous mice were grossly normal at birth, but after 1–2 months they developed thin skin that tore after gentle handling. Although the gene was inactivated, a large fraction of the N-propeptides of type I procollagen in skin and the N-propeptides of type II procollagen in cartilage were cleaved. Therefore the results suggested the tissues contained one or more additional enzymes that slowly process the proteins. Electron microscopy did not reveal any defects in the morphology of collagen fibrils in newborn mice. However, in two-month-old mice, the collagen fibrils in skin were seen as bizarre curls in cross-section and the mean diameters of the fibrils were approx. half of the controls. Although a portion of the N-propeptides of type II procollagen in cartilage were not cleaved, no defects in the morphology of the fibrils were seen by electron microscopy or by polarized-light microscopy. Female homozygous mice were fertile, but male mice were sterile with a marked decrease in testicular sperm. Therefore the results indicated that ADAMTS-2 plays an essential role in the maturation of spermatogonia.

Key words: collagen, fibrils, metalloproteinase, propeptides, spermatogonia.

INTRODUCTION

Procollagen *N*-proteinase (ADAMTS-2; where ADAMTS stands for **a** **d**isintegrin **a**nd **m**etalloproteinase with **t**hrombo**s**pondin repeats) was initially discovered by the presence of partially-processed type I procollagen in the skin of cattle with a homozygous genetic defect that produced extremely fragile skin; the phenotype was defined as dermatosparaxis [1]. The partially-processed type I procollagen consisted of monomers in which the C-propeptides were removed, but a large part of the N-propeptides were uncleaved. The persistence of the N-propeptides on the monomers gave rise to highly irregular collagen fibrils with decreased cross-linking and decreased tensile strength [1–4]. Similar defects were subsequently seen in patients with Ehlers–Danlos syndrome type VIIC and in inbred strains of sheep and cats (see [5]). Isolation of the enzyme required for cleavage of the N-propeptides from type I procollagen demonstrated that it was a zinc metalloproteinase with a molecular mass of approx. 110 kDa [6,7]. The enzyme cleaved specific-Pro-Gln- and -Pro-Ala-bonds in both type I and type II procollagen, but had an unusual conformational requirement for the cleavage [8]. The procollagens were not cleaved if the proteins were partially denatured; denaturation unfolded the hairpin conformation in which the propeptides are folded back across the major triple helix of the monomers [5,8]. Isolation of cDNAs for the protein [9] demonstrated that the *N*-proteinase belonged to the new class of enzymes [10–16] termed ADAMTS. The enzyme ADAMTS-2 was similar to other proteins in the same family in that it had a pro-domain, a characteristic zinc-binding domain, and a distinctive distribution of cysteine residues [9]. It differed from the other ADAMTSs in that it had a distinct RGD sequence for integrin binding. Analysis of the genes from cattle with dermatosparaxis and patients with Ehlers–Danlos syndrome type VIIC demonstrated that they all contained mutations that produced premature termination codons for translation of the mRNA and led to decay of the transcripts and no functional protein [17]. Assays for mRNAs in mice demonstrated that the gene was expressed at high levels in all tissues that are rich in type I collagen (S.-W. Li, A. L. Sieron, M. Arita, A. Colige, B. V. Nusgens, C. M. Lapière and D. J. Prockop, unpublished work). However, the mRNA transcripts from ADAMTS-2 were inappropriately high relative to mRNAs for type I and type II procollagens in several tissues, such as testes, lung, spleen and kidney. Also, the mRNAs were found in inappropriately high levels in 7-day-old mouse embryos.

In the present study we have used homologous recombination to prepare transgenic mice that are homozygous for inactive alleles of ADAMTS-2. The mice developed fragile skin similar to the fragile skin seen in cattle with dermatosparaxis and in patients with Ehlers–Danlos syndrome type VIIC. The phenotype is mild, apparently because one or more additional proteinases partially cleave the N-propeptides from type I procollagen. The phenotype became more severe as the mice became older, apparently because type I collagen fibrils did not increase in diameter with age as they did in control mice. In addition, the male mice were sterile with a decrease in testicular sperm. Therefore the enzyme apparently has an unexpected role in the maturation of spermatogonia.

Abbreviations used: ADAMTS, **a** **d**isintegrin **a**nd **m**etalloproteinase with **t**hrombo**s**pondin repeats; ADAMTS-2, procollagen *N*-proteinase; ES, embryonic stem; G418, Geneticin; pCcollagen, collagen containing C-propeptides; pNcollagen, collagen containing N-propeptides.

