Localization of Type II Collagen mRNA Isoforms in the Developing Eyes of Normal and Transgenic Mice With a Mutation in Type II Collagen Gene

Mikko Savontaus, Tapio Ihanamäki, Marjo Metsäranta, Eero Vuorio, and Minna Sandberg-Lall

Purpose. To elucidate the function of type II collagen in the development and diseases of the eye by analyzing the temporospatial expression of the long (IIA) and short (IIB) isoforms of type II collagen in the normal and transgenic Dell mice.

Methods. Normal and Dell transgenic embryos harboring a deletion mutation in the proal (II) collagen chain were studied from day 10.5 of embryonic development up to day 10 postpartum. Northern and in situ hybridizations and RNase protection assays were used to study the developmental and temporospatial expression of type II collagen isoforms.

Results. Expression of type II collagen mRNAs was observed at all developmental stages with maximum expression at 16.5 days of embryonic development. RNase protection analyses confirmed that both wild type and transgene-derived mRNAs underwent similar alternative splicing of exon 2 in the eye. By in situ hybridization, both isoforms were observed in the cornea, sclera, vitreous, ganglion cell layer of retina, developing ciliary body–iris, and in the retinal pigment epithelium–Bruch's membrane as well as in the lens and conjunctiva. Differences were observed between eyes of Dell mice and of control subjects in the levels and temporal expression patterns of type II collagen mRNA, which resulted in structural abnormalities in histologic analysis.

Conclusions. Widespread expression of type II collagen mRNAs in ocular structures suggests an important role for type II collagen in structural development of the eye. As the expression patterns observed correspond to structural abnormalities in the eyes of Dell mice, the current results offer a promising basis for further development of mouse models for arthroophthalmo-pathies.


Type II collagen, a member of the subfamily of fibrillar collagens, is the major component of the fibrils responsible for the structural strength of cartilage and the vitreous of the eye. In addition to these structures, type II collagen or its mRNA or both also have been detected in many nonchondrogenic regions during embryonic development, including the notochord, brain and chondrocranium, heart, and many embryonic basement membranes. 1–5 Distribution of type II collagen in the eye has been studied mainly in the chick, where the protein or its mRNA or both have been found in cornea, neural retina, sclera, and the ciliary body by immunohistochemistry and in situ hybridization. 6–8 In contrast, little is known about type II collagen expression in mammalian eyes; one in situ hybridization study available suggests that the expression pattern in the mouse eye resembles that in the chick eye. 2

Type II procollagen, a homotrimer of proal (II) chains, is synthesized in two forms, resulting from alternative splicing of exon 2, encoding a 69-amino acid cysteine-rich region of the amino-terminal propeptide. 9 The shorter form, type IIB, does not contain exon 2-encoded sequences and is the predominant isoform of mature cartilage. The longer splice variant, type IIA, containing exon 2-encoded amino acids is expressed by prechondrogenic cells at the onset of
chondrocytes, and by the cells of the perichondrium. Type IIA also has been detected in various noncartilaginous tissues during embryonic development. In developing avian cornea, type IIA has been found to be the predominant isoform; both isoforms also have been detected in bovine vitreous. Some of these diseases, namely the Stickler and Wagner syndromes and Kniest dysplasia, exhibit changes in the ocular system as well and therefore also are called arthroophthalmopathies. Specific mutations in the type II collagen gene have been characterized in several patients with these diseases. The ocular findings in these diseases are related to the structural weakening of connective tissue and include myopia, vitreoretinal degeneration, retinal detachment, glaucoma, and cataract.

