Autosomal Recessive Cornea Plana: In Vivo Corneal Morphology and Corneal Sensitivity

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PURPOSE. Autosomal recessive corneal plana (RCP) is a rare corneal anomaly with unknown pathogenesis and a high incidence in Finland. The aim was to examine corneal sensitivity and the morphology of different corneal layers and subbasal nerves in RCP patients.

METHODS. Three patients with a diagnosed autosomal recessive corneal plana were examined. Corneal sensitivity to different modalities of stimulation was tested in four corneas using noncontact esthesiometry. Tissue morphology of three corneas was evaluated, and in two corneas thickness of corneal layers was measured using in vivo confocal microscopy.

RESULTS. Corneas of RCP patients appear to have mechanosensory, polymodal, and cold-sensitive nerve terminals. RCP patients had normal sensation thresholds for chemical, heat, and cold stimulation but a high threshold for mechanical stimulation. Their capacity to discriminate increasing intensities of stimulus was reduced, except for cold stimuli. Thickness of the epithelial layer was reduced, whereas total corneal and stromal thicknesses were slightly reduced or close to normal values. In all cases Bowman’s layer was absent. Subbasal nerves had abnormal branching patterns. The arrangement of anterior keratocytes was altered, showing clustered and irregularly shaped nuclei. Increased backscattering of light in confocal microscopy through focusing (CMTF) profiles was observed throughout the stroma. Epithelial and endothelial cells appeared to be regular in shape.


Autosomal recessive corneal plana (RCP), or flat cornea, is a rare hereditary anomaly with a high incidence in the northern part of Finland. To date a total of 78 RCP cases have been found in Finland.1 RCP is characterized by extremely low corneal refractive power leading to strong hyperopia, slight microcornea, a widened limbal zone, a marked arcus senilis, a shallow anterior chamber, and a deep central peripera. Slight microcornea, a widened limbal zone, a marked arcus senilis, a shallow anterior chamber, and a deep central peripera. Slight microcornea, a widened limbal zone, a marked arcus senilis, a shallow anterior chamber, and a deep central peripera. Slight microcornea, a widened limbal zone, a marked arcus senilis, a shallow anterior chamber, and a deep central peripera. Slight microcornea, a widened limbal zone, a marked arcus senilis, a shallow anterior chamber, and a deep central peripera.

A gene locus for RCP has been assigned to the long arm of chromosome 12,2 but the functional significance of the gene is not yet characterized. A gene for autosomal dominant cornea plana (DCP), with distinct clinical features, has been mapped to a separate locus on chromosome 12.3 To the best of our knowledge, histopathologic features of cornea plana have only been reported in connection with two family members undergoing penetrating keratoplasty due to complications in DCP corneas.4

The aim of our study was to characterize whether RCP patients exhibit changes in the morphology of corneal cell layers by using in vivo confocal microscopy5,6 and in sensitivity by testing the response to different modalities of stimulation with noncontact esthesiometry.7 In addition, epithelial thickness, total corneal thickness, and backscattering of light was measured using in vivo confocal through focusing microscopy (CMTF).5,6

METHODS

Patients

Left corneas of three patients (two men, 54 and 49 years of age; and one woman, 48 years of age) with RCP, previously ascertained,1 were enrolled in our descriptive case series and received confocal microscopy. Sensitivity was tested in four corneas of three patients. All patients had been diagnosed at an early age in the 1950s and followed by one of us (HF) throughout the decades. The clinical signs had remained essentially the same during the years. None of the corneas had a history of corneal erosions or neovascularization. Family histories were consistent with autosomal recessive inheritance. The patients belong to the families showing linkage to the disease locus CNA2 on chromosome 12.2,5 The subjects signed an informed consent to a protocol approved by The Ethical Review Com...
mittee of Helsinki University Eye and Ear Hospital, and the research plan followed the tenets of the Declaration of Helsinki.

