Mice With a Deletion in the First Intron of the *Col1a1* Gene Develop Age-Dependent Aortic Dissection and Rupture

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Abstract—The functional significance of the first intron of the *Col1a1* gene in regulation of type I collagen synthesis remains uncertain. A previous study in mice established that a mutated *Col1a1* allele that lacked a large fraction of the first intron, but retained the sequences required for normal splicing, was subject to an age- and tissue-dependent decrease in expression. In this study, we report that mice homozygous for this deletion are predisposed to dissection and rupture of the aorta during their adult life. Aortic dissection was not detected in autopsies of heterozygous animals or their littermate controls. Electron micrographs revealed fewer collagen fibrils and less compacted, irregular elastic lamellae in the aortic walls of homozygous mutant animals. Northern analysis of aortic RNA from 2.5- and 12-month-old homozygous mutant mice revealed that *Col1a1* mRNA levels were decreased by 29% and 42%, respectively, relative to those of control littermates. In 12-month-old heterozygotes, the decrease was 32%. Allele-specific amplification of heterozygous cDNAs demonstrated that this reduction was limited to transcripts from the mutant allele. The collagen content of the aortas of homozygous mutant mice was also significantly lower in comparison to that of age-matched, control animals. These data establish that the integrity of the aortic wall depends on an adequate content of type I collagen, and that continued synthesis of collagen in the aorta as a function of age is critically dependent on sequences in the first intron of the *Col1a1* gene. **(*Circ Res*. 2004;94:83-90.)**

Key Words: collagen ■ gene regulation ■ targeted mutation ■ RT-PCR ■ homologous recombination

Type I collagen is the main component of fibrils that provide tissues with tensile strength. To achieve the highly variable levels of type I collagen in different tissues during development, growth, aging, and tissue repair, the genes encoding the constituent α1 and α2 chains of type I collagen (*Col1a1* and *Col1a2*) are likely to be under complex transcriptional and posttranscriptional control. Both positively and negatively acting genomic elements that are involved in tissue-specific transcriptional control have indeed been identified in the upstream promoter region of the *Col1a1* gene,[1–3] and also exist in the first intron.[4,5] These elements mediate the complex effects of cytokines and growth factors.[6–8]

Hormuzdi and coworkers[9] generated a mouse line with a large deletion in the first intron of the *Col1a1* gene (herein termed Col-IntΔ or Δ/Δ mice) that did not impair normal splicing of the shortened intron. As a function of age, the expression of the mutated allele was reduced by about 50% from its control level in lung and skeletal muscle. Nevertheless, both in cell culture and in response to bleomycin-induced fibrosis, cells expressing the mutant allele were able to increase collagen gene transcription to levels approaching that of the wild-type allele.[9,10] Thus, the mutant allele in Δ/Δ mice can respond to physiological signals, and these signals can override the inhibitory effects of the loss of intronic sequences.

In contrast to the role of elastic fibers, the contribution of type I collagen to the mechanical properties of the aorta is not fully appreciated. Type I collagen is the major component of the aortic adventitia,[11] whereas type III collagen comprises the majority of the collagen in the medial layer[12] and is also the major collagen synthesized by smooth muscle cells.[13] In accord with these findings, a number of genetic defects in collagen in humans and mice are associated with rupture of the aorta and other large vessels. Patients with type IV Ehlers-Danlos syndrome, which is caused by mutations in type III collagen, are at risk for rupture of the aorta and other large arteries.[14] Similarly, mice that are null for type III collagen develop dissecting arterial aneurysms that rupture prenatally or in early adulthood.[15] Osteogenesis imperfecta in humans is characterized primarily by a propensity for bone fractures, and is caused by mutations in either the *COL1A1* or *COL1A2* genes encoding type I collagen. However, patients occasionally develop dissection of the aorta[16] and other large vessels.[17]

Original received February 20, 2003; revision received November 5, 2003; accepted November 11, 2003.

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Circulation Research **is available at http://www.circresaha.org** **DOI: 10.1161/01.RES.0000108263.74520.15**

Although a targeted disruption of the *Col1a1* gene has not been performed, the random insertion of the Molony murine leukemia virus into the first intron of *Col1a1* inactivated the gene in most tissues, including blood vessels.[18,19] These mice die at day 11 to 14 of embryonic age from rupture of blood vessels. The availability of Δ/Δ mice, in which an age-dependent reduction in collagen synthesis in a number of tissues had been demonstrated, has made it possible to study the function of the first intron in a more systematic manner. In this study, we describe the phenotype of aging adult mice that are homozygous for the Col-IntΔ allele. These mice develop aortic dissection that leads to rupture in a high percentage of the animals. Data obtained by Northern analysis, allele-specific expression of the *Col1a1* gene, determination of collagen content, and visualization of collagen fibrils by electron microscopy demonstrated that the defect was associated with reduced synthesis of type I collagen in the aortic wall. These findings suggest that mutations in intronic *COL1A1* sequences could contribute to age-dependent aortic dissection and rupture in humans.