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MATERIALS AND METHODS

Preparation of transgenic mice

A clone for ADAMTS-2 was isolated from a 129/Sv mouse lambda phage library (129SVJ, liver, 6–8-week-old female, 9–23 kb; Stratagene, La Jolla, CA, U.S.A.) using a human cDNA as a probe [17]. The clone contained a 14 kb insert from the gene (see Figure 1A). Two separate fragments were subcloned into the plasmid (pBluescript Ski; Stratagene): (1) a 4.2 kb *Bgl*II–*Spe*I fragment and (2) a 2 kb *Xba*I–*Xba*I fragment. A neomycin-resistance gene (PMC-I Neo; Stratagene) was cloned into the *Spe*I site of the first fragment and the second *Xba*I–*Xba*I fragment was inserted into the *Xba*I site at the 3′ end of the neomycin-resistance gene. A 3 kb *Bam*HI–*Bam*HI fragment containing the herpes simplex virus thymidine kinase gene (pHSV-106; Gibco BRL, Rockville, MD, U.S.A.) was then inserted into the *Bgl*I site. The targeting vector was linearized at a *Not*I site in the plasmid, purified by agarose gel electrophoresis, and ethanol precipitated. The targeting vector was electroporated into embroyic stem (ES) cells from 129/Sv mice with 35 μg/ml DNA and 10^7 cells/ml at 830 V and 3.0 μF [18]. The cells were plated on to a feeder layer of mouse fibroblasts that had been transfected with a neomycin-resistance gene and selected for resistance with Geneticin (G418). The ES cells on the feeder layer were selected for 7–10 days with 400 μg/ml G418 and 10 μM gancyclovir (Syntex Chemicals, Inc., Boulder, CO., U.S.A.). Resistant clones were expanded for Southern-blot analysis. One correctly targeted ES clone was co-cultured overnight with a 2.5-day-old morula [19] removed from pregnant C57BL/6 mice. The blastocysts were inserted into the uterine horn of pseudo-pregnant CDI mice. Mice that were chimaeric by coat colour were bred to wild-type mice. The transgenic offspring were identified for germline transmission of the mutated allele by Southern-blot analysis.

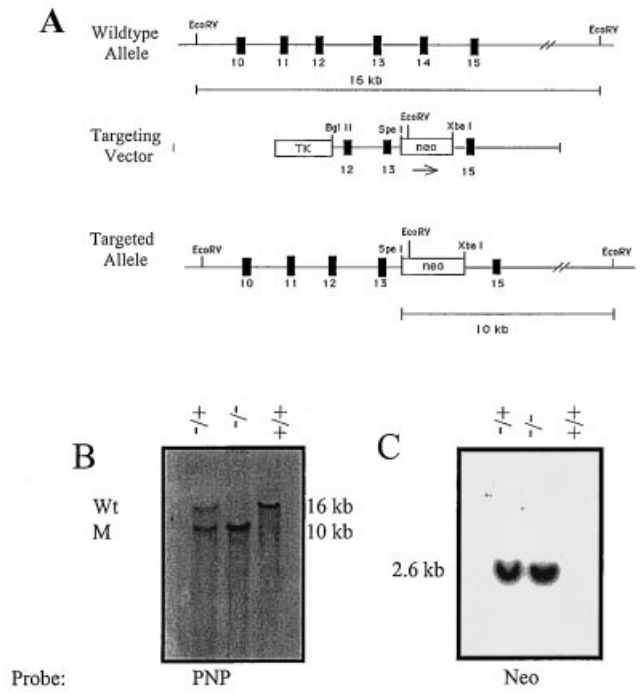

Figure 1 Gene constructs and assay for homozygous recombination in transgenic mice

(**A**) Diagram of a segment of the ADAMTS-2 gene and the targeting vector. As indicated, homologous recombination was predicted to generate a new 10 kb *Eco* RV fragment. Neo, neomycin; TK, thymidine kinase. (**B**) Southern-blot analysis of transgenic mice demonstrating that both homozygous and heterozygous mice were produced. Wt, wild-type; M, mutant. (**C**) Southern-blot assay demonstrating the presence of the neomycin-resistance gene in both heterozygous and homozygous mice.

Southern-blot analysis

DNA extracted from tail or toe was digested with proteinase K, phenol extracted and propan-z-ol precipitated. The DNA was digested with *Eco*RV, separated on an agarose gel, and transferred on to a nylon membrane (Biotrans; ICN, Costa Mesa, CA, U.S.A.). The membrane was probed with two DNA fragments that were labelled with [^{32}P]dCTP by random primer extension: an *Xba*I–*Eco*RV fragment from the 3′ end of the genomic clone and the neomycin–resistance gene.

Northern-blot analysis

For extraction of mRNA, primary cultures of skin fibroblasts were prepared and polyadenylated mRNA was extracted (Micro-polyA Pure Kit; Ambion, Austin, TX, U.S.A.). The RNA was separated on a 1 % (w/v) agarose gel. The gel was blotted on to a nylon filter and the filter was probed with a ^{32}P-labelled fragment from a human cDNA clone [17], and a mouse cDNA for the Collα1 gene (encoding the α1 chain of type I collagen) [20].