The wide range of clinical manifestations of type II collagen mutations is not well understood, although characterization of the basic defects has provided some clues for establishing genotype–phenotype correlations. Limited availability of cartilage and ocular tissues from patients with arthroophthalmopathies makes systematic analyses of their pathogenic mechanisms difficult. To circumvent this obstacle, we and others have produced transgenic mice harboring mutations in the type II collagen (Col2al) gene as models for such diseases. The most informative mice we have produced harbor a Col2a1 transgene with a 150-bp deletion removing the 45-bp exon. The transgenes thus code for proα(II) collagen chains, which are shortened by 15 amino acids at the amino-terminal end of the triple helical domain. Because their carboxytermini are not affected, the mutant chains appear to associate with the wild-type proα(II) chains and to produce a dominant negative effect. This is seen as dose-dependent reduction of normal cartilage collagen fibrils, dose-dependent accumulation of proteinaceous material in the rough endoplasmic reticulum of chondrocytes, and dose-dependent severity of the resultant phenotype. In line Dell, six copies of the transgene result in a mild phenotype of early-onset osteoarthritis and slight growth retardation. Because these mice reproduce normally, it has been possible to obtain offspring homozygous for the Dell transgene locus (12 transgene copies), which exhibit a perinatally lethal phenotype consistent with severe human chondrodysplasia with short limbs, abnormal craniofacial development, spina bifida and cleft palate, and respiratory distress. Transgenic mice of line Del3 (15 transgene copies) exhibit a similar, but even more severe, phenotype of short-limbed dwarfism and also die at birth. Histologic analysis of the eyes of these mice showed several genotype-dependent structural abnormalities, proving that Dell mice could serve as models for arthroophthalmopathies. In this study, we followed the temporospatial expression of type II collagen isoforms in the eyes of normal and Dell transgenic mice during embryonic development by in situ and Northern hybridizations and RNase protection analyses to better understand the role of type II collagen in ocular development and in arthroophthalmopathies.

**MATERIAL AND METHODS**

**Transgenic Mice**

This study was performed on the offspring of one transgenic founder mouse Dell carrying six copies of a mouse type II collagen transgene with a 150-bp deletion, including the 45-bp exon. Mice heterozygous for the transgene locus were mated and the embryos were delivered by Cesarean section at days, 14.5, 16.5, and 18.5 of gestation. This experimental setup produced embryos homozygous (+/+), heterozygous (+/−), and nontransgenic (−/−) littermates that served as control subjects. DNA was extracted from the tails of the animals for genetic analysis by polymerase chain reaction and Southern hybridization to determine the genotypes. Additional tissue samples were obtained from nontransgenic control subjects at 10.5 and 12.5 days of embryonic development and from 1-, 5-, 7-, and 10-day-old mice. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Preparation and Analysis of Total RNA**

Total RNAs were extracted as described previously from the whole eyes of 1-, 5-, and 10-day-old mice, from the conjunctiva, the lens, and whole eyes minus lens of 1-week-old mice, as well as from the whole eyes of mouse embryos at days 10.5, 12.5, 14.5, 16.5, and 18.5 of gestation. Aliquots of 7.5 to 15 μg of total RNA were fractionated on 1% agarose gels containing 2 M formaldehyde, blotted overnight onto Pall Biodyne transfer membranes, and hybridized with 32P-labeled cDNA inserts as suggested by the supplier of the membrane. The bound probe was detected by autoradiography at −70°C using X-ray films and intensifying screens.

Determination of the ratios of long and short isoforms of transgene-derived and wild type mRNAs for proα(II) collagen was performed using an RNase protection assay. Solution hybridization was performed at 55°C using 5 × 106 cpms of probe and 10 μg of total RNA. After hybridization, the samples were digested with RNase A and T1, and proteinase K, and...
were exposed with X-ray films and quantified on a
preparation for electrophoresis on denaturing 4.5%
precipitated for electrophoresis on denaturing 4.5%
for type II collagen. The coding potential of the type II collagen mRNA is shown below. UT, untranslated sequence; N-pro and C-pro, amino- and carboxyterminal propeptides, respectively.