Materials and Methods

Experimental Animals

Col-IntΔ mice, containing a 1283-bp deletion in the 1462-bp first intron of the *Col1a1* gene, have been described.[9] A silent mutation, which created a new *Xho*I site, was introduced in the *Col1a1* gene to assist in the detection of the mutant allele in genomic DNA, and to determine the relative levels of expression of the wild-type and mutant alleles.[9] Homozygous Δ/Δ mice were mated with mice sharing the same 129/OlaHsd /C57BL/6 background to produce a population of control (+/+), heterozygous (Δ/+), and homozygous mutant (Δ/Δ) mice. The mice were genotyped by PCR amplification and restriction digestion of DNA.[9] A group of animals (n=121) was studied for a period of up to 18 months. All animals whose deaths were estimated to occur no more than 12 hours previously were autopsied, and tissue samples were taken for examination by light microscopy. In addition, aortas from age-matched pairs of asymptomatic, Δ/Δ, and +/+ mice at 3 and 12 months of age were used for transmission electron microscopy. Kaplan-Meier survival analysis was used to assess all causes of mortality in the different groups. The statistics of survival curves were compared using the Mann-Whitney rank-sum test. This study was approved by the Committee for Animal Welfare at the University of Turku.

Histology

Samples were collected from a number of tissues, including brain, liver, lung, muscle, heart, kidney, and skin, to detect possible histological alterations in mutant animals. The aorta was dissected to the level of the renal arteries and was subjected to more extensive analysis. Specimens were fixed in 4% buffered paraformaldehyde, treated with graded concentrations of ethanol, dried with xylene, and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E), Verhoeff-van Gieson (for elastic tissue), or van Gieson stains. For detection of fibrillar collagens, sections were stained with Masson's trichrome or Picrosirius red and, in the latter case, were examined under polarized light.[20]

Electron Microscopy

The aortas of apparently normal mice were perfused with 0.1 mol/L phosphate-buffered 2.5% glutaraldehyde by injection through the left ventricle. The descending aortas of Δ/Δ and age-matched control mice were cut into small pieces and fixed overnight in the same solution. Specimens were post-fixed in 1% osmium tetroxide and 1.5% potassium ferrocyanide, dehydrated in ethanol, and embedded in Epoxy resin. Ultrathin sections were stained with 5% uranyl acetate and examined by transmission electron microscopy.

Extraction of mRNA and Northern Analysis

Aortas from apparently normal Δ/Δ, Δ/+, and +/+ mice, aged 2.5 and 12 months, were cut into small pieces. Total RNA was isolated by extraction with 4 mol/L guanidinium isothiocyanate, followed by sedimentation through 5.7 mol/L cesium chloride.[21] For Northern analysis, 10 μg aliquots of total RNA were run on 1% formaldehyde-agarose gels, stained with ethidium bromide, and transferred to membranes. Purified cDNAs were labeled by random priming with ^{32}P-dCTP to specific activities of approximately 1×10^9 cpm/μg DNA, and the membranes were hybridized with probes for proα1(I) and proα1(III) collagen,[22] and subsequently with a probe for 28S rRNA[23] at 42°C in 50% formamide, 1 mol/L NaCl, 1% sodium dodecyl sulfate (SDS), 10% dextran sulfate, 5× Denhardt's, and 100 mg/L sonicated calf thymus DNA. High stringency washes were performed at 55°C in 0.1× SSC, 0.1% SDS. The bound probes were detected and quantified with a phosphorimager, and the results normalized to the 28S rRNA signal on the same membranes.

RT-PCR

The relative expression of the wild-type and mutant *Col1a1* alleles in aortic RNA, extracted from heterozygous mice, was determined by RT-PCR.[9] The synthesis of the first strand was performed with 2 μg RNA and 300 ng of primer P1R (5′-CCGGGCTTGC-CAGCTTCCCCATCATC-3′). Five microliters of the reaction products was then subjected to 26 amplification cycles at an annealing temperature of 55°C in the presence of 100 ng of primers P1 (5′-CCACGCATGAGCCGAAGCTAACCCC-3′) and P2R (5′-CTTCCCCATCATCTCCATTCTT-3′) and 2 mmol/L $MgCl_2$. For quantification of allele-specific transcripts, as determined by the relative abundance of the *Xho*I-resistant and -sensitive fragments,[9] [^{32}P]dCTP (1 μCi/sample) was added and a 27th amplification cycle was run. Addition of [^{32}P]dCTP to only the final amplification cycle prevents erroneous estimates of transcript abundance that result from heteroduplex formation between the two species of transcripts. Labeled cDNAs were restricted with *Xho*I, purified, and electrophoresed on a 6% acrylamide gel. The gels were then dried and analyzed by phosphorimaging to determine the radioactivity of the *Xho*I-resistant 736-bp and *Xho*I-generated 589-bp fragments. To correct for the number of dCTP nucleotides in the smaller 147-bp *Xho*I fragment, the counts obtained for the 589-bp DNA fragment that was derived from the Col-IntΔ allele were multiplied by 1.3. The values are expressed as a ratio of the abundance of the *Xho*I-sensitive fragment to that of the *Xho*I-resistant fragment. Thus, a value of 1 is expected if the two alleles are transcribed equally well, and a value of 0.5 if the mutant allele is transcribed half as well as the wild-type allele. The statistical significance of the values was tested by analysis of variance.