Protein analysis

Skin from newborn mice was frozen in liquid nitrogen, crushed, and extracted overnight with ice-cold 0.5 M acetic acid and 10 mM EDTA. The sample was neutralized rapidly in the cold with 0.1 M NaOH, followed by boiling in SDS and separation by SDS/PAGE [7.5 % (w/v) polyacrylamide gel]. The gel was then silver stained (Silver Stain Kit; Bio-rad, Hercules, CA, U.S.A.). To isolate cartilage, sterna from the mice were dissected, stripped of perichondrium and washed in Hanks solution. The samples were then processed with the same protocol used for samples of skin. Standards were type I procollagen from human skin fibroblasts [20], and pepsin-digested type II collagen from sternal cartilages of 17-day-old chick embroyos. For standards of collagen containing N-propeptides (pNcollagen) and C-propeptides (pCcollagen), the type I procollagen was processed with C-proteinase and N-proteinase from chick embryo tendons [6,21].

Light and electron microscopy

Tissues from newborn and adult mice were fixed by immersion overnight in half strength Karnovsky's fixative [2 % (w/v) paraformaldehyde and 2.5 % (w/v) glutaraldehyde buffered with 0.1 M phosphate (pH 7.4)] at 4 °C. The solution was changed every other day for 10 days. Samples were post-fixed in 1 % (w/v) osmium tetroxide, dehydrated in an ascending series of ethanol solutions and embedded in LX-112 epoxy resin (Ladd Research Industries, Williston, VT, U.S.A.). Vertical sections with a silver-grey interference colour were cut from the skin and cartilage specimens. Sections were first stained manually with 1 % (w/v) tannic acid for 30 min, followed by staining with uranyl acetate for 1 h and lead citrate for 2 min using a Leica Reichert Ultrostainer (Leica). The sections were examined with a JEM 1200EX transmission electron microscope (JEOL, Peabody, MA, U.S.A.).

Micrographs (4) were systematically photographed from the extracellular matrix of the dermis of each animal at magnification 30000 ×, starting from the epidermis–dermis junction of the skin

and moving towards the dermis–subcutis junction. The magnification of the microscope was verified with a cross-grating replica (54864 lines/inch; Bal-Tec, Baleers, Lichtenstein). Micrographs were digitized and stored as 8-bit grey-scale images using an Apple Power Macintosh 7100/80 computer equipped with a Photometrics 250 thermal-stabilized CCD-camera (Photometrics, Trenton, NJ, U.S.A.). The images were analysed with the IPLab image processing software (v. 2.5.5; Signal Analytics, Vienna, VA, U.S.A.). Final pixel size was 1.8 nm. To determine the average collagen fibril diameter, the minimum diameter of each collagen fibril profile in 0.22 μm^2 area of each micrograph (an average of 91 profiles/animal were analysed) was systematically measured with PRISM (v. 3.5; Analytical Vision, Raleigh, NC, U.S.A.) image analysis software [22]. For statistical analysis of the average collagen fibril diameter, the two-tailed non-parametric Wilcoxon matched-pair signed rank test was used with SPSS (v. 6.1.1; SPSS, Chicago, IL, U.S.A.) software.

Polarized light microscopy of the collagen of articular cartilage and subchondral bone

Polarized light microscopy of the collagen network was performed using unstained sections [23]. The sections were examined with a Leitz Ortholux 2 Pol microscope equipped with crossed polarizer and analyser, strain-free optics, a 16×0.45 N.A. Fluotar objective (Leitz, Wetzlar, Germany) and an interference monochromator ($\gamma = 595 \pm 10$ nm; Optometrics, Inc., Ayer, MA, U.S.A.). A square pixel size corresponded to 1.4 μm and the dynamic range was 12 bit. Linearly-polarized light was used to determine the orientation of collagen fibrils that were (1) parallel, (2) oblique and (3) perpendicular to the cartilage surface as previously described [23]. Histologically, the fibrils were located in the superficial, intermediate and deep cartilage zones respectively. The whole cartilage and subchondral bone plate thicknesses were measured. Area-integrated retardation was used to estimate the birefringement collagen fibril parallelism and content [23]. Measurements were also made from semi-circularly polarized light microscope images to reveal fibrils situated in the direction of extinction as follows: the original, linearly-polarized image was subsequently combined with another image of the specimen taken after parallel rotation of both the polarizer and the analyser by 45°. Then, the two images were combined using the Boolean maximum-function with the IPLab software (v. 2.2.5; Signal Analytics) for measurements. Zone thickness-weighted area-integrated retardation values were determined for zones (1) to (3), as well for subchondral bone [23]. The volume fraction (%) of subchondral bone was estimated from semi-circularly polarized light images by binarizing the bone structures using a constant threshold value. Based on the preferential collagen orientation assessed with linearly-polarized light, the average cartilage thickness and the thickness of the superficial, intermediate and deep zones were calculated from the control and knockout mice.

Examinations of sterile males

To examine sperm from the sterile males, the cauda epididymis was dissected free of the testes and squeezed to express a suspension of sperm into 1 ml of M16 medium. The suspension was then examined under a stereological microscope.