Preparation of Probes
The mRNA for procollagen mRNA isoforms (IIA and IIB) was detected using the reverse transcription–polymerase chain reaction technique with mouse cartilage RNA as template and two oligonucleotides based on the published mouse genomic sequence29 as amplification primers (Fig. 1). The resultant fragment was cloned into pBluescript KS vector, and its identity was confirmed by sequencing using the Sequenase kit (United States Biochemical, Cleveland, OH). The probe for detection of the ratios of long and short isoforms of transgene-derived and wild type mRNAs for procollagen II collagen was constructed using the reverse transcription–polymerase chain reaction technique with mouse cartilage RNA as template and two oligonucleotides based on the published mouse genomic sequence29 as amplification primers. The obtained fragment was cloned into pGEM-T vector and identified by sequencing (Fig. 1). The hybridizations were performed at 52°C for 18 hours. Hybridization was followed by washes and autoradiography for 2.5 to 94 days, and the sections were stained with hematoxylin.

RESULTS
Developmental Expression of Type II Collagen mRNAs in Mouse Eyes
To characterize the expression levels of type II collagen isoforms in the eye during mouse embryonic development, total RNA was extracted from eyes of normal mice at 10.5, 12.5, 14.5, 16.5, and 18.5 days of embryonic development. Specific mRNA levels were detected using probes pMCol2a1-1 (detecting isoforms IIA and IIB) and pMCol2a1-2 (detecting only the IIA isoform) (Fig. 1). The hybridizations showed that type II collagen mRNA was detected with both probes from embryonic day 10.5 onward. After correction for variations in total RNA loaded, densitometric analyses showed the highest mRNA levels with both probes at 16.5 days after which the expression levels declined (Fig. 2). To further characterize the structures of the eye expressing type II collagen, total RNA was extracted from whole eyes of 1-day-old normal pups as well as from the eyes of 1-week-old normal pups dissected into the following fractions: lens, conjunctiva, and eyes without lens. The subsequent Northern analysis confirmed the expression of type II collagen mRNAs also in the lens and conjunctiva of normal mice at this developmental stage (Figs. 3A, 3B, 3C). As the expression of type II collagen gene was higher in the eyes of 1-day-old mice than in any of the samples from 1-week-old mice, ocular RNA extracted from 5- and 10-day-old mice also was analyzed for total type II collagen mRNA levels (Figs. 3D, 3E, and 3F). This confirmed that the mRNA levels peak at embryonic day 16.5 and then gradually decline at least until the age of 10 days.
Type II Collagen in Developing Mouse Eye

Distribution of Type II Collagen mRNAs in Different Structures of Mouse Eyes

To identify the cellular sites of type II collagen synthesis, we localized type II collagen mRNAs by in situ hybridization of Del1 +/+ and −/− eyes using probes pMCol2a1-l (detecting both isoforms) and pMCol2a1-2 (detecting only the IIa isoform) at embryonic days 14.5 (Fig. 6), 16.5 (Fig. 7), and 18.5 (Fig. 8). At all developmental stages, hybridization with both probes was more intense in Del1 homozygous embryos in all positive tissues. Hybridization was observed in the vitreous space, the region of the internal limiting membrane and the ganglion cell layer. In the neuroepithelial layer of retina, no labeling was observed except in the outer edge of the neuroepithelium, which is detached artificially from the pigment epithelium in tissue sections because of the dehydration processes while the eyes are being embedded. At low magnification.

Differential Expression of Type II Collagen Isoforms in Normal and Dell Mouse Eyes