Clinical Examination
On slit lamp all three corneas manifested with identical clinical signs typical of cornea plana1: strong hyperopia due to reduced corneal curvature, a widened limbal zone, a marked arcus senilis, a shallow anterior chamber, and a central corneal opacity (Fig. 1). The current best corrected visual acuities were 20/125, 20/16, and 20/25, and the refractions 1\(^{13.0 \text{ cyl}}\) 2\(^{3.0 \text{ ax 70}}\), 1\(^{11.0}\), and 1\(^{10.0 \text{ cyl}}\) 2\(^{0.75 \text{ ax 30}}\), respectively. The lens and vitreous body of each eye showed no pathology, and the fundi appeared normal.

Esthesiometer
A gas esthesiometer described previously allowed us to perform selective mechanical, chemical, and thermal stimulation of the corneas.7 Gas jets of 3 seconds' duration were applied to the corneal surface, separated by 2-minute pauses. The selective mechanical stimulation consisted of a series of six pulses of air at flows varying from 0 to 300 ml/min. For selective chemical stimulation, five pulses of a mixture of air and CO\(_2\) at different concentrations (0–80% CO\(_2\)) were used. For selective thermal stimulation seven pulses of air at different temperatures (−10°C to +80°C) were applied, inducing corneal surface temperature variations between −5°C and +3°C around the control value (34.4°C).7 For selective chemical and thermal stimulation, flows below mechanical threshold of each subject were used. To prevent changes in corneal temperature during selective mechanical and chemical stimulation, the air was heated up to 50°C at the tip of the probe.7

Psychophysical Studies
The subject was seated in front of a slit lamp, and the head was supported by a holder. The tip of the probe was placed perpendicular to the center of the cornea, at a distance of 5 mm from the corneal surface. The subject was asked to blink immediately before each pulse and to keep the eye open during the 3-second stimulus. He/she identified the onset and offset of the stimulus by the click produced by the opening of the valve in the probe. Selective mechanical, chemical, and thermal stimulation was performed on the left eye in two subjects and on both eyes in one subject. Pulses were applied at random.

![Figure 1](image1.png)

**Figure 1.** Clinical photographs of a 49-year-old man with recessive autosomal cornea plana. (A) A widened limbal zone and a marked arcus senilis are clinical signs typical of cornea plana. Unfortunately the central corneal opacity is not very well reproduced in the photograph. (B) A side view of the same cornea. A greatly reduced corneal curvature and a shallow anterior chamber are shown here.

![Figure 2](image2.png)

**Figure 2.** Response to mechanical stimulation of the corneal surface of RCP patients. (A) Subjective intensity. (B) Irritation. (C) Stinging pain component. (D) Burning pain component. (E) Warming sensation. (F) Cooling sensation. Data are mean ± SEM of the VAS values indicated after each stimulus (n = 4 stimulated corneas of 3 subjects).
Immediately after each stimulus, the subject was asked to indicate the intensity of the stimulus in a continuous, 10-cm visual analog scale (VAS) where 0 was “no sensation” and 10 was the “maximal sensation ever experienced.” The lowest intensity of stimulus that evoked a response $0.5$ VAS units was considered to be the sensation threshold. After each stimulus the following components of the experienced sensation were evaluated in five separate VAS: degree of irritation, burning pain, stinging pain, warming sensation, and cooling sensation. The subjects were also asked to describe the quality attributes of the sensation evoked by each stimulus.

**In Vivo Confocal Microscopy**

A tandem scanning confocal microscope (TSCM, model 165A; Tandem Scanning Corporation, Reston, VA) was used for examining the central cornea of the patients. One eye per subject was examined. The setup and operation of the confocal microscope has been described previously.$^5,6$ Briefly, a $\times 24$, 0.6 numeric aperture (NA) variable working distance objective lens was used. The field-of-view with this lens is $450 \times 360$ μm, and the z-axis resolution is 9 μm. Images were detected using a Dage VE1000 low-light level camera and recorded on SVHS tape. Video images of interest were digitized using a PC-based imaging system with custom software (University of Texas Southwestern Medical Center at Dallas) and printed using an Epson Stylus Color 800 printer (Seiko Epson Corporation, Nagano, Japan). In addition, CMTF scans were obtained as previously described.$^5,6$ Using the custom software, the CMTF data were digitized and CMTF profiles (image intensity versus focal depth) were calculated. In one subject six and in another subject three acceptable CMTF profiles were obtained. One patient was not able to fixate steadily and no acceptable profiles were produced. The morphology of the cellular structures could, however, be evaluated. The thickness of the epithelium, Bowman’s layer, stroma and total cornea was calculated as the average of the values obtained from each in and out scan. A quantitative estimate of corneal haze (backscattering) was obtained by calculating the area below the haze peak in the CMTF profiles.$^6$