To examine sections of testes, mice were anaesthetized with ketamine/xylazine (150 mg/kg) and then slowly perfused with 10 ml of PBS, followed by 10 ml of 4% (w/v) buffered paraformaldehyde, using a 27-gauge butterfly catheter attached to a 10 ml syringe. The testes were then dissected and placed back in the same fixative at 4 °C on a low-speed rotator for 24 h. Following fixation, the samples were washed several times with PBS (pH 7.4), and then transferred to PBS/7% (w/v) sucrose in a 14 ml conical tube and placed on a low-speed rotator in a cold room for 6 h. Specimens were then removed and dehydrated in cold 70% (v/v) ethanol for 4–5 h, 95% ethanol overnight and two changes of 100% ethanol, each for 4 h. Samples were infiltrated and then embedded in glycol methacrylate as described by the manufacturer (Technovit 8100; Energy Beam Sciences, Agawam, MA, U.S.A.). The following day, blocks were transferred to a vacuum desiccator at room temperature to dry. Sections were cut on a Porter–Blum brand JB-4 microtome at 1.5 μm, placed on positively-charged slides, air-dried and heated at 56 °C for 5 min. Sections were stained with 1% (w/v) Toluidine Blue for several seconds, and then rinsed in tap water. Sections were subsequently dehydrated and mounted.

RESULTS

The targeting construct was synthesized so that a neomycin-resistance gene replaced sequences that were part of intron 13, all of exon 14 and part of intron 14 (Figure 1A). The construct was used to carry out homologous recombination of ADAMTS-2 gene into ES cells that were used to produce transgenic mice [18,19,24,25]. As indicated in Figures 1(B) and 1(C), both heterozygous and homozygous mice were obtained. Northern-blot analysis (Figure 2) demonstrated that neither the 7.0 nor the 3.7 kb mRNA from the gene was present in fibroblasts from homologous transgenic mice. However, protein assays of tissues demonstrated that the N-propeptides of both type I and type II procollagen were partially processed in tissues of the mice. As indicated in Figure 3 (left-hand panel), tissue extracts of the skin from the homozygous transgenic mice demonstrated the presence of both $pN\alpha_1(I)$ chains of type I procollagen and fully processed $\alpha_1(I)$ and $\alpha_2(I)$ chains. To examine the processing of type II procollagen, sternal cartilages were dissected from the mice. As noted previously [24–26], the cartilage of transgenic mice is readily obtained free of adjacent tissues so that it does not contain any evidence of type I procollagen or type I collagen as

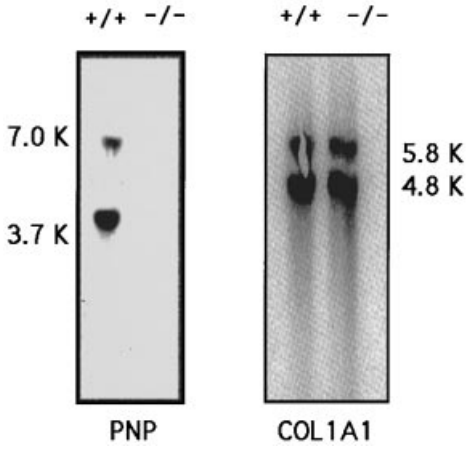

Figure 2 Northern-blot analysis of skin fibroblasts for expression of mRNA from the ADAMTS-2 gene

Left-hand panel: Northern-blot analysis using a cDNA probe [17] for the human ADAMTS-2 gene (PNP). The major transcripts of the gene were 7.0 and 3.7 kb (K). There is no evidence of either the 7.0 kb or the 3.7 kb transcript in skin fibroblasts from homozygous mice. Right-hand panel: Hybridization of the same blot with a cDNA probe for the mouse Col1α1 gene (COL1A1) [20].