Hydroxyproline Assay

Aortic hydroxyproline contents were determined by a modification of Woessner's protocol.[24] Aortic tissues were lyophilized, weighed, hydrolyzed in 6 N HCl at 125°C for 3 hours, evaporated, and dissolved in H_2O. Samples and hydroxyproline standards were oxidized with Chloramine-T, which was subsequently destroyed with 3.15 mol/L perchloric acid. The color reaction was performed with *p*-dimethylaminobenzaldehyde at 60°C, and absorbance was measured at 557 nm. The protein contents of aortas were calculated from nitrogen values determined from the same hydrolyzates.[25] Relative contents of collagen were then estimated from hydroxyproline to nitrogen ratios, and the values were subjected to statistical analysis with Student's *t* test.

Results

Survival of Δ/Δ Mice

As the colonies of mice aged, spontaneous deaths started to occur at 4 months of age and continued more or less

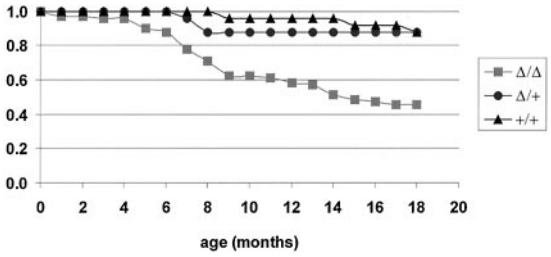

Figure 1. Cumulative survival of control (+/+), heterozygous (Δ/+), and homozygous Col-Int Δ (Δ/Δ) mice during an observation period of 18 months. Vertical axis indicates the fraction of surviving mice. $P<0.0001$ for homozygous mutant vs heterozygous and control animals.

constantly to the conclusion of the study at 18 months (Figure 1). At autopsy, a common pathology was observed: a large hematoma, which was associated with an aortic dissection, surrounded the heart or pericardial sac and extended through the diaphragm into the peritoneal cavity. All of the mice that died with this phenotype were found to be Δ/Δ, and more than half of all Δ/Δ mice (39/72) died during the 18-month observation period (online Table 1, in the online data supplement available at http://www.circresaha.org). Among those 72 animals, 33 were male and 39 were female. Aortic rupture was detected and verified histologically in 15 (8 males and 7 females) of the 17 autopsied animals. Survival curves for the three genotypes are presented in Figure 1, and additional related data are summarized in online Table 1.

Characterization of Aortic Pathology in Δ/Δ Mice

No significant morphological changes were detected by inspection or by light microscopy in any tissue other than blood vessels. Analysis of aortas by light microscopy confirmed the presence of dissection of the aortic wall in Δ/Δ mice (Figures 2 and 3), but the histology of other large blood vessels in these mice was normal. Dissection of the aortic adventitial and medial layers is shown in Figures 2B and 2C. In most cases, the hematoma that accompanied the dissection almost completely obliterated the aortic lumen. Despite substantial effort, we could not locate the exact site of the dissection and rupture in many of the animals. However, histological evidence indicated that, in some cases, the dissection started proximally, at or close to the aortic root. A suspected site of rupture of the medial layer is depicted in Figure 2D.

The aorta of an apparently healthy Δ/Δ mouse showed a hematoma in the immediate vicinity of the heart (Figure 3A, 3C, and 3E). The aortic lumen was narrowed. Sections of this aorta, stained with Picrosirius red and Masson's trichrome, demonstrated the presence of reduced collagen in the adventitia. Thus, less fibrillar collagen was detected in the homozygous Col-Int Δ mouse (Figures 3C, 3E, and 3G) than in the control aorta (Figures 3D and 3F). Collagen fibrils that are interspersed among elastic fibers in the medial layer appeared to be more randomly distributed in the aortas of Δ/Δ mice than in control aortas, but their amount and structure could not be judged in these sections. However, there was fibrosis with deposition of collagen throughout the associated hematoma (Figures 3A, 3C, and 3E). Thus, in apparent contrast to adventitial fibroblasts, the cells responsible for this fibrosis are able to synthesize considerable quantities of collagen, presumably type I.