**FIGURE 3.** Response to chemical stimulation of the corneal surface of RCP patients. (A) Subjective intensity. (B) Irritation. (C) Stinging pain component. (D) Burning pain component. (E) Warming sensation. (F) Cooling sensation. Data are mean ± SEM, $n = 4$ corneas of 3 RCP patients.

**FIGURE 4.** Response to thermal stimulation of the corneal surface of RCP patients. (A) Subjective intensity. (B) Irritation. (C) Stinging pain component. (D) Burning pain component. (E) Warming sensation. (F) Cooling sensation. Data are mean ± SEM, $n = 4$ corneas of 3 RCP patients. No change in corneal temperature was evoked with air at $50^\circ$C (arrow in A).
**Statistical Analysis**

Intensity-response curves were obtained for mechanical, chemical, heat, and cold stimulation. Pearson correlation was used to determine stimulus-response relationship. Data were expressed as mean ± SEM.

**RESULTS**

**Sensation Threshold**

The flow of air (at 50°C) required to evoke a sensation in the cornea was established at 260 ± 0 ml/min (n = 4 eyes of 3 patients). The sensation threshold for mechanical stimulation was defined by all subjects as “irritation.” Concentration of CO₂ in air necessary to evoke a sensation varied among individuals (range, 20%–80% CO₂); the mean threshold was 40% ± 14% CO₂ (n = 4). Threshold sensation evoked by CO₂ was also defined by RCP patients as “irritation.” Heat threshold was 80°C ± 0°C (n = 4); heat threshold sensation was defined by subjects as “slightly irritant.” Cooling threshold was established at 15°C ± 5°C (range, 25°C to 10°C; n = 4). The threshold sensation for cold stimulation was always defined as “cooling and slightly unpleasant."

**Discrimination of Stimulus Intensity**

Figures 2A through 4A illustrate the intensity-response curves for selective mechanical, chemical, and thermal stimulation of the cornea of RCP patients. The average VAS values of subjective intensity of the sensation were plotted against the intensity of the applied stimulus. Except for cold stimulation, no significant correlation was found between the intensity of the stimulus and the experienced intensity reported by the subjects (Pearson correlation coefficients = 0.829, 0.390, 0.945, and −0.986; P = 0.08, 0.517, 0.212, and 0.014; for mechanical, chemical, heat, and cold stimulation, respectively).

**Components of Sensation**

After the four modalities of stimulation (mechanical, chemical, heat, and cold), low VAS values were given to irritation, stinging, and burning pain components of the sensation (Figs. 2–4, panels B–D). Thermal components of the sensation (warming and cooling) were also evaluated after each stimulus. Subjects assigned low VAS values to thermal components when mechanical, chemical and heat stimulation were performed (Figs. 2–4, panels E and F). Cooling was the predominant component of the sensation evoked by cold stimulation, its magnitude being proportional to the intensity of stimulation (Pearson coefficient, −0.960; P = 0.040; Fig. 4F).

**Figure 5.** Images of epithelial cells and keratocytes of patients with RCP. The surface epithelial cells (A) and basal epithelial cells (B) present with normal morphology. Bowman’s layer is absent, and the most anterior keratocytes are imaged at the same level with subbasal nerves (C). The most anterior keratocyte nuclei (arrows) are visible among increased reflectivity from pathologic extracellular matrix (D). Midstromal keratocytes (arrows) were embedded in increased extracellular matrix (E). Note the stromal nerve (open arrow). Posterior keratocyte nuclei were obscured due to opaqueness of the stromal matrix (F). Size of all images is 390 × 290 μm.
In Vivo Confocal Microscopy