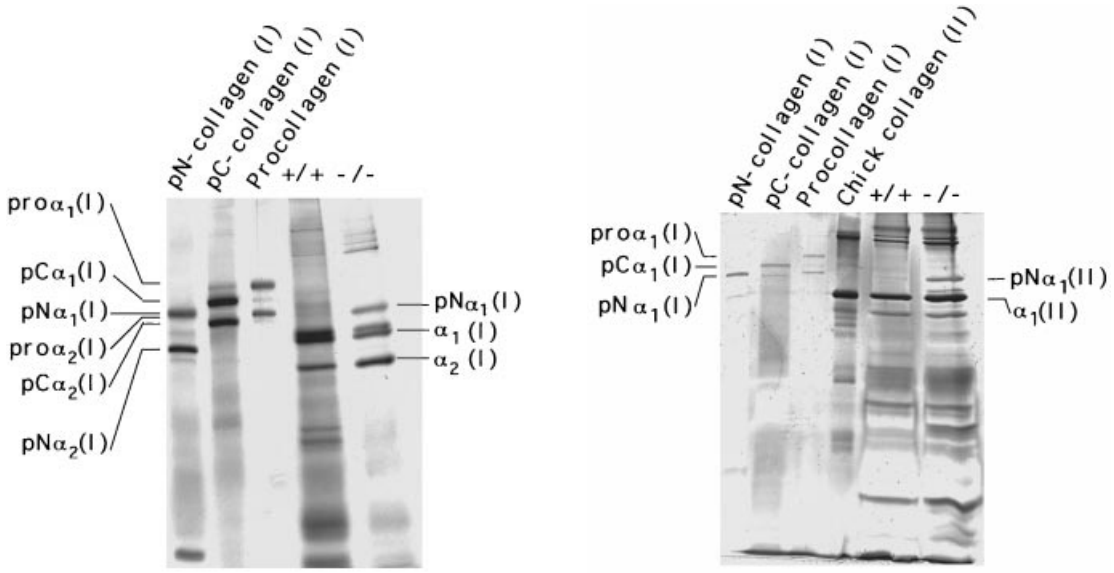

Figure 3 Protein analysis by SDS/PAGE

Left-hand panel: Presence of type I pNcollagen in skin from homozygous transgenic mice (—/—). Skin from newborn mice was frozen, crushed and extracted overnight in cold 0.5 M acetic acid. Both pNα_1(I) chains and fully processed α_1(I) and α_2(I) chains were present in skin extracts from the homozygous mice. The pNα_2(I) chains probably co-migrated with the α_1(I) chains under the conditions used. Right-hand panel: Both pNα_1(II) chains and fully processed α_1(II) chains were present in cartilage from the homozygous transgenic mice.

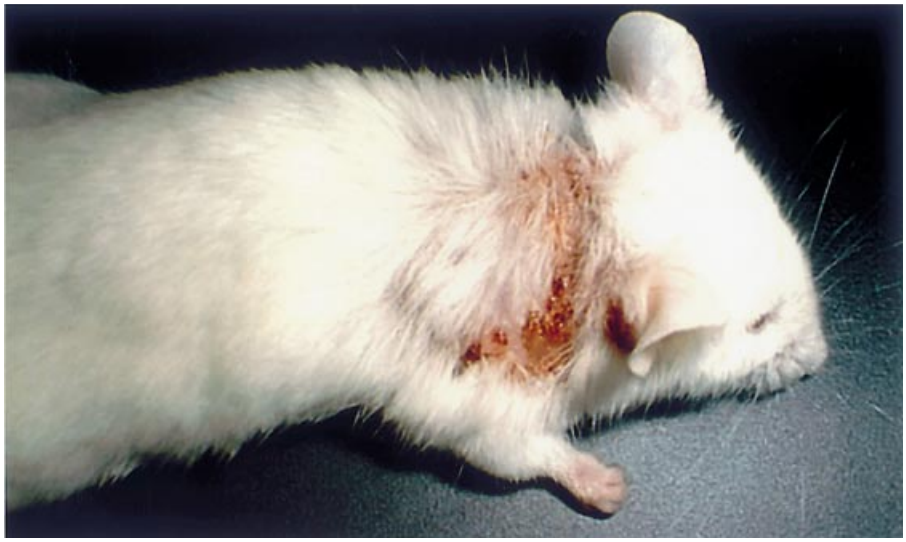

Figure 4 Picture of a homozygous transgenic mouse that was 4 months old and in which skin at the neck tore after an attempt to lift it gently by the scruff of the neck

assayed by light and electron microscopy, by PCR assays for mRNA transcripts, and by labelling experiments with ^{14}C-labelled amino acids. As indicated in Figure 3 (right-hand panel), protein extracts of sternal cartilage from the homozygous mice demonstrated both the presence of pNα_1(II) chains of type II procollagen and fully processed α_1(II) chains.

Phenotype of the mice

At birth, the heterozygous and homozygous mice were difficult to distinguish from control littermates. The only apparent difference was that in collecting specimens of tail for DNA assays, the skin of homozygous mice was prone to separate from the rest of the tail. At birth, the mean weight of a homozygous transgenic mouse was 1.85 g ($n = 15$) and the mean birth weight of a control littermate was 1.93 g ($n = 15$). The transgenic mice grew at the same rate as littermates so that at 2 months, the mean weight of a homozygous transgenic mouse was 23.1 g ($n = 15$) and that of a control littermate was 23.1 g ($n = 14$). X-ray analyses of the skeleton showed no differences between the transgenic mice and the control littermates at either 2 days or 2 months (results not shown).