The aortic media consists of elastic lamellae that can be clearly distinguished in light microscopic sections (Figures 2B, 2C, and 2E). These lamellae are connected by interlamellar fibrils that course radially through this layer,[26] and much of their structure could correspond to collagen fibers that are seen in the media by Masson's stain (Figures 3F and

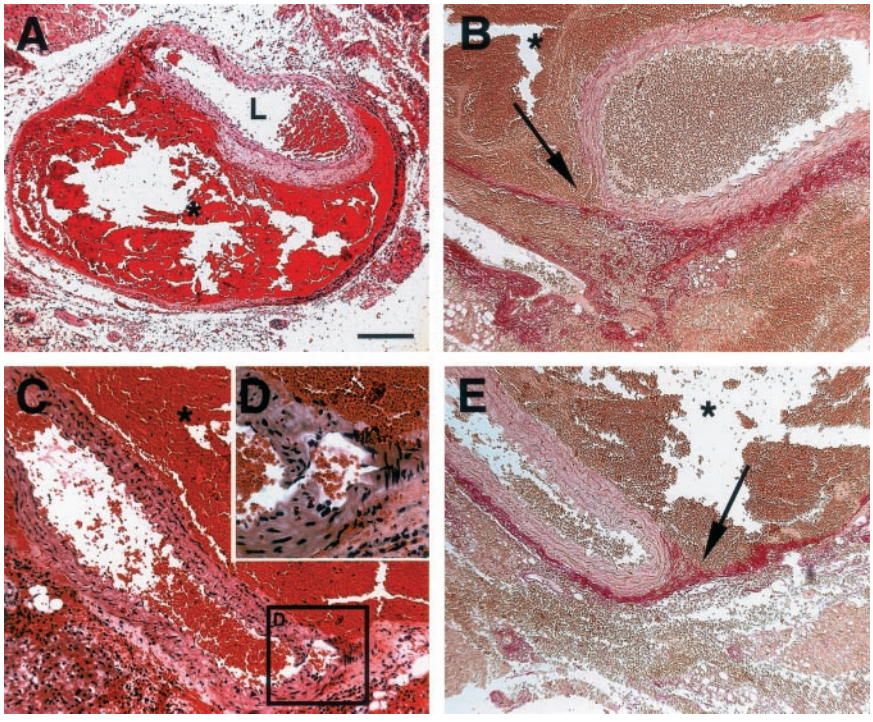

Figure 2. Histopathological appearance of a typical ruptured aorta. A pseudolumen (asterisk) filled with extravasated blood and narrowing of the aortic lumen (L) are observed in a Δ/Δ animal (A). At higher magnification, the site of dissection in the adventitia is clearly visible (arrows in B and E). Possible rupture site of the medial layer is shown in C and D (inset). A, C, and D, H&E stain; B and E, van Gieson stain. Bar in A=200 μm (in A), 100 μm (in B, C, and E), and 50 μm (in D).