To quantify the differential expression of type II collagen mRNA isoforms in normal and transgenic embryos, RNase protection assays were performed using total RNAs from the eyes of normal mice at 12.5, 14.5, 16.5, and 18.5 days of embryonic development, and from the eyes of Del1 +/− and +/+ mice at the age of 18.5 days of embryonic development (Fig. 4B). The results were in accordance with the Northern analyses with the highest expression of both isoforms at 16.5 days of embryonic development (Fig. 5A). The assay confirmed that the transgene-derived type II collagen transcripts also underwent alternative splicing of exon 2. In the heterozygous Del1 eyes, the amount of transgene-derived mRNA was equal to the endogenous mRNA, and in the homozygous eyes, the amount of transgene-derived mRNA was approximately twofold higher (Fig. 5B). The two endogenous isoforms were expressed in roughly the same amounts during normal development (Fig. 5A), with the type IIB isoform slightly predominating (52% to 58% of total). In the heterozygous and homozygous Del1 mice, the relative level of type IIB mRNA was somewhat higher (67% of total) (Fig. 5B).
FIGURE 4. Characterization of type II collagen expression in mouse embryonic eyes by RNase protection. (A) A schematic presentation of the probe used in the assay, pMCol2al-(2→7), and the four different type II collagen isoforms and the lengths of the respective protected fragments. The alternatively spliced exon 2 and the deleted exon 7 are shown with a gray and a black box, respectively; tg = transgenic, wt = wild type. (B) Detection of endogenous and Ex transgene-derived proal(II) mRNAs by RNase protection assay. The 32P-CTP-labeled antisense probe, pMCol2al-(2→7), was hybridized to 10 μg of total RNA from the eyes of normal and transgenic embryos followed by electrophoresis on denaturing 4.5% polyacrylamide gel with end-labeled Mspl fragments of pBR322 as standards. The sizes of the protected endogenous fragments are 465 bp (wt IIA) and 365 bp (wt IIB) and those derived from the transgene 420 bp (tg IIA) and 320 bp (tg IIB).

FIGURE 5. Quantitation of proal(II) mRNA transcripts by RNase protection assay shown in Figure 4. Relative levels of type II collagen transcripts were determined using a phospho-imager. The relative densities have been corrected for the difference in specific activity because of the varying guanine content in the protected fragments (wt IIA:tg IIA:wt IIB:tg IIB = 147:133:124:110). (A) Expression of type II collagen isoforms during normal embryonic eye development. The black columns represent type IIA and white columns type IIB. (B) Expression of type II collagen isoforms in normal and transgenic eyes at 18.5 days of development. The black and white columns represent transgene-derived types IIA and IIB, respectively.
FIGURE 6. Localization of type II collagen mRNAs by in situ hybridization in normal (A,D,G,K) and Del1+/+ (B,C,E,F,H,J,L,M) mouse eyes at 14.5 days of embryonic development. Signal was detected with probe pMCol2a1-2 for procollagen IIA collagen mRNAs (A to F) and with probe pMCol2a1-1 for both forms of procollagen IIA collagen mRNAs (G to M) in the region of the internal limiting membrane of retina (open arrow), ganglion cell layer (gcl), vitreous (v), pigment epithelium–Bruch’s membrane (pe), sclera (scl), cornea (c), and lens (le). Dark-field views (D,E,F,K,L,M) and the corresponding bright-field micrographs (A,B,C,G,H,J). The corneal hybridization signal is illustrated (C,F,J,M). The other panels shown in this figure are not sectioned through cornea. The anatomic structures are indicated in bright-field micrographs, A and C. Bar = 100 μm.

mation, the autoradiographic grains in the area of pigment epithelium (and detached neuroepithelium) are difficult to illustrate because pigment granules cause a similar dark field reflection. At higher magnification, however, the two signals can be identified easily by comparing the patterns obtained with sense and antisense probes (Figs. 8J1 and 8J2). A strong signal was detected in the developing ciliary body and iris region at days 16.5 and 18.5 of embryonic development as well as in the retinal pigment epithelium–Bruch’s membrane region at all developmental stages studied.

An intense hybridization was observed in the scleral wall at all timepoints. The strongest scleral signal
FIGURE 7. Localization of type II collagen mRNAs by in situ hybridization in normal (A,C,E,G) and Del1+/+(B,D,F,H) mouse eyes at 16.5 days of embryonic development. Hybridization signal with probe pMCol2a1-2 for pro-α1(IIA) collagen mRNAs (A to D) and with probe pMCol2a1-1 for both forms of proα1(II) collagen mRNAs (E to H) is best shown in dark-field micrographs. Note the strong labeling in cornea and sclera in the animals negative for the transgene (C and G) and even stronger signal in the respective areas in the transgenic animals homozygous for the transgene (D and H). Anatomic structures are indicated in bright-field micrograph (E). Arrow = developing ciliary body and iris, open arrow = internal limiting membrane of retina, arrow head = conjunctiva, c = cornea, gcl = ganglion cell layer of retina, le = lens, nel = neuroepithelial layer of retina, pe = pigment epithelium-Bruch’s membrane, scl = sclera-choroid, v = vitreous. Bar = 100 μm.
Type II Collagen in Developing Mouse Eye