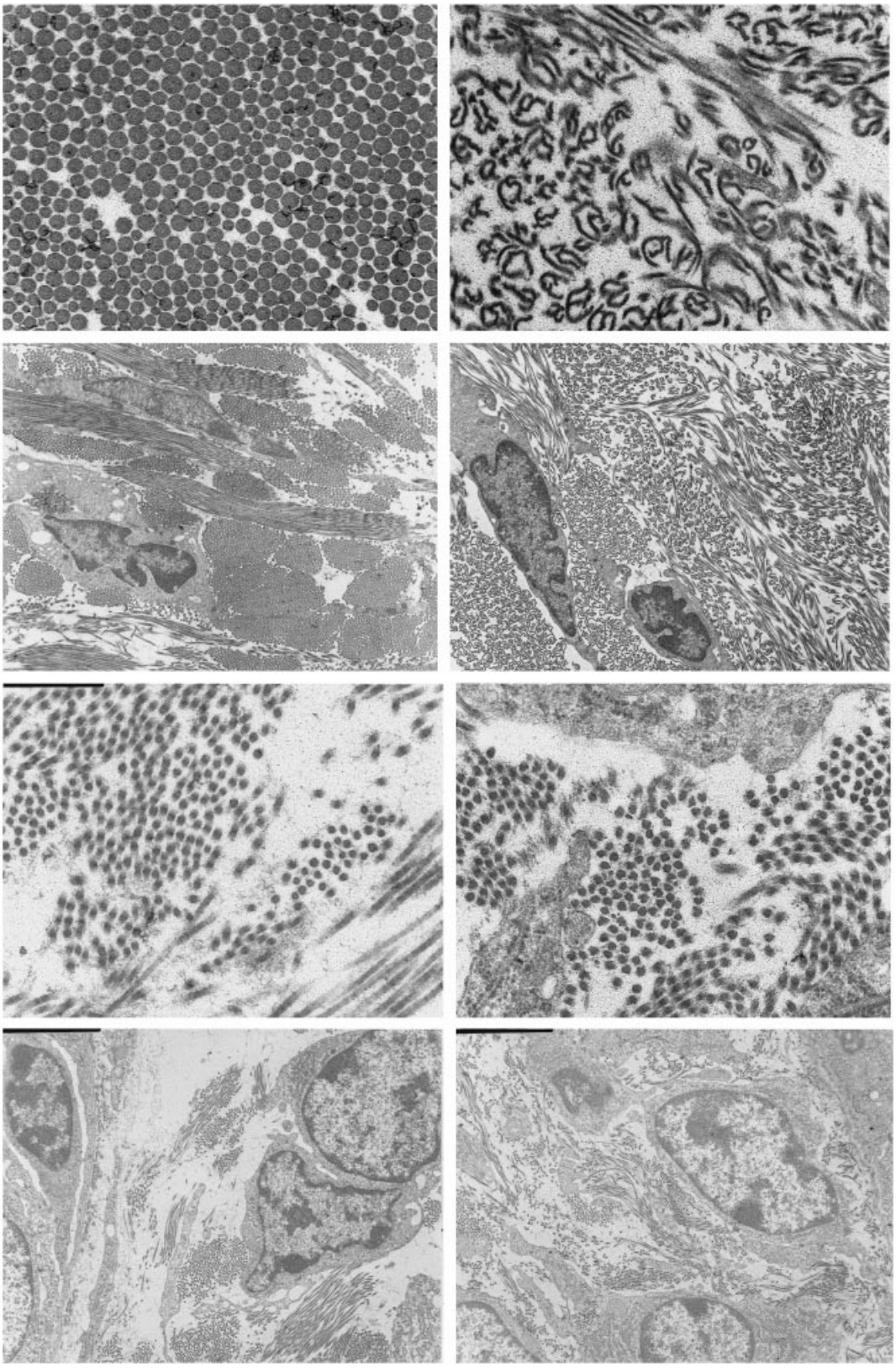

Figure 5 Electron microscopy from wild-type and homozygous transgenic mice

Top two left-hand panels: skin from a control 2-month-old mouse. Top two right-hand panels: skin from a 2-month-old homozygous transgenic mouse. Bottom two left-hand panels: skin from a control 2-day old mouse. Bottom two right-hand panels: skin from a 2-day-old homozygous transgenic mouse.

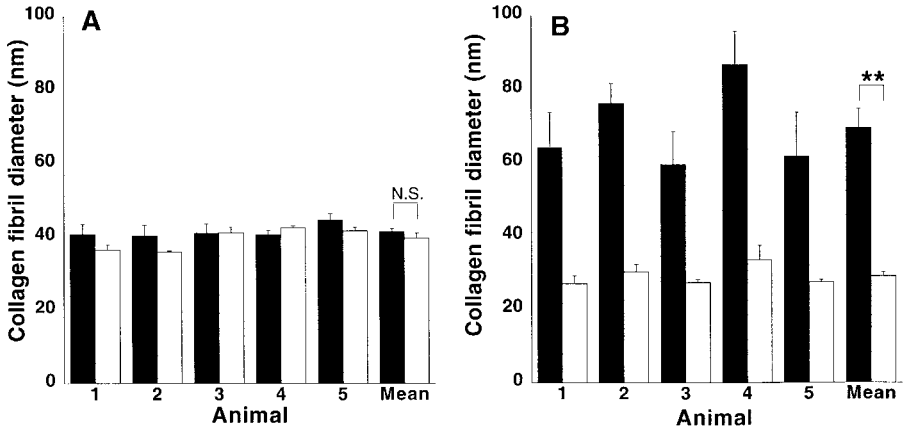

Figure 6 Fibril diameters from control and homozygous transgenic mice

Skin from 2-day-old mice (**A**). Skin from 2-month-old mice (**B**). Solid bars, control mice; open bars, transgenic mice; $^{**}P < 0.01$, Wilcoxon matched-pair signed rank test.