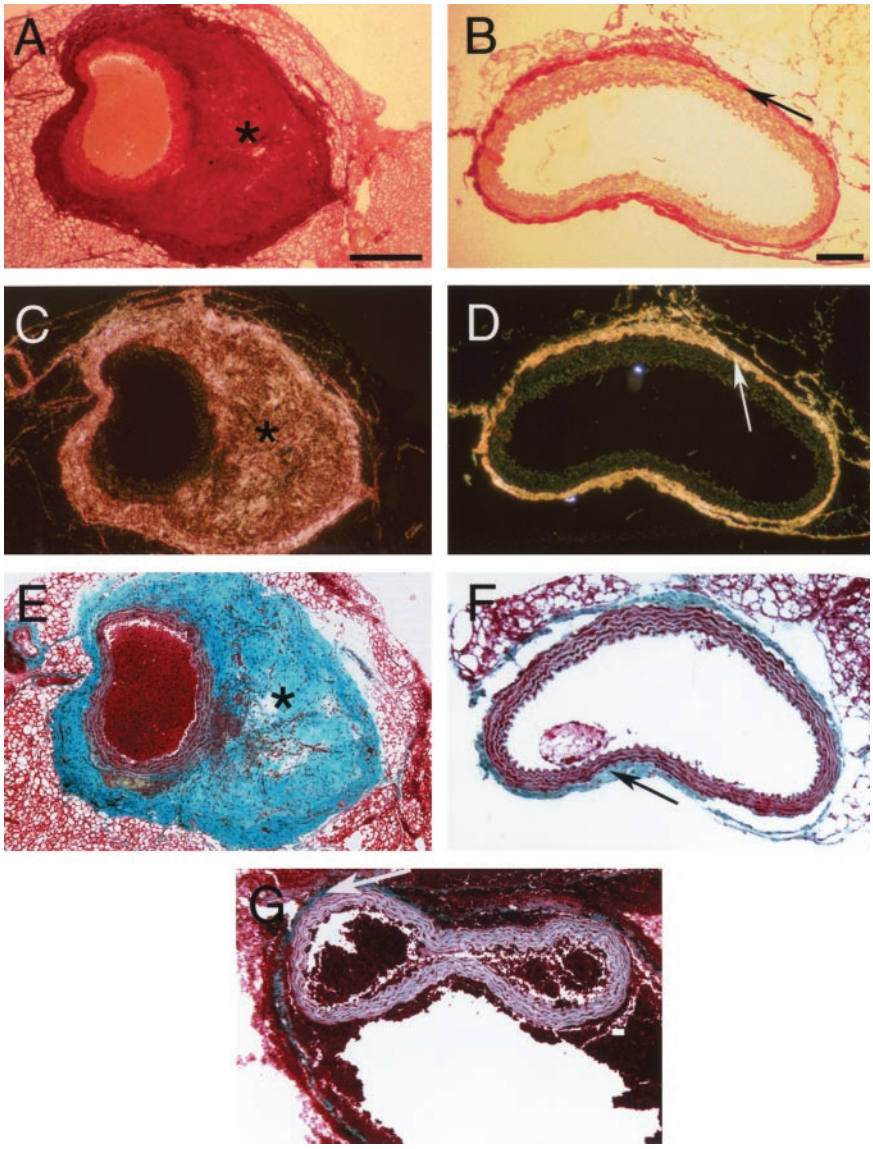

Figure 3. Picrosirius red (A through D) and Masson's trichrome (E through G) staining of aortas from Δ/Δ (A, C, E, and G) and from control mice (B, D, and F). Sections A, C, and E are from an animal with a nonfatal hemorrhage and an organized hematoma (asterisk). Section G is from another animal that was found dead with a ruptured aorta, in which the hematoma was more recent and not fibrosed. Sections C and D, photographed under polarized light, show fibrillar collagen in Δ/Δ and control aortas. With Masson's trichrome stain, blue collagen fibrils are clearly seen in the adventitia (arrows in F and G), in the organized hematoma (asterisk in E), and between the elastic lamellae (F and G). Arrows in B, D, F, and G point to the adventitia. Bar in panel A=100 μm (in A, C, and E) and 150 μm (in B, D, F, and G).

3G). Examination of elastic lamellae in Δ/Δ aortas by Verhoeff-van Gieson stain did not reveal fragmentation, although the width of the lamellae appeared to vary more widely than controls, and in some areas of the wall, the number of lamellae was reduced (data not shown). However, electron micrographs of perfused aortas from 12-month-old, randomly selected, and outwardly normal Δ/Δ mice showed distinct abnormalities. Specifically, the elastic lamellae of Δ/Δ animals were less compacted and more variable in width, displayed irregular borders, and were surrounded by less dense collagen fibrils than the corresponding lamellae in control animals (Figures 4A and 4B). Thus, the organization of elastic lamellae could depend on the integrity and quantity of interlamellar collagen fibrils. In contrast, there were no significant changes in elastic lamellae in micrographs of aortas from 3-month-old mice (data not shown).

Analyses of Aortic Collagen mRNA Levels

mRNA levels for types I and III collagens in heart and aortic tissues were determined by Northern analysis. Densitometric measurements showed that the levels of proα1(I) collagen mRNA were reduced by 29% in the 2.5-month-old homozygous mutant mice and by 32% and 42% in 12-month-old Δ/+ and Δ/Δ mice, respectively, in comparison with those of littermate controls (Figure 5). The differences in the levels of transcripts encoded by the Col-IntΔ and wild-type alleles were also analyzed by allele-specific RT-PCR of aortic mRNAs from heterozygous mice. In these experiments, the expression of the mutated allele was reduced by 26%, in comparison with that of the wild-type allele (Figure 6). This result is in general agreement with those of Northern analyses. A faint band migrating in the position of the 736-bp fragment was often present in Δ/Δ samples and is attributed to incomplete digestion of the fragment by *Xho*I. No differences were detected in type III collagen mRNA levels between mutant and control animals (data not shown).

Hydroxyproline Content of Aortas

Hydroxyproline and total nitrogen contents were measured in aortas of Δ/Δ, Δ/+, and +/+ mice (Figure 7). The collagen