was detected in the eyes of transgenic animals at day 16.5 of embryonic development; at day 18.5 of embryonic development, the signal was markedly weaker and most intense near the choroidal area (Figs. 6, 7, and 8).

At day 14.5 of embryonic development, a strong signal was detected in the corneas of Dell homozygous mice with both probes used (Figs. 6C, 6F, 6J, and 6M), and a weaker but clear signal was detected in the corneas of the nontransgenic animals at the same developmental stage (data not shown). The most intense hybridization was observed in Dell +/+ corneas with the probe detecting both type  IIA and IIB mRNAs at day 16.5 of embryonic development. At this developmental stage, both transgenic and normal corneas showed strong hybridization signal with both probes used (Fig. 7). At day 18.5 of embryonic development, the nontransgenic corneas showed hardly any hybridization with either probe, but in the Dell homozygous corneas, hybridization still was detected with both probes used (Fig. 8).

Some signal also was detected with both probes in the lens and conjunctiva of Dell homozygous and control animals at all developmental stages (Figs. 6, 7, and 8). The in situ hybridization results are summarized in Table 1.

Structural Abnormalities in the Eyes of Dell Mice

The histologic changes observed in Dell +/+ eyes are summarized in Figure 9 for 18.5 day embryos. The most apparent structural abnormalities were observed in the vitreous architecture of homozygous Dell mice where reduced filament density was observed without any clear changes in their structure (Figs. 9C1 and 9C2). In some Dell mice, the lens-iris diaphragm was displaced anteriorly and the anterior chamber was shallow or absent. In contrast, the anterior chamber was well formed in the normal animals of the same gestational age. In the corneal stroma, the lamellar structure appeared more disorganized with larger interfibrillar spaces in the transgenic animals as compared with the control subjects (Fig. 9). Similarly, the fibrillar structure of sclera of Dell mice was arranged more loosely than in the control subjects.

DISCUSSION

In the current study, we have examined the expression of the Col2al gene in normal and Dell transgenic mouse eyes during embryonic development and during a short neonatal period of 10 days using Northern hybridization, RNase protection assay, and in situ hybridization. The Northern blot analyses and RNase protection experiment results on the normal mouse eyes showed the presence of both forms of mRNAs at all stages of embryonic development studied. Northern hybridizations showed type II collagen expression as early as 10.5 days of ocular development continuing at least until 10 days postpartum. RNase protection assays showed that the expression of total type II collagen mRNA continued to increase from embryonic day 12.5 to day 16.5 approximately fourfold; thereafter, it declined at least until neonatal day 10. The peak mRNA level coincides with eye lid closure at embryonic day 16, but presently, there is no direct causal relation between these phenomena.32 The relative expression levels of the two isoforms remained fairly constant during normal embryonic development with the type IIB isoform slightly predominating. The RNase protection experiments on the eyes of transgenic animals at 18.5 days of embryonic development confirmed that both the endogenous and transgene-derived Col2al transcripts underwent alternative splicing of exon 2 in the eye. There was a genotype-dependent increase in the total amount of type II collagen mRNA in the transgenic eyes when compared to that of the control subjects. The expression level of transgene-derived type II collagen mRNA was approximately equal to endogenous mRNA in the heterozygous and approximately twofold in the homozygous eyes. Similar, although somewhat lower, ratios of transgene versus wild type mRNAs have been observed earlier in Dell limb cartilages.39 The expression of the wild-type long-form mRNAs remained at a constant level, but a small increase in the wild-type short-form mRNAs was detected in the transgenic eyes. At present, we do not know whether this observation represents a compensatory increase of endogenous mRNA due to the expression of defective type II collagen mRNA or reflects the retarded development of Dell mice.