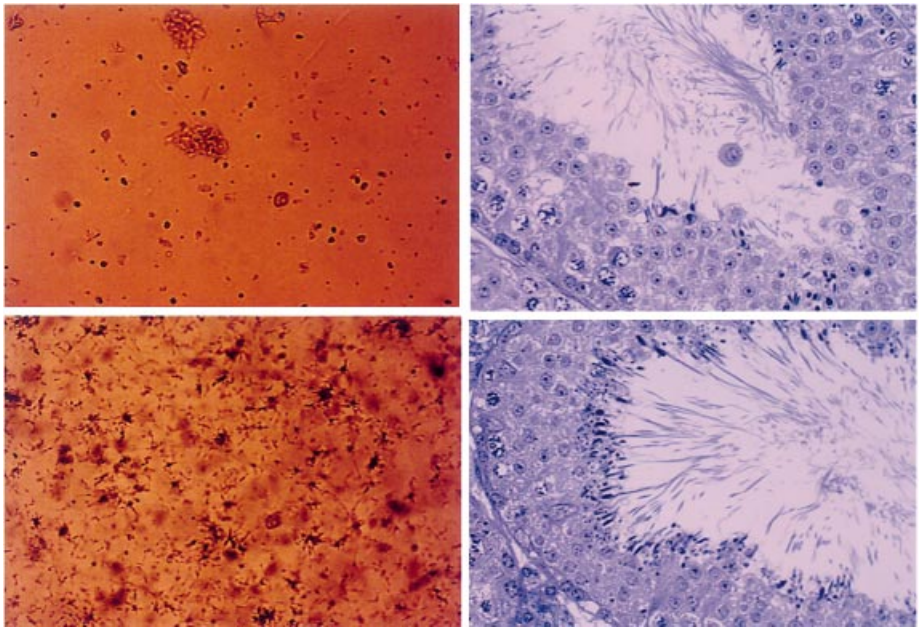

Figure 7 Histology of testes and semen from control and transgenic mice

Top left-hand panel: semen from the epididymis of a homozygous transgenic mouse. Bottom left-hand panel: semen from a control mouse. Top right-hand panel: section of testes from a homozygous transgenic mouse. Bottom right-hand panel: section of testes from a control mouse. Magnification × 100.

After approx. 2 months, the homozygous mice began to develop recognizable differences from control littermates. The facies began to look more triangular with a shorter snout. The hair was less dense with thinner hair follicles. The skin felt thinner and softer, and began to tear with slight trauma. For example, skin near the ears was frequently torn and was scarred from the mice scratching their ears. The skin around the neck was easily torn with gentle efforts to lift the mice by the scruff of the neck (Figure 4). Similarly, the skin of the tail frequently stripped off if attempts were made to lift the mice by their tails.

Microscopy

Tissues were taken for microscopy from five 2-day-old homozygous transgenic mice and five 2-month-old homozygous transgenic mice. Tissues from an equal number of control littermates were examined at the same time. Dissection of the tissues for microscopy did not reveal any phenotypic changes in the 2-day-old mice. In the 2-month-old mice, the skin was thinner. Also, the xiphoid was markedly concave and mineralized.

Electron microscopy of tissues from the 2-day-old mice did not detect any differences in the cellular morphology or the collagen fibril structure in skin (Figures 5 and 6). Also, no differences were found in articular cartilage (results not shown). In the older transgenic mice, however, differences in the collagen fibril structure were apparent in skin. The collagen fibrils in skin were seen as bizarre curls in cross-sections (Figure 5). Also, the mean diameter of the collagen fibrils was approx. half of that in controls (Figure 6). In the same 2-month-old mice, microscopy of articular cartilage and growth plate cartilage did not reveal

any differences from controls. Also, polarized-light microscopy of cartilage showed that there was no difference in the birefringence values obtained from the superficial, intermediate or deep zones of articular cartilage. Similarly, there was no difference in the thickness of the superficial or intermediate deep zones of articular cartilage. In addition, there were no differences in subchondral plate thickness, bone volume fraction, or birefringence values obtained from the subchondral bone.

Mouse sterility

Homozygous female mice were fertile and were readily bred through multiple generations. However, the homozygous male mice were sterile. Each of ten adult homozygous males were placed in cages overnight with two wild-type female mice that had previously been shown to be fertile. The procedure was then repeated a second and a third time with the same ten males and new females that had previously given birth to litters. None of the 60 females developed plugs or became pregnant.