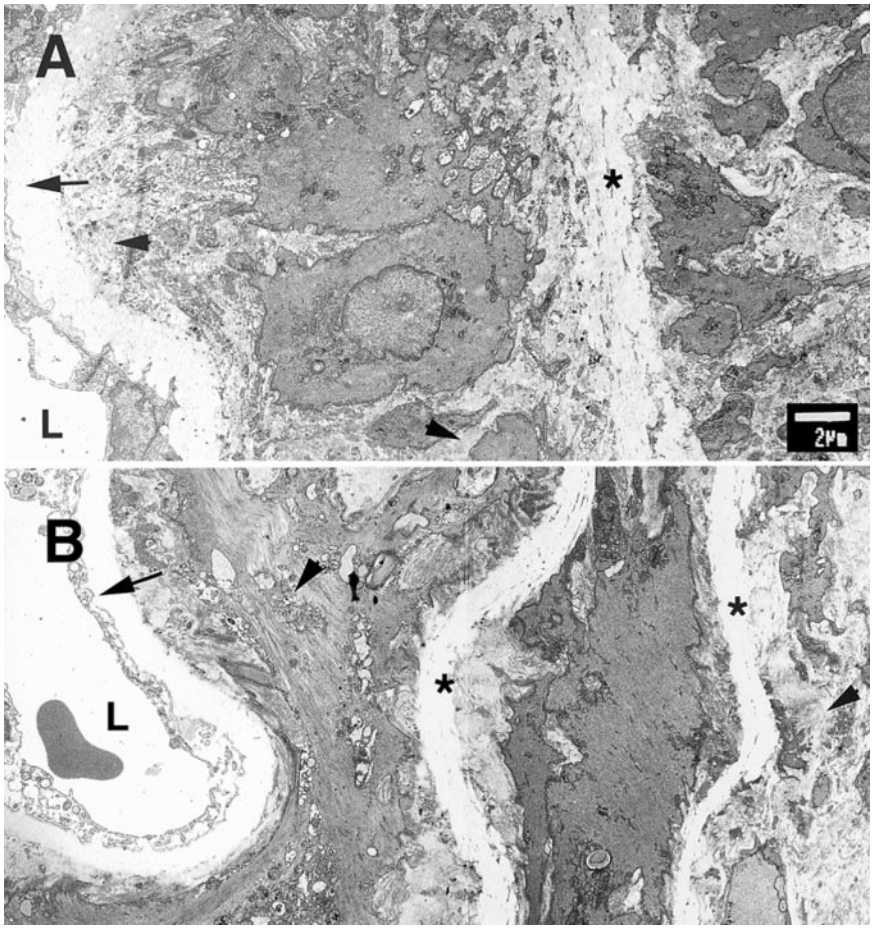

Figure 4. Transmission electron micrographs of cross sections from grossly normal descending aortas from a Δ/Δ mouse (A) and from a littermate +/+ mouse (B). Each micrograph is a composite of 2 individual exposures. Elastic lamellae in the Δ/Δ mouse aorta are less compacted and are irregularly shaped in comparison with those in the control mouse. These sections are representative of sections obtained from 4 aortas of each genotype. Bar in A corresponds to 2 μm. L indicates lumen; asterisk, elastic lamellae; arrowheads, collagen fibrils; and arrows, internal elastic lamina.

content of aortas in homozygous mutant mice, as determined by hydroxyproline analysis, was significantly (20%) lower than that in the aortas of heterozygous or control mice, but this reduction is less than that of the corresponding *Col1a1* mRNA levels (Figures 5 and 6; see Discussion).

Discussion

The possibility that the first intron of the *Col1a1* gene might play a role in the regulation of its expression was first suggested by the studies of Jaenisch and coworkers.[5,27,28] In experiments in which the 9-Kbp Moloney murine leukemia virus was randomly inserted into the genome of mice, they identified a line in which the virus was integrated into the first intron of the *Col1a1* gene, 19 bp downstream from the 5′ splice site. Mice that were homozygous for this mutation died from rupture of large blood vessels, including the aorta, between embryonic days 11 and 14. Subsequent studies showed that the insertional mutation inactivated *Col1a1* in most cells, with the exception of odontoblasts and osteoblasts.[18,19] These initial reports sparked a number of sometimes conflicting studies from laboratories that attempted to define the sequences that were inactivated by this mutation.[5] Eventually, the preponderance of evidence supported the existence of orientation-dependent, cell-specific transcriptional elements in the first intron of the α1(I) collagen gene in both mice and humans.[5,29,30] This evidence was bolstered by consistent data, obtained in transgenic experiments that utilized human α1(I) collagen transgenes with different promoter and intronic sequences.[4,31]

In this report, we describe a striking feature of the phenotype of Δ/Δ mice: these mice are highly susceptible to the development of age-dependent aortic dissection and rupture. Δ/Δ mice were found to be viable, fertile, and apparently healthy during their initial assessment, which rarely extended beyond 6 months, although an age-dependent reduction in expression of the mutant allele in lung and muscle was documented.[9] However, on observation for more prolonged periods of time, as described in this study, spontaneous deaths occurred in these mice. During the course of an observation period of 18 months involving 121 mice, most of the deaths, and all deaths due to aortic rupture, occurred in the homozygous Col-IntΔ mouse colony. Thirty-nine of the Δ/Δ mice (54%) died during the course of the experiment (online Table 1). In some cases, preservation of tissues was poor because of the time lapse between death and autopsy. Gross dissection indicated that most of these animals died because of hemorrhage. We believe that these mice had ruptured aortas, although this was not verified histologically. However, histological examination of the aorta was performed in 17 mice, and an aneurysm was identified in 15 cases (online Table 1). One animal died of unknown causes and another had a tumor in the peritoneal cavity. Three Δ/+ and three +/+ mice also died during this 18-month period. The cause of death in these animals is not known. None of these mice was found to have aortic