As documented previously, the alternatively spliced forms of type II procollagen mRNAs either include (type IIA mRNA) or exclude (type IIB mRNA) exon 2 encoding a cysteine-rich domain in the amino-propeptide.9 During fibrillogenesis, removal of this propeptide is considered to be essential for synthesis of normal collagen fibrils.39 In mouse, type IIA mRNA has been shown to represent the major form of type II procollagen transcripts in both nonchondrogenic and prechondrogenic cells.12 In the mouse, a switch from type IIA to IIB mRNAs has been shown to occur as chondrocytes differentiate.37 Type IIA mRNAs have been shown to remain as the major form of type II procollagen transcripts in most nonchondrogenic mouse and human tissues.12,34,35 The expression of type II collagen isoforms in mouse eye has not been studied previously in detail. Our results suggest that both isoforms are expressed in fair amounts in the mouse eye. These findings differ from the data obtained from avian eyes, where type IIA has been found to be the predominant isoform in developing cornea and optic cup with only trace amounts of IIB detected.14
The current observation on the expression of type II collagen in mouse cornea, ciliary epithelium, and retina largely is in agreement with the localization of type IX collagen mRNA in chick and mouse eyes. However, in mouse cornea, Liu et al did not find expression of α1(IX) mRNA, although it has been found in chicken cornea. Type IX collagen associates with type II collagen fibrils and also exhibits short and long forms, resulting from alternative use of promoters in the gene coding for the α1(IX) chain. Developing chicken and mouse eyes have been shown to contain mostly the short form of α1(IX) mRNA. Further studies are needed to determine how well the long and short forms of type IX and type II (pro)collagens are colocalized in the mouse eyes.

In situ hybridization analyses allowed us to locate the cells synthesizing type II collagen mRNAs. A strong signal with both type II collagen probes was observed in the vitreous near the internal-limiting membrane and in the ganglion cell layer of retina at all developmental stages studied and suggests an important role for type II collagen in the growth and development of these structures. The findings also are in accordance with the previous theory of the role for type II collagen in the developing mouse eye.

### TABLE 1. Localization of Type II Procollagen mRNAs in Normal and Del1 +/+ Mouse Eyes During Embryonic Development

<table>
<thead>
<tr>
<th></th>
<th>14.5 Days</th>
<th>16.5 Days</th>
<th>18.5 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II A + B</td>
<td>II A</td>
<td>II A + B</td>
</tr>
<tr>
<td></td>
<td>N D</td>
<td>N D</td>
<td>N D</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitreous</td>
<td>+ +</td>
<td>++ ++</td>
<td>++ ++</td>
</tr>
<tr>
<td>Ganglion cell layer</td>
<td>+ +</td>
<td>++ ++</td>
<td>++ ++</td>
</tr>
<tr>
<td>Neuroepithelial layer</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>RPE/Bruch’s membrane</td>
<td>++ ++</td>
<td>++ + +</td>
<td>++ + +</td>
</tr>
<tr>
<td>Gliarial body/iris</td>
<td>na na na na</td>
<td>++ + +</td>
<td>++ + +</td>
</tr>
<tr>
<td>Sclera</td>
<td>++ ++</td>
<td>++ + +</td>
<td>++ + +</td>
</tr>
<tr>
<td>Conjonctiva</td>
<td>++ + +</td>
<td>++ + +</td>
<td>++ + +</td>
</tr>
<tr>
<td>Cornea</td>
<td>I + + +</td>
<td>I + + +</td>
<td>I + + +</td>
</tr>
<tr>
<td>Lens</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
</tbody>
</table>

RPE = retinal pigment epithelium; na = data not available.