To examine the cause of the infertility, the testes were dissected from mature homozygous mice. The semen was expressed from the epididymis and examined by light microscopy. As indicated in Figure 7 (left-hand panels), few, if any, active sperm were seen in semen from the homozygous transgenic mice, whereas many active sperm were seen in controls. Sections of testes from the transgenic mice showed a marked decrease in mature sperm (Figure 7, right-hand panels).

DISCUSSION

The transgenic mice with inactive alleles for ADAMTS-2 showed many similarities to animals with dermatosparaxis and patients with Ehlers–Danlos syndrome type VIIC [1,17,27–30]. However, the transgenic mice had several unexpected features.

The transgenic mice were similar to other animal models and patients with defects in ADAMTS-2 in that a significant proportion of the N-propeptides of type I procollagen were cleaved even in the absence of the apparently specific ADAMTS-2. Assays of skin from cattle with dermatosparaxis indicated that approx. 43 % of the type I procollagen was cleaved to collagen [4]. Assays of skin from the transgenic mice carried out in the present study indicated large variations among samples, but the amounts of cleaved and uncleaved protein were approx. equal in some samples. Therefore skin apparently contains one or more additional enzymes that slowly cleave the N-propeptides.

One unexpected feature of the transgenic mice was that the phenotype became more severe with age. Age-dependent changes were not reported in animals with defects in ADAMTS-2 [1,5,27], apparently because severe lacerations of skin prompt either euthanasia of the animals or death from infections. Age-dependent changes have also not been reported in patients with similar defects [5,17,29], perhaps because the patients learn to protect themselves from trauma or the changes are too difficult to evaluate objectively. The phenotype became more severe with age in the mice, apparently because there was a large increase in the diameter of type I collagen fibrils during the first few months after birth in control mice, but not in the transgenic mice (see Figure 6).

A second unexpected feature was that no defects in the structure of cartilage or collagen fibrils of cartilage were found in the transgenic mice even though a portion of the N-propeptides of type II procollagen was not cleaved. Therefore the additional bulk of the N-propeptides did not have any apparent effect on the assembly of type II collagen into fibrils, whereas the presence of the N-propeptides of type I collagen extensively distorted both the morphology and the strength of type I collagen fibrils [2–4]. The difference may be explained by the fact that fibrils of type II collagen are normally thinner than fibrils of type I collagen and, therefore, the N-propeptides can more easily be displaced to the surface without significantly altering the cross-sectional packing of the monomers (see [2–4]). One caveat, however, is that the apparent processing of type II procollagen reflects the synthesis of a splice variant of the monomer lacking the amino acids encoded by exon 2 and, therefore, contains a shortened N-propeptide that makes $pN\alpha_1$(II) chains difficult to distinguish from α_1(II) chains [31].

A third unexpected finding in the present study was the sterility and decreased spermatogenesis seen in the homozygous male mice. Male sterility has not previously been associated with any defect in type I collagen or any other collagen (see [29,30]). Other animals with defects in ADAMTS-2 generally do not survive to a reproductive age, and few of the small number of patients with Ehlers–Danlos type VIIC have been followed for long periods of time. Testes has been shown to contain a series of ADAM proteins [32–38] but none has the structural features characteristic of an ADAMTS. Moreover, the ADAMs identified in testes and semen appear to be involved in the binding of sperm to oolema and fusion of gametes [33,35–37]. Transgenic mice with an inactive allele for cyritestin, one of the ADAMs found in testes, showed normal spermatogenesis and sperm migration, but the sperm were unable to bind to the zona pellucida of ova [38]. Therefore the results in the present study indicate that ADAMTS-2 has a different role than most disintegrins of the ADAM class in testes. The results are consistent with recent indications that the ratio of mRNA for ADAMTS-2 to mRNAs for fibrillar procollagens is unexpectedly high in a number of tissues ([17], and S.-W. Li, A. L. Sieron, M. Arita, A. Colige, B. V. Nusgens, C. M. Lapière and D. J. Prockop, unpublished work). Therefore ADAMTS-2 may be similar to procollagen C-proteinase (see [5]) in having biological functions that are additional to the role of the enzyme in the processing of procollagens to collagens.

The transgenic mice prepared in the present study should provide useful models for exploring the effects of inhibitors of collagen synthesis in wound healing and in fibrotic conditions. Excessive fibrosis in the form of irreversible deposition of collagen fibrils is the pathological consequence of injury to most tissues in man (see [39]). Therefore extensive attempts have been made to develop agents that can modulate the amount of collagen fibrils deposited in response to tissue injury. One target of the efforts has been ADAMTS-2, since the enzyme is extracellular and its activity is essential to generate fibrils of normal tensile strength and metabolic stability [5,39]. Inhibition of ADAMTS-2 causes synthesis of thin and irregular fibrils that do not become normally cross-linked [2–5]. Therefore fibrils containing pNcollagen probably have a rapid metabolic turnover and are less likely than normal collagen fibrils to form irreversible fibrotic deposition during wound repair. The transgenic mice prepared in the present study should provide a means of testing whether partial and specific inhibition of ADAMTS-2 will decrease the fibrotic reactions to tissue injury.

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