The outer and basal epithelial cells presented with normal shape and reflectivity (Figs. 5A, 5B). The mean epithelial thicknesses in the center of the cornea, measured in two RCP subjects, were 35.5 and 40.7 μm. Because Bowman’s layer was absent in all three corneas, the most anterior keratocyte nuclei had a displaced location immediately below the epithelium and were observed in the same image as the subbasal nerve fiber bundles (Fig. 5C). The anterior keratocyte nuclei were different in shape, reflectivity, and arrangement from those in normal subjects (Fig. 5D). None of the corneas showed the circular arrangement of keratocytes as described by Müller et al. (1995). Midstromal keratocyte nuclei were embedded in an opaque matrix (Fig. 5E), and posterior keratocyte nuclei were obscured due to opaqueness of the stromal matrix (Fig. 5F). Endothelial cells were regularly distributed, but detailed analysis of the cell sizes was impossible because they were poorly visualized (data not shown).

The nerve fiber bundles forming the subbasal nerve plexus appeared less abundant than in normal individuals (Fig. 6). In one patient, images of both altered nerves and a well-preserved nerve plexus comparable to those of a normal cornea were observed (Fig. 6A). The branching pattern of subbasal nerves was exceptional when compared with healthy corneas, where parallel running thick bundles with thin interconnecting branches are frequently encountered. In RCP corneas abnormal subbasal fiber bundle branching or fusing was perceived (Figs. 6B–6D). In addition, some of the fiber bundles ran among abnormally highly reflecting extracellular matrix (Figs. 6E, 6F). In some fiber bundles, beads corresponding to the location of mitochondria and neurotransmitter-containing vesicles were observed (Figs. 6A, 6C, 6E). Images of stromal nerves were captured occasionally, but they were not analyzed because of the limited number of stromal nerves visualized by in vivo confocal microscopy.

The mean stromal thickness values measured in the central cornea of two subjects were 364.1 and 471.4 μm, and the mean total corneal thickness values were 404.8 and 506.9 μm. Both corneas presented abnormal CMTF profiles. Starting from the level of the most anterior keratocytes, an increased backscattering of light could be noticed (Fig. 7), giving an abnormally high haze estimate in both corneas (mean, 8978 and 2540 U). For comparison, a CMTF profile produced from a healthy cornea is also presented.

**DISCUSSION**

In vivo confocal microscopy has proven to be suitable for the characterization of dynamic cellular responses during corneal regeneration. The ability to observe changes at the cellular level, such as those observed in the epithelial and subepithelial layers, provides valuable insights into the mechanisms of disease progression and potential therapeutic targets. The observation of altered nerve fiber patterns in RCP corneas highlights the importance of neural tissue in the maintenance of corneal integrity and function. Further studies are needed to elucidate the specific mechanisms underlying these changes and to develop effective treatments for RCP.
wound healing,6 and it has been useful in the assessment of structural changes caused by corneal dystrophies.8,11 Furthermore, it can improve the diagnosis of acanthamoebic keratitis.12 In the present study we were able to characterize in vivo qualitative and quantitative alterations in sensitivity and in morphology of corneal structures in RCP corneas.

The cornea is innervated by trigeminal sensory afferents that terminate as free nerve endings in the corneal tissue, a few of them in the anterior and medium part of the stroma and most of them in the corneal epithelium, especially between the epithelial wing cells.10,13 Based on their response stimulation, three types of neurons innervating the cornea have been functionally characterized: mechanosensory, polymodal, and cold-sensory neurons (see Ref. 14 for a review). Selective mechanical, chemical, heat, and cold stimulation was performed to establish the type (and functional state) of the neurons innervating the cornea of RCP patients. The data clearly showed that these subjects present the capacity to recognize the different modalities of stimulation, suggesting that their corneas are innervated by pure mechanosensory, polymodal, and cold-sensitive neurons.