Summary of the expression of type II procollagen mRNAs in normal (N) and Del1 +/+ (D) mouse eyes at 14.5, 16.5, and 18.5 days of embryonic development. The intensity of hybridization is described as very strong labeling (+ + +), strong labeling (+ +), some labeling (+), and no labeling (-). Data not shown in the figures (I). Although not shown in the table, the signal intensities for the type II A + B mRNAs were higher than those for type II A mRNA. This was confirmed by RNase protection analyses (Fig. 5A).
collagen in guiding the axonal processes of the developing retinal ganglion cells as they traverse the retinal surface and converge to form the optic nerve bundle. The observed expression of type II collagen mRNAs in the lens and ciliary body also is in accordance with the previous assumption of the role of these structures in the production of vitreous collagens together with the cells of retina and hyalocytes. The observed expression of both transcripts in the cells of the outer edge of neuroepithelium and of retinal pigment epithelium—Bruch’s membrane suggests a role for type II collagen in maintaining the structural strength of the attachment area of retina and pigment epithelium. The expression of type II collagen in the vitreous, retina, and the attachment area of retina and pigment epithelium can at least partly explain the vitreoretinal degeneration frequently resulting in retinal tears and retinal detachment in arthroophthalmopathies with mutations in type II collagen gene. This is further supported by the previous and current observations on the structural changes in vitreous and retina in transgenic mice with mutations in type II collagen gene. The expression of type II collagen in the lens and ciliary body—iris region also is in accordance with the alterations in lens capsule and the lens—iris diaphragm in severely affected transgenic mouse lines Dell (carrying 15 copies of the same transgene as in Dell line) and Gly-85-1 (carrying approximately 50 copies of glycine-to-cystein mutation in the mouse al(II) collagen chain) and may partly be connected to glaucoma and early formation of cataracts in arthroophthalmopathies linked to mutations in type II collagen gene. Compared with Dell mice, the somewhat milder ocular phenotype in homozygous Dell mice is in agreement with our earlier observations on the transgene expression and severity of skeletal abnormalities, which also are greater in Dell mice than in homozygous Dell mice.
We also observed transient expression of type II collagen mRNAs in the developing sclera and cornea. A strong signal was detected with both probes used at day 14.5 of embryonic development; at 16.5 days, the signal was even stronger, but dramatically decreased by day 18.5 of development. In Del1 +/+ animals, the scleral and corneal signals still clearly were detectable at this timepoint, whereas in the control animals, they essentially had disappeared. Although this phenomenon partly could be because of overexpression of the transgene, we think it mainly reflects the developmental delay in cartilages of these animals described previously.3,5,6 Alterations in cartilaginous and fibrous sclera have been described in chicken models of form-deprivation myopia.41 Presently and previously observed histologic changes in sclera and cornea of transgenic mice harboring mutations in type II collagen gene22 suggest an important role for type II collagen in the structure and development of cornea and sclera. This is further supported by the current observation on the expression of type II collagen gene in cornea and sclera. In accordance with these findings are also the myopic changes characteristic of syndromes, which are caused by mutations in the type II collagen gene.11,25,30,42,48 Altogether, these findings suggest that type II collagen might play a role in the determination of the axial length of the eye and possibly also the corneal curvature, which in part influence the final refraction of the eye.

Our current and previous25 observations of structural abnormalities in the vitreous, retina, lens, lens--iris diaphragm, cornea, sclera, and in the area of Bruch’s membrane of homozygous Del1, heterozygous Del3, and Gly-85-1 mice are in accordance with those of the location of expression patterns of type II collagen mRNAs in this study. These changes are similar to those in human Stickler and Wagner syndromes and in Kniest dysplasia, where mutations in type II collagen gene have been found. The current results offer a promising basis for further development of mouse models for these human diseases.

**Key Words**

arthroophthalmpathy, eye development, in situ hybridization, transgenic mice, type II collagen

**Acknowledgments**

The authors thank Päivi Auho, Merja Lakkisto, Tuula Oiva-nen, and Heli Salminen for expert technical assistance.

**References**