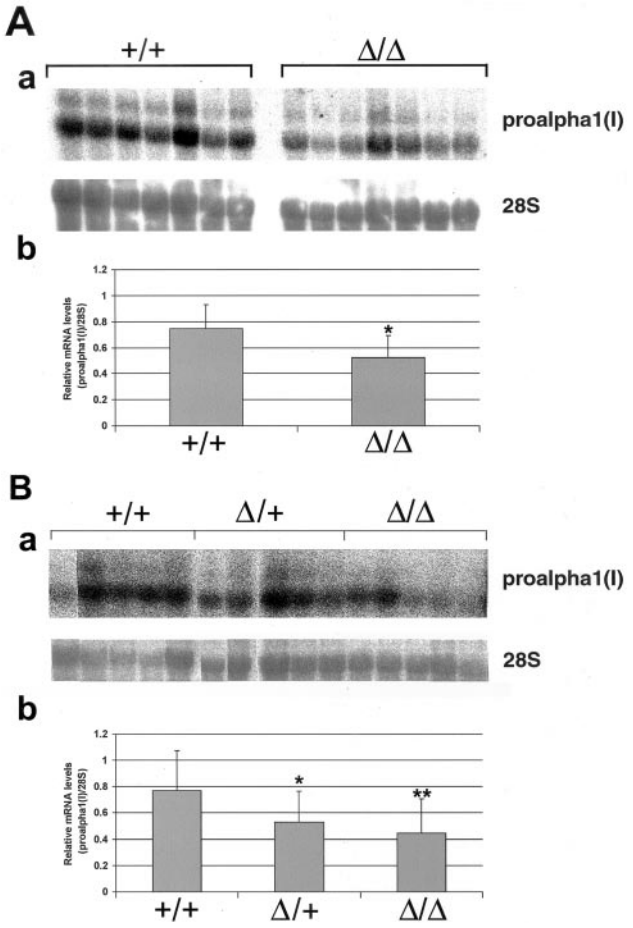

Figure 5. Northern analyses (a) and corrected densitometric measurements (b) of aortic proα1(I) collagen mRNA levels in 2.5-month-old Δ/Δ (A) and 12-month-old Δ/Δ and Δ/+ mice (B), compared with the levels in +/+ littermates. At 2.5 months, the mRNA levels were reduced by 29% in Δ/Δ mice and at 12 months by 32% and 42% in Δ/+ and Δ/Δ mice, respectively, relative to +/+ mice. Statistically significant differences are marked with asterisks (*$P<0.05$, **$P<0.01$; 2.5-month-old mice n=14; 12-month-old mice n=18, Student's *t* test).

pathology. To determine whether increased blood pressure could be a factor in these deaths, systolic blood pressure was measured by the tail-cuff method in six Δ/Δ and five control mice. No significant differences were detected (data not shown). Thus, we conclude that the Col-IntΔ mutation is expressed as a recessive trait, which in the homozygous state predisposes mice to aortic dissection.

Because the targeted deletion is in the *Col1a1* gene, it is reasonable to assume that reduced collagen expression is responsible for the aortic pathology. The results of Northern analyses (Figure 5) and studies of the relative levels of expression of the mutated and control collagen alleles (Figure 6) document that age-dependent reduced expression of *Col1a1* occurs in the aorta of Δ/Δ mice. These results are supported by examination of aortic sections stained with Masson's trichrome and Picrosirius red (Figures 3C through 3H). Hydroxyproline analyses of the aortic tissue also indicated a statistically significant decrease in total collagen in Δ/Δ mouse aortas, compared with that of their littermate

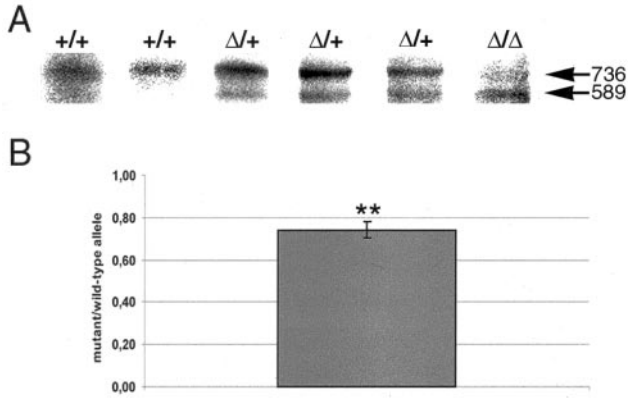

Figure 6. Allele-specific expression of transcripts encoded by the Col-IntΔ and wild-type alleles, as analyzed by RT-PCR of aortic mRNAs from heterozygous mice mRNA. A, Products of the wild-type and mutated alleles were digested with *Xho*I, which selectively cleaves the cDNA derived from the mutated allele, and the bands were separated by polyacrylamide gel electrophoresis. Relative intensity of the larger, 589-bp cDNA was corrected for loss of the shorter fragment (see Materials and Methods). B, Ratio of the intensities of the cDNA products from the mutated and wild-type alleles. **$P<0.01$, n=9.