Thresholds for chemical, heat, and cold sensation of RCP patients were normal or close to the normal range,7 but they were not able to discriminate the intensity of the stimulus. Mechanical threshold measured in these patients was significantly higher than those measured in normal subjects.7 This enhanced mechanical threshold could be due to the reduction of subbasal corneal nerve bundles observed in RCP patients, because straight fibers in this plexus are considered mechanoreceptive in nature.15 Decreased mechanical sensitivity could also be due to the reduction of the number of intraepithelial nerve endings, but the spatial resolution of in vivo confocal microscopy did not allow us to study the density of nerve terminals. A decrease of the number of intraepithelial nerve terminals sensitive to mechanical, chemical, and heat stimulation (i.e., polymodal nociceptors, the most abundant type of corneal nerve fibers)14 could explain the increased threshold and the reduced ability of subjects to discriminate the intensity of mechanical, chemical, and heat stimuli. Cold-sensitive neurons represent a low percentage (less than 10%) of the neurons innervating the cornea.14,15 Nerve terminals sensitive to cold encoded properly the intensity of cold stimuli applied to the cornea in RCP.

When the central epithelial thickness recorded in the present study (35.5 and 40.7 μm) was compared with the thickness of control corneas (50.6 ± 3.9 μm) reported in a previous study using the same technique,5 it was clear that in RCP the epithelial thickness was greatly reduced without changes in morphology of the surface and basal epithelial cells. This is in contradiction to an earlier report in which irregular reparative epithelial cystic proliferation of the corneal epithelium was described in autosomal dominant cornea plana.4 However, these corneas presented with corneal ulceration and subsequent vascularization and cicatrization of the superficial layers of corneal stroma. Just as for the extended number of RCP examined previously,1 no signs of corneal erosions or neovascularization were observed in our patients. Our data support histopathologic findings that Bowman’s layer is absent or defective in cornea plana.4 The anterior regions of excised corneas also showed infiltration of lymphocytes, plasma cells, and polymorphonuclear leukocytes in histologic sections.4 Although it is quite difficult to discriminate between different inflammatory cells in confocal microscopy images, there were no signs of such cells in the corneal stroma of RCP patients. In one cornea, cellular structures possibly reminiscent of Langerhans’ cells were observed associated with subbasal nerve fiber bundles, but such cells are occasionally also observed in healthy corneas.

In a Finnish cohort, thin corneas were observed on slit-lamp examination.1 Outside the central corneal disc they appeared to be thinner than in the center. In the present study,
the central corneal thickness was 507 μm in one eye but strongly reduced to 405 μm in another eye. The first patient had a central stromal thickness (471.4 μm) closely resembling that of normal corneas, whereas the second had a markedly thinner stroma (364.1 μm). This observation could be explained by a progressive loss of superficial corneal stroma in RCP subjects. The loss of stroma induced by corneal lesions can be a trigger to activate keratocytes. RCP patients did not show the highly reflective nuclei and visible cellular processes that are characteristic of activated keratocytes. However, it is possible that the keratocyte processes are obscured by a highly reflective extracellular matrix.

In CMTF of normal corneas two major intensity peaks are detected, one for the surface epithelium and one for the endothelium. Our RCP corneas produced CMTF profiles with two additional haze peaks. The first peak started behind the basal epithelial cells and extended to the posterior stroma, and a second haze peak was located just in front of the endothelial peak. In RCP, corneal opacities are mainly found in the central disc and attached to Descemet’s membrane. The second haze peak likely corresponds to this clinical finding. Opacities were not restricted to the outermost part of the stroma because increased backscattering was found throughout the whole corneal stroma.

In conclusion, the in vivo findings in RCP can be summarized as follows: a thin epithelium; disappearance of Bowman’s layer; different location and arrangement of the most anterior keratocyte nuclei; a reduction in the number of subbasal nerve fiber bundles and a change in their branching pattern; increased backscattering due to abnormal extracellular matrix occurring in two peaks: one posterior to the epithelium and one anterior to the endothelium; sensitivity to selective stimulation of the corneal surface with several modalities of stimulus, indicating the presence of mechanosensory, polymodal, and cold-sensitive neurons innervating the cornea; and increase of the mechanical threshold and absence of discrimination of the stimulus intensity, except for cold stimulation, suggesting a reduction of mechanosensory and polymodal nociceptive nerve terminals and the presence of functional cold-sensitive nerve endings.

References