controls (Figure 7), although this reduction (20%) is less than that predicted by measurements of mRNA levels. This apparent discrepancy can be explained by the fact that collagen content measures accumulation over a long period of time, whereas mRNA levels are more likely to reflect the synthetic rate at the time the measurement was made. It is likely that the turnover rate of collagen in the aortas of older adult mice is quite low. Thus, a significant fraction of the collagen in older animals could have been laid down at a younger age, when the expression of the mutant *Col1a1* allele was higher (Figure 5). Furthermore, hydroxyproline values do not discriminate between the amounts of types I and III collagens; the latter, which is a major collagen in the aorta, is expressed at a normal rate in mutant mice, as judged by equal mRNA levels in mutant and control animals (data not shown). Thus, the reduced hydroxyproline values in Δ/Δ aortas reflect a highly significant reduction in type I collagen, and this finding is supported by EM micrographs. We suggest that there may be a threshold effect whereby a modest decrease in collagen I expression, as seen in heterozygous mice, has a very limited effect on survival, but a further decrease, as seen

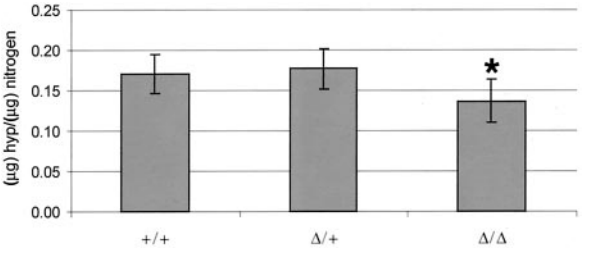

Figure 7. Hydroxyproline concentration (μg/μg total nitrogen) in the aorta of Δ/Δ and Δ/+ mutant mice compared with the concentration in +/+ littermates. Difference between the values in Δ/Δ mutant aortic tissue and controls is statistically significant. *$P<0.05$.

in the homozygous mutant animals, strongly predisposes to aortic rupture.

In contrast to the fibroblasts and smooth muscle cells in the aortic wall, the cells responsible for the fibrosis of the hematoma, shown adjacent to the aorta of a Δ/Δ mouse (Figures 3C and 3E), are able to synthesize considerable quantities of collagen, which is presumably type I. This result is consistent with that observed in bleomycin-induced lung fibrosis in Δ/Δ mice,[10] and supports the earlier suggestion that cells are capable of increasing expression from the mutant allele in response to injury.

Disorders of collagen biogenesis, other than those that cause a reduction in synthesis, can also result in weakness of the aortic wall. Lysyl oxidase, a member of a family of five homologous proteins, is a copper-dependent enzyme that catalyzes the first step in the formation of covalent crosslinks in collagens and elastin.[32] Mice that are deficient in lysyl oxidase, either on the basis of a targeted disruption of the gene[33] or because of a spontaneous mutation at the *mottled* locus that impairs copper transport,[34] develop aneurysms. The latter disorder is the murine equivalent of Menkes disease.[35] However, because cross-linking of both collagens and elastin is likely to be compromised in lysyl oxidase-deficient mice, the defective protein primarily responsible for aneurysm formation cannot be ascertained with certainty.

An intriguing aspect of our findings is the age-related reduction of expression of the *Col1a1* gene in Δ/Δ mice. Although we currently have complete data only for lung, muscle, and aorta, it seems probable that other tissues will also manifest reduced collagen content with age. A recent study has found that Δ/Δ mice show geometric changes and altered biomechanical properties in long bones.[36] We currently do not understand the basis for the impact that age has on collagen gene expression, and this remains a subject for future study. A partial explanation for the finding that the aorta may be particularly sensitive to the reduction in collagen synthesis in our mutant mice comes from studies in rats, which show that the collagen content of the aorta normally increases with age. Thus, collagen represents 8% of the dry weight of the vessel at birth, 23% at 2 months, and 30% at 6 months.[37] These findings suggest that there is a requirement for an increasing collagen content in the aortas of older animals. This requirement could make the aorta susceptible to rupture if a reduced level of synthesis compromised replacement of the protein that was lost by normal turnover.

In conclusion, these studies provide additional evidence for an age-dependent role of intronic sequences in the *Col1a1* gene in the regulation of collagen gene expression. Aging mice that are homozygous for a deletion in the first intron of the gene are subject to a substantial risk of aortic dissection and rupture. Although the DNA sequences that are responsible for these effects have not yet been identified, the surprisingly high degree of sequence conservation between rodent and human first introns of the α1(I) gene[38] suggests that the human gene may be subject to similar regulation. Intronic mutations and deletions in COL1A1 should therefore be considered in individuals who have a family history of aortic hemorrhage without evidence for the presence of any of the subtypes of osteogenesis imperfecta or other known causes of dissection or aneurysm formation.

Acknowledgments

This study was supported by the Academy of Finland (Project 52940), the Turku University Foundation, the Finnish Heart Foundation, and by a grant (AR11248) from the NIH (to P.B). O.R. and I.K. are recipients of fellowships from the Turku Graduate School of Biomedical Sciences. The authors thank T. Oivanen and M. Lakkisto for expert technical assistance and Drs David Dichek and Helene Sage for helpful discussion and comments on the manuscript.

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